

Study on the mechanisms of water ecological remediation integrated by attapulgite and submerged macrophyte

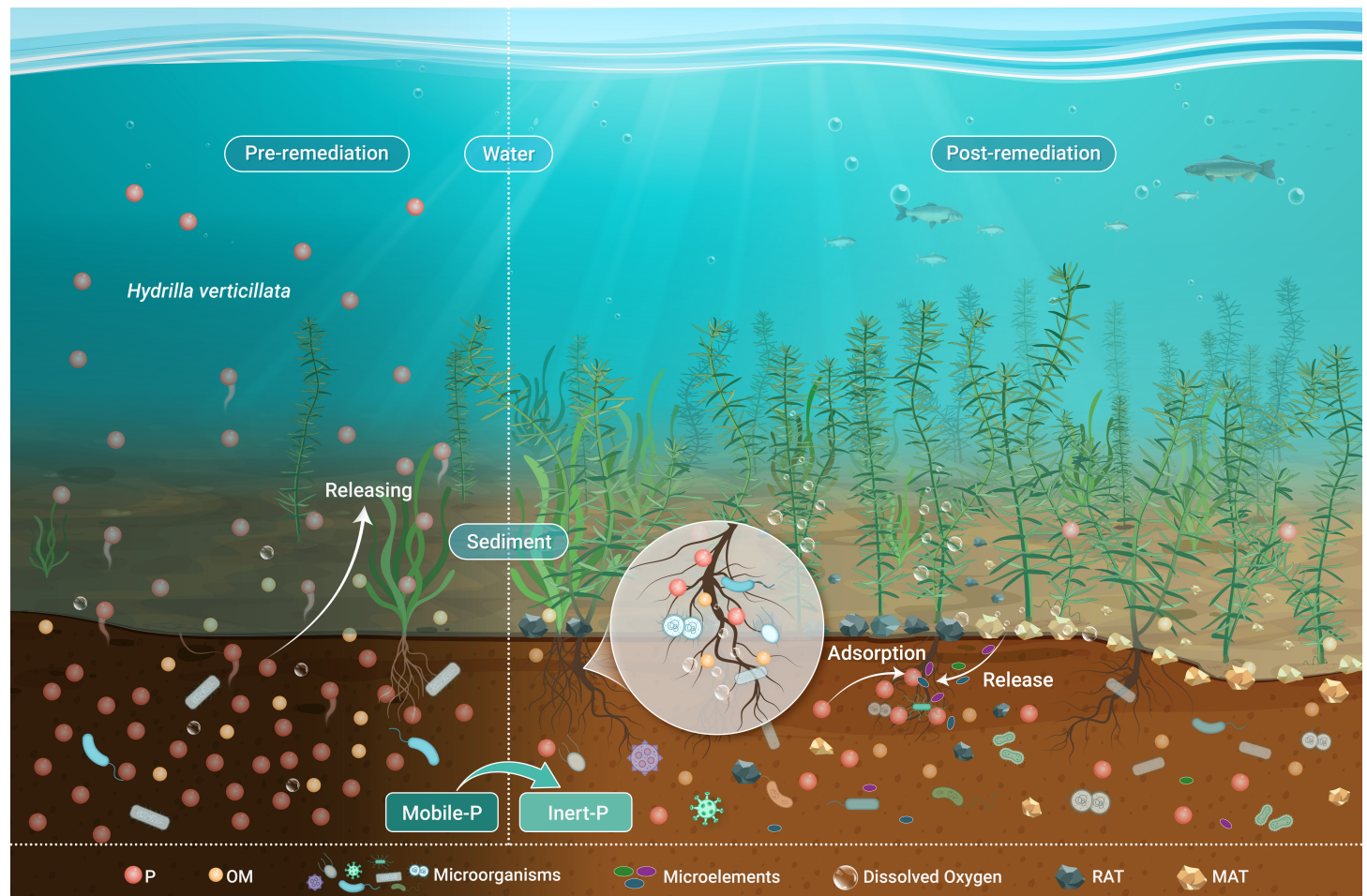
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Received: March 3, 2025; Accepted: June 28, 2025; Published Online: July 4, 2025; <https://doi.org/10.59717/j.xinn-geo.2025.100157>

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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Synergistic control of endogenous phosphorus release by submerged macrophytes and modified mineral materials.
- Mineral materials strengthen antioxidation enzyme and decrease reactive oxygen content.
- The materials increase complexity and stability of rhizosphere microbial community.
- Sediment physicochemical conditions were optimized for submerged macrophyte.

Study on the mechanisms of water ecological remediation integrated by attapulgite and submerged macrophyte

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Received: March 3, 2025; Accepted: June 28, 2025; Published Online: July 4, 2025; <https://doi.org/10.59717/j.xinn-geo.2025.100157>

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Citation: Du X., Wang R., Liu Y., et al. (2025). Study on the mechanisms of water ecological remediation integrated by attapulgite and submerged macrophyte. *The Innovation Geoscience* 3:100157.

Phosphorus is considered to be a limiting nutrient that affects primary productivity, and sediment is an important source and sink of phosphorus in lakes. This study investigates how raw attapulgite (RAT) and thermally modified attapulgite (MAT) interact with submerged macrophyte *Hydrilla verticillata* to control sediment endogenous phosphorus. Attapulgite is rich in essential trace and major elements for plant growth, with properties such as non-toxicity, strong adsorption, and chemical stability. Results show that calcination at 500°C significantly enhances attapulgite's phosphorus adsorption capacity, increasing its specific surface area and pore volume. The growth of *Hydrilla verticillata* was improved by MAT, which enhanced its stress resistance through increased antioxidant enzyme activity. The addition of 10% and 20% RAT notably increased sediment redox potential, reduced sediment pH to neutral, and created favorable conditions for biological proliferation. Additionally, the findings indicate that the addition of attapulgite markedly reduces the organic matter and phosphorus content in sediments, helping to mitigate the risk of internal nutrient release in eutrophic lakes. In summary, attapulgite can accelerate the recovery of lake ecosystem by enhancing the stress resistance of submerged macrophyte, reducing the content of organic matter and phosphorus in sediments, improving the oxygen content of sediments, and reducing the release of endogenous phosphates. This study provides scientific evidence and theoretical support for the application of attapulgite in the restoration of eutrophic lake sediments.

INTRODUCTION

Submerged macrophytes are integral to the functioning of aquatic ecosystems due to their ability to regulate key nutrient cycles, particularly nitrogen and phosphorus, enhance water transparency, and inhibit algal proliferation—functions that are critical for ecological restoration. The successful re-establishment of submerged macrophyte across a lake is widely recognized as a key indicator of the recovery of eutrophic lake systems.¹⁻⁴

Lake sediments, which serve as primary sites for nutrient cycling and biogeochemical processes, provide essential support for the growth of submerged macrophytes by offering nutrients and minerals.⁵⁻⁷ However, the accumulation of pollutants, including toxic contaminants, organic matter, and excessive nitrogen and phosphorus, has led to highly imbalanced sediment microecologies that inhibit plant growth.⁸⁻¹⁰ The physicochemical properties of sediments, therefore, play a decisive role in determining the success of macrophyte colonization, root establishment, and proliferation. In light of this, the selection of appropriate in-situ immobilization materials to remediate polluted sediments has become a crucial strategy for restoring aquatic vegetation and facilitating the recovery of degraded lake ecosystems.¹¹

Attapulgite, also referred to as palygorskite, is primarily produced in the provinces of Anhui, Jiangsu, Shandong, and Liaoning in China.¹² Attapulgite is a naturally occurring porous silicate mineral characterized by its abundant availability, low cost, and favorable properties, such as high adsorption capacity, thermal stability, surface activity, and ion exchange potential. These attributes have led to its widespread application across diverse sectors, including agriculture, environmental remediation, petrochemical industries, construction, and pharmaceuticals.¹³⁻¹⁴ Distinct from other immobilization materials, attapulgite possesses a unique nanometer-scale porous crystal

structure, which, when applied as a soil conditioner, significantly increases soil porosity, alleviates compaction, and improves both water retention and permeability.¹⁵ Furthermore, attapulgite is composed of non-toxic, environmentally benign components, ensuring that it poses no harm to benthic organisms or large invertebrate communities. Its potential to enhance soil fertility and agricultural productivity has also been demonstrated.¹⁶ In addition, attapulgite exhibits exceptional phosphorus adsorption and retention capabilities, effectively controlling the release of endogenous nutrients for extended periods, with studies showing sustained phosphorus sequestration over 124 days.¹⁷ Moreover, the mineral is enriched with essential trace elements beneficial to plant growth. When utilized as a substrate in crop fertilizers, it has been shown to increase yields and improve crop quality.¹⁸⁻¹⁹ Based on these properties, this study hypothesizes that attapulgite can fully utilize its phosphorus adsorption capacity in the sediments of eutrophic lakes, thereby alleviating high salt stress on plants. Additionally, attapulgite can improve plant growth under adverse conditions and strengthen their repair capacity through nutrient supply and the improvement of the physicochemical properties of sediments.

To fill the research gap regarding the impact of attapulgite on the stress adaptability of submerged plants and the synergistic effects of submerged plants on the physicochemical properties of lake sediments, this study investigates the application of environmentally sustainable and cost-efficient attapulgite, known for its high adsorption capacity, for in-situ restoration of submerged macrophyte. The primary objectives of this study are to examine: (1) the physicochemical properties of attapulgite before and after thermal modification; (2) the effects of attapulgite on the physiological responses of the submerged macrophyte *Hydrilla verticillata*; and (3) the influence of attapulgite on the sediment microenvironment, particularly in terms of nutrient availability and redox potential. By systematically exploring these aspects, this research aims to offer valuable technical insights for enhancing submerged macrophyte restoration in sediments with complex conditions and contribute to the broader understanding of aquatic ecosystem reconstruction.

MATERIALS AND METHODS

Study site and sediment collection

West Lake, situated in Hangzhou, Zhejiang Province, China, represents a typical urban shallow lake. The sediment from Xiaonan Lake, a sub-lake of West Lake, is characterized by elevated total phosphorus levels, high mobility, and significant organic matter content, conditions that severely hinder the growth and survival of submerged macrophytes.^{10,20} Given these challenges, this study focuses on the central sediment of Xiaonan Lake as the primary subject of investigation. The sediment sampling was conducted at coordinates 120°8'53" E and 30°14'7" N. The collected sediment, which appears gray-black, exhibits physicochemical properties indicative of severe nutrient enrichment and pollution. Detailed chemical composition and physical characteristics are provided in Table S1. To ensure the integrity of subsequent co-cultivation experiments, large particulate impurities were removed from the sediment samples, which were then stored at 4°C to preserve their original condition prior to experimentation. These sediments serve as the foundation for analyzing the influence of attapulgite on sediment properties and the growth dynamics of submerged macrophytes under eutrophic conditions.

Materials

The submerged macrophyte utilized in this experiment was *Hydrilla verticillata*, a member of the Hydrocharitaceae family and a submerged species known for its strong adaptability, rapid reproductive capacity, and high resistance to pollution. Studies have demonstrated that *Hydrilla verticillata* can quickly dominate aquatic environments, even under unfavorable conditions, and plays a significant role in mitigating eutrophication in lakes.^{21–22} Prior to experimentation, the plants were thoroughly rinsed with distilled water and acclimated under controlled conditions of 4000 Lux light intensity, with a 14:10 light-to-dark photoperiod for a duration of 7 days. For transplantation, plants exhibiting similar growth characteristics were selected and trimmed to a uniform length of 15 cm to ensure consistency in experimental conditions.

The attapulgite used in this study was sourced from Gansu Wudi Mining Co., Ltd., with the raw material originating in Jingyuan, Gansu Province. The particle size of the attapulgite ranged from 2 to 4 mm. Prior to use, the attapulgite was thoroughly cleaned, soaked in ultrapure water for 24 hours, and subsequently dried. The crystalline structure of attapulgite is characterized by its needle-like, rod-like, or fibrous form, with a honeycomb-like lattice that contains extensive internal and external pore channels, providing a large specific surface area.²³ It has been demonstrated that thermal activation can significantly modify this crystalline structure, facilitating the release of additional calcium, iron, and aluminum ions from the crystal matrix, thereby improving the material's phosphorus adsorption capacity.²⁴ In this study, the phosphorus adsorption efficiency of attapulgite was enhanced through thermal modification. The raw attapulgite (RAT) particles were uniformly distributed in a crucible and calcined in a muffle furnace at 500°C for 2 hours, resulting in thermally modified attapulgite (MAT). After calcination, the material was allowed to cool to room temperature, after which it was ready for experimental use.¹⁸

Experiments method

Material characterization and data analysis. After thermal modification of attapulgite, the following tests are conducted:

1. **Physical Structure Characterization:** The modified attapulgite's physical and chemical attributes were assessed through Scanning Electron Microscopy (SEM), which provided detailed imaging of the material's surface morphology. Additionally, a fully automated Brunauer-Emmett-Teller (BET) surface area and porosity analyzer was employed to quantify specific surface area and pore volume. Elemental composition was further analyzed using X-ray Fluorescence (XRF) spectroscopy, allowing for the identification of major and trace elements.

2. **Leaching Experiment Design:** A leaching experiment was performed to determine the concentration of elements released from the modified attapulgite. In the experimental procedure, 150 grams of both modified and unmodified attapulgite were separately mixed with 300 milliliters of deionized water. On the 180th day of the experiment, 10 milliliters of the supernatant were collected using a microsyringe, subjected to centrifugation, and subsequently preserved in a -4°C refrigerator with the addition of 0.2 milliliters of electronic-grade nitric acid. This was achieved using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) with a PerkinElmer 8300, in conjunction with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using a PerkinElmer NexION 300X, to accurately quantify the leached elemental content. These tests provided crucial insights into the material's environmental behavior and its potential applications in sediment remediation.

Phytoremediation experiments. The experimental setup consisted of glass tanks with dimensions of 25 cm × 25 cm × 50 cm. Sediment, *Hydrilla verticillata*, and water were all collected from Xiaonan Lake in West Lake, Hangzhou. Raw attapulgite (RAT) and thermally modified attapulgite (MAT) were incorporated into the sediment at varying mass ratios of 10%, 20%, 40%, and 60%. Grouping settings details for co-culture experiments are shown in Figure 1. The mixtures were evenly distributed across the bottom of each tank to form an 8 cm-thick sediment layer. *Hydrilla verticillata* plants, pre-trimmed to uniform lengths, were then transplanted into the tanks. Each experimental group contained 20 plants and was replicated across three independent glass tanks. The water level in each tank was maintained at a constant height of 45 cm, and the tanks were exposed to natural light condi-

tions with direct air interaction to simulate a lake-like environment. The experimental period spanned 3 months, with macrophyte and sediment samples collected on a monthly basis. In situ measurements of sediment oxidation-reduction potential (ORP) and pH were taken using a portable analyzer (Thermo Scientific A326). Microbial samples were collected in the first and third months to assess changes in the microbial community. Sediment organic matter (OM) was quantified using the combustion method,²⁵ while sediment phosphorus fractions were analyzed according to the SMT phosphorus fractionation method.²⁶ This experimental design enabled a comprehensive analysis of the effects of attapulgite on sediment properties and *Hydrilla verticillata* under controlled conditions.

Physiological and biochemical analysis. For *Hydrilla verticillata* sample collection, three randomly selected specimens were harvested from each experimental setup. The collected samples were immediately wrapped in dry ice to preserve their physiological state and transported to the laboratory for the prompt analysis of physiological and biochemical parameters. Chlorophyll content in the leaves was quantified using a modified version of the Lichtenthaler-Arnon method.¹⁷ Malondialdehyde (MDA) levels, which serve as an indicator of lipid peroxidation and oxidative stress, were measured using the thiobarbituric acid (TBA) method.²⁷ The activity of superoxide dismutase (SOD), a critical antioxidant enzyme involved in plant defense against oxidative damage, was determined through the nitroblue tetrazolium (NBT) photoreduction assay.²⁸ These analyses provided insights into the physiological responses and stress tolerance of the plants under varying experimental conditions.

Microcosm experiments. Sediment samples for microscopic and microbial analysis were collected using an improved small columnar sediment sampler following standardized rhizosphere sampling protocols.²⁹ The collected sediment was immediately placed into sealable bags for secure storage. Sampling was conducted at two time points: 30d and 90d. All samples were transferred into 10 mL sterile centrifuge tubes and stored at -80°C to preserve their integrity for subsequent analyses. Microbial community structure was assessed by Shanghai LingEn Biotechnology Co., Ltd., using Illumina MiSeq high-throughput sequencing technology. Genomic DNA was extracted from the sediment samples, and the V4-V5 hypervariable regions of the 16S rDNA were amplified using specific barcode-labeled primers. The primer sequences employed were 515F (GTGCCAGCMGC-CGCGG) and 806R (GGACTACNCGGTGTCTAAT).^{30–31} The resulting PCR products were purified using the AxyPrep DNA Gel Extraction Kit (AXYGEN), followed by quantification with the QuantiFluor™-ST Blue Fluorescence Quantification System. The purified products were then pooled in appropriate ratios based on the sequencing requirements for each sample. Sequencing adapters were ligated, and sequencing libraries were constructed. High-throughput sequencing was performed using Illumina PE250 technology.³² The resulting microbial data were analyzed to determine the alpha diversity and richness of the microbial communities present in the sediment, providing insights into the effects of attapulgite treatment on microbial ecology.

Data analysis

Image processing was conducted using Origin Pro 8.0 (Origin Lab Corp.), while all statistical analyses were performed using SPSS 18.0 software (SPSS Inc., IBM, USA). The visualization of the correlation heatmap, principal component analysis (PCA), and redundancy analysis (RDA) was performed using R version 3.5.1. Statistical differences between experimental groups were evaluated at a significance level of $\alpha = 0.05$. Data are reported as mean values ± standard deviation, with each treatment consisting of three replicates. Statistical significance was determined through one-way Analysis of Variance (ANOVA) and multiple comparisons to identify differences among groups, which multiple comparisons were performed by the least significant difference (LSD) with Bonferroni correction to reduce type I errors. Additionally, Pearson correlation analysis was employed to assess the relationships between variables, including *Hydrilla verticillata* parameters, sediment characteristics, and the effects of attapulgite. This comprehensive statistical approach enabled the identification of significant patterns and correlations, providing a robust framework for interpreting the experimental results.

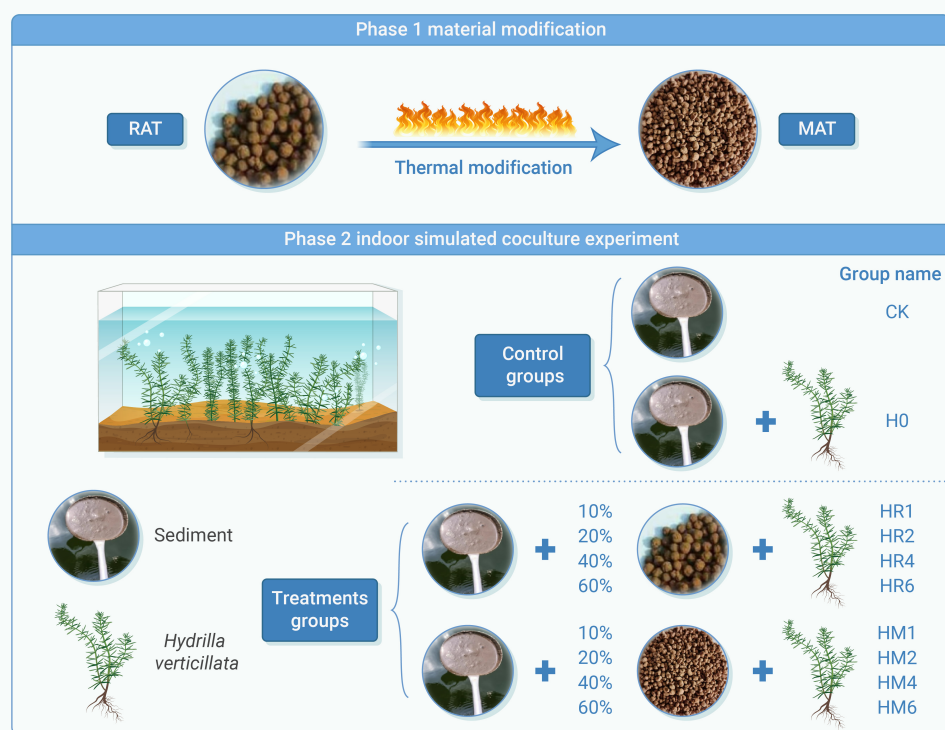


Figure 1. Grouping settings for co-culture experiments.

leachable amount of attapulgite on 180d was 4.01 mg/g for unmodified attapulgite and 5.63 mg/g for thermally modified attapulgite (Figure 3). Both types of attapulgite can leach beneficial microelements for plant growth, such as Ca, Na, S, Mg, and K. Specifically, the leachable amounts of Ca, Na, S, Mg, and K in RAT are 1.27, 2.33, 1.59, 0.34, and 0.09 mg/g, respectively, while in MAT, the corresponding leachable amounts are 1.44, 1.34, 0.98, 0.04, and 0.20 mg/g. Additionally, attapulgite also releases trace elements beneficial for plant growth, such as Zn, Mo, Se, and Mn.³³⁻³⁴ Among these elements, K and B are involved in the development of plant branches and roots,³⁵⁻³⁶ Mn and Mg are related to chlorophyll synthesis,³⁷ S and Mo aid in protein synthesis,³⁸ and Zn plays a crucial role in plant development by promoting the synthesis of growth hormones.³⁹⁻⁴⁰

RESULTS

Effects of thermal modification on the physicochemical properties of attapulgite

Figure 2 illustrates the morphological and structural changes in attapulgite resulting from thermal modification. Prior to modification, the surface of the unmodified attapulgite exhibits elongated, rod-like crystal bundles with distinct profiles and well-defined fibrous morphology. These rod-like crystals are irregularly embedded within an amorphous, block-like structure, indicating the presence of significant impurities alongside the attapulgite crystals. Following thermal treatment at 500°C, the rod-like crystals undergo disaggregation, forming irregular particles, predominantly ranging in size from 1 to 20 µm, though some dispersed rod-like crystals persist in certain regions. BET analysis further reveals that the specific surface area and pore volume of the raw attapulgite are 110.19 m²/g and 0.25 mL/g, respectively, with an average pore diameter of 9.22 nm. In comparison, thermal modification results in a 23.38% increase in specific surface area and a 20.00% increase in pore volume, while the average pore diameter decreases slightly. These changes suggest that the thermal activation significantly enhances the adsorption properties of attapulgite, primarily by increasing surface area and pore volume, critical factors for its efficacy in environmental applications.

To assess the composition and leachable elements of attapulgite, X-ray fluorescence (XRF) spectroscopy and leachable element analysis were performed. These analyses were designed to evaluate attapulgite's chemical profile and potential environmental impact, particularly in the context of ecological remediation. The detailed findings are presented in Table 1 and Figure 3. Attapulgite was found to exhibit a complex composition, predominantly comprising SiO₂, Al₂O₃, CaO, Fe₂O₃, and MgO, along with various impurities. SiO₂, Al₂O₃, and CaO were identified as the major constituents.

Following thermal modification, a notable reduction in SiO₂ content was observed, accompanied by a corresponding increase in the proportion of alkaline metals. The phosphorus content remained low, ranging from 0.20% to 0.23%, which suggests that phosphorus present in attapulgite would not significantly affect its adsorption capacity in phosphorus-related experiments. This compositional analysis provides essential insights into the material's chemical stability and its suitability for long-term use in environmental applications, especially in nutrient management and sediment remediation.

By comparing the leachable element content of attapulgite before and after modification, we can further analyze its role and potential hazards in ecological remediation applications. The experimental results reveal that the total

Effects of attapulgite on the physiological and biochemical indicators of *Hydrilla verticillata*

Impact of attapulgite on the photosynthetic pigment content of *Hydrilla verticillata*

The effects of attapulgite on the photosynthetic pigments of *Hydrilla verticillata* are illustrated in Figure S1. Correlation analysis reveals that the trends in chlorophyll a and total chlorophyll content (Chl-a + Chl-b) across different groups are generally consistent ($R^2 = 0.99$, $P < 0.001$). On 30d, the chlorophyll a content in the HM2, HR2, and HR6 groups is significantly lower than that of the control group ($P < 0.05$). By 60d, chlorophyll b content in the HR4 group is significantly lower than in the control group, while differences in other groups are not significant compared to the control. The chlorophyll a, the chlorophyll b, and total chlorophyll content in *Hydrilla verticillata* reach their maximum values on 60d, with the highest levels observed in the HM4 and HR1 groups. By 90d, the total chlorophyll content decreases across all groups. These results suggest that the effect of attapulgite on photosynthetic pigment levels is time-dependent, with the most pronounced effects observed at intermediate stages of the experimental timeline, followed by a subsequent reduction in pigment concentration over time. This trend highlights the complex interactions between attapulgite and the photosynthetic mechanisms in submerged macrophytes, potentially driven by varying nutrient availability or stress responses.

Effects of attapulgite on MDA and SOD content in *Hydrilla verticillata* leaves

The effects of attapulgite on malondialdehyde (MDA) and superoxide dismutase (SOD) levels in the leaves of *Hydrilla verticillata* exhibit contrasting trends, as shown in Figure S2. During the first month of the experiment, MDA content and SOD activity in the modified attapulgite (MAT) groups were found to be inversely correlated with the attapulgite mixing ratio. Specifically, SOD activity was significantly higher, while MDA content was markedly lower compared to the control group ($P < 0.05$). This suggests that MAT effectively mitigated *Hydrilla verticillata* oxidative stress by enhancing antioxidant enzyme activity in the early stages, with the most pronounced protective effects observed in the HM1 group. Except for the HM4, HM6, and HR2 groups, MDA content in the other attapulgite-treated groups initially increased and then decreased over time. Throughout the experiment, SOD activity in the attapulgite-treated groups consistently remained higher than in the control, with the HM1 group showing the highest SOD activity ($P < 0.05$). By the conclusion of the experiment, both MDA content and SOD activity were positively correlated with the attapulgite mixing ratio in both the raw and modified attapulgite groups. Notably, SOD activity in all treated groups

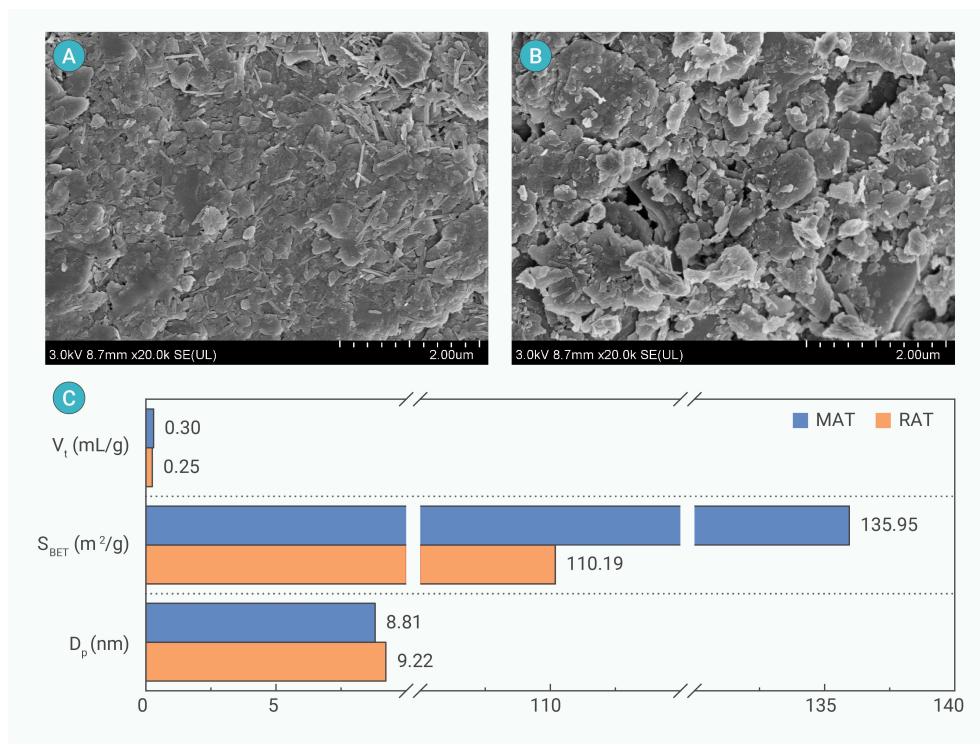


Figure 2. Scanning electron microscope (SEM) pictures and BET parameter (A) SEM of RAT. (B) SEM of MAT. (C) SEM of BET parameter.

the appropriate ratio of attapulgite enhances sediment redox potential and pH balance, which are crucial for maintaining sediment quality and preventing nutrient release in lake ecosystems.

The effect of attapulgite on sediment organic matter. Lake sediments enriched with high levels of organic pollutants often create anaerobic or hypoxic conditions, which can inhibit plant root respiration and significantly hinder ecological restoration efforts. Therefore, reducing the organic matter (OM) content in such sediments is critical for successful lake restoration. Figure S4 presents the results of a one-way ANOVA analysis on the organic matter content of sediments treated with varying proportions of attapulgite mixtures. Throughout the experimental period, sediments treated with modified attapulgite (MAT) displayed only minimal differences in OM content compared to the control group. However, sediments treated with raw

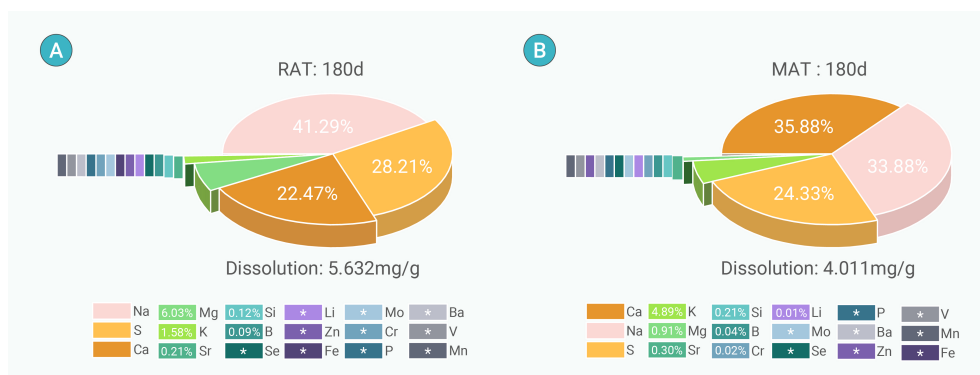


Figure 3. The content of elements dissolved by attapulgite (A) Element leaching of RAT on the 180th day. (B) Element leaching of MAT on the 180th day. (*: percentage of dissolution < 0.01%)

exceeded that of the control group. However, in high-dose attapulgite treatments, the accumulation of MDA indicated that submerged macrophyte were experiencing membrane lipid peroxidation, suggesting oxidative stress. Conversely, low-dose attapulgite appeared more effective in helping submerged macrophyte resist oxidative damage, reinforcing the importance of dosage in determining the efficacy of attapulgite in mitigating oxidative stress.

The impact of attapulgite on sediment

The effect of attapulgite on the physical properties of sediment. Once the system reached stabilization, measurements of sediment oxidation-reduction potential (ORP) and pH were taken, as depicted in Figure S3. The ORP provides a quantitative assessment of the oxidation-reduction conditions within lake sediments.⁴¹ All sediment samples remained in an oxidized state throughout the experiment. The addition of raw attapulgite (RAT) notably improved the ORP values compared to the control group (H0). Specifically, a positive correlation between the ORP values and attapulgite mixing ratio was evident at $\geq 20\%$, but this trend reversed as the mixing ratio below 20%, indicating a negative correlation.

Regarding pH, the sediments in the control group exhibited slight acidity, which may increase the risk of Fe/Al-bound phosphorus (Fe/Al-P) release. In contrast, the application of modified attapulgite (MAT) significantly elevated the sediment pH ($P < 0.05$), stabilizing it at near-neutral levels. This stabilization of pH is critical in reducing the potential for phosphorus release, thereby contributing to improved sediment conditions. The results demonstrate that

substantially lowering the organic matter content of sediments. This reduction in OM content plays a crucial role in mitigating anaerobic conditions, thus facilitating improved sediment quality and promoting more favorable conditions for ecological restoration in eutrophic lakes. The data underscore the potential of raw attapulgite as a viable solution for reducing sediment organic pollutants and enhancing the overall restoration process.

The effect of attapulgite on sediment phosphorus forms. Throughout the experimental period, the total phosphorus (TP) content in sediments treated with raw attapulgite (RAT) consistently remained lower than that in the control group (H0). At 90d, Figure S5 shows that the HR6 group exhibited the most substantial reduction in TP content (0.87 ± 0.01 mg/g), compared to the control group (1.31 ± 0.03 mg/g). Similarly, higher proportions of thermally modified attapulgite (MAT) also resulted in significant reductions in sediment TP content.

Among the various phosphorus forms present in the sediments, inorganic phosphorus (IP) represented the largest proportion, as illustrated in Figure 4. By 30d, IP accounted for 87.12% and 67.58% of the total phosphorus in the HR6 and H0 groups, respectively, reflecting trends consistent with overall TP content. The HR6 group also exhibited the lowest levels of Fe/Al-bound phosphorus (Fe/Al-P), with Fe/Al-P content decreasing as the attapulgite mixing ratio increased. In contrast, the levels of calcium-bound phosphorus (Ca-P) increased, particularly in the RAT treatment groups, where Ca-P concentrations were higher than in the control group.

Total phosphorus content in the H0 sediments was comparable to that in the CK group. However, the application of RAT in conjunction with *Hydrilla*

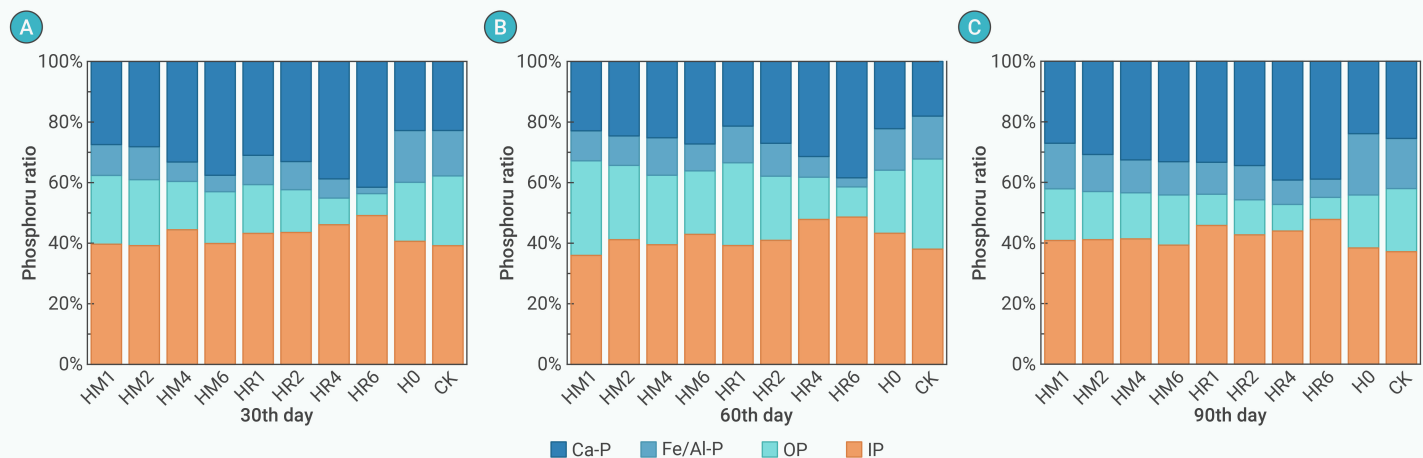


Figure 4. Phosphorus fractional composition of sediments (A: 30d. B: 60d. C: 90d)

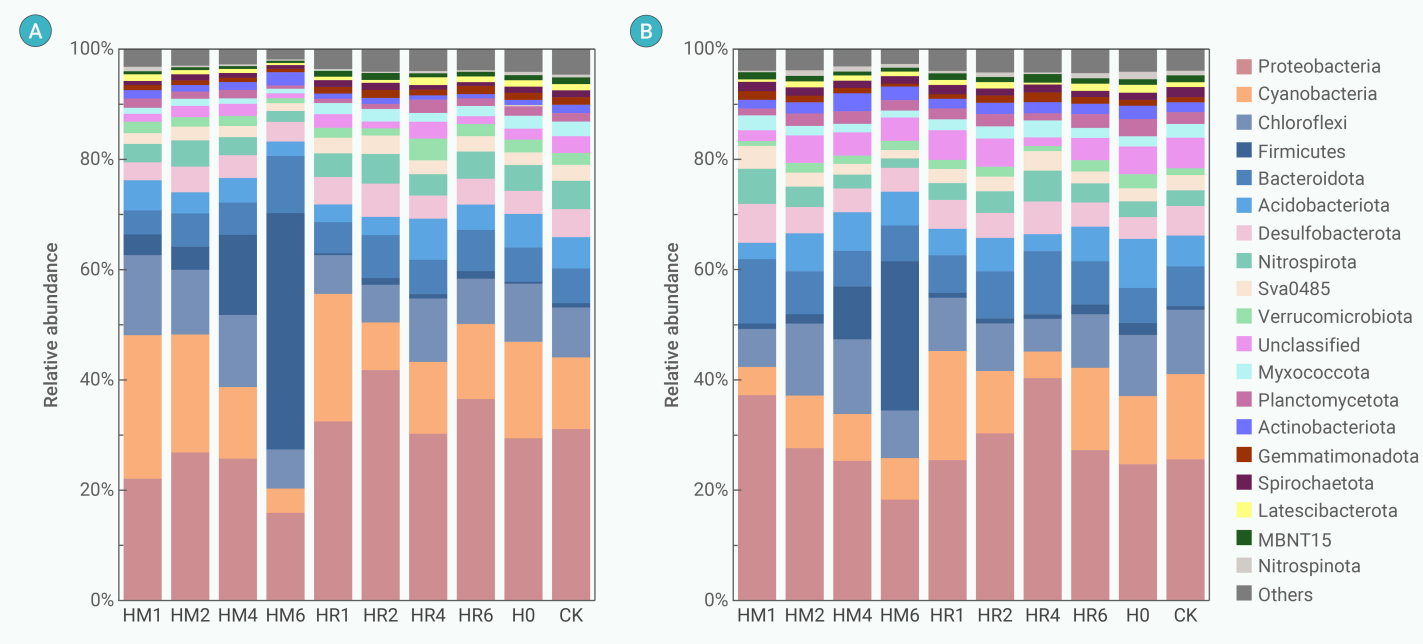


Figure 5. Relative abundance of microbial community at phylum level in groups of *Hydrilla verticillata* (A: 30d. B: 90d)

verticillata significantly reduced TP levels in the sediments. Both RAT and MAT treatments were effective in decreasing Fe/Al-P while increasing Ca-P content, with the magnitude of these effects positively correlated with the attapulgite mixing ratio. Organic phosphorus (OP) concentrations increased from 30d to 60d, followed by a slight decline at 90d. By the later stages of the experiment, the HR6 group had the lowest OP content (0.11 ± 0.01 mg/g), significantly lower than that of the control group (0.41 ± 0.03 mg/g). These findings underscore the capacity of attapulgite, particularly in higher proportions, to mitigate phosphorus release in sediments by altering its chemical speciation and reducing nutrient availability.

DISCUSSION

The effect of attapulgite on sediment microbes

Microbial community sequencing analyses were performed on sediment samples collected at 30d and 90d, with the alpha diversity indices presented in Table S2. At 30d, the number of operational taxonomic units (OTUs) in the raw attapulgite (RAT) treatment groups ranged from 75374 to 81644, all of which exceeded those observed in the control group (H0). Specifically, the Shannon index values for the HR2, HR4, and HR6 groups were higher than those in the H0 group, while both the Chao and ACE indices for HR4 were

also elevated compared to the control.

By 90d, with the exception of the HR1 group, which displayed a higher OTU count than the control, all other groups exhibited a decrease in OTU numbers relative to H0. The Shannon index for HR1 (10.05) was marginally higher than that of H0 (10.04), whereas the Shannon index values for the remaining groups were lower than H0. These findings suggest that the addition of lower proportions of RAT can enhance microbial diversity in sediments, as reflected by increased OTU numbers and diversity indices. However, the application of higher RAT concentrations appears to lead to a reduction in microbial diversity, indicating a potential threshold beyond which attapulgite may negatively impact the microbial community. This outcome highlights the importance of optimizing the attapulgite dosage to balance microbial ecosystem health and restoration efficacy.

Figure 5 presents the relative abundance of microbial phyla across different treatment groups. At 30d, the dominant phyla in all groups were *Proteobacteria*, *Cyanobacteria*, *Chloroflexi*, and *Firmicutes*, with average relative abundances of 29.22%, 15.38%, 9.95%, and 7.01%, respectively. By 90d, the relative abundance of *Cyanobacteria* had decreased by 4.45%, and the thermally modified attapulgite (MAT) treatment groups exhibited significantly lower *Cyanobacteria* abundances compared to the control groups (H0 and

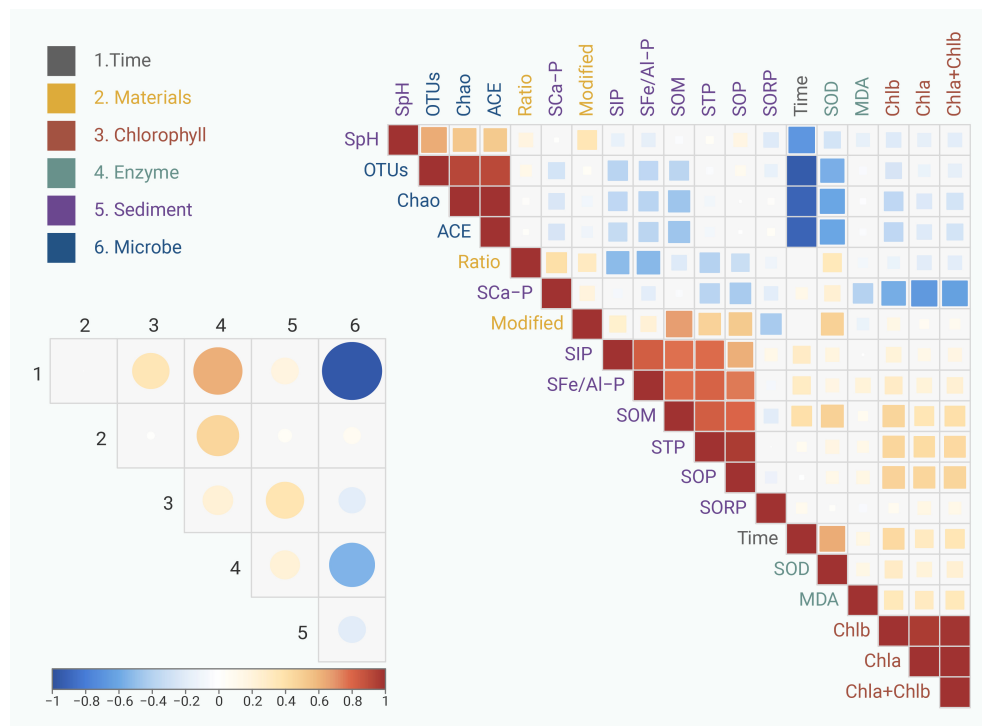


Figure 6. Pearson correlation coefficients for attapulgite, hydrilla verticillata and sediments.

total phosphorus content, while showing positive correlations with SOD activity and calcium-bound phosphorus (Ca-P). Organic matter content in the sediment was negatively correlated with ORP but positively correlated with total phosphorus, organic phosphorus, inorganic phosphorus, and Fe/Al-bound phosphorus.

The increasing proportion of attapulgite, along with the application of modified treatments, led to a significant increase in SOD enzyme activity in *Hydrilla verticillata* leaves. This suggests that the growth of submerged macrophyte enhances sediment organic matter utilization and contributes to an improved redox potential by releasing oxygen into the rhizosphere, thus enhancing nutrient availability. Lower proportions of modified attapulgite (MAT) and raw attapulgite (RAT) contributed to higher sediment ORP, whereas higher proportions appeared to exert excessive mechanical pressure on plant roots, potentially hindering

oxygen diffusion and release.^{33,50}

CK). This finding suggests that the combined application of MAT and submerged macrophytes, such as *Hydrilla verticillata*, is more effective in controlling nutrient-rich sediments than the use of *Hydrilla verticillata* alone.

The abundance of *Firmicutes* in the MAT treatment groups was also higher than in the raw attapulgite (RAT) and control groups. On 30d, *Firmicutes* accounted for 3.79% to 42.83% of the microbial community, and although its abundance significantly decreased by 90d, it remained relatively high in the HM4 and HM6 groups. This sustained presence of *Firmicutes* may be associated with reduced sediment moisture due to excessive MAT addition. Given *Firmicutes*' known tolerance to extreme conditions, such as drought, their relative abundance could serve as an indicator of sediment suitability for the growth of submerged macrophytes.⁴²

As the co-cultivation period progressed, attapulgite-modified sediment showed an increased abundance of sulfate-reducing bacteria (SRB), particularly *Sva0485* and *Desulfobacterota*, compared to the plant control group (H0). SRB play a vital role in ecological remediation by immobilizing heavy metals through the reduction of sulfates to sulfides, making them important in the restoration of heavy metal-contaminated aquatic ecosystems.⁴³ Additionally, both 10%–20% MAT and 10%–60% RAT treatments led to an increased relative abundance of *Nitrospirae*, a phylum of nitrifying bacteria. In the *Hydrilla verticillata* groups, the average increases in *Nitrospirae* were 2.17% and 1.15%, respectively. Nitrifying bacteria, such as *Nitrospirae*, are essential for macrophytes, as they oxidize nitrites to nitrates, facilitating nutrient uptake and playing a critical role in the nitrogen cycle.^{44–46} Furthermore, the relative abundances of *Proteobacteria*, *Bacteroidota*, and *Nitrospirota* in HR2, along with *Cyanobacteria* in HR1, were consistently higher than those in the control group CK at both 30 d and 90 d. This observation indicates that low-ratio attapulgite amendments may promote plant growth by enhancing nutrient cycling, improving sediment microstructure, and eliminating toxic substances.^{47–49}

Correlation analysis between attapulgite, *Hydrilla verticillata*, and sediments

Correlation analysis (Figure 6) reveals several key relationships between experimental variables. Sampling time was found to be positively correlated with superoxide dismutase (SOD) activity in *Hydrilla verticillata* leaves and negatively correlated with sediment pH. The modified treatments displayed positive correlations with sediment organic matter (OM) and various phosphorus forms. Notably, the attapulgite mixing ratio exhibited negative correlations with sediment oxidation-reduction potential (ORP), organic matter, and

oxygen diffusion and release.^{33,50}

Elemental leaching analysis further indicated that calcium accounted for 22.47% and 35.88% of the total leached elements in RAT and MAT, respectively. The dissolution of calcium-rich minerals is typically associated with increased sediment pH and calcium ion concentration,⁵¹ which may explain the observed pH fluctuations in the early stages of the experiment. Over time, the secretion of organic acids from plant roots likely contributed to further reductions in sediment pH.⁵² These correlations emphasize the complex interactions between attapulgite amendments, plant activity, and sediment chemistry, which collectively influence sediment nutrient dynamics and ecological restoration outcomes.

In addressing the issue of internal nutrient loading in eutrophic lakes, attention is typically directed toward nutrient elements involved in biogeochemical cycling within the aquatic ecosystem. Organic matter functions as the primary carrier of these nutrients, and the growth of macrophytes is closely linked to nutrient uptake and organic matter accumulation.⁵³ As sediments undergo mineralization and early diagenesis, the degradation of organic matter releases nutrients back into the system.

Thermal modification of attapulgite has been shown to reduce its SiO₂ content while increasing the proportion of alkaline metals, which enhances its capacity for phosphorus adsorption—phosphorus being a critical nutrient in eutrophic systems. The incorporation of thermally modified attapulgite (MAT) has been demonstrated to effectively reduce both organic matter and total phosphorus content in sediments. It is postulated that MAT promotes the transformation of Fe/Al-bound phosphorus (Fe/Al-P) into calcium-bound phosphorus (Ca-P). This process is likely facilitated by the high calcium content in attapulgite, as Ca²⁺ ions interact with phosphate ions, converting unstable phosphorus forms into more inert Ca-P forms, thus achieving "phosphorus passivation".⁵⁴ This conversion is critical for limiting the bioavailability of phosphorus and mitigating the internal nutrient loading that drives eutrophication.

Principal component analysis (PCA) and redundancy analysis (RDA)

To gain a more comprehensive understanding of the relationships between the variables, a principal component analysis (PCA) was performed on the dataset. The results of this analysis are illustrated in Figure 7A. The first and second axes of the PCA yielded eigenvalues of 0.36 and 0.19, respectively. The analysis reveals that sediment pH, calcium-bound phosphorus content, and superoxide dismutase (SOD) activity are positively correlated with the attapulgite mixing ratio. In contrast, variables such as chlorophyll content,

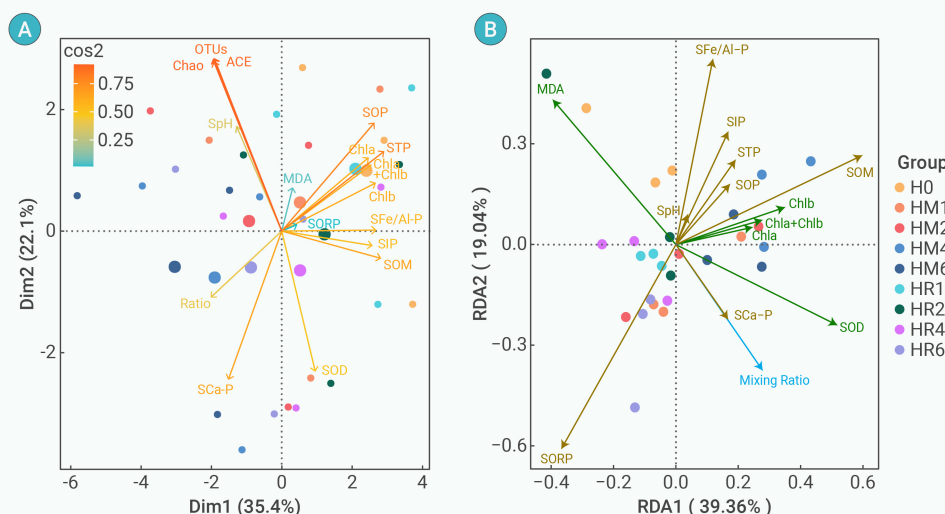


Figure 7. PCA (A) and RDA (B) of sediment properties and submerged macrophyte characteristics in response to attapulgite mixing ratio.

internal nutrient loading and improving sediment quality.

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organic matter, total phosphorus, organic phosphorus, and inorganic phosphorus show negative correlations with the attapulgite mixing ratio. This pattern suggests that the incorporation of attapulgite effectively decreases phosphorus content in the sediment, a key factor in controlling eutrophication. However, excessive attapulgite application may negatively affect plant growth by hindering nutrient availability.

Redundancy analysis was performed on the test results to explain the relationship between submerged macrophytes characters and substrate conditions. The RDA results showed that the explanation between submerged macrophytes characters and substrate conditions was 19.04% and 39.36%, respectively (Figure 7B). It is evident that the mixing ratio positively influenced the total phosphorus (TP), organic phosphorus (OP), inorganic phosphorus (IP), Fe/Al-bound phosphorus (Fe/Al-P) content, sediment organic matter, and chlorophyll content. This suggests that groups with a higher mixing ratio are more effective in alleviating phosphorus loading in the substrate within the context of this study.

CONCLUSIONS

This study comprehensively investigated the effects of modified attapulgite on submerged macrophyte and the sediment microenvironment, yielding several key findings.

1. Attapulgite was found to contain a rich array of trace and major elements essential for plant growth, with thermal modification increasing its specific surface area and pore volume to 135.95 m²/g and 0.30 cm³/g, respectively—enhancements of 23.38% and 20.00% over the raw material.

2. The combination of attapulgite and *Hydrilla verticillata* proved more effective in removing phosphorus from sediments than the use of *Hydrilla verticillata* alone. Phosphorus reduction was significant, with the HR6 group showing a 33.69% decrease in total phosphorus (TP) compared to the control ($P < 0.05$). The attapulgite proportion was negatively correlated with TP, inorganic phosphorus (IP), organic phosphorus (OP), and Fe/Al-bound phosphorus (Fe/Al-P), while positively correlated with calcium-bound phosphorus (Ca-P), highlighting its potential for phosphorus stabilization.

3. Attapulgite enhanced antioxidant enzyme activity in *Hydrilla verticillata*, contributing to higher superoxide dismutase (SOD) activity and maintaining sediment pH and oxidation-reduction potential (ORP) at levels conducive to biological growth. This improvement was evident in the reduction of malondialdehyde (MDA) levels, particularly in groups with higher attapulgite concentrations ($P < 0.05$), suggesting that appropriate attapulgite dosages can promote plant root oxygenation.

4. While the addition of lower proportions of attapulgite enhanced sediment microbial diversity, excessive amounts led to a reduction in diversity. Notably, Firmicutes abundance in the MAT treatment groups was significantly higher, ranging from 3.79% to 42.83% at 30d.

These findings collectively indicate that the combined application of attapulgite and *Hydrilla verticillata* effectively mitigates the risk of phosphorus release, contributing to the remediation of eutrophic lakes by addressing

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FUNDING AND ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China (No. 31830013; 32201384; 42307336); National Natural Science Foundation of Hubei Province Youth A (2025AFA070); Key Research and Development Program of Hubei Province, China (2022BCA066; 2024BCB062); China Postdoctoral Sciences Foundation (2024M763481); The project of cross-disciplinary research team of state key laboratory of breeding biotechnology and sustainable aquaculture (2024BBSA09). We thank Ms. Liping Zhang for the experimental help. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

Xiaopei Du: Writing – original draft, Visualization, Formal analysis. Rou Wang: Investigation, Methodology, Data curation. Yunli Liu: Writing – review & editing, Conceptualization, Data curation, Formal analysis. Hang Yang: Formal analysis, Investigation. Qi Feng: Methodology, Formal analysis, Data curation. Guoliang Bai: Conceptualization, Formal analysis. Qiaohong Zhou: Conceptualization, Writing – review & editing, Supervision, Project administration. Zhenbin Wu: Conceptualization, Writing – review & editing, Supervision, Project administration. Yi Zhang: Methodology, Conceptualization, Writing – review & editing, Funding acquisition.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

Date will be made available on request.

SUPPLEMENTAL INFORMATION

It can be found online at <https://doi.org/10.59717/j.xinn-geo.2025.100157>