The effectiveness of novel lanthanum-modified bentonite products in reducing phosphorus in water and sediment

M.Sc. Thesis

M. Al-Homsi

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Supervisors: M.F.L.L.W. Lurling
M. Nunes Teixeira Mucci

Project leader: M.F.L.L.W. Lurling

Table of Contents

Abstract:

Eutrophication has been one of the most serious ecological challenges for waterbodies. Reducing nutrient internal loading using in-lake interventions showed remarkable results in controlling this phenomenon. Lanthanum-Modified Bentonite (LMB), under the commercial name Phoslock, has been tested in 200 lakes around the world and its efficiency was proved. New LMB products (LMB2, LMB3 and LMB4) with enhanced phosphorus (P) binding capacity were developed, therefore, testing their efficiency compared to Phoslock became a necessity. This study explores the effectiveness of the novel LMB products in immobilizing phosphorus in aquatic environments. Utilizing Phoslock as a reference to which the three novel LMB products were compared in effectiveness, the study investigates their impact on P immobilization under two different conditions of temperature cold (7°C) and warm (21°C). Two experiments were conducted; the first experiment used sediment cores to evaluate the effectiveness of two novel products in reducing P release from the sediment. The second experiment assessed the binding effectiveness of three novel products with P in the water column, employing two different application methods; slurry and non-slurry, assessing their influence on P binding rates and the overall effectiveness. The novel LMB products exhibited comparable effectiveness to Phoslock with P fluxes signifying their potential as equally efficient alternatives. The results for P fluxes from the sediment to water column were 144.07, -26.52, -92.55 and -34.54 in the cold phase and 1461.23, -320.17, 3.83 and -303.72 $mg.m^{-2}.day^{-1}$ in the warm phase for control, LMB1, LMB2 and LMB3 groups respectively. Results indicated distinct patterns in phosphorus immobilization in water column, with the slurry method demonstrating faster binding rates, particularly for LMB3 and LMB2. Turbidity levels, highest in LMB2 and LMB4 groups during the slurry method, normalize after three days, comparable to the non-slurry method's turbidity after one week. Furthermore, the study examines products' influence on electrical conductivity, dissolved oxygen, and pH levels. Despite slight fluctuations, these environmental abiotic parameters remain within acceptable ranges. The significant decrease in P concentrations in the novel LMB cores compared to control in the warm phase along with the increase in Ca-bound P in the sediment in the cores that were treated with LMB, and the enhanced potential of the novel products in binding P in the water column supports the hypothesis of the enhanced phosphorus immobilization by the novel products.

1. Introduction:

Agriculture, urbanization, and industrialization have been causing excessive nutrient loads to seep into surface waters through fertilizers, fecal matter from livestock, and untreated wastewater (Harper, 1992). This has led to an over-enrichment of surface waters, which in turn has caused a proliferation of algae and cyanobacteria, known as harmful algal blooms (HABs) (Hallegraeff, 2003). This overgrowth can be harmful, as it leads to the deterioration of water quality. Some cyanobacteria produce toxins that threaten aquatic life, as well as the health of people and animals (Osorio-Reyes et al., 2023). Exposure to cyanotoxins can cause a range of health issues in both humans and animals, from skin irritation and respiratory problems to gastrointestinal distress. As well as serious health problems such as liver and kidney damage (Codd et al., 2005). As algae and cyanobacteria grow, they reduce water clarity, depriving the aquatic plants of sunlight and making it difficult for aquatic species to thrive, which can cause further disruption to the ecosystems (Wang & Zhang, 2020). Hence, algal blooms in freshwater bodies can lead to adverse economic implications such as the decline of property values and loss of tourism (El-Shehawy et al., 2012), detrimental sanitation repercussions and environmental hazards, affecting crucial ecosystem services such as potable water supply, agricultural irrigation, as well as the reduction of water's usefulness for recreational activities like swimming and fishing. Consequently, this may put people and animals at risk, affecting their quality of life and overall well-being. The process of increased enrichment of inland and coastal waters with nitrogen (N) and phosphorus (P) inputs that are driven by human activities during the Anthropocene era is referred to as eutrophication (Withers et al., 2014). Mainly the nutrients responsible for eutrophication are nitrogen and phosphorus. Although other elements such as silicone, potassium, iron, manganese and calcium can play a role there (Harper, 1992). Choosing between N and P to limit HABs has been a topic of debate. One important aspect of that debate is that many cyanobacterial species are able to fix gaseous nitrogen (N_2) and use it as a source of N (Reynolds, 1984), contrary to P that does not have an atmospheric cycle, which makes it more difficult to control. Studies also showed that oxyanions (including phosphate PO_4^{-3}) can be effectively immobilized using metal oxides/hydroxides. These materials, synthesized using metals such as aluminum (Al), calcium (Ca), cerium (Ce), iron (Fe), lanthanum (La), magnesium (Mg), zinc (Zn), and zirconium (Zr), have demonstrated successful uptake of oxyanions from water (Bacelo et al., 2020). Freshwater bodies contain sediments that act as sinks for organic and inorganic matter and minerals (Christophoridis & Fytianos, 2006). Sediments in lakes are significant in the overall phosphorus metabolism as they act as both reservoirs and sources of phosphorus (Boström et al., 1988a). The release of P from the sediment is a complex phenomenon that involves several processes chemical, environmental and physical (Christophoridis & Fytianos, 2006). However temperature, pH and redox potential appear to be among the major drivers of phosphorus release from the sediment (mobilization) (Boström et al., 1988b). During summer months, phosphorus is released from the bed sediment into the water column, leading to concentrations that are 200-300% in some lakes, higher than those seen in winter. This process is called internal loading (Søndergaard et al., 2003).

To address the issue of internal P loading, a variety of actions aimed at mitigation may be undertaken. Different interventions (physical, chemical and biological) may have varying degrees of success in terms of alleviating the consequences of eutrophication in the lake (Jørgensen, 2009). Those interventions differ from each other depending on many factors, which requires tailored solutions for each specific case. Some interventions may

be more costly and may come with other consequences for the ecosystems (as in sediment dredging and high-energy ultra-sound) (Lürling & Mucci, 2020), may be associated with ecological consequences such as algaecides and herbicides (Jančula & Maršálek, 2011). In addition to that algal blooms may reoccur if algae is removed without a wise management for P loading (Han et al., 2024). Consequently, the sustainability and long-term endurance of these measures must be considered to ensure that the eutrophication does not reoccur soon. P-binding products are found to yield more immediate and sustainable results compared to other techniques when applied in the appropriate time (Lürling & Mucci, 2020). Overall, managing eutrophication requires careful planning and implementation of strategies that take into account the unique characteristics and challenges of each affected body of water. When source-oriented measures such as external nutrient reductions pose economic or technical difficulties, such as in cases where nutrient pollution comes from diffuse sources, or where political or economic factors are unfavorable, effect-oriented (symptoms-oriented) measures may be indispensable (Lürling et al., 2016). Moreover, even when external nutrient loads are reduced, excessive legacies of internal nutrient load may be released from bed sediments causing prolonged recovery periods lasting from decades to even centuries (Carpenter, 2005). Geo-engineering interventions, which involve the manipulation of lake processes to facilitate recovery including P-removing materials (Waajen et al., 2016), appear to be the most promising among available in-lake interventions; while they are cost-effective and efficient for mitigating eutrophication, they can also produce immediate results (Spears et al., 2013). In addition to that, the longevity of their effects can last for a considerable period up to a decade, when the external load is low, before an introduction of reapplication is required, as demonstrated in the case of lake Rauwbraken where external P load was low (Van Oosterhout et al., 2021). The focus of those interventions is on reducing and sequestering internal nutrient loads in the bed sediment, as well as the nutrients from the water column. Chemical treatments based on metals like Iron (Fe) and Aluminum (Al) have been used to control phosphorus in lakes, but there is growing interest in Lanthanum-modified bentonite (LMB) as an engineered compound due to its distinctive properties and minimal environmental impact. (Dithmer et al., 2016a).

Lanthanum-modified bentonite (LMB), that was first used under the commercial name Phoslock, is among the most effective geo-engineering chemical compounds available for stripping soluble reactive phosphorus (SRP) from both the water column and sediment (Waajen et al., 2016; Lürling et al., 2016b). Phoslock has been implemented in approximately 200 aquatic ecosystems globally and has been subjected to comprehensive assessments at different scales, including laboratory, mesocosm, and whole lake experiments (Copetti et al. 2016). Phoslock has unique characteristics such as its exceptional ability to effectively bind P over a broad range of pH values, exhibiting a high P removal efficiency between pH 5 and 9 (Ross et al., 2008). The maximum binding capacity was at PH=6, while at PH=10 the binding capacity was 2 times lower (Kang et al., 2022). Additionally, its P-binding capacity remains unaffected by anoxic conditions, making it a crucial advantage (Waajen et al., 2016). There was no apparent impact of temperature on the ability of LMB to bind with Phosphorus (Kang et al., 2022). Phoslock, was originally developed by the Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia. Phoslock contains 5% lanthanum and 95% bentonite clay (Afsar & Groves, 2009). Phoslock had been the exclusive commercially-available product for Lanthanum Modified Bentonite (LMB) until recently. However, there are now companies that are actively developing new LMB products with higher levels of Lanthanum (ie 8 and 10%), that is the active ingredient, yet the carrier (bentonite) is different, from there the need emerges to investigate their performance. Nevertheless, there is

not much information in the scientific literature about their efficiency. Thus, the aim of this research is to test the effectiveness in binding mobile P of three newly developed LMB products (named LMB2, LMB3 and LMB4) that vary in their lanthanum content 10, 8 and 10% respectively. An experiment was carried out using LMB1, LMB2 and LMB3 on sediment samples collected from De Vijver, a eutrophic urban pond. And a second experiment was done using water collected from Lumen pond using LMB 1, 2, 3 and 4. The findings of this study will assist in determining the most effective form of LMB in sequestering P in freshwater bodies, which can be helpful in informing future applications of the adequate product to mitigate eutrophication. The chemical inactivation of P using LMB is a promising approach, and further research in this area will help to advance our understanding of how to manage nutrient loads in freshwater ecosystems.

Research objectives research questions:

The objective is to provide knowledge on the effectiveness of novel LMB products (LMB2, LMB3 and LMB4) in order to help water authorities and lake managers make more informed decisions regarding the restoration of eutrophic waterbodies.

This study aims at investigating the difference in the effectiveness of phoslock (LMB1) and the novel LMB products in reducing mobile P from water column and the sediment P release.

General research question:

How does the effectiveness of the novel LMB products (namely; LMB2, LMB3 and LMB4) compare to Phoslock in reducing mobile P concentration from water column and controlling sediment P release?

Specific research questions:

1- How does the effectiveness in the amount of mobile P inactivated from the sediment by LMB2 and LMB3 products compared to LMB1?
2- How fast can LMB2, LMB3 and LMB4 inactivated mobile P in water column compared to LMB1?
3- How do the products LMB1, LMB2 and LMB3 affect the chemistry (turbidity, pH and EC) of the water column?
4- Is the slurry application method of the material more effective in mobile P inactivation than adding the material on top of water column?

Hypothesis:

It is hypothesized that all LMB products will perform similarly in their effectiveness in binding with phosphate, leading to the immobilization of mobile P from both the sediment and the water column in all the sediment cores. On the other hand, due to the higher percentage of La in the novel products, it is expected that they will bind to mobile P at faster rates than Phoslock in the water column when applied in similar quantities. The chemistry indicators of the water (turbidity, pH and EC) is hypothesized to change due to the introduction of LMB products in the water. Since the slurry method implies more area contact of the particles of the products with phosphate in the water, the products are expected to perform better using slurry method compared to adding the material on the top of the water column.

2. Methods and materials:

2.1. Description of study site:

The urban pond De Vijver can be found in Beek en Donk town, situated in Laarbeek, a municipality located in the southern province of North Brabant in the Netherlands. It spans 135.93 m in length and 107.23 m in width, covering an approximate area of 10,503.15 square meters according to the Area Calculator Using Google Earth (n.d.). The pond has an average depth of around 1.5 meters. The pond is surrounded by vegetation and large trees and boasts a small island in the center, where large trees grow. In addition to a high number of waterfowls. Unfortunately, the pond has been a source of concern for the local residents due to eutrophication-related issues. Particularly during the summer season, the pond suffers from severe eutrophication driven by cyanobacterial blooms (Lürling et al., 2010), rendering it unsuitable for recreational activities such as swimming or boating. Experiments were conducted in the pond by students from the AEW group, yielding the following findings: on January 30th, 2023, nutrient concentration samples were collected from the pond and measured. The measurements revealed ortho P at 0.15mgP/L, and TP at 0.18mgP/L. Dissolved oxygen levels were approximately 11mg/L. The dissolved oxygen data was obtained on March 6th, 2023. I used the pond as a model for my experiment and collected sediment cores as illustrated in figure (1), the sampling of the cores took place in April 2023.

Figure 1: Pond De VIjver from a satellite view (Google Earth) with the location of the collection of the sediment as indicated by the white arrow.

2.2. Materials

LMB 1, commercially known as Phoslock, was provided by Phoslock environmental technology and contains around 5% Lanthanum. LMB 2, commercially known as Eutrosorb G, was provided by SePRO corporation and

contains around 10% La. LMB 3 does not have a commercial name and it is not available commercially, the La content is around 8%. LMB 4, commercially known as Zeofixer, was provided by EasterNode and contains around 10% La.

2.3. Experimental Setup:

The sampling plan involved collecting 22 sediment core samples from the pond, using gravity sediment corer (UWITEC), the cores were allocated as follows after sampling:

- 5 replicates for the negative control group.
- 5 replicates for the positive control group, where Phoslock (LMB1) was applied. The effectiveness of Phoslock in P reduction has been previously tested and documented.
- 5 replicates for the LMB2 treatment group.
- 5 replicates for the LMB3 treatment group. (LMB materials were made available by Wageningen U&R).
- 2 sediment cores that were collected to be used in the case of loss of any sediment core during the transportation of the cores to the laboratory.

After sediment cores were collected from the pond, they were transported immediately to the laboratory and stored in storage fridge in the dark at 7 °C. In the day of sampling the following procedure was followed:

- Samples of the initial concentrations of P were taken from each sediment core by taking water samples of 30 ml from the top of the water column using a syringe then it was filtered using a 1.2 µm filter into 50ml PE bottles subsequently the bottles were frozen at -20^{o} C and stored for a later phosphorus content analysis using Autoanalyser (Skalar SAN^{+} segmented flow analyzer) following the Dutch standards protocol NEN 6663 (NNI,1987).
- 2 PE bottles of 500 ml of pond water were collected to use them in the case of loss in water column in the sediment cores.

Before the experiment had been started, various measurements in the sediment cores were taken to evaluate the water quality of the study area such as pH, EC (electrical conductivity) using the multi-meter (Multi 340i). After the measurements, the products (LMB 1,2 and 3) were applied. The dosing of the products were calculated for each core individually according to its water P content as well as the quantity of mobile P in the first 3 cm of the sediment (the sum of the loosely adsorbed P, redox sensitive P (BD-P) and organic-P) in addition to the binding capacity of each product. The amount of mobile P in both the water column and the first 3 cm of the wet sediment was obtained from the previous work of AEW-group students on the same pond. In order to apply a customized quantity of each product, the volume of the water column in each sediment core was calculated. As well as the volume of the first 3cm of the sediment in each core, based on the radius of the core. Thereafter, by multiplying the mobile P wet content µg/g by the density we get the concentration of mobile P in (µg/ml). Then we get the mobile P content of the sediment (µg) in the core by multiplying mobile P (µg/l) by the volume (in cm^3=ml) of the first 3 cm of the sediment. The lanthanum content of each product were provided by the manufacturers of each product, thus the binding capacity, as follows: LMB1: 10 mg P/g, LMB2: 20 mg P/g, LMB3: 17 mg P/g. The total mobile P considering the first 3cm of the sediment was 38.02 mg, which makes the dose of the materials as 1550.14, 775.47, and 913.52 g/m^2 for LMB1, LMB2 and LMB3,

respectively. The products were put in aluminum cups after which, using a syringe, water from the top layer of the water column was poured into the cups in order to make a slurry out of the material and the water before they were sprayed on the water surface so that the whole amount is distributed evenly in the water column and thereafter on the top of the sediment.

The cores were then incubated for 6 weeks in the dark at 7 °C then for 5 weeks at room temperature (21°C) to imitate cold and warm conditions, weekly samples were taken as described before then were stored in the freezer immediately after sampling, in order to measure the P concentration, thereafter, using AutoAnalyser. During the experiment the cores were put under anoxic conditions (Dissolved oxygen (DO) less than 0.5 mg) to imitate a worst-case scenario in which the redox effect is low enough to stimulate the release of P from the sediment, this was achieved by bubbling nitrogen in the cores and measuring DO (using Oxyguard meter) until oxygen level went below 0.5 mg, the bubbling took place only at the beginning of the experiment and it was not needed over the course of the experiment since the cores maintained the anoxic conditions. Other factors were measured such as pH, EC and DO on weekly basis to ensure that the experiment is conducted under controlled conditions and to help interpret the results. One week after application, water samples of 30 ml were taken from the top of the water column of each sediment core, the samples were filtered using the syringe and a 1.2 µm unit filter (Aqua 30/1.2CA, Whatman, Dassel, Germany) and were put in PE bottles of 50 ml after which they were stored in the freezer at -20 °C for a later P content analysis utilizing the AutoAnalyser following the Dutch standards protocol NEN 6663 (NNI,1987). The water samples were taken from the top of the water column every week for 11 weeks. The cores were then filled back to the top with milliQ water to compensate for the loss in water caused by sampling.

The phosphorus (P) flux (mg P/m^2/day) between the sediment and the water column was determined by calculating the variance in P concentrations in the water column at two specific points of time after the start of the experiment following the equation: $P_{flux}= \frac{P\ final - P\ initial}{A*t}$, where P_{final} is the concentration at the end of the phase, $P_{initial}$ is the concentration of P at the beginning of the phase, A is the cross-sectional area of the core and t is the time, in days, between the initial and the final sampling. This calculation resulted in the identification of two distinct fluxes: the first flux was derived from the variance in the concentrations of P between the beginning of the experiment and the moment prior to any alterations in core temperature (named the cold flux), while the second flux was determined from the moment the cores were transitioned to room temperature until the conclusion of the experiment (named the warm flux). At the end of the experiment, the sediment in the cores were sliced until 6cm with 3cm interval using sediment slicer, then stored in zipper storage bags at 7 °C to be subjected later to P sequential extraction protocol, in order to measure the mobile P that had been immobilized and transferred from mobile forms in the water column and the sediment to the apatite form in the top layer of the sediment.

The P sequential fractionation protocol (Paludan& Jensen 1995) (see appendix I) involved immersing the sediment in a series of chemical procedures enabling the extraction of different fractions of phosphorus from the sediment. Each step helps disrupt the bonds between phosphorus and a specific mineral bound to it, this includes the extraction of P from the most bioavailable P fractions to the least bioavailable fractions. The process begins with P loosely adsorbed in pore water, P bound to Fe, organic P, P bound to Al, P bound to Ca.

At the end the process finalizes with the refractory P. All steps except for the last one, involved horizontally shaking tubes and centrifuging them at 3000rpm. The initial process entailed extracting the water fraction using oxygen-free nano-pure water, which released both soluble reactive phosphorus (SRP) of the interstitial water that is loosely adsorbed on the surfaces of Fe and $CaCO_3$. This estimates the immediately available phosphorus. Next extraction was carried out using BD-reagent, the BD-reagent is known for its strong reducing properties and comprising Bicarbonate 0.11 M $NaHCo_3$ and Dithionite 0.11 M $Na_2S_2O_4$. The reagent underwent N_2 bubbling to help release the phosphorus bound to Fe hydroxides, the supernatant was then oxidized by aeration. The third phase involved using a 0.1 M NaOH solution which is assumed to extract SRP sorbed to clay and oxides of Al and also organic P, the supernatant was dark brown due to the organic matter. After acidification the supernatant was slightly colored with precipitate which is perceived as humic acids. The fourth phase releases SRP that is bound to carbonates and apatite-P which is hardly bioavailable, that can be done using 0.5 M HCl. The remaining pellet contains the refractory P, this extraction requires the use of a strong acid, in this case sulfuric acid 2M H_2SO_4 was used after drying the pellet at 105°C then ignition at 550°C.

The determination of both the sediment's dry weight and the organic matter content is an essential procedure at the end of the P fractionation protocol. This is specially necessary to calculate the concentration of each P fraction in the sediment in each g of dry weight, as well as controlling the comparability of the organic matter content between all the cores, allowing for precise quantification of organic P and ruling out any differences in P coming from different organic matter contents. To carry out this procedure, sediment from each interval was precisely weighed in aluminum cups. The aluminum cups were initially weighed both before and after the transferring the sediments from the zipper bags. Subsequently, the cups were placed in the oven at 105°C overnight, with the exact weight recorded afterwards. This step allowed for the determination of the sediment's dry weight and, consequently, its water content. Following that, the aluminum cups containing the dried sediments were ignited in furnace at 505°C. The difference in weight between the dried sediment and the weight after ignition was used to calculate the organic matter content present in the sediment.

2.4. Extra experiment on binding rate of the products:

For this experiment, water from the Lumen pond at Wageningen university was used. The analysis of the filtered pond water using the AutoAnalyzer, revealed an Ortho-P concentration of 0.006 mg/l. Based on two factors the decision was made for the required ortho-P concentration in the cylinders. The factors are ortho-P content in the filtered lake water and the maximum binding capacity of all the products which is LMB2 and LMB4 (that binds 20 mg P for each 1 g of the material). Hence it was decided that the total P concentration in the cylinder should be 25 mg/l which ensured that at least 5 mg/l of P remained unbound, maintaining a P concentration above 0 mg/l once the material reached its full binding capacity. This approach was chosen to maintain experiment realism by preventing complete binding of all available P to the material in order to make valid comparisons between the products. To achieve a spiked P concentration of 25 mg/l in the cylinders, a total of 24.994 mg of phosphorus was added. The water was spiked with 0.14 grams of potassium hydrogen phosphate (K2HPO4) for each liter of filtered pond water.

One gram of each product was added to 1l cylinders of filtered Lumen pond water. The samples were filtered using a vacuum filtration using a 1.2µm glass fiber filters (Whatman GF/C, VWR International B.V.,

Amsterdam, The Netherlands), the experiment was conducted in room temperature (21°C). This experiment was approached in two different methods:

In the first method the products were not mixed with water prior to application and were added directly to the water column. A total number of 12 cylinders was used for this experiment, divided as 3 replicates for each treatment group (LMB1, LMB2 and LMB3) as well as the control group. Aliquots of 30 ml of water samples were taken from the cylinders using a syringe and a 1.2 µm unit filter in order to obtain the actual P content and filter out the products and any micro-organisms that may contain P, and then put into 50 ml PE bottles, and stored in the freezer at -20°C, to be measured later using AutoAnalyser to evaluate the difference in P concentrations, the measurement points for this method were: 1 hour, 3 hours, 1 day, 3 days, 1 week post application. To evaluate the settling rate of the products in water, measurements of turbidity were taken by drawing samples from the top layer of the water using a syringe and subsequently measuring turbidity using turbidity meter (Hach 2100) (Hach, Tiel, The Netherlands) at the following post-application measurement points: 10 minutes, 30 minutes, 1 hour, 3 hours, 1 day, 3 days, 1 week. In the second method another new experiment was conducted using 13 cylinders. The products were mixed in aluminum cups with filtered lake water in order to replicate the actual conditions where a slurry was made out of the products and the water, mimicking the real process prior to application to an actual lake. After conducting the first method a new product (LMB4) was made available. So the distribution of the cylinders was made as follows: 3 replicates for each product (LMB1, LMB2 and LMB3) including the new product (LMB4) in addition to one cylinder for the control group. The water samples of 30ml for P analysis were taken after application using a syringe and a 1.2 µm unit filter, then the samples were put into 50 ml PE bottles. The water samples were taken in the following timing: 1 hour, 3 hours, 1 day, 3 days, 1 week, 2 weeks, 3 weeks. The samples were then stored in the freezer at -20°C, to be analyzed for P later using AutoAnalyser. Turbidity was measured after: 10 minutes, 30 minutes, 1 hour, 3 hours, 1 day, 3 days, 2 weeks, 3 weeks of products application. The binding rates of the products then were calculated based on the initial and final P concentrations in the water divided by the total duration of the experiment.

The unit filters of 1.2µm that were used in the sediment cores and in the binding rate in the water column, after the experiments were finished, the filters were tested for P contamination. The test was carried out using two groups of MiliQ water samples: one control group of 3 samples in which MiliQ water was not filitered using the filters and another group of 3 samples that involved using the filters. For this analysis, a manual phosphate analysis was performed (see appendix II for more details).

2.5. Data process and analysis:

The data obtained from the experiment's measurements were analyzed using the Statistical Package for the Social Sciences (IBM SPSS) software version 25. The analysis included the application of repeated measures ANOVA to assess the influence of the products applied to the sediment cores on phosphorus concentration over time. The same analysis was used to asess the changes in water chemistry over time due to the introduction of the products. The null hypothesis for the main experiment assumes that, on average, there is no difference in the effectiveness of the treatments compared to LMB1 and control groups. It implies that any observed differences in the sample data are due to random variation, and there is no true effect of the

treatments. The alternative hypothesis implies that novel products (LMB2 or LMB3) have more effectiveness in reducing mobile P compared to LMB1 and control groups.

3. Results:

3.1. Phosphorus concentration in the sediment cores:

3.1.1. Cold phase:

Figure 2 presents a visual representation of the weekly measurements of ortho-phosphate (ortho-P) concentrations across the treatment groups, with each line corresponding to a specific treatment group. Notably, the graph reveals trends and fluctuations in ortho-P levels over time. A notable observation is the evolution of error bars accompanying the data points. Initially, the error bars appeared relatively small, indicating lower variability in ortho-P measurements which represents the initial concentrations. However, as the experiment progressed, these error bars noticeably increased in size, which means a growing variability in the cores over time. Each treatment group's line exhibits distinct patterns in relation to the control group (depicted as a reference line). Specifically, LMB1 and LMB3 Group: The lines for the LMB1 and LMB3 treatment groups show significant fluctuations in ortho-P concentrations throughout the experiment. Notably, there are periods where ortho-P levels rise above and drop below the control group line, suggesting dynamic changes in this group's response to the treatment. LMB2 group appear to keep low concentrations consistently over the course of this phase of the experiment. This observation indicates a unique response. However, all the mean concentrations of ortho-P for LMB groups exhibited a decline, transitioning from 14.15 (±0.76), 12.10 (±0.61) and 10.85 (±1.12) µg/l at the initiation of this phase to 11.08 (±7.70), 1.38 (±0.33) and 6.85 (±1.83) µg/l at the conclusion, for LMB1, LMB2 and LMB3 respectively. In contrast, the mean concentration of the control group increased from 9.39 (±0.55) to 26.07 (±5.84) µg/l.

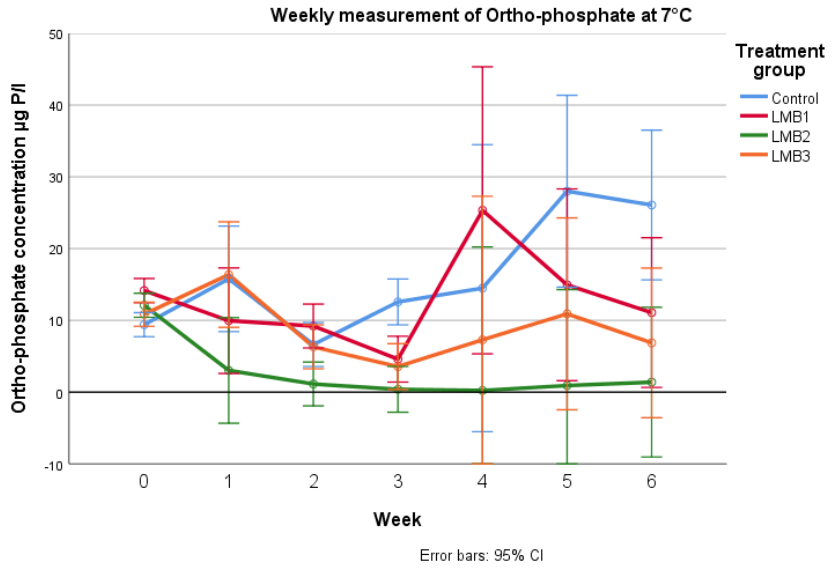

Figure 2 weekly concentrations of ortho-P in the cold phase

The results of multiple measurement ANOVA function for ortho-P concentrations of the part of the experiment conducted at 7°C are summarized in the table 1. The table shows that there was no statistically significant difference ($p > 0.05$) in the means between the control group and two of the treatment groups, LMB1 and LMB3. However, a significant difference ($p < 0.05$) was observed when comparing the control group to LMB2. Additionally, there was no significant difference between the means of the treatment groups themselves. Variance analysis between pairs of treatment groups (LMB1, LMB2), (LMB1, LMB3), and (LMB2, LMB3) did not yield significant results, which resulted in two homogeneous groups (LMB1, LMB2 and LMB3) and (Control, LMB1 and LMB3) .

Multiple Comparisons

Measure: MEASURE_1

Bonferroni

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	LMB1	3.381714286	3.680550269	1.000	-7.69060968	14.45403825
	LMB2	13.39800000	3.680550269	.013	2.325676038	24.47032396
	LMB3	7.257142857	3.680550269	.397	-3.81518111	18.32946682
LMB1	LMB2	10.01628571	3.680550269	.091	-1.05603825	21.08860968
	LMB3	3.875428571	3.680550269	1.000	-7.19689539	14.94775253
LMB2	LMB3	-6.14085714	3.680550269	.688	-17.2131811	4.931466820

Based on observed means.
The error term is Mean Square(Error) = 33.866.

Table 1 Pairs comparison from Post Hoc test for ortho-P measurements in cold conditions

The bar chart depicted in Figure 3 illustrates ortho-P fluxes from the water column to the sediment within the treatment groups. Notably, LMB2 stands out as having the most prominent downward flux among all the tested LMB products under these conditions. In contrast, the control group shows a notably high positive flux, indicating flux from the sediment to the water column. The ortho-P fluxes for the control, LMB1, LMB2, and LMB3 treatment groups are 144.07 (±5.87), -26.52 (±7.74), -92.55 (±0.70), and -34.54 (±2.15) µg P/m2/day, respectively. Positive values signify a flux from the sediment to the water column, while negative values denote a flux in the opposite direction.

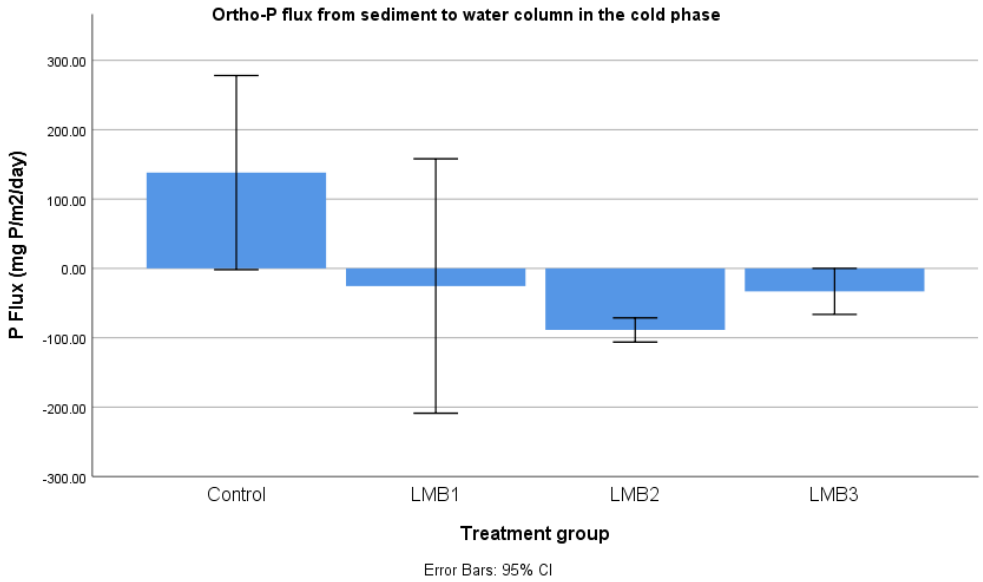

Figure 3 ortho-P fluxes from the sediment to the water column in the cold phase

At the commencement of this phase, the mean pH for all sediment cores was 7.96 (±0.006). Over the initial 6 weeks of the experiment, depicted in Figure 4, the pH averages for the control, LMB1, LMB2, and LMB3 groups were 7.79 (±0.2), 7.22 (±0.25), 7.98 (±0.14), and 7.67 (±0.27), respectively. Using the Post Hoc test in the repeated measures ANOVA indicated statistical significance when comparing the means of the LMB1 group against all other treatment groups. The mean difference results suggest a lower pH value for the LMB1 group throughout the cold phase compared to the other treatment groups. However, no significance was observed in the difference in means between the other LMB groups and the control group ($p>0.05$) suggesting no change in pH for those groups (see table 2 and figure 4).

Multiple Comparisons

Measure: MEASURE_1

Bonferroni

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Con	LMB1	.5693	.06866	.000	.3608	.7777
	LMB2	-.1890	.06866	.089	-.3975	.0195
	LMB3	.1186	.07282	.746	-.1025	.3397
LMB1	LMB2	-.7583	.06866	.000	-.9667	-.5498
	LMB3	-.4507	.07282	.000	-.6718	-.2296
LMB2	LMB3	.3076	.07282	.004	.0865	.5287

Based on observed means.
The error term is Mean Square(Error) = .012.

Table 2 Pairs comparison from Post Hoc test for pH measurements in cold conditions

The mean values for EC over time were 430.72 (±22.57), 470.07 (±20.88), 453.95 (±19.91), and 398.35 (±85.05) µS/cm for control, LMB1, LMB2 and LMB3 respectively. Post Hoc multiple comparisons among all groups

(except for the pair LMB1-LMB2) showed statistical significance ($p<0.05$) which indicates for differences in the electrical conductivity between those groups, yet the decrease in EC across all the groups was notable in the EC graph (see table 3 and figure 4).

Multiple Comparisons

Measure: MEASURE_1

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Con	LMB1	-39.3500	7.42005	.000	-61.6720	-17.0280
	LMB2	-23.2250	7.42005	.039	-45.5470	-.9030
	LMB3	22.4250	7.42005	.049	.1030	44.7470
LMB1	LMB2	16.1250	7.42005	.271	-6.1970	38.4470
	LMB3	61.7750	7.42005	.000	39.4530	84.0970
LMB2	LMB3	45.6500	7.42005	.000	23.3280	67.9720

Based on observed means.
The error term is Mean Square(Error) = 137.643.

Table 3 Pairs comparison from Post Hoc test for EC measurements in cold conditions

Over the course of this phase of the experiment, the 20 sediment cores maintained anoxic conditions consistently, with dissolved oxygen (DO) values below the pre-defined threshold for anoxic conditions (DO<0.5 mg/l). The mean DO values for the treatment groups were 0.14 (±0.04), 0.15 (±0.06), 0.13 (±0.04), and 0.11 (±0.02) mg/l for the control, LMB1, LMB2, and LMB3, respectively. Additionally, Post Hoc comparisons revealed no statistical significance between the control group and all LMB groups ($p<0.05$) (see table 4 and figure 4).

Multiple Comparisons

Measure: MEASURE_1

Bonferroni

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Con	LMB1	-.0083	.01114	1.000	-.0418	.0252
	LMB2	.0063	.01114	1.000	-.0272	.0398
	LMB3	.0297	.01114	.101	-.0038	.0632
LMB1	LMB2	.0146	.01114	1.000	-.0189	.0481
	LMB3	.0380	.01114	.021	.0045	.0715
LMB2	LMB3	.0234	.01114	.310	-.0101	.0569

Based on observed means.
The error term is Mean Square(Error) = .000.

Table 4 Pairs comparison from Post Hoc test for DO measurements in cold conditions

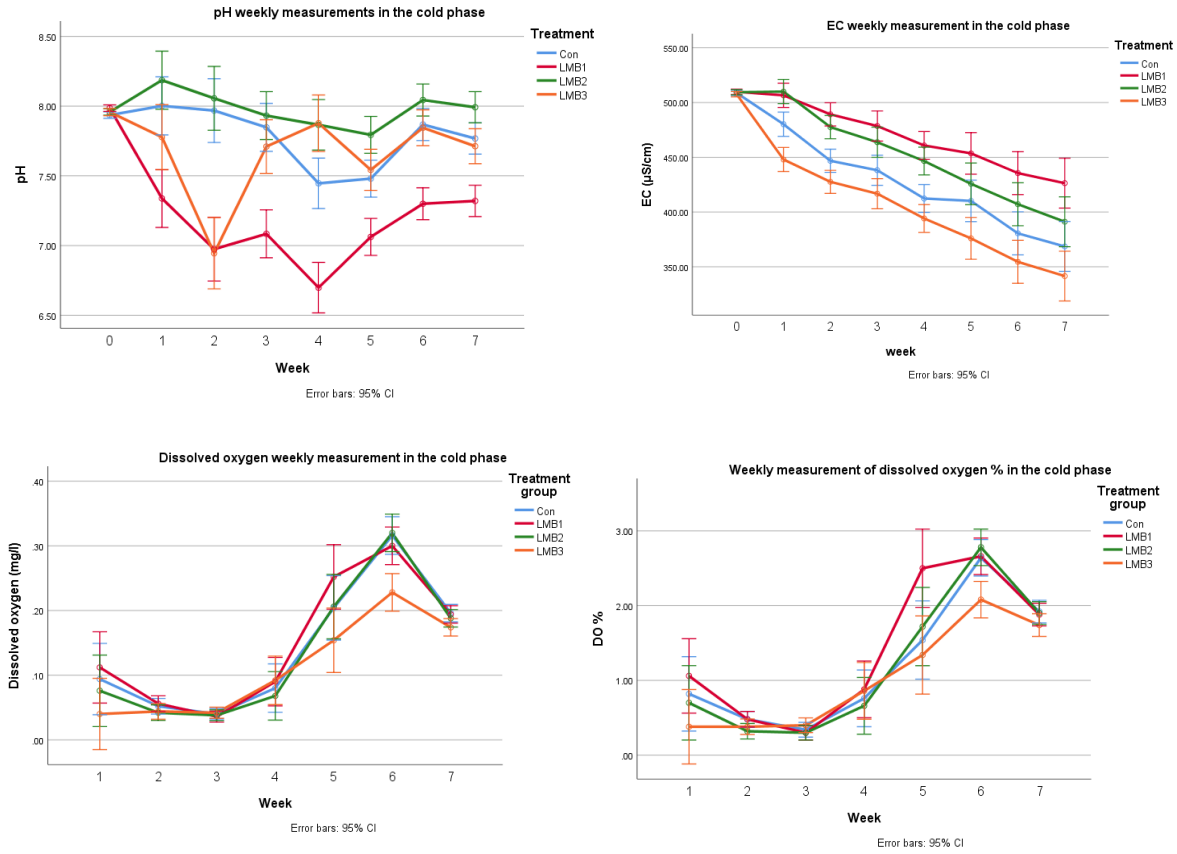

Figure 4 Weekly measurements of PH, EC and DO in the cold phase

3.1.2 Warm phase:

In contrast to the observations under cold conditions, the data presented in Figure 3 for warm conditions revealed a substantial divergence between the control and the other LMB treatment lines. While control mean concentrations for ortho-P increased to high concentrations from 74.68 (±11.55) to 175.452 (±30.81) µg P /l, while the means of the LMB groups maintained low concentrations LMB2 5.41 (±0.87) to 5.67 (±3.45) LMB1 and LMB3 means decreased from 45.79(±20.52) and 26.08 (±4.66) to 23.71 (±12.20) and 5.13(±0.99) µg P/l, respectively. The distinct separation of these lines indicates a significant effect of the treatment in warm conditions underscoring the role of temperature as a contributing factor in the observed outcomes.

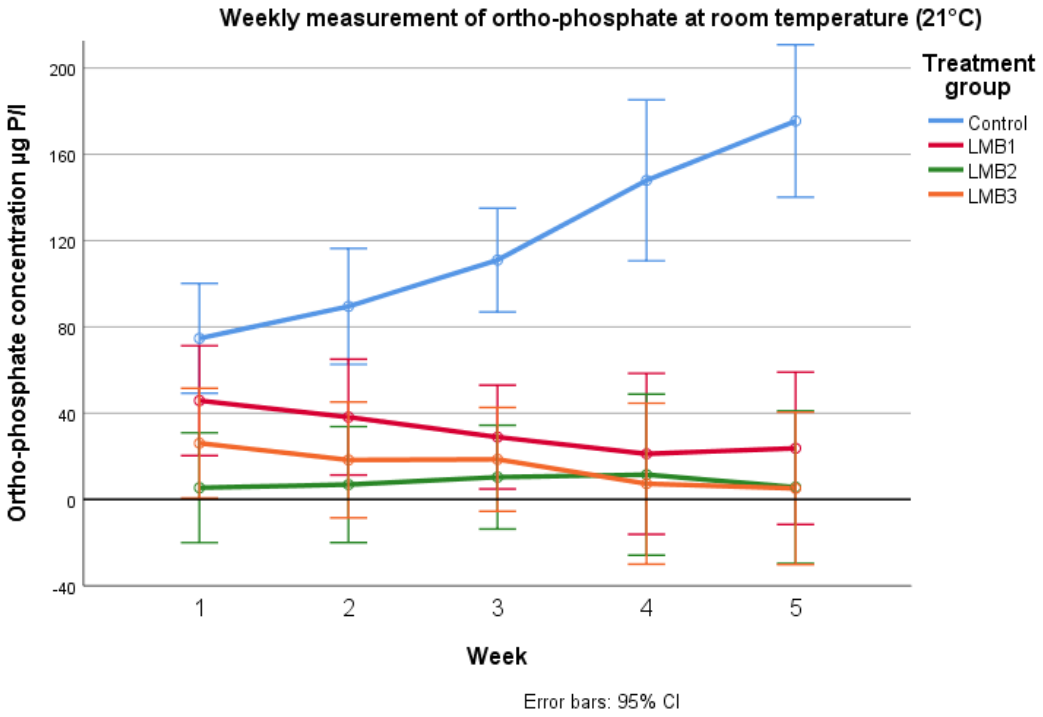

Figure 5 weekly measurement of ortho-P in warm conditions. Error bars: 95% CI.

The variance between the control group and each of the treatment groups demonstrated a statistically significant difference ($p<0.05$). This underlines the treatment's capacity to induce changes in ortho-P concentrations under warmer environmental conditions. Conversely, when we examine the variance between pairs of treatment groups, a different pattern emerges. In this case, the differences between treatment group pairs showed no statistical significance. This suggests that, within the various treatment groups, the responses are comparable, and there are no pronounced variations between them regarding ortho-P concentrations (see table 5).

Multiple Comparisons

Measure: MEASURE_1

	(I) Product	(J) Product	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bonferroni	Con	LMB1	88.16800000	18.44358325	.001	32.68354421	143.6524558
		LMB2	111.7548000	18.44358325	.000	56.27034421	167.2392558
		LMB3	104.6340000	18.44358325	.000	49.14954421	160.1184558
	LMB1	LMB2	23.58680000	18.44358325	1.000	-31.8976558	79.07125579
		LMB3	16.46600000	18.44358325	1.000	-39.0184558	71.95045579
	LMB2	LMB3	-7.12080000	18.44358325	1.000	-62.6052558	48.36365579

Based on observed means.
The error term is Mean Square(Error) = 850.414.

Table 5 Pairs comparisons from Post Hoc test for ortho-P measurements in warm conditions

LMB2 exhibited the lowest flux from the water column to the sediment, reaching a level that could be considered negligible at 3.83 (±3.56) µg P/m2/day. On the other hand, both LMB1 and LMB3 displayed more substantial negative fluxes during this phase, measuring -320.17 (±23.87) and -303.72 (±4.77) µg P /m2/day

respectively. In contrast, the control group demonstrated the highest positive flux, indicating a significant movement from the sediment to the water column at 1461.23 (±32.90) µg P/m2/day (refer to figure 6).

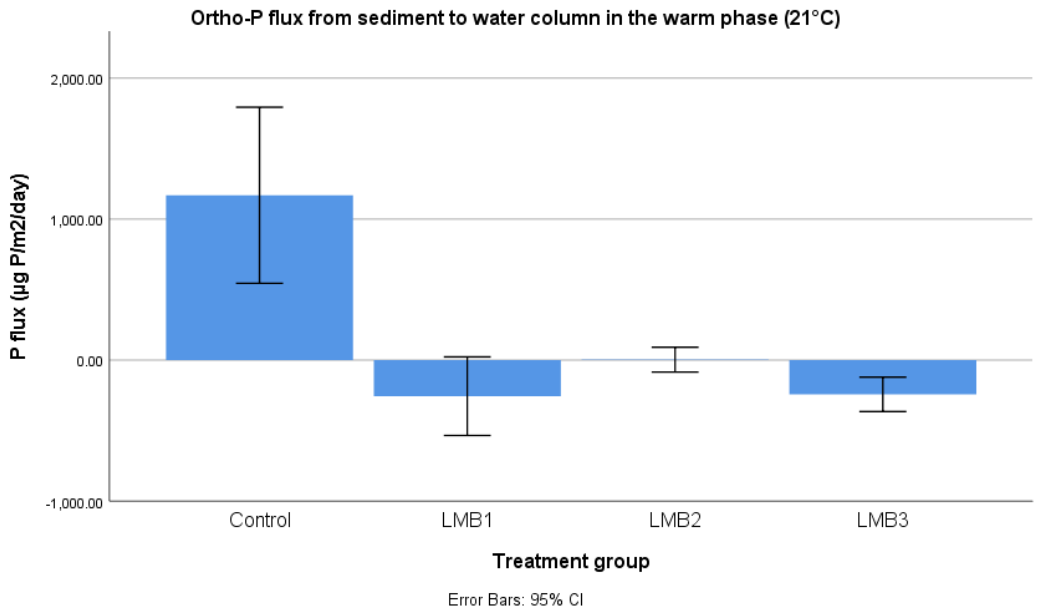

Figure 6 Ortho-P fluxes in the warm phase from the sediment to the water column. Error bars: 95% CI.

Since this phase is an extension of the previous one, distinct shifts in the initial pH readings among the treatment groups were observed. However, these readings ranged between the neutral pH value (pH=7) and slightly above it (pH=8). Initially, the pH means were recorded for the control, LMB1, LMB2, and LMB3, respectively as 7.72 (± 0.024), 7.39 (±0.035), 7.9 (±0.036), and 7.45 (± 0.07) as well as the average pH measurements over time 7.46(±0.07), 7.15 (±0.15), 7.75 (±0.07), 7.37(±0.13). Similar to the first phase, significant differences in pH means were highlighted by a repeated measures ANOVA, particularly between the LMB1 group and all other groups (LMB1-other group) resulted in $p<0.05$ (LMB1-LMB2: $p<0.001$,LMB1-LMB3: $p<0.001$, LMB1-Control: $p<0.001$). The differences in the means of the pairs (LMB1-other group) were negative, indicating that lower pH values were exhibited by the LMB1 group compared to the other groups. Similarly, statistical significance emerged in the comparison of LMB2 means with all other treatment groups ($p<0.05$), with positive differences implying higher pH values in LMB2 than in the other treatment groups. Notably, no statistical difference in means was observed between LMB3 and the control ($p>0.05$) (see table 6 and figure 7).

Multiple Comparisons

Measure: MEASURE_1

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Con	LMB1	.3060	.05565	.000	.1386	.4734
	LMB2	-.2940	.05565	.000	-.4614	-.1266
	LMB3	.0828	.05565	.937	-.0846	.2502
LMB1	LMB2	-.6000	.05565	.000	-.7674	-.4326
	LMB3	-.2232	.05565	.006	-.3906	-.0558
LMB2	LMB3	.3768	.05565	.000	.2094	.5442

Based on observed means.
The error term is Mean Square(Error) = .008.

Table 6 Pairs comparison from Post Hoc test for pH measurements in warm conditions

Unlike the cold phase, there were no statistical significant differences in EC means between the pairs comparing the control group to the LMB groups except for the LMB3 group where P value was below 0.05. In fact, the statistical significance ($p<0.05$) was present in all pairwise comparisons involving the LMB3 treatment group with the other treatment groups in Post Hoc test. The pairs comparing the means of (LMB3 – other groups' means) always resulted in negative values, indicating lower values of EC mean for LMB3 compared to the means of the other groups. This implies a significant alteration in EC due to the introduction of LMB3 treatment. The mean EC values for the control, LMB1, LMB2, and LMB3 groups were 368.28 (±30.18), 376.52 (±35.39), 365.36 (±20.55), and 312.88 (±28.84), respectively (table 7 and figure 7).

Pairwise Comparisons

Measure: MEASURE_1

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Con	LMB1	-8.240	13.798	1.000	-49.749	33.269
	LMB2	2.920	13.798	1.000	-38.589	44.429
	LMB3	55.400	13.798	.006	13.891	96.909
LMB1	LMB2	11.160	13.798	1.000	-30.349	52.669
	LMB3	63.640	13.798	.002	22.131	105.149
LMB2	LMB3	52.480	13.798	.009	10.971	93.989

Based on estimated marginal means

b. Adjustment for multiple comparisons: Bonferroni.

Table 7 Pairs comparison from Post Hoc test for EC measurements in warm conditions

A slight elevation in DO levels was recorded in all the sediment cores in the last week of this phase. Despite that elevation DO levels remained in the tolerated range for the pre-defined anoxic conditions DO< 0.5 mg O_2/l (in fact no treatment group mean exceeded 0.15 mg O/l). Post Hoc test comparisons showed no statistical significance ($P \leq 0.05$) in the comparisons of LMB groups means against control means, except for LMB2 group which exhibited a statistical significance against all other treatment groups, with the LMB2 DO mean below the

means of the other groups. Nevertheless, the DO means for all treatment groups were below 0.5 mg O/l with values of 0.03 (±0.01), 0.03 (±0.02), 0.02 (±0.004), and 0.03 (±0.01) for control, LMB1, LMB2 and LMB3 respectively (table 8 and figure 7).

Multiple Comparisons

Measure: MEASURE_1

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bonferroni	Con	LMB1	.0020	.00253	1.000	-.0056	.0096
		LMB2	.0124	.00253	.001	.0048	.0200
		LMB3	.0048	.00253	.456	-.0028	.0124
	LMB1	LMB2	.0104	.00253	.005	.0028	.0180
		LMB3	.0028	.00253	1.000	-.0048	.0104
	LMB2	LMB3	-.0076	.00253	.050	-.0152	.0000

Based on observed means.
The error term is Mean Square(Error) = 1.60E-005.

Table 8 Pairs comparison from Post Hoc test for DO measurements in warm conditions

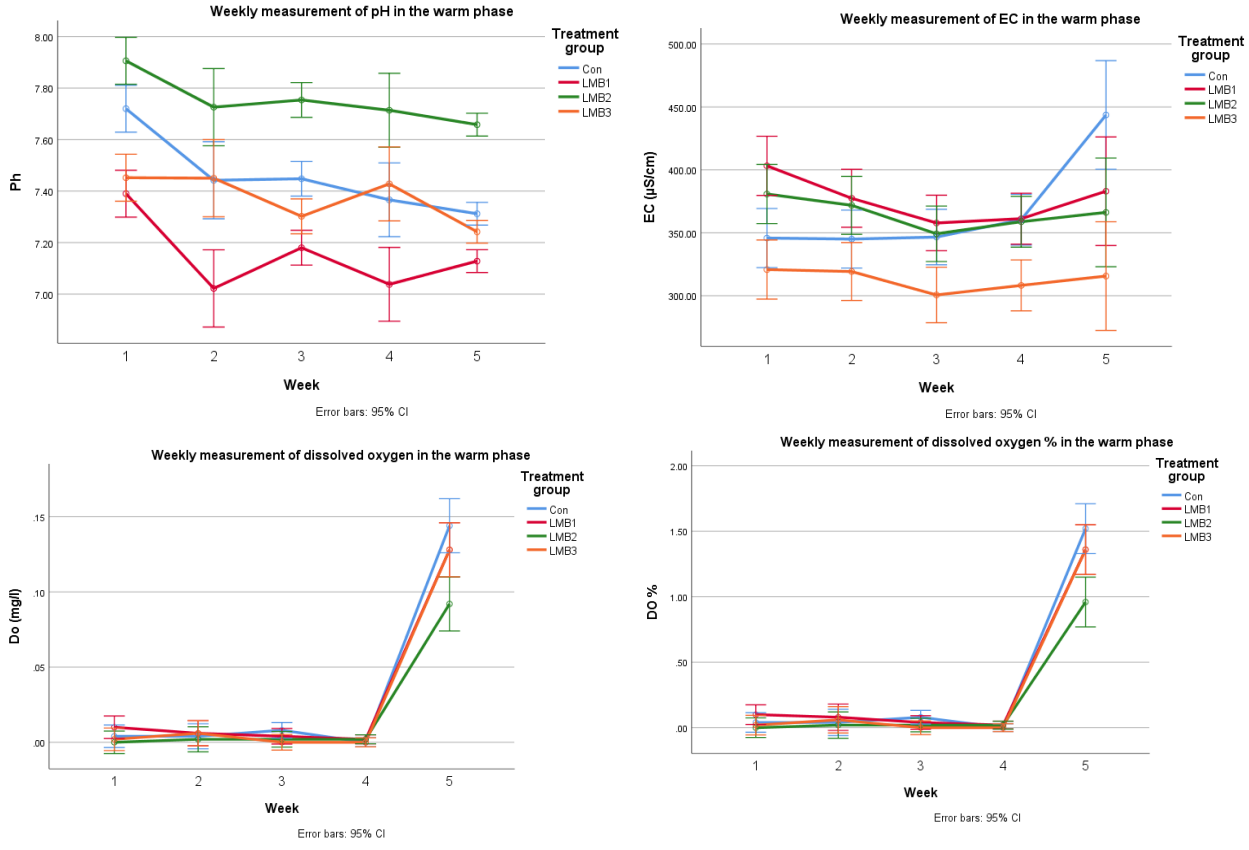

Figure 7 Weekly measurements of pH, EC and DO in the warm phase. Error bars: 95% CI.

3.1.3. The comparison between cold and warm phases:

The warm and cold graphs were integrated into a single graph (Figure 8) to facilitate a visual comparison between the two phases. Before exposing the sediment cores to warmer conditions, the graph illustrates a phase where the data points remain relatively steady, indicating no significant differences in phosphorus concentrations between the control and other treatment groups. However, once the sediment cores are subjected to warmer conditions, a distinct divergence becomes apparent between the control and treatment groups. Concentrations in the control group show a gradual increase, while the treatment groups consistently exhibit a decrease.

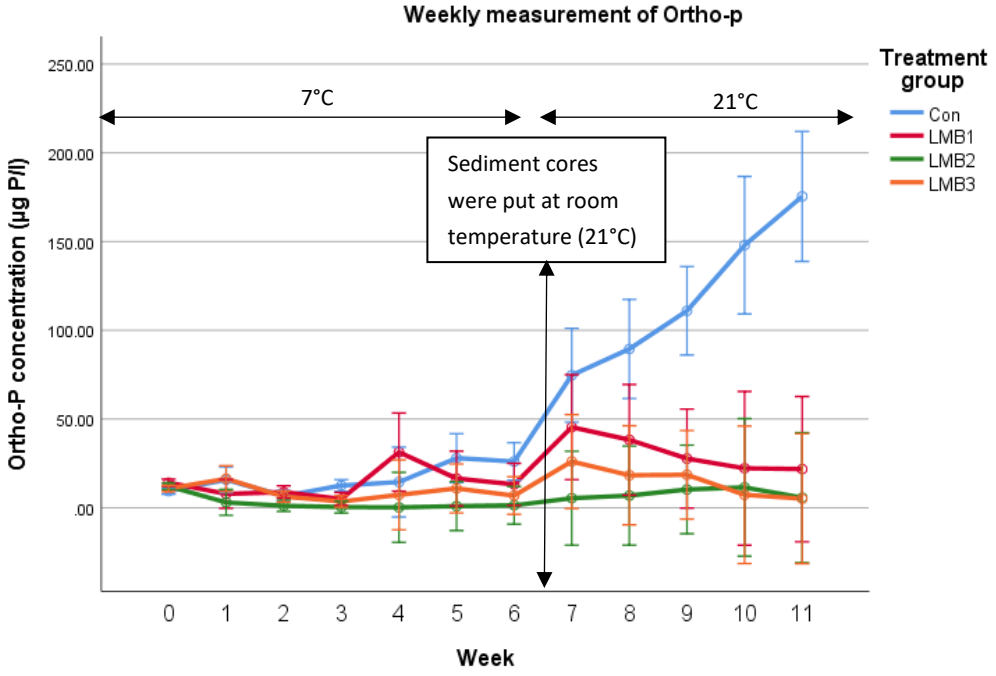

Figure 8 weekly measurement graph for the whole period of the experiment combining cold and warm phases. Error bars: 95% CI.

3.2. Sediment Psenner protocol:

The average values of total phosphorus (TP) content in the upper 6cm layer of sediment were 0.98, 0.90, 0.84, and 0.90 mg P/g dry weight (DW) sediment for the control, LMB1, LMB2, and LMB3 groups, respectively. Considering the first 6cm the mobile phosphorus fraction, which includes: labile P = (TP from water fraction), organic P =((TP from NaOH-P fraction) – (SRP NaOH-P fraction)), and Fe-P = (TP from BD fraction), constituted 52.56%, 48.14%, 46.69%, and 44.47% while the Immobile P (Ca-P =(TP HCl), Al-P =(SRP NaOH) and residual P = (TP H_2SO_4)) constituted 47.44%, 51.86%, 53.31%,and 55.53% of TP for control, LMB1, LMB2 and LMB3 respectively. Notably, the mobile P fractions had decreased values in the first 3 cm in the LMB treated sediment cores compared to control cores. Water fraction constituted 0.0018% for control sediment of the TP while in LMB treatment groups LMB1=0.0007%, LMB2=0.0008% and LMB3=0.0006%. Similarly, the percentage of Fe-P was lower in LMB sediment samples (44.83%, 43.03%, and 39.25% for LMB1, LMB2, and LMB3, respectively) compared to the control group (52.68%). As well as in organic-P control= 1.26%, LMB1=1.17%, LMB2=0.74%,

LMB3=0.67% . Conversely, HCl-P in the first 3cm had an increased value in the LMB samples (LMB1=24.57%, LMB2=29.71%, and LMB3=33.10%) compared to the control sample 15.55%. It is worth mentioning here that the calculations of organic-P fraction resulted in negative values in some of the samples, which was attributed to the detection limit of the Autoanalyser which is 10 µg/l, therefore the negative values were substituted by 10 µg/l. The stacked-clustered bar chart (figure 9) depicts the magnitude of P content in all the P fractions. The organic matter content exhibited uniformity across all sediment samples, hovering around approximately 10% (figure 10).

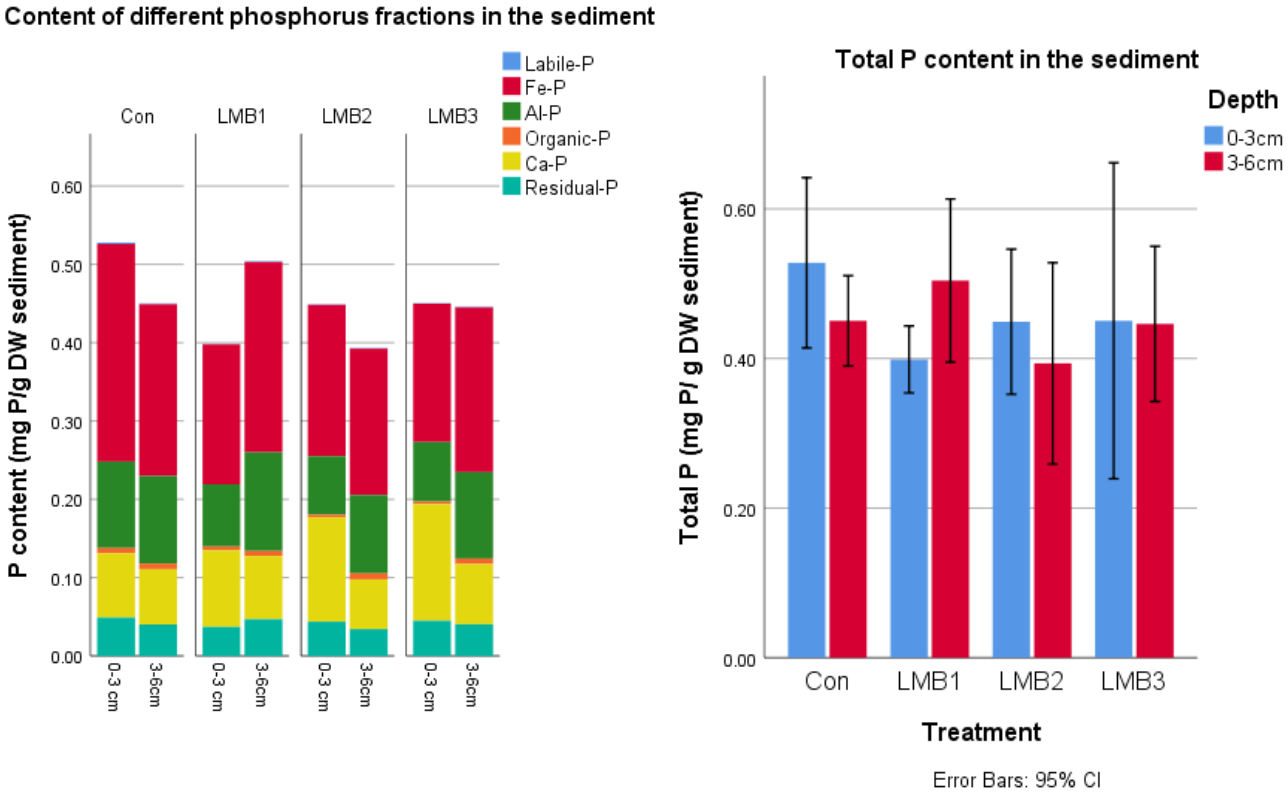

Figure 9 Content of P extracted from different fractions two different depths of the sediment (0-3 and 3-6 cm) sliced at the end of the experiment of all the treated sediment cores.

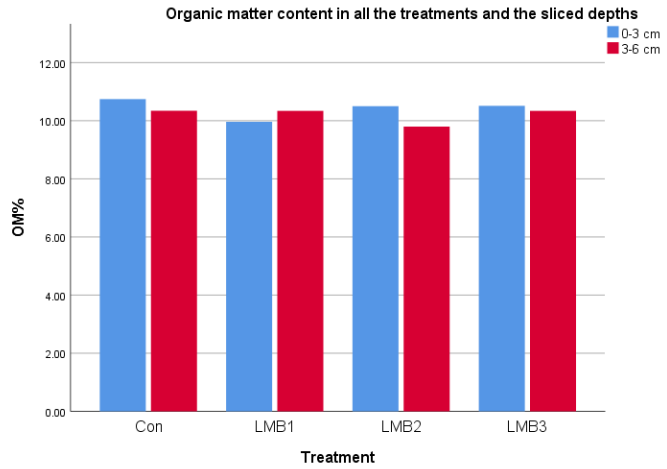

Figure 10 Organic matter content % in the sediment samples for treatment groups, each clustered on the two sliced depths (0-3cm and 3-6cm).

3.3. Binding rate experiment:

3.3.1. First rate experiment:

In this experiment in which the material was not mixed with water before application and the powder was added to the surface of the water, the binding rates for the materials were for ortho-P 0.984, 1.028 and 2.228 mg P/ g material /day and for total P 1.810, 0.898, 1.381 mg P/g material /day for LMB1, LMB2 and LMB3, respectively (figure 11) after one week of application. Hence, the order of the products according to the binding rate is as follows from fastest to slowest: LMB3, LMB2 and LMB1. There was a strong statistical significance in the difference of the means only in the pairs that include LMB3 group ($p<0.05$), the differences in the means indicate low ortho-P concentrations in LMB3 group compared to the other groups (table 9).

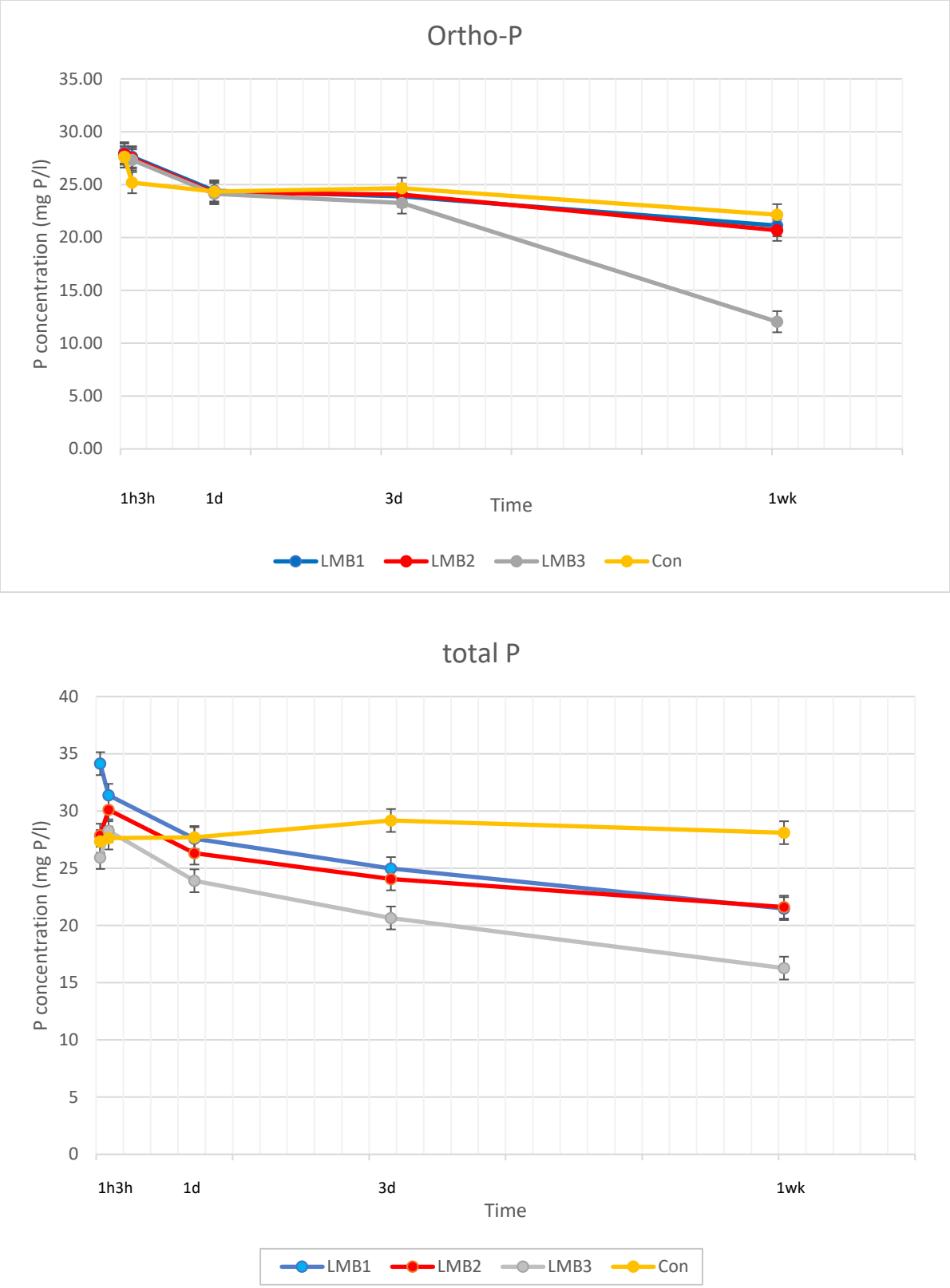

Figure 11 ortho-P and total P concentrations for treatment groups 1 hour, 3 hours, 1 day, 3 days, and 1 week after applying the materials

Multiple Comparisons

Measure: MEASURE_1

	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bonferroni	Con	LMB1	-.2207	.34927	1.000	-1.4357	.9944
		LMB2	-.0873	.34927	1.000	-1.3024	1.1277
		LMB3	1.9113	.34927	.004	.6963	3.1264
	LMB1	LMB2	.1333	.34927	1.000	-1.0817	1.3484
		LMB3	2.1320	.34927	.002	.9169	3.3471
	LMB2	LMB3	1.9987	.34927	.003	.7836	3.2137

Based on observed means.
The error term is Mean Square(Error) = .183.

Table 9 multiple comparisons for the treatment groups

Turbidity: After 10 minutes of applying the materials in the cylinders the order of the materials in their turbidity was LMB2, LMB1, LMB3 and control with the values of 22.66, 4.1, 3.34, 1.19 NTU respectively. However the material turbidity had settled down after 3 days of the application of the materials with approximately similar values to that of the control group (figure 12).

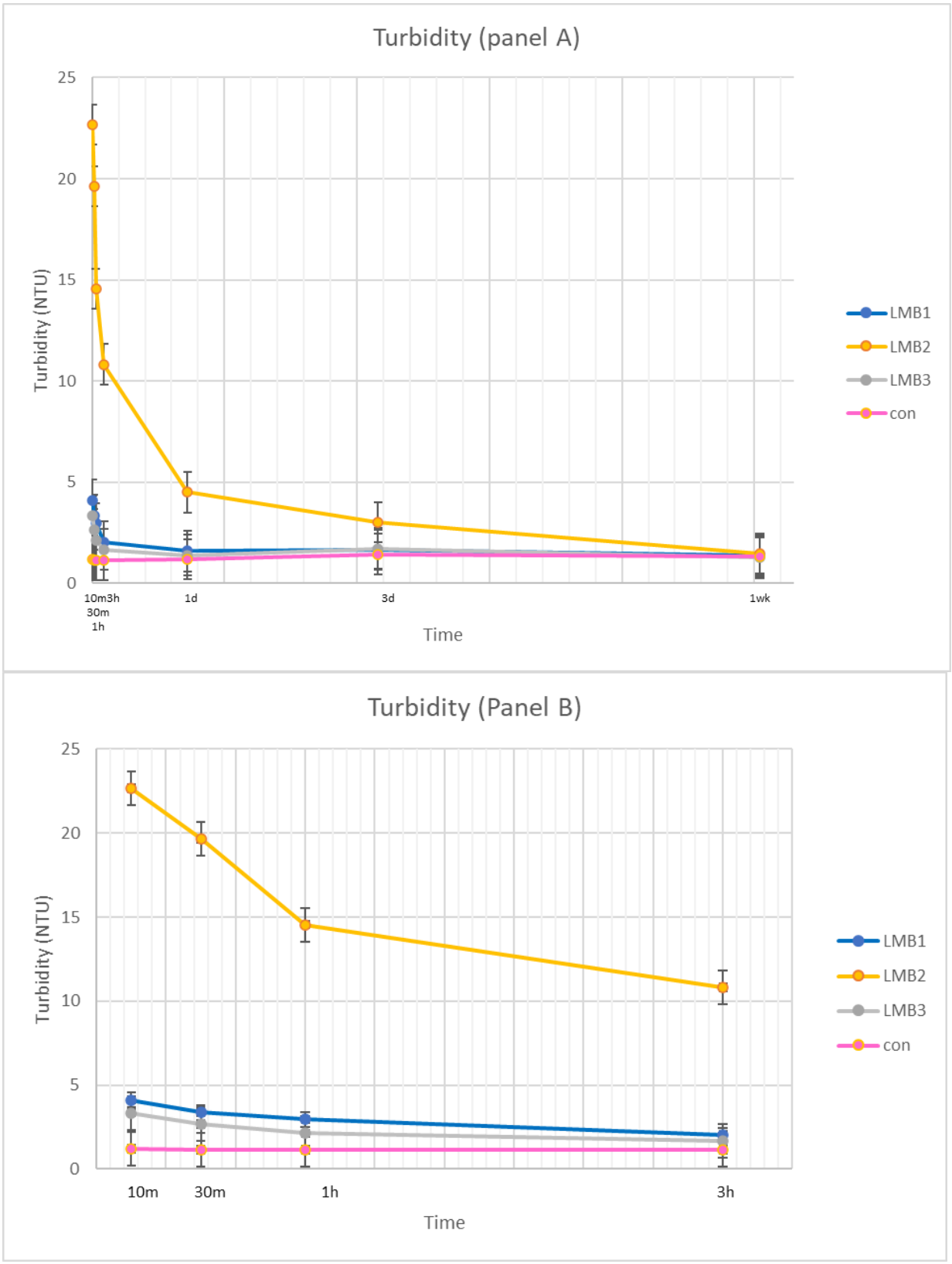

Figure 12 Turbidity in the cylinders after 10 minutes, 30 minutes, 1 hour, 1 day, 3 days and 1 week after applying the materials (Panel B is a zoomed version of the first 3 hours in Panel A)

3.3.2. Second rate experiment:

The binding rate for the materials resulted as follows: 1.056, 1.054, 1.071, 1.555 mg P/g material / day for ortho-P and 1.002, 0.973, 0.791, 1.443 mg P/ l / day for total P for LMB1, LMB2, LMB3 and LMB4 respectively. Consequently, the order of the material based upon their binding rate was as follows beginning from the highest: LMB4, LMB3, LMB1 and LMB2 (figure 13).

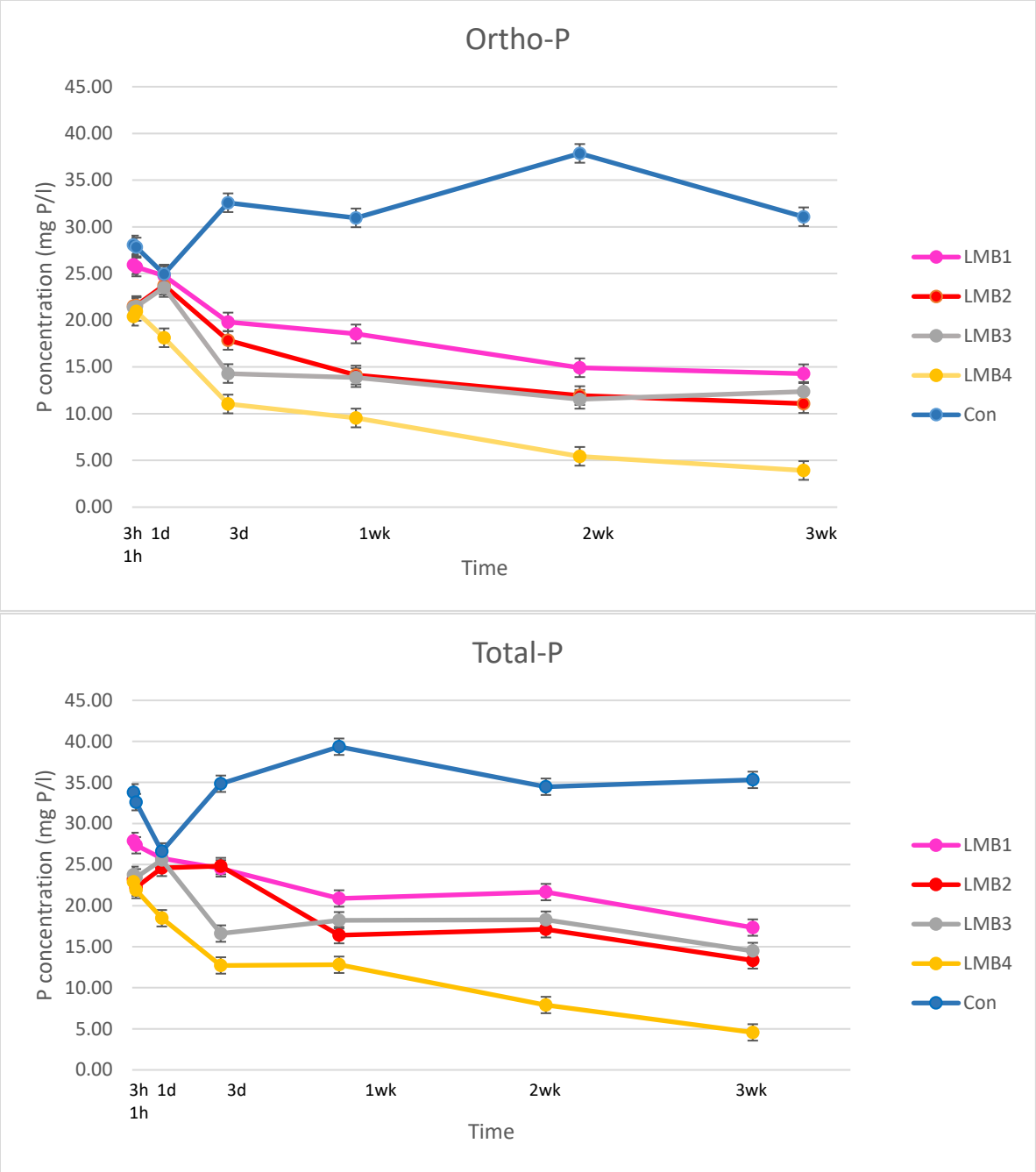

Figure 13 Ortho-P and total P concentrations for the treatment groups 1 hour, 3 hours, 1 day, 3 days, 1 week, 2 weeks, and 3 weeks after applying the materials.

Pairwise comparisons based on estimated marginal means showed strong significance between the control group and all LMB treatments with positive value of the difference, indicating lower concentrations of P as a result of the introduction of LMB products, as well as a strong significance in the pairs involving LMB4 with all the other LMB treatments with values indicating a relatively lower concentrations of P in LMB4 samples compared to the other LMB samples (Table 10).

Pairwise Comparisons

Measure: MEASURE_1

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Con	LMB1	9.909	.691	.000	7.261	12.556
	LMB2	13.068	.691	.000	10.420	15.716
	LMB3	13.562	.691	.000	10.915	16.210
	LMB4	17.698	.691	.000	15.050	20.345
LMB1	LMB2	3.160	.489	.002	1.287	5.032
	LMB3	3.654	.489	.001	1.782	5.526
	LMB4	7.789	.489	.000	5.917	9.661
LMB2	LMB3	.494	.489	1.000	-1.378	2.367
	LMB4	4.630	.489	.000	2.757	6.502
LMB3	LMB4	4.135	.489	.000	2.263	6.008

Based on estimated marginal means

b. Adjustment for multiple comparisons: Bonferroni.

Table 10 pairwise comparisons of treatment groups based on the estimated marginal means

Turbidity: Blending the substances prior to use led to notably elevated turbidity levels, particularly for LMB2 and LM4. The turbidity values recorded were 9.68 NTU for LMB1, 261.67 NTU for LMB2, 12.61 NTU for LMB3, 190.33 NTU for LMB4, and 1.94 NTU for the control. 3 days post application, these groups had a significant decrease in turbidity levels, while LMB1 and LMB3 values had approached similar values of the control. The turbidity measurement 1 week after application did not take place because of a technical problem. However, by the end of a 2-week period, all treatment groups had stabilized in terms of turbidity (figure 14).

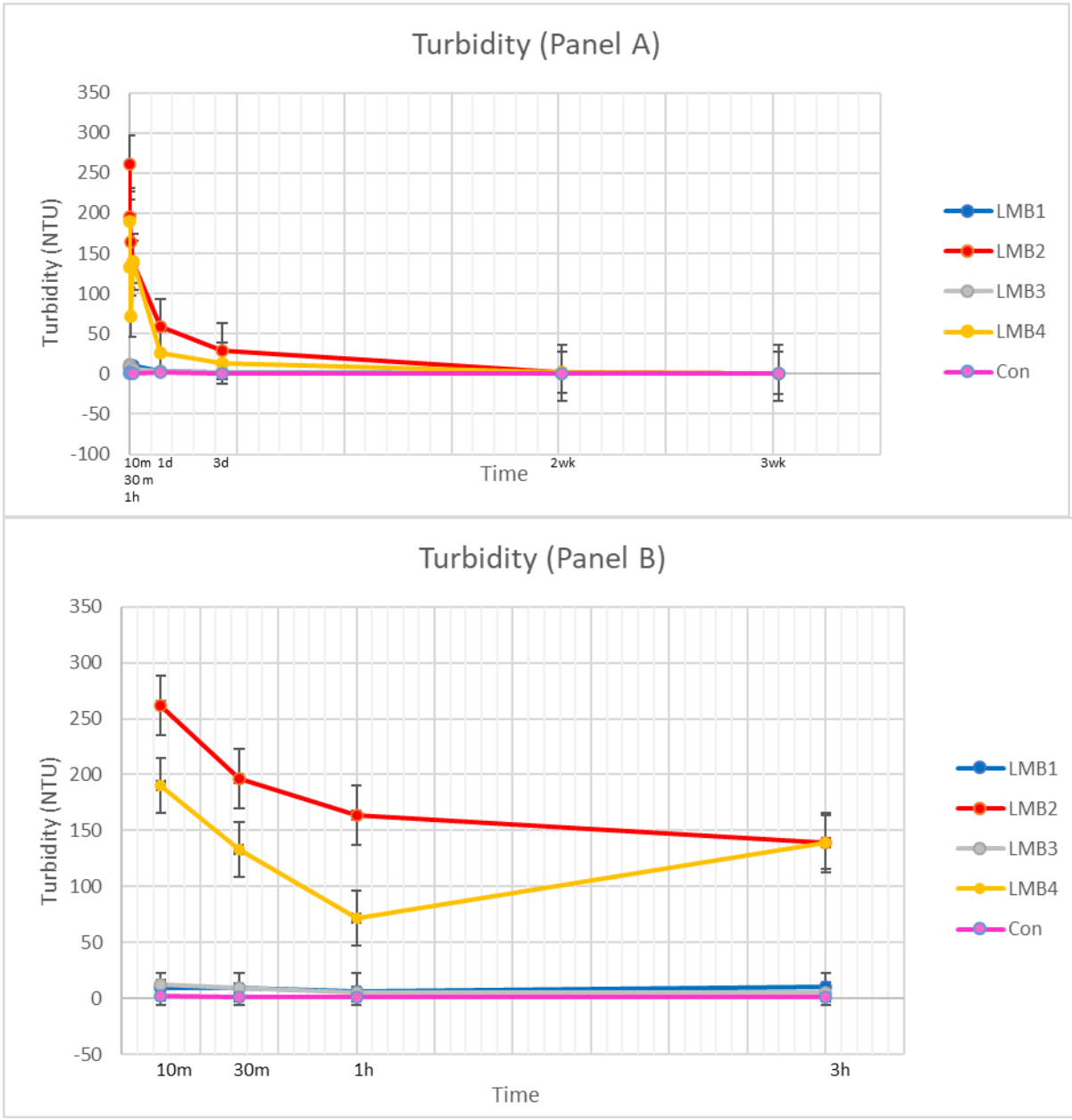

Figure 14 Turbidity in the treatment groups after 10 minutes, 30 minutes, 1 hour, 3 hours, 1 day, 2 weeks and 3 weeks of application (Panel B is a zoomed version of the first 3 hours of Panel A)

4. Discussion:

In light of the existing literature, the main focus revolved around the assessment of the effectiveness of Phoslock which had been the exclusive lanthanum-modified bentonite (LMB) product that is commercially available before LMB2, LMB3 and LMB4 were introduced to the market. Phoslock has a 5% lanthanum content and a binding capacity of 10 mg P/g, examples from literature on Phoslock can be found in (Akinnawo, 2023; Copetti et al., 2016; Reitzel et al. 2013; Vargas & Qi, 2019). Since the novel LMB products were made commercially available just recently and only in some countries, there has been an urge to expand the current understanding on the efficiency of those products. This study aims at filling this gap by conducting a thorough investigation of the abovementioned recently introduced LMB variants. These novel products, characterized by enhanced binding capacities of LMB3 =17 mg P/g and LMB2= 20 mg P/g, served as focal points in this study for comparison with LMB1. The primary aim is to evaluate whether the novel LMB products with higher lanthanum content, thus binding capacities, than Phoslock exhibit superior effectiveness in binding with phosphate from water column and sediment, as well as the effect of those products on some environmental abiotic parameters, like pH and EC, and the turbidity caused by those products. To examine the hypotheses, the study adopted a multifaceted approach. The approach evaluated the effectiveness of the products under two different temperature conditions, focusing on the immobilization of phosphorus in the water column, besides the decrease of mobile P release from the sediment. Additionally, the study explored the repercussions of this immobilization on the different fractions of phosphorus present in the sediment. Furthermore, the binding rate of the LMB products accompanied with the turbidity underwent scrutiny through two distinct application approaches within cylinders of lake water. This analysis aimed to contribute insights into the relative effectiveness of these LMB products in tackling the challenges associated with eutrophic aquatic environments.

4.1. Temperature and P immobilization interaction in water column and the decrease in sediment P release:

The interaction of temperature and the functionality of Lanthanum-Modified Bentonite (LMB) products revealed a noteworthy contrast. In the cold phase, the immobilization pattern of phosphorus (referred to as a negative P flux), specifically for the cores treated with both of LMB1 and LMB3 was lower compared to that of the warm phase which can be attributed to the low P release from the sediment in the former phase. This was evident in the P flux of the control cores in this phase compared to the warm phase. Hence, the low negative P flux (the flux from the water column to the sediment) in the LMB cores in this phase does not seem to be a result of the impact of temperature on the functionality of the LMB products as shown by Kang et al. (2022), but rather as a result of the decreased P release from the sediment to the water column in the first place due to the low temperature. However, in the warm phase, all LMB products exhibited a distinctive pattern compared to control, transforming phosphorus from its mobilized form to an immobilized state. This transformation is evident in the results of phosphorus fluxes, with negative fluxes observed throughout the combined effect of both phases for all LMB groups. Notably, LMB2 was able to maintain the expected immobilization process of P in two different conditions of temperature. In the preceding cold phase LMB2 kept P flux at negative values. Those values remained negligible in the warm phase despite the high P release from the sediment. Contrastingly, the control cores exhibited a positive upwards phosphorus flux in both phases, indicating movement of mobile P from the sediment to the water column which can be explained by the

internal loading processes in the sediment. This aligns with the findings in literature (Søndergaard et al., 2003; Yang et al., 2022). It is worth mentioning here that the detection limit for autoanalyzer is 10 µg P/l, which indicates that most of the values in the cold phase for LMB1 and LMB3 were below detection limit. The negative P flux in the LMB treated sediment cores was further supported by a decrease in the percentages of mobile P (water-, BD-, and organic-P fractions in the sediment) which were relatively lower in LMB groups by 35.73% in LMB1, 31.12% in LMB2 and 37.04% in LMB3 compared to control group (0.29 mg P/ g DW sediment). This decrease was accompanied with an increase in the percentage of HCl-P fraction in the first 3cm compared to control cores, which was illustrated clearly in the results in which HCl-P fraction was higher than control (0.082mg P/g DW sediment) by 19.03% for LMB1, 62.5% for LMB2 and 81.59% for LMB3. That also comes in agreement with the literature (Meis et al., 2013). These changes are likely to have resulted from the precipitation of mobile phosphorus after binding to Lanthanum (La) forming Rhabdophane ($LaPO_4.nH_2O$) (Copetti et al., 2016; Dithmer et al., 2015), then the breakdown in the bonds between La and phosphorus due to the use of HCl in the fractionation procedure (Bishop et al., 2014; Meis et al., 2012) which explains the negative fluxes in the LMB-treated cores.

Yet the mobile P content in the treatment cores was still high, which means that not all the mobile P was released to the water column yet. This suggests the need for a longer period of incubation for all the mobile P to be transferred the water column or to immobilized forms (Wang et al., 2017). The fractionation data that was presented by AEW students was examined by the start of the experiment, which was used to estimate the doses of the products that were applied to the cores in my experiment. The data was used as a reference to compare it to the fractions obtained from the cores at the end of my experiment. When comparing the students' fractionation data to the data of my cores, the total mobile P in the sediment had decreased by 55%, which may explain the high flux of mobile P in the water column that was demonstrated as an increase in the water fraction in control cores by 32.5% compared to the students' data. The same fraction suffered a decrease in LMB1, LMB2 and LMB3 by 45.7%, 42.6%, 54.25% respectively compared to the student's data. A notable decrease in BD fraction in all cores by 55.5% for control, 71.4% for LMB1, 69% for LMB2 and 71.7% for LMB3, that may be a consequence of the release of BD-P caused by the redox reactions in the sediment due to the anoxic conditions (Boström et al., 1988a; Sørensen, 1982). In addition to that, a decrease in organic-P was noticed by 65.3% in control, 75.6% LMB1, 82.6 LMB2 and 84.3% LMB3 which may be caused by the degradation processes of organic matter in the sediment specially under high temperature (Ahlgren et al., 2011). Total mobile P showed a substantial decrease in all LMB-treated cores by LMB1 71.5%, LMB2 69.4%, LMB3 72,01% compared to control as a result of binding with LMB products, which thereafter was transferred to more refractory fractions (Ca-P).

Hence, remarkable findings were observed. The study's two phases demonstrated significant reduction in P in water column for the cores treated with LMB compared to control cores. Furthermore, there is a distinct contrast between the negative fluxes observed in all LMB-treated cores and the positive flux in control cores, which all suggests a decrease in P release from the sediment caused by the introduction of LMB products. Lastly, the increase in immobile P within the Ca-P fraction in the sediment fractionation procedure in the treated cores in comparison to control cores. All those observations reinforce the proposed hypothesis in respect to the effectiveness of the novel products in reducing mobile P in the water column and the sediment as effectively as Phoslock.

4.1.1. Implications for the pond De Vijver:

An estimation was made considering the first 3cm of the sediment, based upon the data of P fractionation of the initial mobile P concentrations with which the doses of the products were calculated for my experiment, in addition to the binding capacity of each one of the products. With mobile P content of 38 mg in the first 3 cm, the estimation suggests that the pond De Vijver might require the following quantities of each material in order to reduce internal P release from the sediment: LMB1= 16213 kg, LMB2= 8017kg and LMB3=9735kg, this might have important economical implications as additional transportation and application costs may arise in case a decision is made to use LMB with lower La content.

4.2. Investigating the influence of LMB on the environmental abiotic parameters:

The experiment results show that introduction of LMB is unlikely to have significantly influenced pH variations. Similar to what other studies found (Kasprzyk et al., 2018; Van Oosterhout & Lürling, 2012). Despite significant differences with control, mainly in LMB1 cores in both phases, the differences between the cores were minor in respect to pH and ranged between 0.2 and 0.7. The measured pH levels in the cores remained within a specific range between 6.5 and 8.5, however, anoxia tends to be responsible for the increase in pH in sediment cores(Meis et al., 2013). LMB was expected to cause an increase in EC (Van Oosterhout & Lürling, 2012) as it promotes the release of ions of metals such as lanthanum and aluminum as suggested by Lürling & Tolman, 2010. However despite the high doses of LMB (4.2 g/l for LMB1, 2.1 g/l for LMB2 and 2.5 g/l for LMB3) compared to the doses applied in field experiments in Dutch waters that range between (0.046 and 0.085 g/l) (Van Oosterhout & Lürling, 2012), no major increases were noticed. On the contrary, a decrease in EC was noticed in the cold phase for all cores including controls. Also no significant differences between LMB cores and control cores were found in the warm phase except for LMB3 which suffered a decrease in EC. This may have happened as a result of the presence of humic substances in the sediment and in the water column, leading to the formation of chelates that surround the metal ions and prevent them from reacting (Omoike & vanLoon, 1999; Reitzel et al., 2017; Van Oosterhout & Lürling, 2012). Anoxia conditions are unlikely to have influenced LMB functionality, as suggested by studies (Funes et al., 2021; Ross et al., 2008). This may be explained by the fact that La is not sensitive to redox reactions and maintains its stability under anoxic conditions (Funes et al., 2021; Gibbs et al., 2010; Li et al., 2019). This indicates no influence of the anoxic conditions on the results of the study. Despite slight increases in DO concentrations, attributed to a change in the measuring device, the levels remained within the anoxic range (DO<0.5 mg/l). The differences in DO concentrations between the control group and LMB groups were statistically not significant in both phases excluding LMB2 in the warm phase, indicating comparable anoxia conditions for all the groups. Furthermore, the organic matter content across all treatment groups, hovering around 10%, suggests similar organic matter decomposition under similar anoxic conditions. This implies that differences in organic-P release rates are not expected due to variations in organic matter decomposition process.

4.3. Binding and settling rate of the material:

The application technique of the material plays a pivotal role in the interaction between LMB and P in the water column, as evidenced by the notable differences in binding rates observed between the first and second application methods. In the former, no statistical distinctions were observed between the control group and all LMB-treated groups. In contrast, significant statistical differences emerged between the control group and the LMB treatment groups when a slurry made out of the material before application. That is most likely

attributed to the larger contact area as LMB particles blend with water in advance. However, a discernible trend in the slurry method demonstrated a faster binding rate for LMB3 and LMB2 compared to LMB1 in both application methods. The slurry method exhibited an accelerated binding rate, such that phosphorus concentrations were as low as those achieved by the slurry method after only 3 days of application, a timeframe equivalent to 1 week in the non-slurry method.

The dose used in my turbidity experiment of 1g/l of each product came in agreement with the dose used in literature for similar experiments (Van Oosterhout & Lürling, 2012). Turbidity levels were highest in the LMB groups when the slurry method was employed, yet all materials exhibited a settling trend, approaching values close to the control group after 3 days of application. These turbidity values were comparable to those recorded after 1 week in the method that did not involve a slurry. However some products caused more turbidity than others. LMB2 showed the highest level on of turbidity in both slurry and non-slurry, it was followed by the LMB4 in the slurry method, while both LMB1 and LMB3 were closer to control in their turbidity than to the other two products. In field experiments, Phoslock (LMB1) 2is usually applied as a slurry then it is sprayed using a spray boom attached to the rear side of the barge to allow for the even and widespread application of the material (Lürling & Van Oosterhout, 2012). Environmental implications of turbidity of LMB products on the growth of all kinds of phytoplankton in the lake are plausible due to light limitation (Kirk & Gilbert, 1990; Van Oosterhout & Lürling, 2012), as well as effects related to feeding zooplankton (Lürling & Tolman, 2010). This is assumed to be an issue in shallow lakes where the sediment-water interactions are higher leading to resuspension of sediment which may cause more turbidity (Funes et al., 2021). It is believed that having suspended particles of LMB in water column is essential as this ensures enhancing the effectiveness in binding with mobile P in water column compared to fast sinking (Van Oosterhout & Lürling, 2012). Therefore, it is essential to follow similar methods to slurry when applying LMB in whole lake experiments, with due consideration to the turbidity-inducing properties in such products. While the experimental setup in this study was conducted under static conditions, it is crucial to take into account the dynamic nature of aquatic environments. This entails a comprehensive understanding of the potential implications, including but not limited to an extended sinking rate of the material, and uneven distribution of the material across the sediment surface.

5. Conclusions:

- The novel LMB products with enhanced binding capacities exhibit promising effectiveness in phosphorus immobilization in the water column and the sediment.
- The binding rates of the novel products are higher compared to Phoslock.
- Application method affects P binding rate of LMB products.
- Some LMB products (i.e. LMB2 and LMB4) cause more turbidity than others but the turbidity caused settles down in a short period of time.
- LMB2 and LMB3 exhibit comparable or potentially superior efficacy compared to LMB1, suggesting a potential cost-saving advantage due to higher Lanthanum (La) content in a more concentrated form, reducing the need for transporting large quantities of material for application in expansive lakes.

6. Recommendations:

- LMB4 requires additional research to elucidate its performance characteristics in sediment core conditions, since it was only tested for binding rate with P in the water column in lake-water cylinders that do not contain sediment.
- Turbidity demands a more focused examination, especially in shallow lakes, to assess the environmental impact of turbidity associated with the application of LMB2 and LMB4, specially on non-target organisms.
- Extending research to field-scale studies is recommended to validate outcomes obtained from controlled laboratory experiments. This approach ensures a more realistic portrayal of environmental conditions and the practical performance of LMB in natural settings.
- Future studies focus on incubation of sediment cores in warm conditions is advised for obtaining more predictable results on the performance of LMB, mainly in shallow water bodies.

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Appendices:

Appendix I: Sequential P fractionation of the sediment protocol.

P0803 Sequential P fractionation sediments

Date: modified 2015/2019 (ML/MM) Update: dec 2022 (WB)

Reference: Modified by Miquel Lurling from Paludan & Jensen.1995, Wetlands 15 365-373 Sequential Extraction of Phosphorus in Freshwater Wetland and Lake sediment: significance of Humic Acids.

Materials:

- 50 ml centrifuge tubes
- dispenser 25 mL
- Eppendorf pipettes/tips
- shaker
- centrifuge
- air-flush installation
- glass Pasteur pipets
- PE bottles 50 and 100mL
- filtration device and GF/C filters
- crucibles with lid and tongs
- drying Oven
- Furnace/ignition oven 550 °C/hot-plate (120°C

Chemicals:

Oxygen-free nanopure water
bubble Milli-Q water 15 minutes with N_2
BD-reagent : 0.11 M $Na_2S_2O_4$ / 0.11 M $NaHCO_3$
*(38 g $Na_2S_2O_4$/**2**L +18,4 g $NaHCO_3$/**2**L)*
0.1 M NaOH-solution
*(8,0 g NaOH/**2**L)*
0.5 M HCL-solution
*(83,3 mL HCl-37%/**2**L)*
2M H_2SO_4
(54.5 mL H_2SO_4- 95-97%/500mL)

Sediments collected from field **Keep collected sediments frozen upon analysis**

Method

See: Schematic overview of sequential P fractionation of sediment

Shaking
use shaker ~140 rpm
Shake tubes horizontally

Centrifugation
5 minutes at 5000 rpm

Decant carefully
don't let the silt slip out pellet sample

Filtration
1,2 µm (GF/C)filter, vacuum filtration

Bubbling N_2 / aeration
Please ask technician

➢ ***Analysis***

1. Twan (sived) sediments and mix thoroughly
2. Take 50 mL centrifuge tubes with cap. Weigh in 1.0 g of sediment to each tube

➢ ***First phase:*** *Oxygen-free nanopure water -->It aims to release the available P, which is the interstitial water loosely adsorbed to surfaces of Fe and $CaCO_3$*

1. add 25 mL of oxygen-free nanopure water. Replace air with N_2 and close tube.
2. Shake tubes for 30 minutes.
3. Place the tubes in centrifuge, centrifuge
4. Decant supernatant ***carefully*** into labelled 100mL PE bottle, save pellet
5. Repeat extraction with 25 mL oxygen-free water for 5 minutes and centrifuge.
6. Decant and combine supernatant.
7. Filter the supernatant over a GF/C filter and collect 30 mL filtrate in a labelled 50 mL PE bottle.

Store this fraction in freezer immediately

Measure PO4-P

➢ **Second Phase:** *BD-reagent (bicarbonate-dithionite mixture) -->aim: stronger reductant that will release the SRP bound manly to Fe-hydroxides and Mn-compounds*

1. Add 25 mL BD-reagent, oxygen-free, to sediment pellet *(from previously phase)*. Replace air with N2 and close tube.
2. Shake tubes for 30 minutes.
3. centrifuge
4. Decant supernatant into 100 mL PE bottles.
5. Add 25 mL BD to pellet, replace air with N_2 .
6. Shake tubes on shaker for 5 minutes,
7. centrifuge
8. decant and combine supernatant. Save pellet
9. Put sample bottles in Aeration-set-up
10. Aerate for 30 minutes
(removal dithionite; keeps Fe and PO_4 in solution)

The fumes that are released during this process must be extracted,
install aeration-set-up in fume hood

10. Add 3 mL 2M H_2SO_4 to the combined supernatants and mix.
11. Filter combined supernatants, collect 30 mL filtrate into 50 mL PE bottle. Label well and store at 4 ^{0}C

Measure PO4-P and TP (total fraction of the dissolved)

- **Third Phase:** *Sodium Hydroxide (NaOH)--> It will extract SRP sorbed by clay- and metal-oxides (Al)*

1. Add 25 mL 0,1 M NaOH to the sediment pellet and close tube.
2. Shake tubes 30 minutes on shaker.
3. centrifuge
4. Decant supernatant into 100 mL PE bottle.
5. Add 25 mL NaOH to sediment pellet.
6. Shake again 30 minutes on shaker and centrifuge.
7. Combine supernatants into 100 mL PE bottle.
8. Add 25 mL of **milli-Q water** to the sediment and shake for 5 minutes, centrifuge
9. Decant the third supernatant in the same 100 mL PE bottle. Save pellet
10. Add 1.5 mL of 2M H_2SO_4 in the supernatant and mix.
11. Filter the supernatant and collect 30 mL filtrate into 50 mL bottle

Measure PO4-P and TP (total fraction of the dissolved)

- **Fourth Phase:** *Hydrochloric acid --> Aim to release P bound to carbonates and apatite-P. This is hardly bioavailable*

1. Add 25 mL 0,5 M HCl and close tube. Shake for 1 hour
2. Centrifuge
3. Decant supernatant into 100 mL PE bottle
4. Add 25 mL Milli-Q water to the sediment, shake for 5 minutes.
5. centrifuge
6. Combine supernatants and filter through GF/C. collect 30 mL filtrate and store at 4 ^{0}C.
7. Save pellet

Measure PO4-P and TP (total fraction of the dissolved)

- **Fifth Phase:** *Finally, the sediment pellet will be extracted using stronger acid and higher temperature. This will give the refractory P*

1. Move the pellet to crucibles *use little milli-Q water*
2. Dry crucibles at 105 ^{0}C in drying oven, over-night
3. Ignite pellet at 550 ^{0}C for 2 hours
4. Cool down and "crumble" pellet in crucible
5. Add 10 mL 2M H_2SO_4 and mix
6. Place crucibles with lid on hot-plate *(in fume hood)* and boil 10 minutes at 120 °C
7. Take-up sample by 10 mL syringe, add filter-disc.
8. Filter sample in a labelled 15 mL tube

Measure PO4-P

- **Also conduct digestion on dry sediment to determine the total-TP, see protocol: P0802** *determination of total N&P in sediments*
- **Determine the moisture content of the sediments used, see protocol: P0602** Dry matter and moisture content of sediments

Calculation

Calculate P in each fraction using results Phase 1 -5

$$[P_{sed}] = \frac{[P_f] \times V_{extract}}{DW}$$

In which P_{sed} = [P] in sediment (µg P g^{-1} DW), P_f = [P] in fraction (µg P mL^{-1}), $V_{extract}$ = volume extractant (mL), DW = dry-weight sediment (g).

Appendix II: Protocol for Ortho-Phosphate (PO_4-P) in water
By Wendy Beekman-Lukassen and modified by Maíra Mucci

Materials:
Filtered lake water or pore water
phosphate reagent solution
12 ml glass tubes and caps in rack
pipets 5ml and 1ml and tips
spectrophotometer set at 690 nm

Caution
The reagent solution contains sulphuric acid, wear lab coat and safety glasses. Clean up spilled material immediacy.

Procedure:

Solution A:
Dissolve 2.4 g of Ammonium Molibdate (CAS Number: **12054-85-2**) in 150 ml of Nano pure water and add 28 ml of sulfuric acid (CAS Number: **7664-93-9**) and add Nano pure water until a final volume of 250 ml.

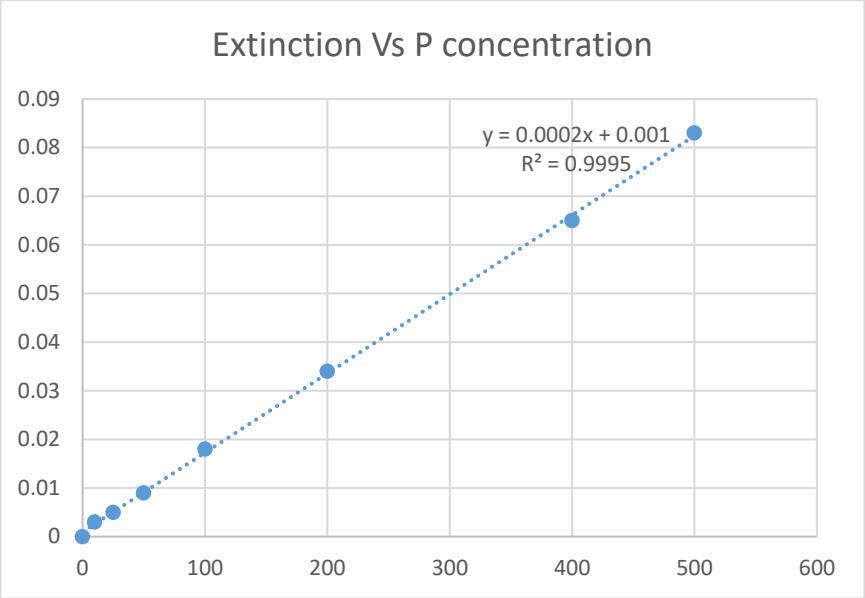
Solution B:
Dissolve 110 mg of Antimony Potassium Tartrate (CAS Number: **28300-74-5**) in 250 ml of Nano pure water.

Phosphate reagent solution:
Mix 100 ml of Solution A with 50 ml of Solution B and dissolve 845 mg of Ascorbic acid (CAS Number: **50-81-7**). Add Nano pure water until a final volume of 200 ml. This solutions needs always to be fresh.

Calibration Curve
- Make 8 standards with known concentration of P (see table below). The concentrations needs to be chosen according to the concertation range of your samples. If P concentration is higher than 1mg P l^{-1} it is advice to dilute the samples and re-measured.
- Pipet 4.5 ml of each standard into glass tubes (8 in total), add 0.5 ml Phosphate reagent solution, close tubes and mix.
- Wait 12.0 minutes and measure the extinction at 690 nm using a spectrophotometer. Important: samples need to be measure after 12 min but before 40 min.

On excel, plot P concentration with extinction and ask for a linear trend line. Later, use will use the equation to calculate the P concentrations in your samples, in which y is P concentration and x is extinction, see example bellow:

µg P/l	ads
0	0
10	0.003
25	0.005
50	0.009
100	0.018
200	0.034
400	0.065
500	0.083



Samples

- Pipet 4.5 ml of your samples in a glass tubes, add 0.5 ml Phosphate reagent solution, close tubes and mix. The volume can also be 9 ml of sample and 1 mg of reagent if the cuvette of the spectrophotometer is bigger.
- Wait 12.0 minutes and measure the extinction at 690 nm using a spectrophotometer.

Important: samples need to be measure after 12 min but before 40 min.

Use the equation from the calibration curve to calculate the P concentrations in your samples.