COMMON CARP *CYPRINUS CARPIO* L. WEAKING THE ACTIVITY OF TWO COMMONLY USED WATER-CLEARING AGENTS FOR LAKE EUTROPHICATION CONTROL: A MESOCOSM STUDY

GAO, J.[1*] – DAI, Q.[1] – FEI, Q.[1] – GUO, F.[2] – MIAO, T.[3] – ZHANG, H.[1] – LIAO, M.[1] – WANG, H.[1]

[1]Key Laboratory of Intelligent Health Perception and Ecological Restoration of Rivers and Lakes, Ministry of Education, Hubei Province Key Laboratory of Ecological Restoration of Lakes and Rivers and Algal Utilization, Innovation Demonstration Base of Ecological Environment Geotechnical and Ecological Restoration of Rivers and Lakes, School of Civil and Environmental Engineering, Hubei University of Technology, Wuhan 430068, China

[2]Gezhouba Group Eco-Environmental Protection CO., LTD., Wuhan 430033, China

[3]Hubei Water Resources Research Institute, Wuhan 430070, China

**Corresponding author*
e-mail: jgao13@hotmail.com; phone: +86-186-7295-0309

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Abstract. Polyaluminum chloride (PAC) and anionic polyacrylamide (APAM) are widely used to control phosphorus in lakes and have demonstrated good levels of efficiency. However, the ability of PAC and APAM to control phosphorus in some eutrophic lakes, in which the fish community is dominated by benthivorous fish, remains unclear. In this study, we investigated the effect of benthivorous common carp (*Cyprinus carpio* L.) on the water-clearing efficiency of PAC and APAM in eutrophic lakes. We performed a 21-day experiment in 12 mesocosms (3 containing carp without PAC or APAM; 3 containing carp with PAC; 3 containing carp with APAM; and 3 controls without carp and agents). Compared to control treatments, the presence of common carp disturbed sediment and promoted the release of phosphorus and suspended solid in the groups with and without water-clearing agents. Significantly lower concentrations of total phosphorus (TP) and total dissolve phosphorus (TDP) were detected in the PAC-Carp and APAM-Carp groups than in the Carp-only group on day 7 ($P < 0.05$), although the concentrations of TP and TDP had not reduced significantly by the end of the experiment. The concentrations of total suspended solids (TSS), inorganic suspended solids (ISS), and organic suspended solids (OSS) were all significantly lower in the PAC-Carp and APAM-Carp groups than in the Carp-only group at the end of the experiment ($P < 0.05$). Collectively, our results indicated that the ability of PAC and APAM to remove phosphorus was unable to offset the negative effect of disturbance caused by the presence of juvenile common carp on phosphorus control. Therefore, it is necessary to consider monitoring and implementing measures to reduce the number of juvenile common carp when controlling eutrophication in shallow eutrophic lakes.
Keywords: *Cyprinus carpio L., polyaluminum chloride, anionic polyacrylamide, phosphorus, bioturbation*

Introduction

Lake restoration efforts have traditionally focused on the reduction of nutrient inputs from the catchment, including point and non-point source pollution (Zamparas and Kyriakopoulos, 2021; Nachtigall and Heim, 2023; Yan et al., 2024). However, on a global scale, there are sufficiently high concentrations of nutrients in lakes to maintain eutrophic conditions (Bhagowati and Ahamad, 2019; Fernandez-Figueroa et al., 2024). Internal nutrient loading is expected to enhance eutrophication and to reduce the

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response time of a lake to external reductions in nutrient load by a decade or even longer (Yang et al., 2020; Kowalczewska-Madura et al., 2022). Therefore, despite successful intervention against external pollution, many lakes worldwide may still be experiencing a slow restoration time due to the excessive loading of internal nutrients (Yin et al., 2016; Wu et al., 2017), particularly phosphorus. The release of phosphorus from sediment into lake water may be so intense that it prevents any improvement in water quality and transparency for a considerable period after the reduction of loading (Qin et al., 2023; Polauke et al., 2024).

Several researchers have demonstrated that the reduction of phosphorus and total suspended solids (TSS) in the water, and the prevention of phosphorus release from the sediment, can be achieved by the application of water-clearing agents (Lu et al., 2013; Agstam-Norlin et al., 2020; Li et al., 2020), such as aluminum salts, polyacrylamide (PAM) and modified zeolite. These are very widespread treatment methods that can restore eutrophic water bodies (Su et al., 2021; Yang et al., 2021). Of the flocculation agents, poly aluminum chloride (PAC) is the most widely used capping agent for water treatment, the restoration of inland waters, and the removal of phosphorus in Europe, the Americas and Asia (Araújo et al., 2016; Kasprzak et al., 2018; Su et al., 2021). PAC exhibits strong flocculation and water-clearing properties (Delgado et al., 2003) and is also able to absorb phosphorus (Huang et al., 2016). PAMs have three different charge types that can promote the flocculation of particles: cationic, anionic and non-ionic (Mason et al., 2005). Polymers can chemically bridge reactive groups, thus increasing floc size and the degree of flocculation; the effect of flocculation depends on the types of polymer and suspended solids in water. However, the efficiency and durability of water-clearing agents remain largely unknown in natural lakes. For example, wind can exert significant influence on the effects of water-clearing agents on the inactivation of phosphorus in sediment due to the surface mixing effect (Liu et al., 2017); furthermore, the role can be destroyed. Benthivorous fish, such as *Prochilodus brevis* and *Carassius Carassius*, can disturb the release of phosphorus from surface sediment through the "top-down effect" following the application of water-clearing agents (Han et al., 2021; Zhang et al., 2022). The removal of benthivorous fish is a priority measure in lake restoration, although these fish populations tend to rebound rapidly in tropical and subtropical lakes (Gao et al., 2014), potentially weakening the efficacy of additional restoration measures. In order to gain a thorough understanding of the efficacy of phosphorus removal agents, it is imperative that we conduct further research that focuses on the impact of disturbances, particularly in the context of applying these agents in eutrophic lakes where benthivorous fish are dominant.

The common carp (*Cyprinus carpio* L.) is a benthivorous fish that is native to Eastern Europe and Asia and has been widely introduced to other continents over the past century (Badiou et al., 2011). Common carp often dominate in degraded shallow lakes, including most Chinese shallow lakes suffering from eutrophication (Gao et al., 2014; Huser et al., 2022). Common carp disturb the bottom sediment while feeding, thereby causing increased water nutrient enrichment that can negatively impact aquatic ecosystems (Vilizzi et al., 2015). Consequently, this disturbance effect cannot be overlooked when applying water-clearing agents for the removal of phosphorus and suspended solids. In this research, we investigated whether common carp disturbance and water-clearing agents can cause antagonistic responses in shallow lakes. We conducted a 21-day mesocosm experiment. We hypothesized that the presence of

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common carp would disturb sediment and promote the release of phosphorus and suspended solids, both with and without the addition of water-clearing agents.

Materials and methods

Fish rearing and experimental design

Our mesocosm experiment was performed in 12 rectangular plastic tanks containing sediment and water (97 cm in length, 65 cm in width, 60 cm in height, with sediment to a depth of 10 cm). The tanks were placed outdoors. Ground sediment (1.1 mg g^{-1} of total nitrogen [TN] and 0.48 mg g^{-1} of total phosphorus [TP]) was obtained from Lake Jinyin, a shallow eutrophic lake in Wuhan City. The sediment was air-dried, and coarse debris was removed. Then, the dried sediment was sieved through a 10.0 mm ×10.0 mm mesh. An approximately 10 cm layer of homogenized sediment was added to each tank, and the tanks were then filled with 250 L of natural eutrophic lake water (TP = 0.42 mg/L). Water was obtained from an outlet channel of Lake Nan in Wuhan City and exposed to natural sunlight and equilibrated for seven days. Experiments involved treatments with the presence of one of two water-clearing agents (PAC or APAM). Twelve tanks were divided randomly into four groups (*Fig. 1*). Three replicate tanks for each treatment were assigned to (1) a CK group (Control: no agent without common carp), (2) a Carp group (common carp but no agent), (3) a PAC-Carp group (PAC with common carp), and (4) a APAM-Carp group (APAM with common carp) (*Fig. 1*). Juvenile common carp were purchased from the fisheries hatchery in Wuhan and all fish were acclimated for 15 days in the same rectangular plastic tanks (at ambient temperature). We did not feed the fish before they were transferred to the fish-holding mesocosms, although several naturally hatched invertebrates, such as snails, zooplankton, and chironomidae larvae were observed in the tanks. The treatments with carp involved only one fish (body length: 10.3 ± 0.6 cm; weight: 7.5 ± 0.8 g). Previous studies suggested that small common carp (<10 cm), that primarily consume zooplankton instead of benthic invertebrates, may not influence nutrient levels (Meijer et al., 1990a, b). Therefore, we selected carp that were approximately 10 cm in size. The selection of fish biomass in this study was based on a similar study relating to a disturbance caused by crucian carp, another benthivorous species (Gu et al., 2016), which involved three densities of fish (0, 10 and 40 g m^{-2}). For our present study, we chose a low density of 10 g m^{-2}. Furthermore, we chose juvenile common carp because these fish were not easily removed during ecological restoration.

PAC and APAM were obtained from the Ecological Restoration Project of Nanhu Lake, a eutrophic lake, in Wuhan. The efficiency of PAC and APAM to reduce phosphorus concentrations has been proven to be effective during the engineering application process. The water-clearing agents (7.5 g, 30 g m^{-3}) were mixed into a slurry with 2 L of water and then added to the water surface in an experiment mesocosm, as described by Ma et al. (2015) and Lin et al. (2019). Water-clearing agents were added at the beginning of the experiment. The experiment lasted from the 19th of April to the 10th of May 2022 and the tanks remained outdoors for the entire experimental duration. We did not feed the common carp during the experiment, although several naturally hatched invertebrates, such as snails, zooplankton, and Chironomidae larvae were observed in the tanks during the experimental period. The tanks were checked daily; we recorded the number of dead common carp and replaced these with individuals of the same size. The trial ran for 21 days in total.

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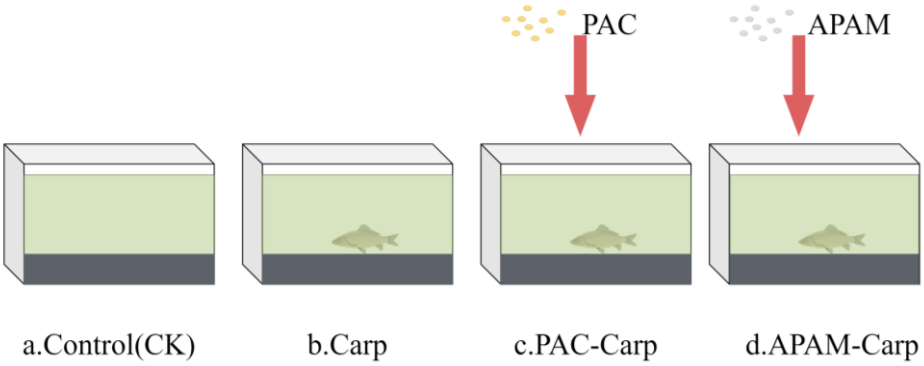

Figure 1. *Diagram showing the experimental set up. (a) CK (Control: no agent and no common carp). (b) Carp (no agent with common carp). (c) PAC-Carp (PAC with common carp). (d) APAM-Carp (APAM with common carp)*

Experimental procedures

At approximately 10 am every seven days, the water temperature and dissolved oxygen (DO) was measured with a YSI meter (YSI ProPlus, Yellow Springs, OH, USA). The water temperatures were 19.8 ± 0.2°C (mean value ± SD), 15.7 ± 0.2°C, 19.0 ± 0.3°C, and 18.0 ± 0.1°C in the four samplings for CK group, Carp group, PAC-Carp group, and APAM-Carp group. The concentration of DO ranged from 6.4 and 8.0 during the experimental period (*Fig. 2*). There were no significant differences among different groups (*Fig. 2*). Simultaneously, water samples were collected 10 cm beneath the water surface in the center of each tank for phosphorus and suspended solid concentrations analyses. Water samples were analyzed using Chinese standard methods (China EPA, 2009) that are equivalent to US standards (APHA, 1998). TP, TDP and SRP concentrations were determined spectrophotometrically upon digestion with persulfate and were measured using the ammonium molybdate method. TSS was determined by filtering suspended material onto GF/C (pore size 1.2 μm) filters, followed by drying at 105°C for 24 h. Subsequently, the used filters were burned in a muffle furnace at 550°C for 4 h to combust all organic matter and then cooled in a desiccator and re-weighed to calculate the concentrations of organic suspended solids (OSS) and inorganic suspended solids (ISS).

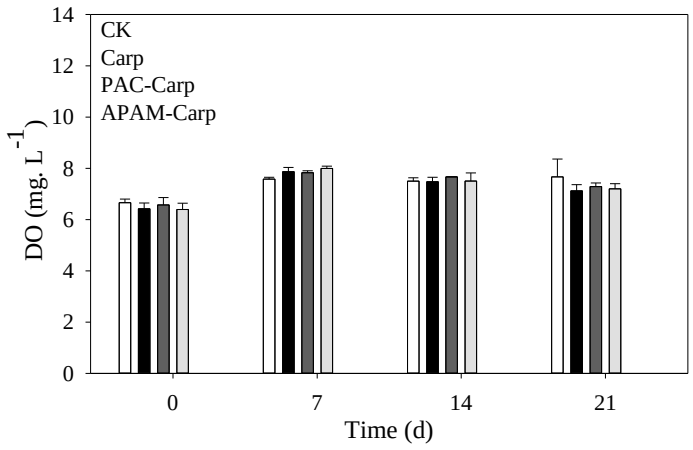

Figure 2. *Mean (±SD, n = 3) concentrations of dissolved oxygen (DO) in different treatments*

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Statistical analysis

We conducted all statistical analyses with SPSS version 19.0 (Statistical Product Service Solutions, USA) at a significance level of $P < 0.05$. One-way analysis of variance (ANOVA) was performed to detect differences between treatments. If the case of significant differences, we then used Tukey's Multiple Comparison Tests to detect significant differences. Prior to analysis, all data sets were examined for normality using the Shapiro-Wilk test; in addition, we tested the homogeneity of variance by applying Levene's test. If data were not normally distributed, then the environmental variables were transformed [ln (x + 1) or square root] to stabilize variance and increase homogeneity. In cases of unequal variance, we used Games-Howell tests to identify differences between groups.

Results and discussion

A significantly lower TP and TDP were detected in the CK, PAC-Carp and APAM-Carp groups than in the Carp group ($P < 0.05$; *Fig. 3i, ii*) on day 7. Both TP and TDP continued to decrease during the experimental period in the CK treatment. However, TP and TDP did not decrease further by day 21 in the PAC-Carp and APAM-Carp groups. These results suggested that PAC and APAM can effectively suppress the increase in phosphorus concentration caused by the presence of common carp in the early stages of PAC and APAM addition. On day 21, higher concentrations of TP and SRP were detected in the Carp, PAC-Carp and APAM-Carp groups when compared to the CK group without carp ($P < 0.05$; *Fig. 3i, iii*). Higher concentrations of TP were detected in the PAC-Carp and APAM-Carp groups than in the Carp group ($P < 0.05$; *Fig. 3i*). These results indicated that common carp destroyed the efficacy of the two water-clearing agents.

Significantly higher concentrations of TSS, ISS and OSS were detected in the Carp, PAC-Carp, and APAM-Carp groups than in the CK group ($P < 0.05$; *Fig. 4i, ii*) on day 7. There were no significant differences between the Carp and APAM-Carp groups with regards to TSS, ISS and OSS, although concentrations were higher in the PAC-Carp group than in the Carp group. However, the concentrations of these parameters were all significantly lower on day 21 ($P < 0.05$, *Fig. 4*). Although the addition of APAM and PAC led to a reduction in particulate matter, the effect on phosphorus was minimal at the end of experiment. Our analyses demonstrated that the removal of phosphorus from the water body had no effect after adding water-clearing agents when common carp were present.

Previous studies also demonstrated the ability of these agents to remove phosphorus (Araújo et al., 2016; Han et al., 2021). APAM is known to be more effective for the removal of organic P (phosphonates and orthophosphate monoesters) than inorganic P, while PAC is known to be more effective for the removal of inorganic P (particularly orthophosphate) (Xu et al., 2020). The concentration of phosphorus in the experimental groups had not reduced significantly by the end of the experiment, thus reflecting the low efficacy of the two types of water-clearing agents on eutrophication control in the presence of benthivorous fish. The presence of common carp has been reported to increase water turbidity *via* feeding activity and the release of sediment (Vilizzi et al., 2014; Huser et al., 2022). The presence of common carp could have destroyed the effect of water-clearing agents, as described previously for *Prochilodus brevis* (Araújo et al., 2016) and crucian carp (*Carassius carassius*) (Han et al., 2021). Common carp feeds mainly on benthic invertebrates and has an inferior mouth that can easily dig into the sediment, thus hampering sediment consolidation; this results in a lower water

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transparency (Fischer et al., 2013; Kowal et al., 2022). Common carp may cause more resuspension than crucian carp because they are more efficient at benthivorous feeding (Lammens and Hoogenboezem, 1991). Large common carp can effectively penetrate up to 12 cm into a substrate while searching for food (Panek, 1987), thus disturbing more than the surface layer of sediment and resuspending fine particles.

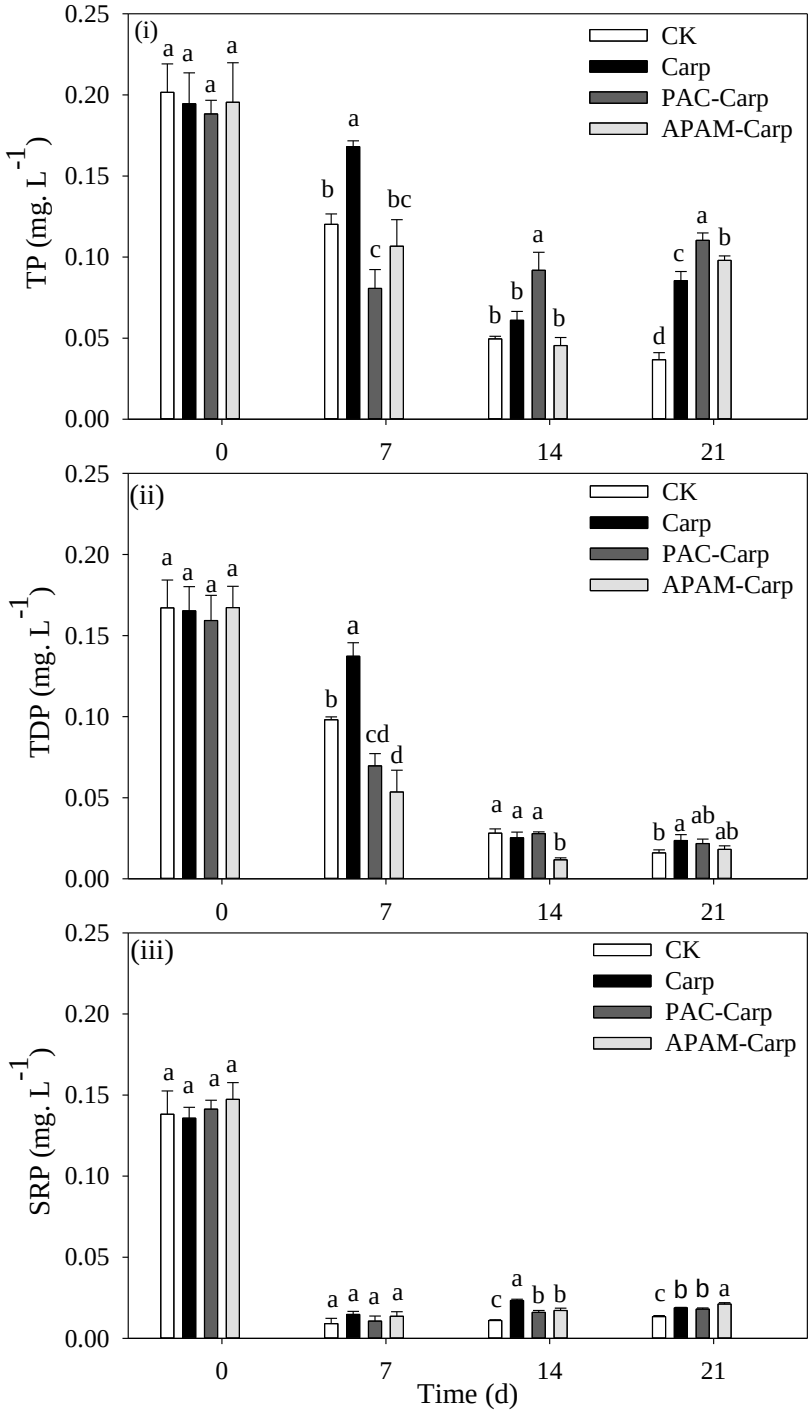

Figure 3. *Mean (± SD, n = 3) levels of total phosphorus (TP), total dissolved phosphorus (TDP) and soluble reactive phosphorus (SRP) in different treatments. (i, ii and iii) depict the levels of TP, TDP and SRP, respectively. Treatment labels sharing a lowercase letter indicate no significant difference between treatments at P > 0.05 using time-series data*

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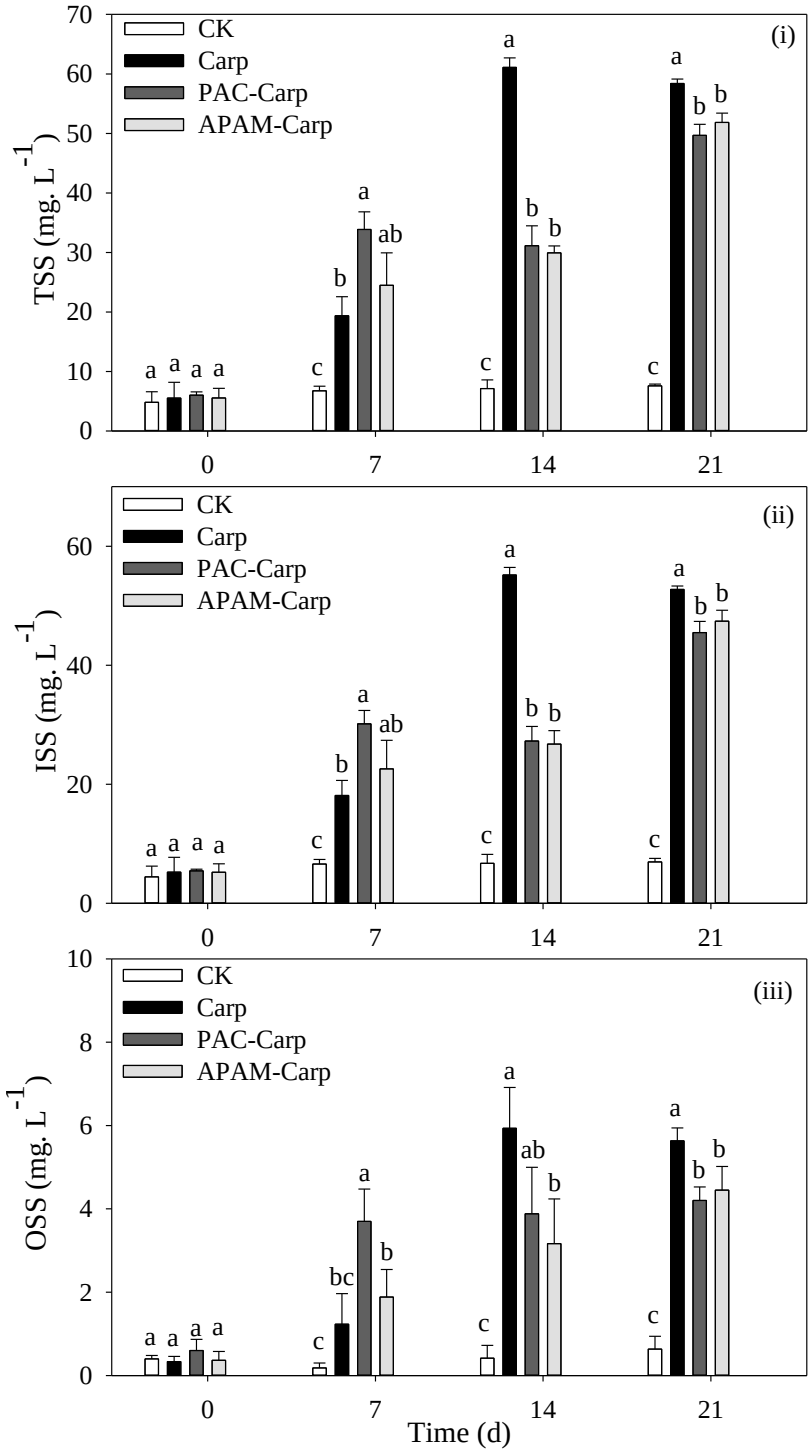

Figure 4. *Mean (± SD, n = 3) concentrations of total suspended solids (TSS), inorganic suspended solids (ISS), and organic suspended solids (OSS) concentrations in different treatments. (i, ii and iii) depict the levels of TSS, ISS and OSS, respectively. Treatment labels sharing a lowercase letter indicate no significant difference between treatments at P > 0.05 using time-series data*

Overall, the efficacy of agents such as PAC and APAM must be assessed in the context of fish activity. Given the dynamic nature of ecosystems and the potential for

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benthivorous fish, such as common carp, to recover, ongoing monitoring and adaptive management strategies are essential to ensure the sustainability of restoration efforts (Weber and Brown, 2009). Further research into the specific behaviors and impacts of various fish species on sediment disturbance and nutrient cycling can guide the development of more targeted and effective restoration measures.

Conclusions

Overall, our results revealed that the disturbance of common carp could destroy the efficacy of PAC and APAM on the removal of phosphorus and suspended solids from a water body. These results suggest that the continual removal of benthivorous fish is a necessary measure to ensure the efficacy of PAC and APAM on the phosphorus control of shallow eutrophic lakes. Reducing the biomass of benthivorous fish like common carp could be a crucial first step in lake restoration.

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Conflict of interests. None declared.

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