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Reduction of phosphate and nitrate in shrimp aquaculture wastewater using microalgae and macroalgae: A comparison of uptake performance

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ABSTRACT

The shrimp farming industry faces environmental challenges, such as the production of wastewater rich in organic matter and nutrients, which can pose a threat to marine ecosystems. The main objective of this research is to treat this wastewater before its release. For this study, three species of macroalgae—*Gracilaria pygmaea*, *Rhizoclonium riparium*, and *Sargassum glaucescens*—were collected from the shores of the Persian Gulf (Bushehr Province). Additionally, a microalgae species, *Nannochloropsis oculata*, was obtained from a cultivation center. The results indicated that *R. riparium* exhibited the highest efficiency among the macroalgae, with a 70% nitrate removal capability within 48 hours. However, *N. oculata* demonstrated the highest overall efficiency, achieving 85% nitrate removal. This study highlights that the examined microalgae species (*N. oculata*) exhibited a greater capacity for absorbing and removing nitrates and phosphates compared to the studied macroalgae species. These findings could contribute to improving wastewater treatment processes associated with the shrimp farming industry.

Keywords: Shrimp farming industry, Wastewater, Persian Gulf, Macroalgae.
Article type: Research Article.

INTRODUCTION

The shrimp farming industry is a key sector of global aquaculture, playing a vital role in food security and the world economy. However, it faces significant environmental challenges, particularly the discharge of large volumes of nutrient-rich wastewater. This effluent contains high levels of nitrogen compounds (such as ammonia, nitrate, and nitrite) and phosphorus, which, if left untreated, can contribute to eutrophication in aquatic ecosystems (Anderson *et al*. 2020). Eutrophication disrupts ecological balance, promotes excessive algal growth, and degrades water quality, posing a serious threat to both aquatic life and human water resources (SEAIRAN, 2023). To tackle these challenges, various methods have been proposed for treating shrimp aquaculture effluents. While conventional physical and chemical methods are effective, they are often costly, require complex infrastructure, and may lead to the production of harmful by-products. Meanwhile, the use of biological methods, especially algae-based systems, has been recognized as a sustainable, cost-effective, and efficient solution (Wang *et al*. 2018). Algae, particularly microalgae, possess unique capabilities for nutrient uptake from their environment. Through the process of photosynthesis, these microscopic organisms utilize nitrogen and phosphorus as essential resources for growth and biomass production. The removal of these nutrients from wastewater not only improves water quality but also prevents environmental issues associated with their accumulation. For instance, algae such as *Chlorella* and *Spirulina* are widely used in bioremediation systems due to their rapid growth rates and high nutrient absorption capacities (Singh & Singh 2015; Tochaii *et al*. 2019; Bibak *et al*. 2023; Bibak *et al*. 2024). Studies have shown that algae-based systems can remove over 80% of nitrogen and phosphorus from shrimp aquaculture effluents. Additionally, algae efficiently absorb toxic ammonia and other nitrogen compounds, storing them as useful substances in their biological structures (Cai *et al*. 2019). Moreover, the oxygen production process of algae can enhance dissolved oxygen levels in water, creating better environmental conditions for other aquatic organisms. Additionally, certain species of algae possess a unique ability to absorb heavy metals and complex organic compounds from effluents. This characteristic makes them highly suitable for treating complex effluents contaminated with various substances (Zhao *et al*. 2022). One of the key advantages of using algae in shrimp farming wastewater treatment is the potential to utilize the produced biomass, making it a highly beneficial and sustainable approach. This method not only mitigates adverse environmental impacts, but also promotes a sustainable resource management cycle by efficiently removing pollutants while generating valuable byproducts. Additionally, algae-based systems offer cost-effective solutions, as they require less energy and have lower infrastructural complexity compared to conventional treatment methods (Gao *et al*. 2014; Venkata *et al*. 2021). Furthermore, integrating wastewater treatment with the production of economically viable products enhances sustainable development, particularly in developing countries where resource efficiency is crucial (Venkata *et al*. 2020; Ravindra *et al.* 2022). Given the environmental and economic challenges associated with managing shrimp farm wastewater, using algae as a sustainable and efficient solution has become increasingly important. Algae's ability to remove nutrients and generate valuable biomass not only helps reduce pollution but also creates opportunities to economically utilize wastewater. This approach can play a significant role in promoting the sustainable development of the aquaculture industry while preserving natural resources (Garima *et al*. 2023; Bibak *et al*. 2024, 2025). The purpose of this study is to compare the ability of micro- and macro-algae to absorb nitrates and phosphates from effluent in shrimp farming ponds. In light of environmental concerns regarding pollution from agricultural and aquaculture runoff, this research aims to assess the effectiveness of these two types of algae in reducing nutrient concentrations. This could help improve water quality and reduce environmental pollution. The results may offer valuable insights for managing waste in aquaculture and related industries.

MATERIALS AND METHODS

Collection and preparation of macroalgae

This study evaluated the effectiveness of three macroalgae species—*Gracilaria pygmaea*, *Rhizoclonium riparium*, and *Sargassum glaucescens*—for wastewater treatment. These macroalgae were collected from the northern coasts of the Persian Gulf in Bushehr City, Southern Iran. They were carefully washed with seawater to remove sediments, epiphytes, and associated microorganisms. Afterward, they were acclimated in controlled laboratory conditions before being introduced into the shrimp aquaculture wastewater. Additionally, *Nannochloropsis oculata* was purchased from aquaculture hatcheries and used in this study. The species, *G. pygmaea* (red algae, family Gracilariaceae, phylum Rhodophyta), *R. riparium* (green filamentous algae, family Cladophoraceae), and *S. glaucescens* (brown algae, family Sargassaceae, class Phaeophyceae) are widely distributed along the coastlines and intertidal zones of the Persian Gulf, with *G. pygmaea* being prevalent along the coast (Sarkar *et al.* 2019), *R. riparium* abundant in intertidal areas (Kumar *et al.* 2018), and *S. glaucescens* thriving in rocky coastal habitats (Zhang *et al.* 2020). *N. oculata*, a green microalga known for its high lipid and protein content, is a promising species for aquaculture feed and biodiesel production (Matsumoto *et al.* 2020), and several research centers in Iran, focus on cultivating it for various biotechnological applications, such as studying its protein content for animal feed (Zarei *et al.* 2019). The choice of these species is motivated by their abundance, ease of access in the region, and their high potential for environmental and industrial applications.

Pre-treatment and filtration of shrimp farm water for experimental use

The water utilized in this study was collected from shrimp farms located in Delvar City, Southern Iran. Before being used in the experiments, the water underwent a pre-treatment process to remove suspended particles and other physical contaminants that could interfere with the accuracy of nutrient analysis and algal growth. Sampling methods for shrimp farm wastewater typically involve selecting various sampling locations along the water flow path, including inflows, outflows, and areas prone to pollution accumulation (Hargreaves *et al.* 2008). Sampling should be conducted at different times of the day and during various seasons to capture temporal variations in water quality, and also during tidal changes or fluctuating water flow periods (Gao *et al.* 2014). Properly cleaned and disinfected glass or plastic containers should be used to avoid sample contamination, and mechanical systems such as pumps may be employed to collect samples from different water depths to ensure a representative water composition (Baker *et al.* 2010). After collection, parameters such as dissolved oxygen, temperature, pH, nutrient concentrations (nitrate, phosphate), and organic matter are typically analyzed to assess wastewater quality (Venkata *et al.* 2021).

Filtration process

The raw shrimp farm water was first allowed to settle in a sedimentation tank for pre-filtration, which helped reduce larger particulate matter. The supernatant was then passed through a Whatman No. 40 filter paper (pore size ≈ 8 µm) for primary filtration to remove fine suspended solids, organic debris, and other physical impurities. After filtration, the water underwent quality control, where it was analyzed for baseline turbidity, total suspended solids (TSS), and nutrient composition (nitrate, phosphate, and ammonia levels) to ensure consistency in the experimental conditions (Jones & Lee 2020). The pre-filtration step was essential in ensuring that the nutrient removal efficiency observed in the study was attributed solely to the introduced macroalgae (*G. pygmaea*, *R. riparium*, and *S. glaucescens*) and the microalga (*N. oculata*), rather than uncontrolled variations in water quality.

Experimental setup for algae cultivation and nutrient removal

The filtered shrimp farm water was then transferred into three 15-liter aquariums with dimensions of 25 × 25 × 25 cm. A total of 30 g wet weight of each algal species was placed in separate aquariums. The algae were maintained under controlled conditions, including regulated light exposure and continuous aeration.

Environmental conditions

Light cycle: The light/dark cycle was set to 16:8 hours (L:D) to ensure optimal photosynthetic activity and growth for the algae.

Temperature: The temperature of the experimental environment was kept at a stable range of 25 ± 2 °C to mimic the natural growing conditions for the algae (Zhang & Li 2019).

pH Monitoring: The pH was monitored and maintained at 7.5 ± 0.2 to ensure that it stayed within the optimal range for algal growth and nutrient uptake. The pH was adjusted daily if necessary, using appropriate buffering agents or acids/bases (Smith & Brown 2020).

Aeration: A continuous aeration system was employed to provide sufficient oxygenation and prevent stagnation in the aquariums, which is essential for both algae and nutrient uptake. These carefully controlled conditions were crucial for maintaining optimal growth rates and nutrient removal efficiencies in the experimental setup.

The nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations in the water samples were measured before and after the experiment using spectrophotometric analysis (Hach-Dr3900), ensuring precise quantification of nutrient removal efficiency by the algae (Doe & Smith 2021).

Sample collection and preparation

Water samples were collected at designated time intervals before and after the experimental treatments to assess changes in nutrient levels. Immediately after collection, the samples were filtered using Whatman No. 40 filter paper (pore size ~8 µm) to remove particulate matter and suspended solids that could interfere with spectrophotometric readings. The filtered samples were then stored at 4 °C in darkness to prevent chemical or biological alterations before analysis.

Statistical analysis

The alteration rates (%) in the NO_3^- and PO_4^{3-}concentrations relative to their initial values were calculated. The data obtained from experiments were analyzed using SPSS software. A One-Way ANOVA test was performed to compare four algal (three macro- and one micro-algae) groups at a significance level of 0.05.

RESULTS AND DISCUSSION

Comparison of nitrate absorption rates

The results of this study demonstrated that the NO_3^- uptake by macroalgae significantly increases with longer exposure to shrimp farm effluent. Specifically, *R. riparium* showed the highest NO_3^- uptake, reaching 70% after

48 hours, compared to *G. pygmaea* and *S. glaucescens*, indicating its strong potential for NO_3^- removal from the environment and its possible application as an effective tool in treating nitrate-contaminated water. In the experiment, macroalgae were subjected to different conditions over varying time periods, and NO_3^- uptake was measured at different intervals (Fig. 1). The results revealed that the NO_3^- uptake initially increased linearly over time. However, after 48 hours, the NO_3^- uptake in the algae reached a plateau, with *R. riparium* absorbing about 70% of the available NO_3^-. This uptake pattern was also observed in the other macroalgal species, though their absorption rates were lower than that of *R. riparium*. These findings suggest that algae, depending on the duration of exposure, are capable of absorbing significant amounts of NO_3^-. This property could be particularly valuable for environmental applications, especially in the treatment of nitrate-polluted water. Therefore, optimizing the timing and environmental conditions for enhanced NO_3^- uptake could be a key step in leveraging algae for both environmental and industrial purposes.

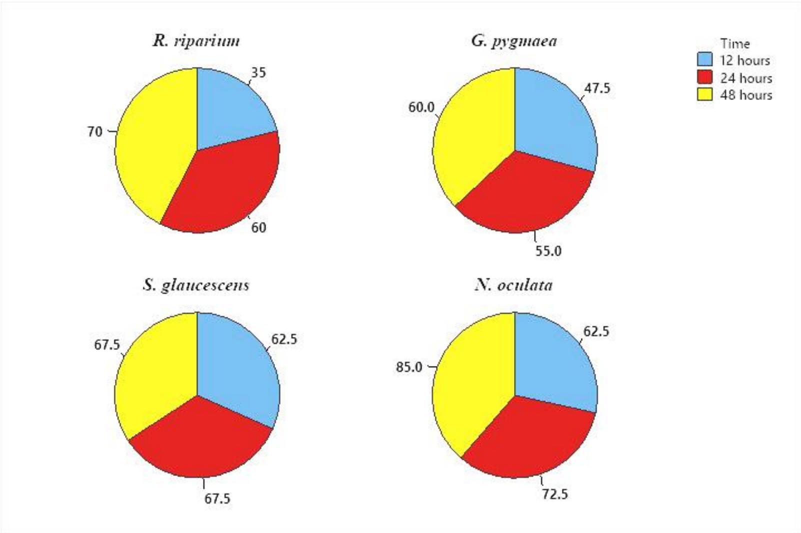

Fig. 1. Nitrate removal rate (%) by the studied algal species.

The results (in Fig. 1) showed that *N. oculata* takes up NO_3^- more quickly (in this treatment, nitrate removal reached 85%.), which can be attributed to its advanced NO_3^- transport mechanisms, the high efficiency of nitrogenase enzymes, and its greater nitrogen demand for faster growth and cell division. In contrast, while macroalgae can absorb NO_3^-, the process is slower due to their more complex structure and physiological constraints. Macroalgae primarily use nitrogen to build their tissue structures, whereas microalgae rely on nitrogen to rapidly produce proteins, nucleic acids, and other essential metabolites (Safi & Rebah 2020; Zhang & Li 2021; Turan & Dönmez 2022). These findings highlight that microalgae are a more effective option for removing excess nitrogen from wastewater and can be utilized in environmental technologies like bioremediation and sustainable agricultural systems. Moreover, their high nitrogen absorption capacity makes them an ideal source for producing nitrogen-rich bioproducts, such as biofertilizers and dietary supplements. These results have potential applications in designing water treatment systems, reducing nitrogen pollution, and managing aquatic ecosystems (Sutherland *et al.* 2020; Nguyen *et al.* 2022; Stedt *et al.* 2022). According to the findings, during the first 12 hours of the experiment, NO_3^- uptake was identical in *S. glaucescens* and *N. oculata* (62.5%; Fig. 2). However, as the experiment progressed to 24 and 48 hours, NO_3^- uptake in *N. oculata* increased significantly. In contrast, *R. riparium* showed the lowest NO_3^- uptake during the initial 12 hours, suggesting that different algal species exhibit distinct NO_3^- uptake patterns over time.

Comparison of phosphate absorption rates

According to the results of this study, *R. riparium* demonstrated the highest PO_4^{3-} uptake efficiency, achieving a removal rate of 67%. This highlights the remarkable capacity of this species to absorb and remove PO_4^{3-} from aquatic environments. However, a temporal analysis revealed that PO_4^{3-} uptake by *R. riparium* reached a saturation point after 24 hours, remaining constant thereafter (Fig. 3). This behavior may be attributed to physiological constraints or the saturation of surface adsorption sites, potentially reflecting the species' limited capacity for PO_4^{3-} transport and intracellular storage.

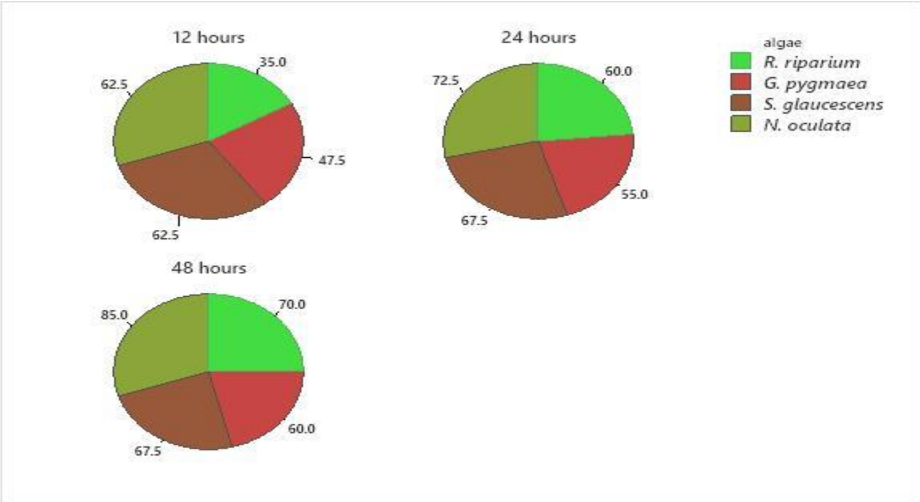

Fig. 2. The comparison of algae over a specific time period (12, 24 and 48 hours) to determine the highest NO_3^- absorption rate (%).

In contrast, *G. pygmaea* and *S. glaucescens* showed a continued increase in PO_4^{3-} uptake beyond 24 hours. This upward trend suggests differences in their metabolic dynamics or more efficient mechanisms for PO_4^{3-} absorption and transport. These mechanisms may involve sustained phosphatase enzyme activity or an increase in cell surface interactions, facilitating the gradual accumulation of PO_4^{3-}. The observed variations in PO_4^{3-} uptake among the studied species are likely linked to differences in cellular architecture, the chemical composition of their cell walls, and regulatory pathways governing PO_4^{3-} metabolism. These findings provide valuable insights for selecting suitable macroalgae species for environmental applications such as biofiltration and phosphorus management in aquatic ecosystems (Smith *et al.* 2023).

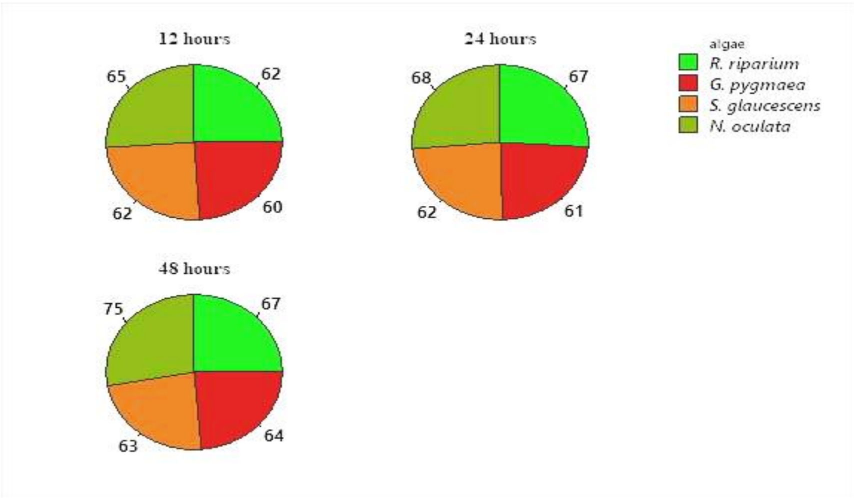

Fig. 3. The comparison of algae over a specific time period (12, 24 and 48 hours) to determine the highest PO_4^{3-} absorption rate (%).

This study showed that *N. oculata* had a significantly higher capacity for PO_4^{3-} uptake compared to the macroalgae examined. It was also indicated that *N. oculata* was able to absorb up to 75% of the PO_4^{3-} in the environment, while macroalgae exhibited much lower absorption levels. This variation is likely due to the distinct biological characteristics of these two algal groups. Microalgae, being smaller in size, have a higher surface-area-to-volume ratio, allowing greater access to environmental nutrients, which likely accounts for their superior PO_4^{3-} uptake efficiency (Powell *et al.* 2008; Zhang *et al.* 2024). In the case of *R. riparium*, extending the time from 24 to 48 hours did not affect PO_4^{3-} uptake levels. This could indicate that PO_4^{3-} uptake sites become quickly saturated in this species or that its physiological and metabolic mechanisms are unable to support increased uptake over a longer duration. This limitation is likely due to either a lack of additional enzymatic activity involved in PO_4^{3-} transport or a reduced cellular ability to respond to alterations in PO_4^{3-} concentrations. In contrast, *S. glaucescens* showed only a slight elevation in PO_4^{3-} uptake over time, suggesting slower uptake mechanisms or lower efficiency in PO_4^{3-} utilization by this species. It might also result from limited enzyme regulation or fewer active uptake sites on the cell surface, thus diminishing the species' ability to effectively exploit environmental PO_4^{3-} (Brown & Green 2018; Smith & Johnson 2019). Unlike these two species, the other two species studied showed a more significant rise in PO_4^{3-} uptake over time. This difference may be attributed to more advanced biological traits or evolutionary adaptations that enable these species to activate more PO_4^{3-} uptake sites at the cellular level or exhibit more efficient enzymatic activity. These species might also have a higher capacity for regulating PO_4^{3-} metabolism or using internal storage and transport strategies for PO_4^{3-} (Nelson & Clark 2017; Williams & Turner 2020). Overall, these findings highlight the importance of each species' biological, metabolic, and regulatory characteristics in determining PO_4^{3-} uptake efficiency. These traits can serve as valuable indicators when selecting species for managing aquatic ecosystems or for biotechnological applications focused on PO_4^{3-} removal from water sources (Lin *et al.* 2021). Fig. 4 illustrates differences in NO_3^- and PO_4^{3-} uptake among various species of algae under study. These variations may stem from physiological and biochemical differences between algal species. Algae employ various mechanisms to absorb these nutrients from their environment, primarily involving active transport across cell membranes and regulation of gene expression related to the NO_3^- and PO_4^{3-} transporters. In many algal species, NO_3^- uptake, as the primary nitrogen source, depends on the activity of NO_3^- reductase, while PO_4^{3-} uptake relies on specialized PO_4^{3-} transporter proteins. These differences may reflect variations in nutritional needs, nutrient availability in the environment, or natural selection pressures (Smith *et al.* 2020). Additionally, variations in NO_3^- and PO_4^{3-} uptake may be influenced by the capacity for nutrient storage in cellular vacuoles or the internal structure of the algae. Species adapted to nutrient-poor environments often develop more efficient uptake and storage mechanisms, whereas those in nutrient-rich environments may have less need for such adaptations (Flynn, 1991; Falkowski & Raven, 2007; Pereira & Yarish 2008). These findings emphasize the significance of choosing specific algae species for environmental and industrial purposes, such as nutrient removal from wastewater or their use as biofertilizers. As a result, this study could serve as a valuable reference for future research focused on optimizing algae-based solutions for environmental management. Increasing the algal biomass from 30 to 60 g L^{-1} enhanced the NO_3^- and PO_4^{3-} uptake across the studied species. After 48 hours at this biomass concentration, *N. oculata* consistently exhibited the highest NO_3^- and PO_4^{3-} absorption compared to the other species. Fig. 5 clearly illustrates the differences in nutrient uptake between the two biomass levels. The results showed a significant relationship between the mean removal of nitrate and phosphate in the micro- and macro-algae ($p < 0.05$). Several studies have explored the potential of algae for NO_3^- and PO_4^{3-} uptake. Ajala & Alexander (2020) investigated the efficiency of three microalgal species—*Chlorella vulgaris*, *Scenedesmus obliquus*, and *Oocystis minuta*—in removing sulfate, NO_3^-, and PO_4^{3-} from wastewater. Their findings revealed that these species effectively absorbed these nutrients, demonstrating their strong potential for use in bioremediation and wastewater treatment systems. Habibi *et al.* (2019) investigated the impact of different light/dark cycles on NO_3^- and PO_4^{3-} removal from synthetic wastewater using *Scenedesmus* sp. cultured in BG11 medium. According to their findings, under optimal lighting conditions, *Scenedesmus* sp. exhibited a high capacity for nutrient uptake, with a 24-hour light cycle providing the best conditions for growth and removal efficiency. Their research highlights the strong potential of *Scenedesmus* sp. for use in the biological wastewater treatment systems. Azizi *et al*. (2021) investigated the removal of NO_3^- and PO_4^{3-} from treated wastewater using *Chlorella vulgaris* under different light regimes in a membrane flat-plate photobioreactor. Their findings revealed that changes in light conditions significantly influenced nutrient uptake efficiency. Their research underscores the strong potential of *C. vulgaris* for advanced bioremediation applications and highlights the effectiveness of membrane photobioreactors in enhancing water quality. Znad *et al*. (2018) investigated the ability of *C. vulgaris* for bioremediation and nutrient removal of NO_3^- and PO_4^{3-} from wastewater, reporting that this microalga effectively removed nutrients from the environment, aiding in the improvement of wastewater quality. Furthermore, their research showed that environmental factors such as light intensity and cell density can influence the efficiency of NO_3^- and PO_4^{3-} removal. These results confirm the high potential of *C. vulgaris* as an efficient tool in wastewater bioremediation systems. The abovementioned studies have demonstrated that microalgae possess a significant ability to remove nitrate and phosphate from water. This phenomenon is attributed to their photosynthetic activity, which facilitates the conversion of carbon dioxide and organic compounds present in water into nutrients for their growth while reducing nitrogen and phosphorus levels (William & Turner 2020). Since most microalgae thrive in aquatic environments, this process can serve as a natural and sustainable approach to controlling nitrate and phosphate pollution, aligning with the findings of the present study. The results of this study indicate that macroalgae play a role in pollutant reduction, and also a more effective option for nitrate and phosphate removal from aquatic sources due to their higher absorption rate, faster growth, and greater adaptability. Therefore, advancing research on optimizing the application of microalgae can contribute to improving biological treatment methods and the sustainable management of water resources.

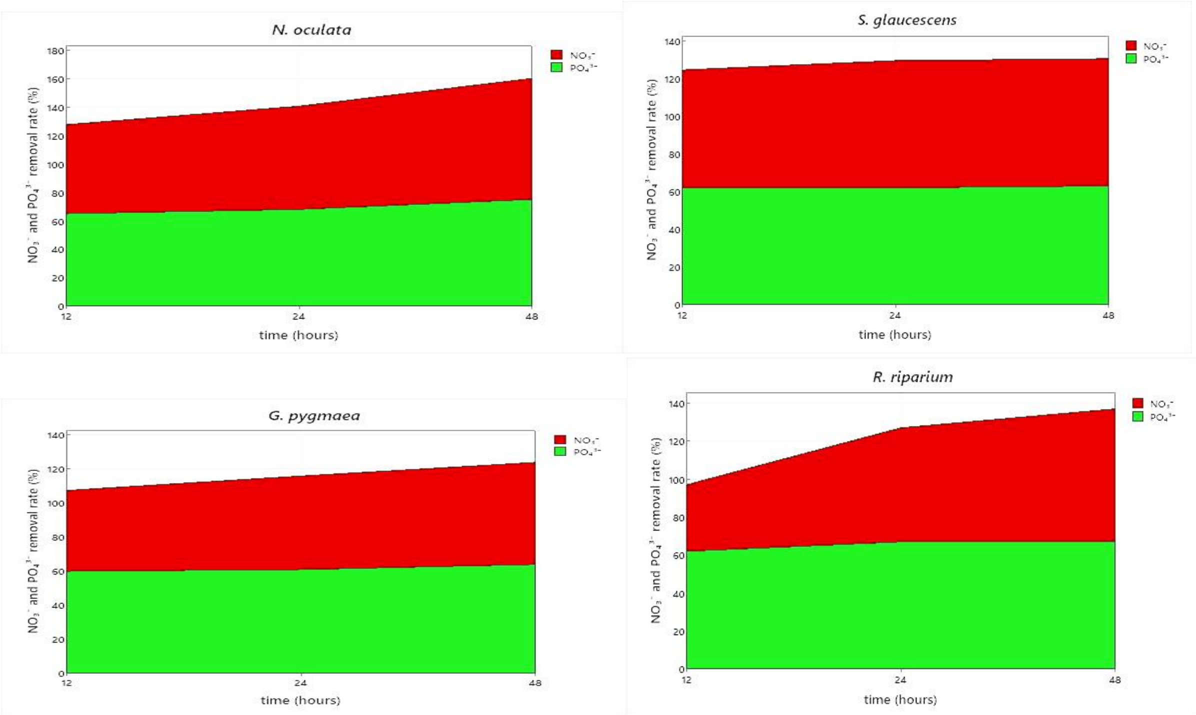

Fig. 4. Comparison of NO_3^- and PO_4^{3-} uptake by each algae species over a period of 12, 24 and 48 hours.

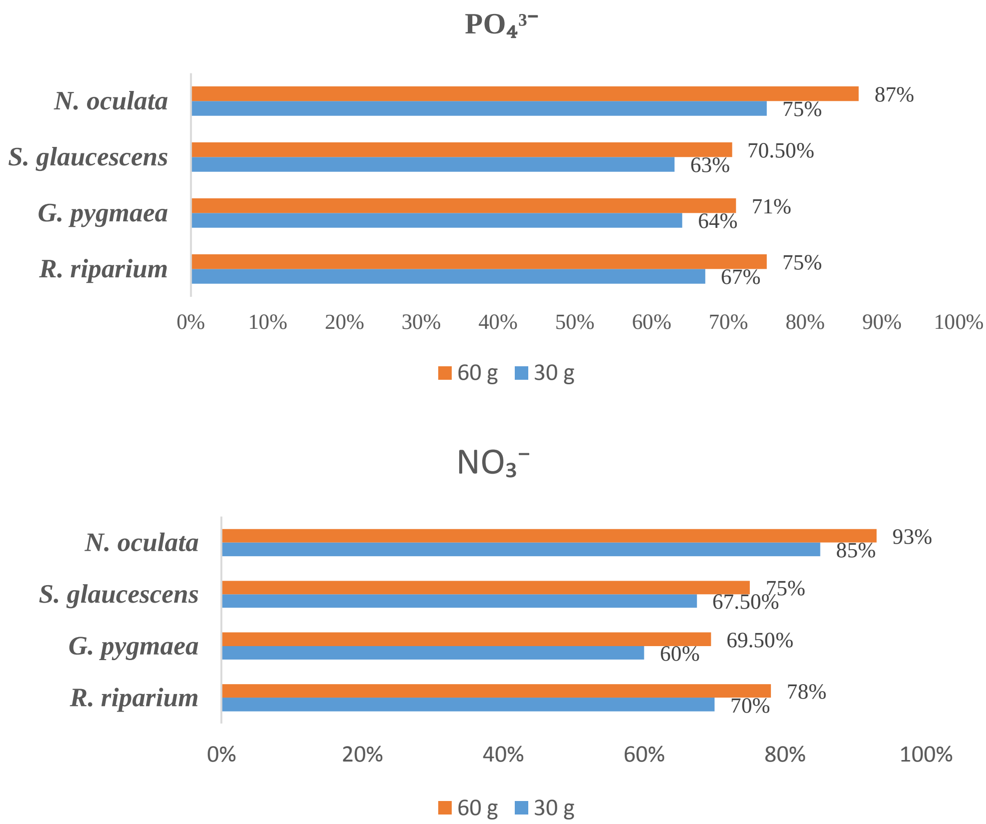

Fig. 5. Effects of two different biomasses (30 and 60 g) of algae on the absorption and removal processes of PO_4^{3-} and NO_3^-.

CONCLUSION

The study on the ability of macro- and micro-algae to remove NO_3^- and PO_4^{3-} from shrimp farm effluents demonstrates their high efficiency in nutrient removal. The results reveal that macroalgae, particularly those collected from the Persian Gulf coast, have a considerable potential for nutrient removal. However, microalgae showed a higher capacity for nutrient uptake compared to the macroalgal species studied. Despite this, the ease of collection, availability, and cost-effectiveness of macroalgae make them a more practical and economically viable option than microalgae. Thus, while microalgae may provide superior nutrient removal performance, macroalgae offer a more feasible and cost-effective solution for large-scale wastewater treatment applications.

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