NM WRRI Student Water Research Grant Final Report

1. **Student Researcher:** Wijayalath Kodige Nimasha Lakshani Abeykoon

   Faculty Advisor: Dr. Yanyan Zhang

2. **Project Title:** Mitigation of Harmful Algal Blooms Using Modified Clays

3. **Research Problem and research objectives**

   Algae are the base of the aquatic food chain and have a significant contribution to aquatic life and photosynthesis. However, when these algae grow without control over freshwater bodies as well as in marine and brackish water environments while releasing toxins that are harmful to humans, birds, fish, shellfish, and marine mammals. The toxins produced by algal blooms, dissolved oxygen depletion, and anoxic conditions followed are not favorable for humans and aquatic life who consume that water. Kidney and liver toxicity, skin rashes, respiratory distress, and neurological symptoms are a few adverse effects due to exposure to harmful algal blooms (HABs). Algal bloom nuisance has severely affected water treatment processes, which increases the demand for coagulants and advanced water treatment processes. HABs are often stimulated by combined phosphorus and nitrogen enrichment. Phosphorus is considered the most significant nutrient in shaping the dynamics of freshwater algal productivity, including HABs. Therefore, effective technologies are urgently needed for HAB control and phosphorus reduction.

   Over the last few decades, biological, physical, and chemical remedies have been investigated for algal bloom mitigation. Clay is one of the promising solutions that was tested in many attempts for HABs treatment. Local availability, low environmental impact, and regeneration potential have made clay an attractive solution for HABs mitigation. In this project, iron-modified clays (sepiolite and kaolinite) have been proposed for HAB mitigation. The specific goals of the research are to (i) investigate the removal of harmful algae by modified sepiolite, (ii) demonstrate the capability of modified clays in the adsorption of algal toxins. (iii) study the capacity of modified clays in phosphate removal to prevent potential future algal blooms, (iv) develop an innovative solution of using dialysis tubes with modified clay inside for phosphate recovery from water bodies to minimize the internal cycling of phosphate.

4. Methodology

Harmful algae culture and modified clays:

In the proposed study, *Microcystis aeruginosa*, one of the most common potentially toxic bloom-formers in eutrophic freshwater, is used as the model microorganism for the HAB study. Two types of clays (kaolinite and sepiolite) will be modified with chitosan and $NH_4Fe(SO_4)_2$ to control HABs. Kaolinite structurally is made by alternative stacking of alumina octahedral sheets and silica tetrahedral sheets. Sepiolite is a porous, light-weight clay with a larger specific surface area and a fibrous structure. The chitosan with a long polymer chain and positively charged groups ($-NH^{3+}$) captured and linked the negatively charged algal cells and other particles; the clay then provided the mass or ballast to carry the flocs to the water sediment. Positively charged iron on the clay could help with the attachment of algal cells while it will react with phosphorus in water to form precipitates on the clay surface.

Removal of cyanobacteria, microcystin, and phosphate by clay flocculation:

Both commercial clays and modified clays, as the flocculants, will be tested for their capabilities of removing algal biomass, microcystin, and phosphate. The mixtures of algal culture and clays were rapidly mixed at 200 rpm for 1 min, followed by slowly mixing at 30 rpm for 20 min. As the flocs form, the samples are allowed to settle for one hour. The supernatant was collected to quantify cyanobacteria, phosphate, and microcystin inside. Unmodified sepiolite and kaolinite were also used as a comparison.

Removal of phosphate and microcystin by dialysis tubes with modified clays:

When modified clay is added directly to a water source, even though phosphate is precipitated, it may be released again to the water body after a few months. Therefore, dialysis tubes with a size threshold of 12-14 kDa were used to recover phosphate from water bodies to minimize the internal cycling of phosphate. Modified clays with a load of 800 mg clay/L were packed in dialysis tubes to remove the phosphate and microcystins in water with algal culture. The phosphate and microcystin in water can enter the semi-permeable membrane of dialysis tubes and reacts with iron-modified clays. Once microcystin is adsorbed, and the phosphate is precipitated on the clays, dialysis tubes can be removed from the water body. Dialysis tubes with modified clays are proposed to be kept submerged and floating in water with the aid of an external floating arrangement.

5. Results, Conclusions, And Recommendations

5.1 Removal of cyanobacteria by clay flocculation

First, both clay types, kaolinite, and sepiolite, were tested for their ability in cyanobacteria removal (Figure 1). Both modified and commercial clays of sepiolite and kaolinite were added to lab-cultured *M. Aeruginosa* samples and flocculated for twenty minutes. Then let it settle for five minutes before sampling. The absorbance of samples at 680 nm was measured as an indicator for algae cell content. Both modified clays reduced the algal cell density after flocculation. The reduction in absorbance at 680 nm further dropped when clay loading was increased from 2 g/L to 5 g/L for modified clays. The samples with commercial clay inside were cloudy, and it explains the reason for the increase in absorbance at 680 nm for these samples. There is no significant difference between modified sepiolite and modified kaolinite in removing *M. Aeruginosa*. All the algae were settled at the bottom of the beakers in which the modified sepiolite and kaolinite were added. The content in the beakers with modified clay was clear, whereas it was still cloudy in beakers with commercial clay and algae.

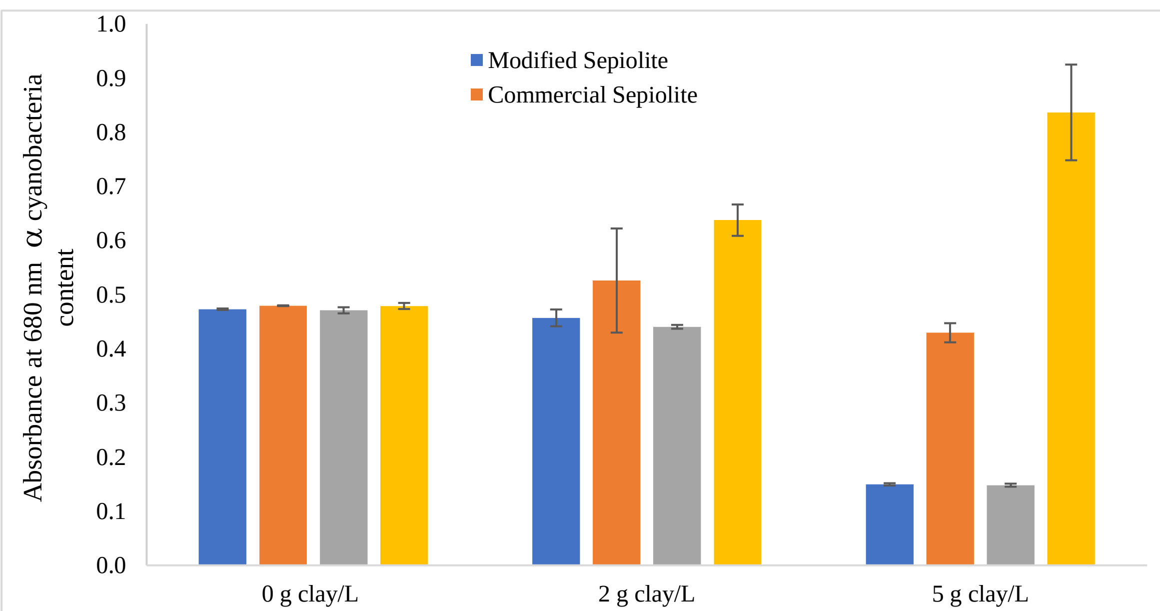

Figure 1: Comparison of cyanobacteria removal by modified and unmodified sepiolite and kaolinite when packed inside a dialysis tube at different clay loadings

5.2 Removal of phosphate by clay flocculation

Initially, the behavior of phosphate removal with different clay loading was studied with both modified and commercial kaolinite and sepiolite. Initial phosphate concentration and phosphate concentration after 20 minutes with clay flocculation were measured. The variation of phosphate removal efficiency with applied clay loading was plotted in Figure 2. The phosphate removal efficiencies of commercial sepiolite and kaolinite were negligible at every clay loading. The maximum phosphate removal efficiency observed for commercial sepiolite and commercial kaolinite were 3.3% and 2.9%, respectively. An increasing correlation (R^2 = 0.85 for modified sepiolite, R^2 = 0.94 for modified kaolinite) between clay dose and phosphate removal efficiency highlights the fact that the phosphate removal was higher when applied clay loading was higher. Phosphate removal efficiency of sepiolite was higher than that of kaolinite at all clay loadings. With the increase of clay loading, the difference between the removal efficiencies of modified kaolinite and modified sepiolite was reduced. The maximum phosphate removal efficiencies for modified sepiolite and modified kaolinite of 96% were achieved with 2.0 g/L clay loading. When the clay loading was higher, there were more sites for iron to attach to clay, thereby increasing phosphate removal efficiencies. There was no significant difference between the PO_4^{3-} removal efficiencies of modified kaolinite and sepiolite, p-value = 0.67 (ANOVA single factor) when clay loading was 2.0 g/L.

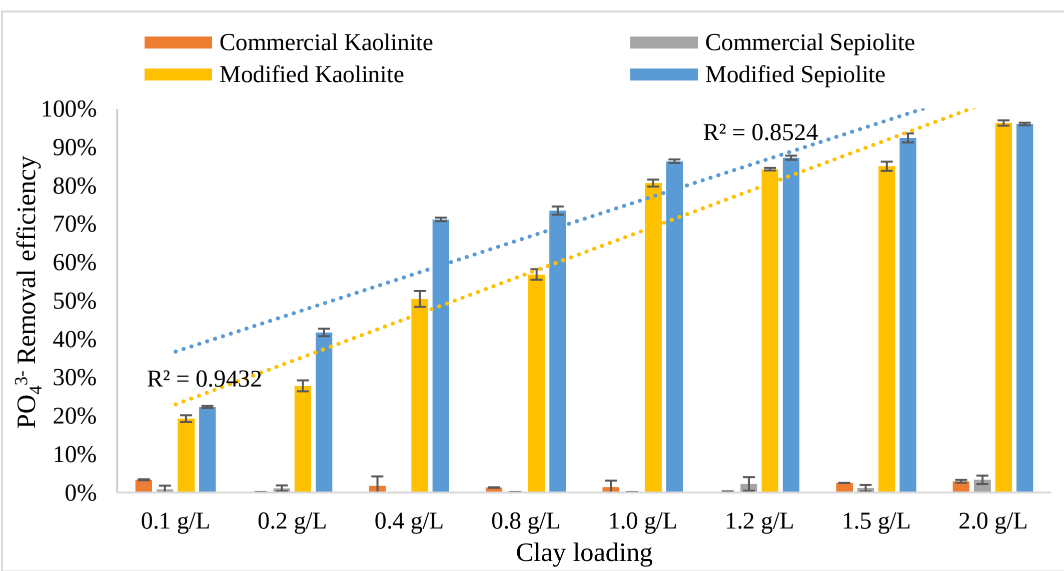

Figure 2: Variation of phosphate removal efficiency with commercial and modified kaolinite and sepiolite clay loading

Next, the two clay types, modified kaolinite and modified sepiolite were compared for their phosphate removal capacity during clay flocculation. 1.6 g of modified kaolinite and sepiolite was added to a phosphate solution with the same initial concentrations of approximately 37 mg/L separately. Total phosphate removal of 24.50 mg/L and 36.30 mg/L were obtained for modified kaolinite and sepiolite, respectively, in 24 hours. Specific phosphate removal of modified sepiolite was 15.31 mg PO_4^{3-} /g clay and that of modified kaolinite was 22.69 mg PO_4^{3-} /g clay. A substantial difference could be observed between the two modified clays in phosphate removal in the first 20 minutes of flocculation study. That is, while kaolinite removed 19.60 mg/L of PO_4^{3-}, sepiolite removed 34.90 mg/L PO_4^{3-} within the first 20 minutes. It indicates that modified sepiolite has a higher phosphate removal capacity and removal rate than modified kaolinite.

Performance of modified sepiolite and modified kaolinite in PO_4^{3-} removal with different initial PO_4^{3-} concentrations were studied. PO_4^{3-} concentrations were measured 20 minutes, 1 hr, 24 hrs, 48 hrs, 72 hrs, and 240 hrs after modified clay application to PO_4^{3-} solutions with different initial concentrations (Figure 3). It was evident that around 50% of PO_4^{3-} was removed within an hour of contact with modified clay. The same trend of PO_4^{3-} removal with time could be observed for all initial concentration conditions. When modified kaolinite and modified sepiolite was compared, modified sepiolite indicated a higher PO_4^{3-} removal than modified kaolinite within the contact time when initial concentrations were similar (Figure 3). It suggests that PO_4^{3-} removal rate of modified sepiolite was higher than that of modified kaolinite, especially in the first 72 hours. The PO_4^{3-} removal is mainly because of the chemical reaction between PO_4^{3-} in the bulk solution and Fe^{3+} ion attached to the modified clay. The primary chemical reaction for the removal of phosphate is given in the following equation (Zhang et al., 2010).

$$Fe^{3+} + PO_4^{3-} + 2H_2O \rightarrow FePO_4{\cdot}2H_2O \downarrow$$

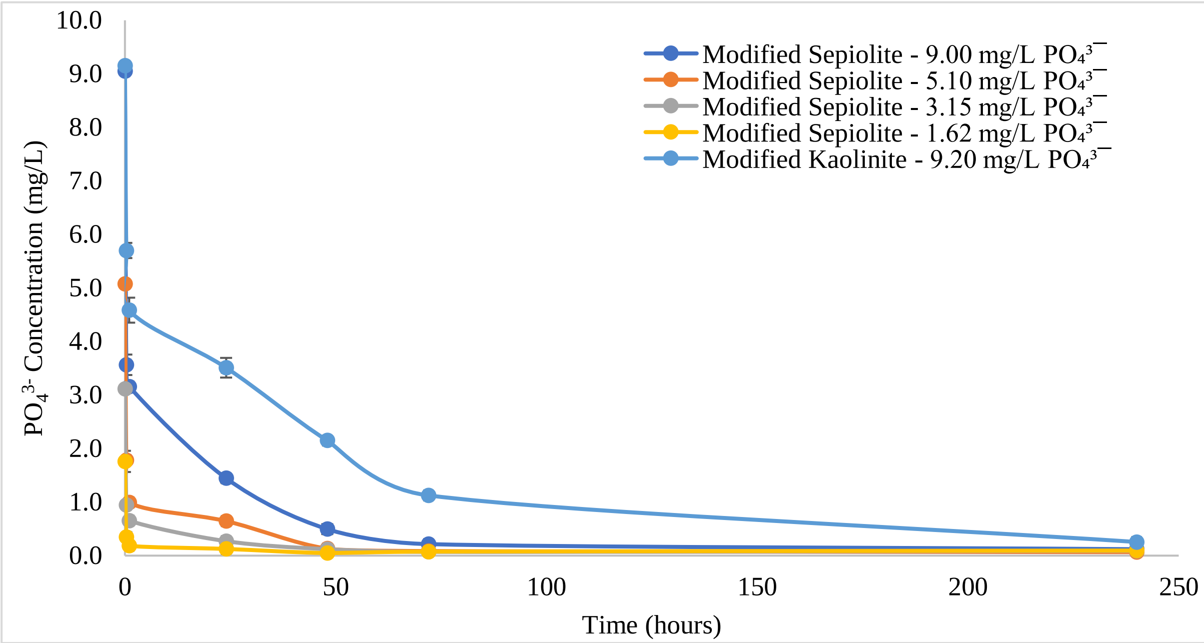

Figure 3: Variation of PO_4^{3-} concentration over time for direct clay application of Modified Sepiolite and Modified Kaolinite for different initial PO_4^{3-} concentrations

5.3 Removal of microcystin from water by clay flocculation

The microcystin, MCLR, was selected for the adsorption experiments to investigate the effectiveness of commercial and modified clays on removing algal toxins. Commercial kaolinite or sepiolite did not show any MCLR removal during the clay flocculation. It was observed that MCLR removal efficiency increased with the initial MCLR concentration (Figure 4). Almost 99.9% of MCLR removal was achieved when the initial MCLR concentration was 200 µg/L. An increasing trend of MCLR removal efficiency was observed when the initial concentration increased from 20 µg/L to 200 µg/L. There was a sudden drop of MCLR removal efficiency to 96.7% when the initial MCLR increased to 400 µg/L (Figure 4). MCLR adsorption around 103 µg MCLR/ g clay could be achieved when the initial MCLR concentration was 400 µg/L.

When modified kaolinite with initial concentrations of 20 µg/L, and 40 µg/L were used for MCLR adsorption, no MCLR adsorption to clay was detected. The highest MCLR adsorption of 87.9% was achieved for MCLR solution with an initial concentration of 400 µg/L, while the lowest adsorption was 54.7% for an initial MCLR concentration of 100 µg/L. As an average, around 70% overall MCLR removal efficiency could be achieved from modified kaolinite.

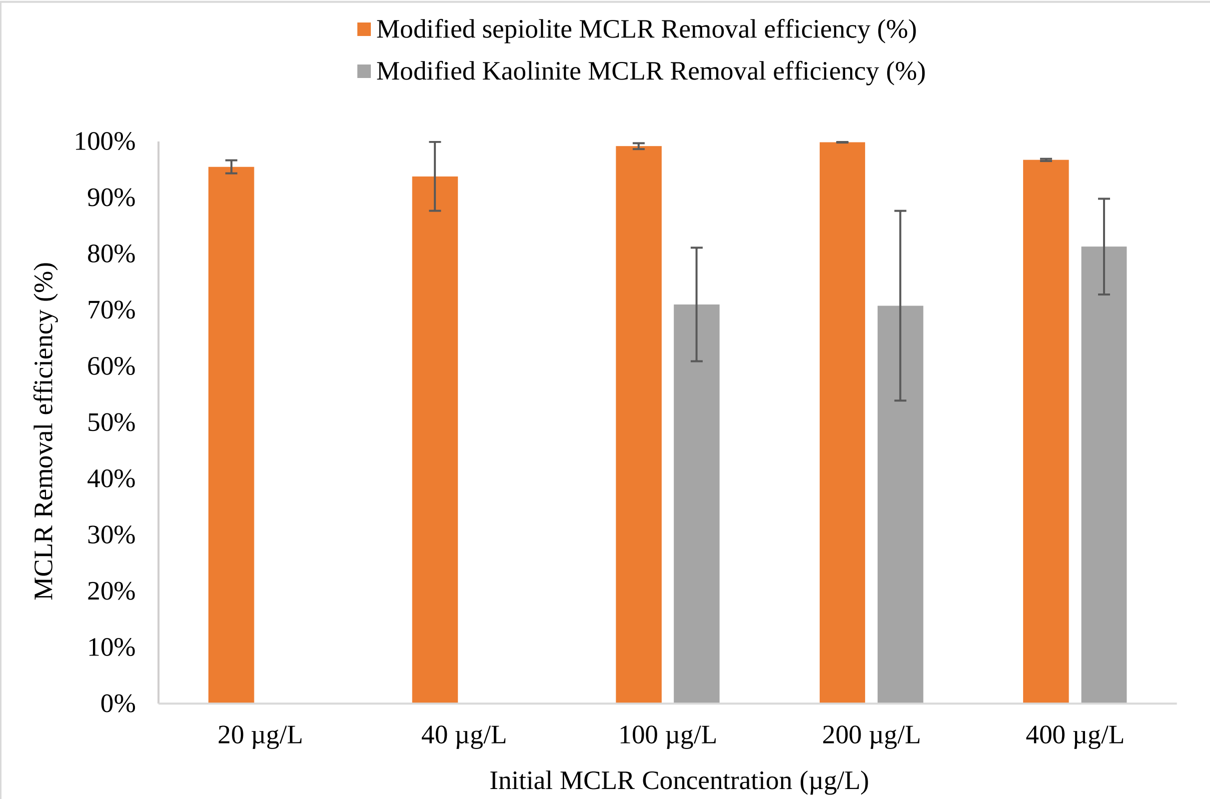

Figure 4: The variation of MCLR Removal by direct clay flocculation of modified sepiolite and kaolinite for different initial MCLR concentration

5.4 Removal of Cyanobacteria and Phosphate by dialysis tubes using modified clays

The PO_4^{3-} removal capacity between two unmodified (commercial) clay types was compared when they were packed inside the dialysis tube. The clay loading was 1.6 g/L for both clays. After adding dialysis tubes filled with clay to PO_4^{3-} solutions, the phosphate concentrations in the solutions were monitored. After 72 hours of flocculation, the phosphate removed by kaolinite was 25.3 mg/L and by sepiolite was 26.8 mg/L. According to this observation, there was no significant difference between phosphate removal for both clay types when it was packed inside the dialysis tubes.

The PO_4^{3-} removal performance of dialysis tubes packed with commercial and modified sepiolite was compared. With a constant clay loading of 800 mg/L, the change of PO_4^{3-} concentrations with time is shown in Figure 5. It was observed that PO_4^{3-} removal was higher when the initial PO_4^{3-} concentration was higher, regardless of the clay type. Compared with direct clay flocculation, the

PO_4^{3-} removal by the clays packed in the dialysis tubes was much slower. The PO_4^{3-} diffusion across the semi-permeable membrane of dialysis tubes was the speed limiting step. The high PO_4^{3-} concentration could accelerate the diffusion and lead to high removal. The highest PO_4^{3-} removal efficiency of dialysis tubes with modified sepiolite was 79% after 8 days of treatment at the initial PO_4^{3-} concentration of 10.87 mg/L. In contrast, the highest PO_4^{3-} removal efficiency for dialysis tubes packed with commercial sepiolite was only 16.6% at an initial PO_4^{3-} concentration of 10.64 mg/L after 8 days of treatment. A maximum PO_4^{3-} removal efficiency of 99% was achieved after 14 days of flocculation of modified sepiolite when the initial PO_4^{3-} concentration was 10.87 mg/L.

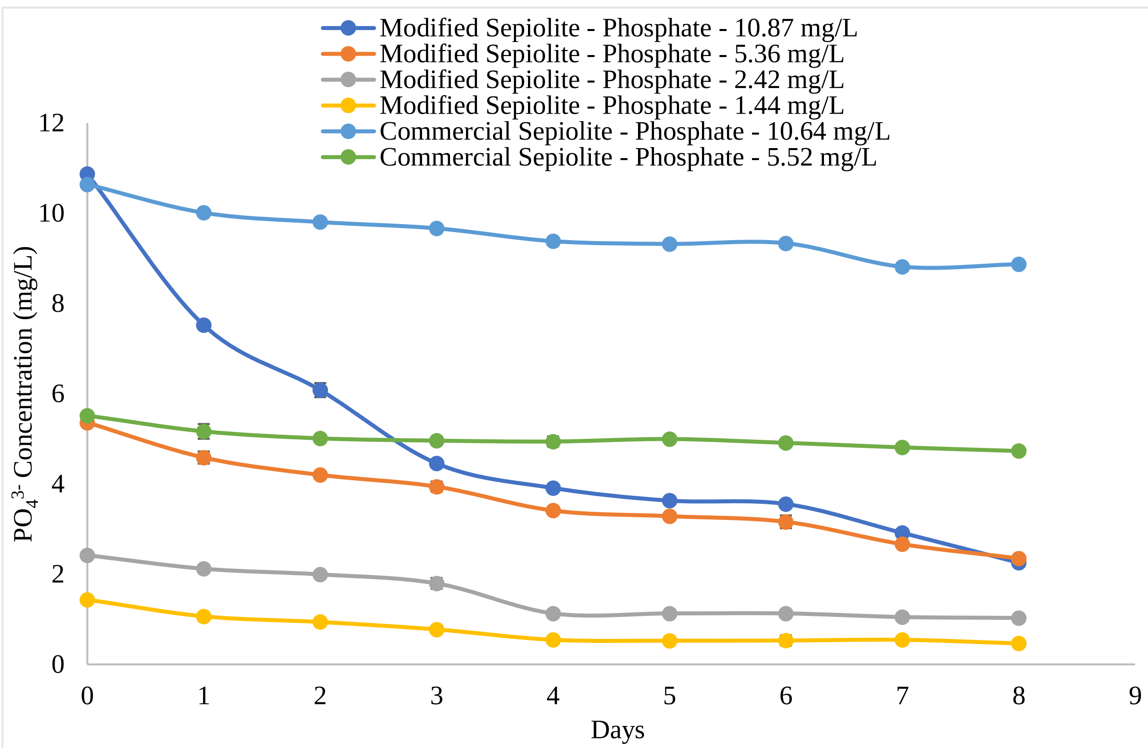

Figure 5: Variation of PO_4^{3-} concentration over time for different initial PO_4^{3-} concentrations with modified and unmodified sepiolite packed inside the dialysis tubes

5.5 Removal of microcystin by dialysis tubes packed with modified clays

The MCLR adsorption experiments were conducted using modified sepiolite and modified kaolinite. Commercial clay was not used for dialysis tube arrangement as it did not show any removal of MCLR, even with direct clay flocculation. When the initial MCLR concentrations were 20 µg/L and 40 µg/L, none of the MCLR was removed by the clay. The removal of MCLR was not observed until day 3. The MCLR removal by modified kaolinite packed inside the dialysis tubes was negligible. Figure 6 shows the variation of MCLR concentration over time for different initial MCLR concentrations with modified sepiolite. Similar to PO_4^{3-}removal, high MCLR concentration in the bulk solution could facilitate diffusion across semi-permeable dialysis tubes, leading to high MCLR removal. The highest MCLR removal efficiency of 97% was achieved at the highest initial MCLR concentration of 800 µg/L applied. An average of 90% MCLR removal efficiency could be achieved by modified sepiolite inside dialysis tube after 10 days.

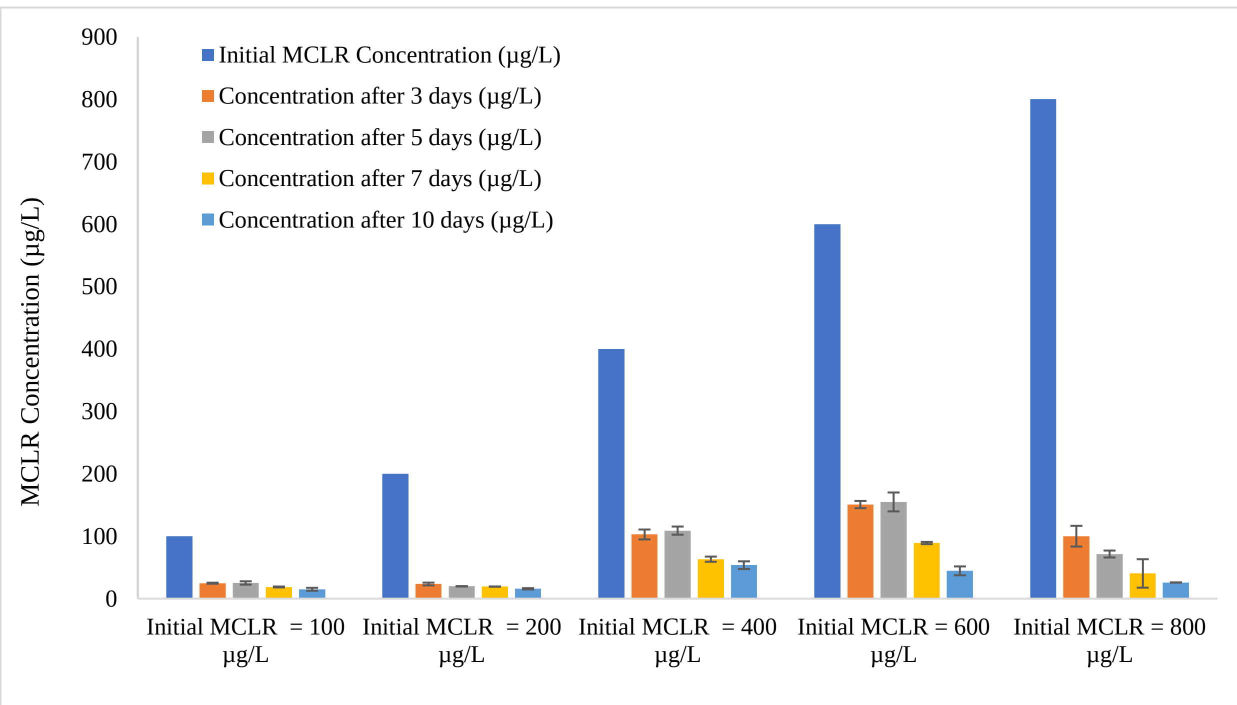

Figure 6: Variation of MCLR concentration over time for different initial MCLR Concentrations with modified sepiolite inside dialysis tubes

6. Benefits from the research

The research was conducted with the aim of developing an effective, economically feasible and environmentally friendly methodology for mitigating harmful algal blooms. It is evident that harmful algal blooms have a huge impact on the country's economy. According to The Woods Hole Oceanographic Institute's estimated calculations, harmful algal blooms cost the United States around $22 million annually in healthcare costs and lost workdays alone. This highlights the importance of this research to the benefit of the economy and health care of the country. Restoration of the aesthetic beauty of algal bloom affected lakes and estuaries would preserve the ecosystem and attract more tourists enhancing the economic benefits.

The proposed solution for HABs mitigation is expected to not only control existing algal blooms in the water body by settling harmful algae and adsorbing algal toxins but also prevent future blooms by precipitating phosphate in water. The innovative method of using dialysis tubes with packed modified clay inside will recover phosphate from water bodies to avoid phosphate release from the sediments. It is also very important to note that since dialysis tubes are biodegradable, non-toxic, and harmless to the environment and organisms, the floating device with packed dialysis tubes can be used in aquatic systems where algal blooms occur. This research is a potential solution for harmful algal blooms, which can remove not only algal cells but also algal toxins. It can also prevent potential future blooms by removing the phosphate from water permanently.

7. How the Grant was put in use:

A total of $2432.7 has been used for lab supplies and nitrogen/phosphate test kits. A total of $4036 was used to hire Ms. Abeykoon for 10 hr per week in Fall 2021 for this project. A total of $1481 and $400 were used for the payment of graduate student tuition and insurance, respectively.

8. List of Presentations:

Mitigation of Harmful Algal Blooms Using Modified Clays, the 66th Annual NM Water Conference, virtual, October 2021. (Poster presentation)

9. List of Publications or Reports:

Master's thesis was completed and submitted to New Mexico State University for the successful completion of Master of Science in Environmental Engineering in December 2021.

10. List of other students or faculty members who assisted me with my project:

Trevor Taylor, a high School student from Arrowhead Park Early College High School, was working with me in this project for one month in the summer of 2021 through the Young Scholars (YS) program conducted by NSF ReNUWIt program,

11. Special recognition awards or notable achievements because of the research, including any publicity such as newspaper articles, or similar:

An article about this project was reported in March 2022 New Mexico Water eNews by WRRI.

12. Future Career Plans:

I successfully completed my master's degree in Environmental Engineering at New Mexico State University in December 2021. I will start my doctorate study at NMSU in Fall 2022 and continue my research in mitigating harmful algal blooms. I would like to pursue a career in either the industry or the academic sector. I hope the knowledge and experience I gained from my research and academic work will pave the path to becoming a successful engineer and scientist. It is my delight to serve my country as an environmental engineer. I am also willing to convey my expertise to the younger generation for their academic knowledge and benefit. I believe that irrespective of the field I choose, I would do my best for society using the knowledge I gathered from NMSU.