JOURNAL OF TROPICAL LIFE SCIENCE
2023, Vol. 13, No. 1, 13 – 22
http://dx.doi.org/10.11594/jtls.13.01.02

Research Article

The Effectiveness of Various Types of Local Hydromacrophytes on the Phytoremediation Process of Catfish Pond Wastewater using a Batch Culture System

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Article history:
Submission June 2022
Revised July 2022
Accepted October 2022

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ABSTRACT

This study aimed to determine the effectivity of various types of local hydromacrophytes on the remediation of wastewater from catfish culture ponds in Gondosuli village, Gondang, Tulungagung, East Java, Indonesia, using a batch culture system. This experiment used a completely random design and was conducted in the glasshouse. The batch culture system was conducted in a 30 L tank containing sand and gravel as the medium for hydromacrophytes. This research consisted of five treatments (emergent, submerged, floating leaf, polyculture of 3 types of hydromacrophytes, and control without hydromacrophytes). This study was repeated three times at the same time. The effectiveness of the phytoremediation model was monitored using indicators of water physicochemical parameters and the biotic index of phytoplankton. The biotic indices were used the Shannon-Wiener diversity index (H') of phytoplankton and the diatom biotic index (Trophic Diatom Index (TDI) and Percentage of Pollution Tolerant Value (%PTV)). After giving the treatment, monitoring was carried out on days 0, 10, 20, 30, and 40. The results showed that the batch culture system for phytoremediation post-harvest catfish pond wastewater with local hydromacrophytes improved the water quality. However, not all water physicochemical parameters after treatments meet Indonesian water quality standards. Abundance showed water quality fluctuations over time, while Shannon Wiener's diversity index (H' value) decreased as water quality decreased. This study's PTV value demonstrated that pollutant levels vary by treatment. Only the control and submerged hydromacrophytes improved their TDI status; another treatment remained moderate. The best treatment was 40 days after acclimatization with polyculture treatment of three types of local hydromacrophytes. Research and other systems like continuous culture are needed for optimal results to improve water quality.

Keywords: Batch culture, Catfish pond, Hydromacrophyte, Remediation, Wastewater, Water quality

Introduction

Gondosuli Village, Gondang District, Tulungagung Regency, is one of the centers of the Minapolitan area where catfish cultivation is the primary fishery sector. Intensive feeding with artificial feed increases the productivity of the fish produced. However, fish metabolism typically only utilizes 50-60% of the food. Consequently, approximately 40% of the feed residues will enter the pond water, impacting water quality. Artificial fish feeding can lead to water pollution, low dissolved oxygen levels, increased physical demand for oxygen, and bacterial loads [1]. Wastewater from catfish ponds in this area contains high organic matter and nutrients from artificial feed residues. This condition is reflected in the catfish ponds' high BOD, turbidity levels, and low DO values [2]. This wastewater has been disposed of directly through drainage that flows into the river, potentially polluting the aquatic ecosystem. Therefore, overcoming and reducing the effects of fish pond wastewater is necessary.

Phytoremediation can be a cost-effective and

How to cite:
Sa'adah U, Retnaningdyah C, Siswanto D (2023) The Effectiveness of Various Types of Local Hydromacrophytes on The Phytoremediation Process of Catfish Pond Wastewater using a Batch Culture System. Journal of Tropical Life Science 13 (1): 13 – 22. doi: 10.11594/jtls.13.01.02.

environmentally friendly technique that uses green plants to contain, sequester, or detoxify contaminants in contaminated water [3]. There are various techniques for phytoremediation, one of which is planting hydromacrophytes in the batch culture system. Previous research in laboratory experiments using hydromacrophytes for phytoremediation demonstrated that some hydromacrophytes, such as *Polygonum hydropiperoides*, *Nymphaea* sp, *Azolla* sp., *Hydrilla verticillata*, *Marsilea crenata*, *Equisetum ramosissium*, *Typha angustifolia*, *Scirpus grossus*, *Ipomoea aquatica*, and *Lymnocharis flava*, which are combined either planted in monoculture or polyculture showed high potential as a nitrate and phosphate remediator agent of wastewater in the aquatic ecosystem [4-7]. Based on the results of this previous study, applying these techniques in catfish farming pond wastewater is essential, especially to compare the effectiveness of the phytoremediation model. It was carried out by planting several local floating leaves, emergent, and submerged hydromacrophytes in catfish ponds through a sustainable aquaculture system to improve wastewater quality before discharge into the environment. Monitoring the water quality can determine the effectiveness of the phytoremediation process.

The success of the process can be measured by the physicochemical quality and the biotic index of the water organisms. One of the organisms that can be used to assess the success of the phytoremediation process is phytoplankton. However, phytoplankton communities are widely used as indicators of ecosystem functions despite their complexity. When evaluating water quality, high volume abundance, seasonal succession, and indicator species are some of the most frequently considered phytoplankton characteristics [8].

The primary objective of this study is to develop an efficient wastewater purification system for catfish ponds in the Gondosuli Minapolitan area using various types of hydromacrophytes. The effectiveness of the phytoremediation model was assessed based on water variations quality and the population of phytoplankton.

Material and Methods

Experimental Design

This experimental study utilized a completely randomized design. Variations in the composition of local hydromacrophytes were used as a treatment with a batch culture system. Variations in the composition of hydromacrophytes consisted of five types of treatment, including emergent hydromacrophyte (*Scirpus* sp., *Acorus calamus*, *Marsilea crenata*), floating leaf macrophytes (*Ipomoea aquatica*, *Azolla* sp.), submerged (*Vallisneria* sp., *Hydrilla verticilata*), polyculture of the three hydromacrophyte types, and control (without hydromacrophyte). Hydromacrophytes cover about 25 to 50 percent of the surface area of the tank. At the bottom of each treatment tank (with 40 cm diameters), 5 cm of sand and 3 cm of gravel were placed as a substrate, and 25 liters of catfish pond wastewater per tank were used for the study (Figure 1). The wastewater utilized is post-harvest water from a 4-month-old Gondosuli Minapolitan catfish pond. The wastewater is generally dischar-

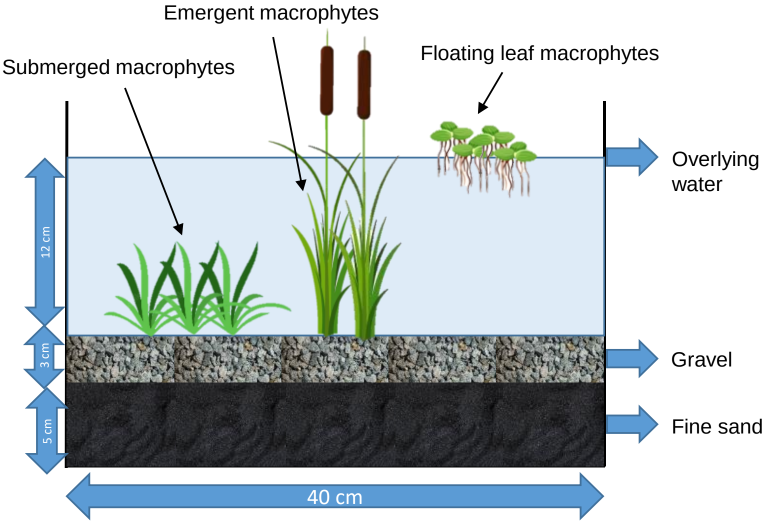

Figure 1. Schematic of phytoremediation experiment set up with batch culture system

ged directly into the water streams. Each treatment was repeated three times so that there were 15 experimental units in the bioreactor simultaneously in the glasshouse of the Biology Department, Universitas Brawijaya.

Monitoring Water Quality

Water quality measurements carried out consisted of water temperature, pH levels, Total Suspended Solid (TSS), conductivity, levels of Dissolved Oxygen (DO) and Biological Oxygen Demand (BOD), nitrate, and orthophosphate based on standard methods for the examination of water and wastewater [9]. Phytoplankton samples were collected by filtering 0.5 liters of water using a plankton net at the tank. The phytoplankton samples were transferred to a flacon container, and then added 10 drops of 4% of formalin and 5 drops of $CuSO_4$. To observe phytoplankton, 1 ml of sample water and placed into the Sedgewick-Rafter cell counting chamber. The counting chamber was then viewed with a light microscope. The identification of phytoplankton was based on the identification handbook published by Bellinger and Sigee [10].

This measurement was carried out in the Ecology and Restoration of Tropical Ecosystem, Biology Department, Universitas Brawijaya. Monitoring of specific biotic indices and physicochemical water quality was conducted in each treatment on day 0 (immediately after incubation), days 10, 20, 30, and 40.

Data Analyses

The physics and chemical parameters measurements and the phytoplankton community in each treatment were compiled. The average value of each parameter between treatments and monitoring time was then calculated. Comparing the average value of water quality obtained with the Water Quality Standard based on Indonesian government regulation No. 22/2021 regarding Water Quality Management and Water Pollution Control can also reveal the level of water pollution. As a bioindicator of water quality in the pond phytoremediation process, Shannon Wiener diversity index, trophic diatom index (TDI), and percentage tolerant value (% PTV) are calculated from phytoplankton data [11, 12]. Mean differences between treatments and biplots (Principal Component Analysis) were analyzed to determine water quality differences between treatments and the most effective phytoremediation model.

Results and Discussion

Physicochemical Properties of Water Quality

Water quality can be reflected in the physicochemical parameters of the water. Pond wastewater used for catfish cultivation aged four months or post-harvest has an average pH value of 7.57, while the pH value of post-harvest pond water was 8.03. This value meets the requirements of Indonesian Government Regulation No. 22/2021 for the Implementation of Environmental Protection and Management class I, which specifies a pH range of 6-9. Planting floating leaf, emergent, or submerged local hydromacrophytes in batch culture can increase the pH level.

The pH level increased in all treatments, including the control (without hydromacrophytes). After 40 days of treatment, the final pH value ranged from 7.95 to 8.70. On the 20 days after incubation, there was a decrease in the pH level, which then increased 30 days after incubation (Figure 2). The pH level of the waters is influenced by the concentration of carbon dioxide (CO_2) and acidic compounds. The rise and fall of pH levels are probably due to submerged hydromacrophytes growth and changes in the abundance of phytoplankton that affects CO_2 levels [13]. When there is photosynthetic activity (both by hydromacrophytes and phytoplankton), CO_2 in the environment will be used, leading to increase pH levels simultaneously.

Following an increase in the pH value, the electrical conductivity (EC) of water decreased. In the post-harvest pond, water has an average conductivity of 117.7 mS/m. After 40 days of incubation, the conductivity decreased to 49.5-90.1 mS/m or 0.495-0.901 dS/m. The conductivity managed to decrease with the time of observation. However, on day 40, after incubation, it appeared to increase the conductivity except for treatment with submerged hydromacrophytes (Figure 2). The reduction in conductivity during the phytoremediation process met the FAO irrigation water quality standard of 0-3 dS/m. The EC value decreased due to the removal of salt from the water by root adsorption or plant uptake. Conductivity measures the ability of the solution to conduct electricity and is correlated with its salt concentration [14]. Typically, the electrical conductivity of water is used to represent the total concentration of charged ionic species. The conductivity is also regarded as a rapid and accurate measure of dissolved solids that reflects an aquatic body's pollution and trophic levels. Low conductivity may account for the softness of the water, and significant conductivity changes may indicate that a discharge or other source of pollution has entered the pond [15, 16].

Dissolved oxygen (DO) is a crucial indicator of water quality, ecological status, productivity, and water body [16]. The DO levels increased in all treatments in post-harvest wastewater from Gondosuli catfish ponds. This increase occurred on day 10 after incubation, decreased on day 20,

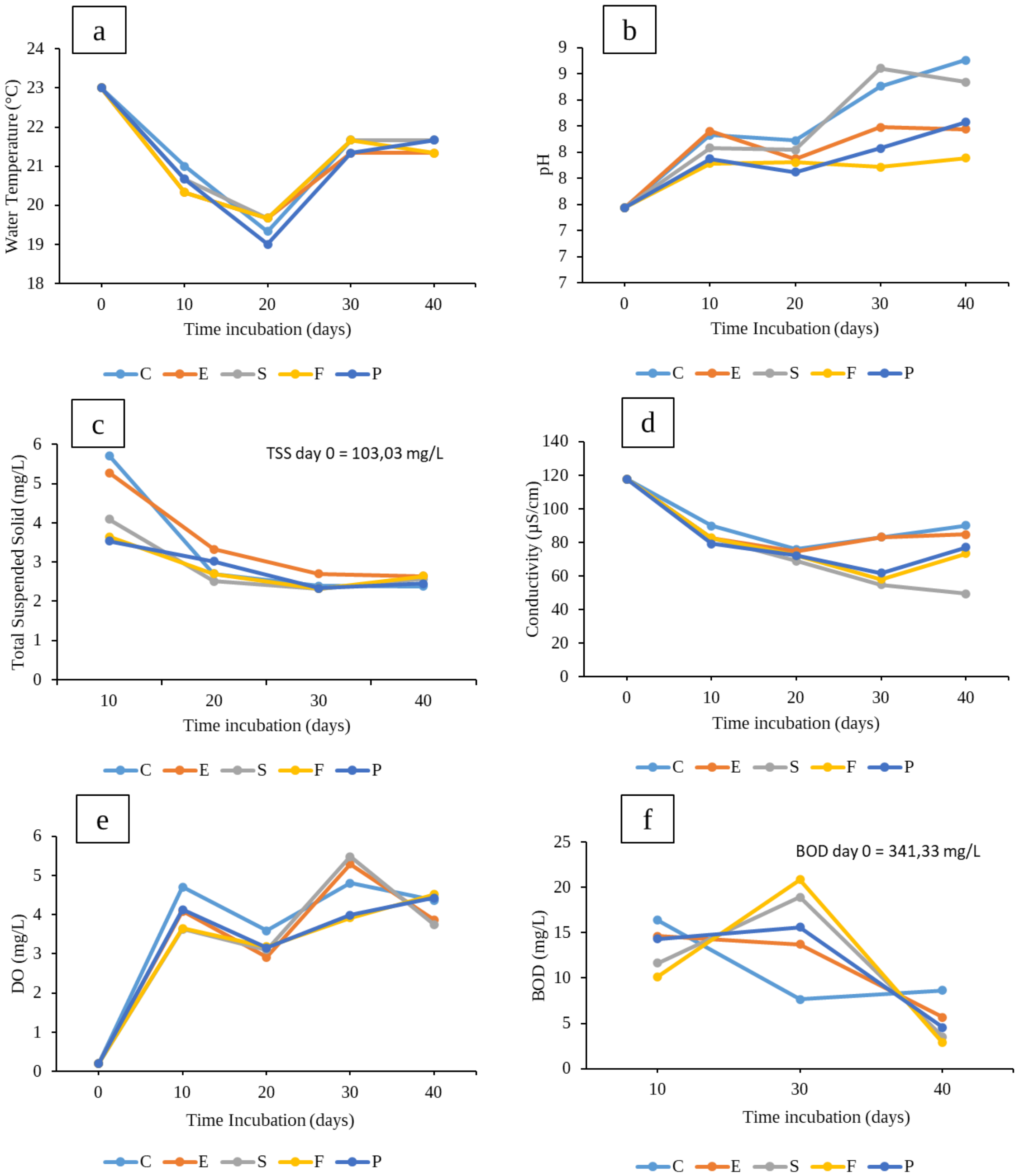

Figure 2. Monitoring the water physics and chemical quality of post-harvest catfish farming pond water in the phytoremediation process using emergent, floating, submerged, polyculture and without hydromacrophytes on days 0 (immediately after acclimatization), 10, 20, 30, 40 after incubation. (a) water temperature, (b) pH, (c) Total suspended solids (TSS), (d) DO, (e) Conductivity and (f) BOD. (Note: n=3; C = control; E = emergent; S = submerged; F = floating leaf; P = polyculture).

and increased again on day 30 until the end of observations on day 40 (Figure 2). Before phytoremediation treatment, preliminary DO levels averaged 0.21 mg/L.

The phytoremediation process in this study using various hydromacrophytes increased DO levels to 3.75-4.82 mg/L after 40 days of incubation. According to government regulation, this meets the minimum requirement for Class II agricultural activities, which is 4 mg/L of dissolved oxygen. Moreover, it also meets the minimum requirements for DO specified by the environmental quality standard (EQS): 5 mg/L for industrial usage, 6 mg/L for drinking, 4 to 5 mg/L for recreation, and 4 to 6 mg/L for fish and livestock [15]. Although, the average DO value in this study is still relatively low. The low DO value in this process indicates that organisms can only use the oxygen produced by photosynthesis in the treatment area for respiration. The level of organic matter in the water strongly influences the low DO level in the catfish farming pond in Gondosuli village. The monitoring results revealed that the level of organic matter pollution in Gondosuli village's catfish farming pond was high [2].

The decrease in DO levels on several days of observation (day 20) could be due to the increase in water surface cover by hydromacrophytes that block sunlight and consequently reduce the release of oxygen from photosynthetic organisms such as phytoplankton. The growth of macrophytes that cover the water surface in excess can reduce water reaeration, block sunlight, and reduce the release of oxygen from photosynthetic organisms in photosynthesis. This process can reduce DO levels at a specific time [17].

The phytoremediation process using hydromacrophytes with a batch culture system in this study reduced biological oxygen demand (BOD) levels in post-harvest catfish pond wastewater. The success of treatments was indicated by the initial BOD value of 341.33 mg/L, which then decreased significantly on day 10 with an average value of 13.42 mg/L. Then on day 30, there was an increase in BOD levels in all treatments except control (without hydromacrophytes). Increased BOD levels may be due to dead or decaying plants, which increases the organic matter content in the wastewater. At the end of the observation on day 40 incubation, BOD levels had decreased with a value range of 2.48-8.54 mg/L, where the control treatment had the highest value compared to other treatments (Figure 2). It occurred because of sedimentation and microbial degradation, especially by aerobic bacteria attached to plant roots. It had the highest BOD level at the end of the control study. The BOD levels from decomposition are more significant than the DO levels in the water, leaving insufficient oxygen for other organisms to survive [18].

Artificial fish feed residues provided by farmers may impact the increasing organic matter. The BOD value can show the presence of organic matter in the waters. When BOD is high, dissolved oxygen (DO) levels decrease because bacteria consume the available oxygen in the water [19]. Because less dissolved oxygen is in the water, fish and other aquatic organisms may suffer and die. Fewer bacteria will decompose if there is no organic waste in the water, and the BOD and DO levels will tend to be lower and higher, respectively.

Total suspended solids (TSS) are organic and inorganic solids suspended in water. By absorbing light, high concentrations of suspended solids can degrade water quality. The water then becomes warmer, diminishing its capacity to retain oxygen, which is essential for aquatic life. Because aquatic plants receive less light, photosynthesis, and oxygen production decrease, so planting hydromacrophytes impacts the sedimentation process of suspended solids in water [18]. The treatment of hydromacrophytes in the batch culture system in this study was proven to reduce TSS levels in the waters on day 10 after incubation (Figure 2). The concentration of total suspended solids (TSS) decreased from 103.03 mg/L to 2.53 mg/L and could also be reduced in a control treatment of gravel and sand without hydromacrophytes. This value meets the requirements of Class I quality standards based on Indonesian government regulation, with a maximum level of 40 mg/L. The temperature of the treatment water during phytoremediation did not show significant differences, ranging between 19°C and 23°C. The value demonstrated that the temperature in the glasshouse during phytoremediation treatment was relatively stable.

The results demonstrated that phytoremediation with various hydromacrophytes in post-harvest catfish pond wastewater reduced nitrate concentrations. Concentration levels of nitrate fluctuate during phytoremediation. The actual increase in nitrate levels occurred 10 days after incubation. Then, on day 20, only emergent and submerged hydromacrophyte treatments decreased. On day 30, nitrate levels decreased in all treatments until the end of this study (except the polyculture treatment, which increased again on day 40 after incubation) (Figure 3). At the end of the study, nitrate concentrations varied between 0.14 and 0.44 mg/L. This value, less than 10 mg/L, meets the requirement for Class I under Indonesian government regulations. The activity of bacteria interacting with hydromacrophytes could lead to variations in nitrate levels in wastewater. According to Ciria *et al*. [20], nutrient removal from wastewater is accomplished through a variety of mechanisms, such as plant uptake, microorganisms living on plant roots that convert nutrients (primarily nitrogen) into inorganic compounds (NH_4^+ and NO_3) that are directly available to plants and physical processes like sedimentation and filtration.

The orthophosphate value fluctuated from the first day to the 30 days after incubation. The lowest level of orthophosphate was observed on the 40 days with an average value of 0.24 -1.54 mg/L, whereas the control treatment had the lowest and highest levels in the polyculture (Figure 3). The low level of orthophosphate in control may occur because orthophosphate is used by phytoplankton for photosynthesis, which has a higher density. It is possible that the addition of water cover caused by the growth of hydromacrophytes also alters the absorption of nutrients in the water. Orthophosphate and nitrate nitrogen are perhaps crucial for several macrophyte species and groups. It showed that eutrophication significantly affected the types of macrophytes [21].

Biotic indices of phytoplankton

Improving the quality of post-harvest catfish farming pond wastewater after the phytoremediation process using various hydromacrophytes can also be seen by monitoring the structure community of phytoplankton as a bioindicator of water quality. Several indices can be used to measure the success of this process, including the Shannon Wiener diversity index, the Trophic Diatom Index (TDI), and the percentage of Pollutant Tolerance Value (%PTV). Based on the results of the study, it is known that the abundance of phytoplankton has decreased compared to before phytoremediation (Figure 4).

As primary producers, phytoplankton serve an important ecological function and are frequently used to indicate water fertility. The abundance of phytoplankton in this study fluctuated during the observation, but the levels tended to decrease for all treatments. This decrease is related to the level of nutrients and organic matter in the waters. According to the literature, one of the most important for plankton's life as water quality parameters is the nutrient content (nitrates and phosphates) [22]. Following the results of nutrient measurements in this study, it was given reason that the decrease in nitrate and orthophosphate levels in wastewater is in line with the reduction of the abundance of phytoplankton.

Shannon Wiener's diversity index (H') was also calculated in this study. H' was predicted to decrease as water quality degraded. Higher H' values would reflect more diverse communities (namely good water quality). Therefore, the H'

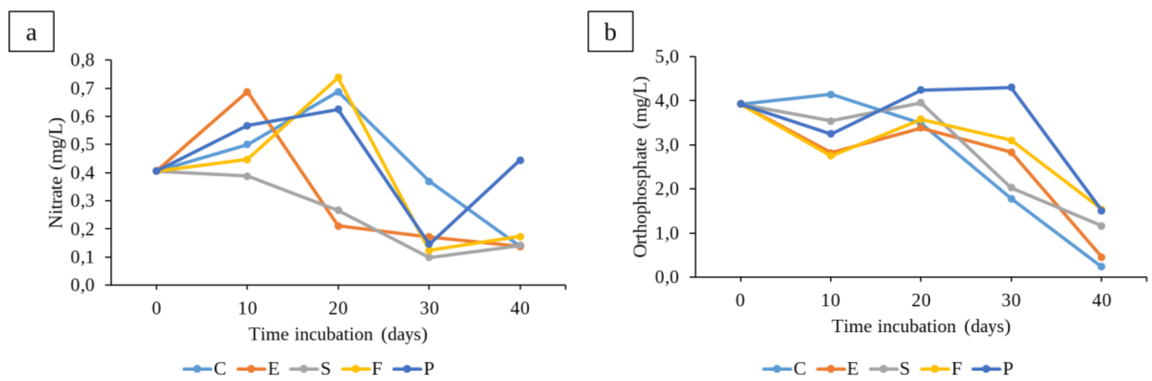

Figure 3. Monitoring nutrient levels of post-harvest catfish farming pond water in the phytoremediation process using emergent, floating, submerged, polyculture and without hydromacrophytes on days 0 (immediately after acclimatization), 10, 20, 30, 40 after incubation. (a) Nitrat and (b) Ortophosphate. (Note: n=3; C = control; E = emergent; S = submerged; F = floating leaf; P = polyculture).

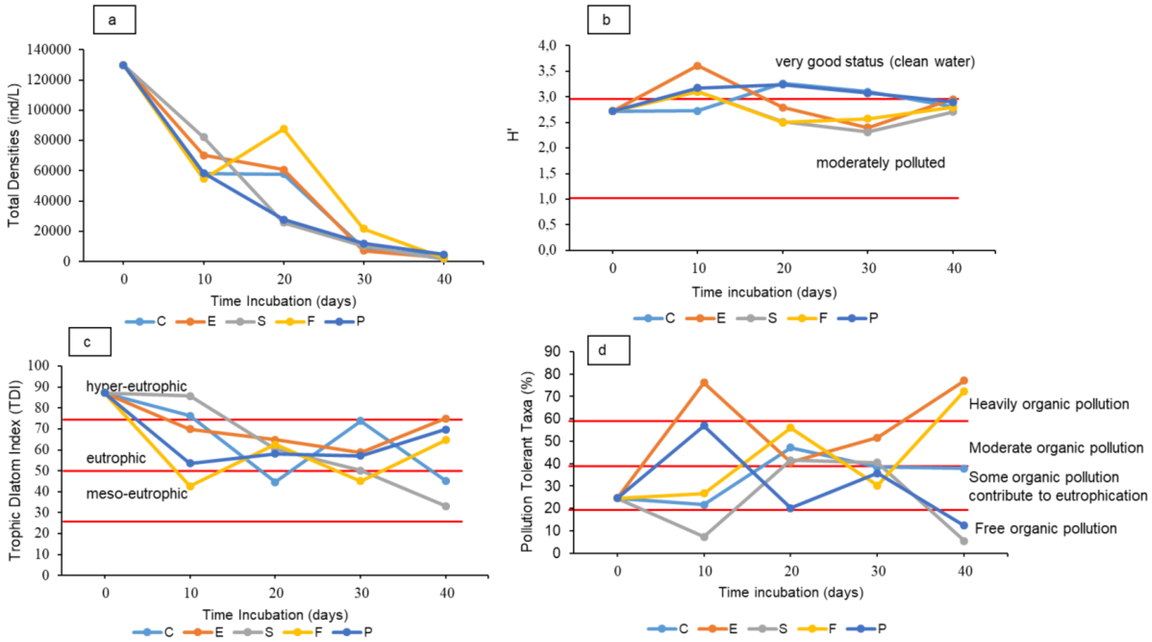

Figure 4. Biotic indices of phytoplankton to monitor the quality post-harvest catfish farming pond water in the phytoremediation process using emergent, floating, submerged, polyculture and without hydromacrophytes on days 0 (immediately after acclimatization), 10, 20, 30, 40 after incubation. (a), (b), (c). (Note: n=3; C = control; E = emergent; S = submerged; F = floating leaf; P = polyculture).

value enabled us to determine the number of species and the abundance distribution of each species within the community [23]. Hydromacrophytes can also affect diversity in plankton organisms by affecting phytoplankton productivity [24]. The results of the calculation of H' in this study indicate that water quality fluctuations occur over a time of observation. On day 10, after incubation, the average of all hydromacrophyte treatments (except control treatment) showed an H value of more than 3, which means the water quality is good (Figure 4). It showed that the hydromacrophyte treatment could increase phytoplankton diversity in the wastewater. However, based on the graph on the following observation day, the value continues to be unstable until, on the 40 days, all H' values are between 1 and 3, which means that the water quality is moderately polluted (moderate status). From the value of H', it is known that the best incubation time to carry out the phytoremediation process for post-harvest wastewater in this catfish pond is 10 days.

The results of the calculation of TDI and %PTV also show fluctuations in value over time of observation (Figure 4). The TDI value decreased, but until the end of the study, only the control and submerged hydromacrophytes decreased to a good status or meso-eutrophic (25-50), while in emergent treatment, floating leaf and polyculture were still in the category of moderate status or eutrophic (50-75). TDI is a comprehensive index that quantifies the degree of organic and inorganic pollution. Furthermore, the percentage of pollution tolerant taxa is used to validate the obtained TDI values, supplementing and verifying the TDI by indicating the levels of organic pollutants that affect the TDI values [25]. Even though there was much organic pollution, it was not easy to distinguish between eutrophication and other effects. As a result, the TDI values are supplemented by an indication of the percentage pollution tolerant values (%PTV), which is calculated as the sum of values belonging to taxa that are generally thought to be remarkably tolerant to organic pollution [12].

The %PTV value from this study shows variations in pollutant levels in each treatment. On the 10th day after the phytoremediation treatment, there was a decrease in the value of %PTV; the submerged hydromacrophyte treatment showed the best value and was categorized as free from significant organic pollution (0-20%), while other treatments were still at a moderate level, even in the hydromacrophyte treatment. Still in the category of heavy pollution (> 60%). Until the end of the incubation period with this batch culture system, only submerged treatment and polyculture of the three types of hydromacrophytes successfully reduced the % PTV value to a level free from organic matter pollution. This result differs from a similar batch culture phytoremediation process in irrigation water contaminated with organic matter and synthetic pesticides that can reduce the level of organic matter pollution from heavily organic to moderately and slightly organic [26].

The difference in results may be due to the significantly higher level of organic matter contamination in the wastewater from catfish farming ponds, as indicated by a higher BOD value compared to the BOD level of polluted irrigation water in earlier studies. According to Xiao et al. [27], pollutant concentrations significantly affect phytoremediation efficiency. Higher levels of organics and nutrients may be indirectly responsible for the declining trend and phytoremediation efficiency decline by inhibiting adaptive physiological regulations.

The effectiveness of the phytoremediation model based on Biplot analysis

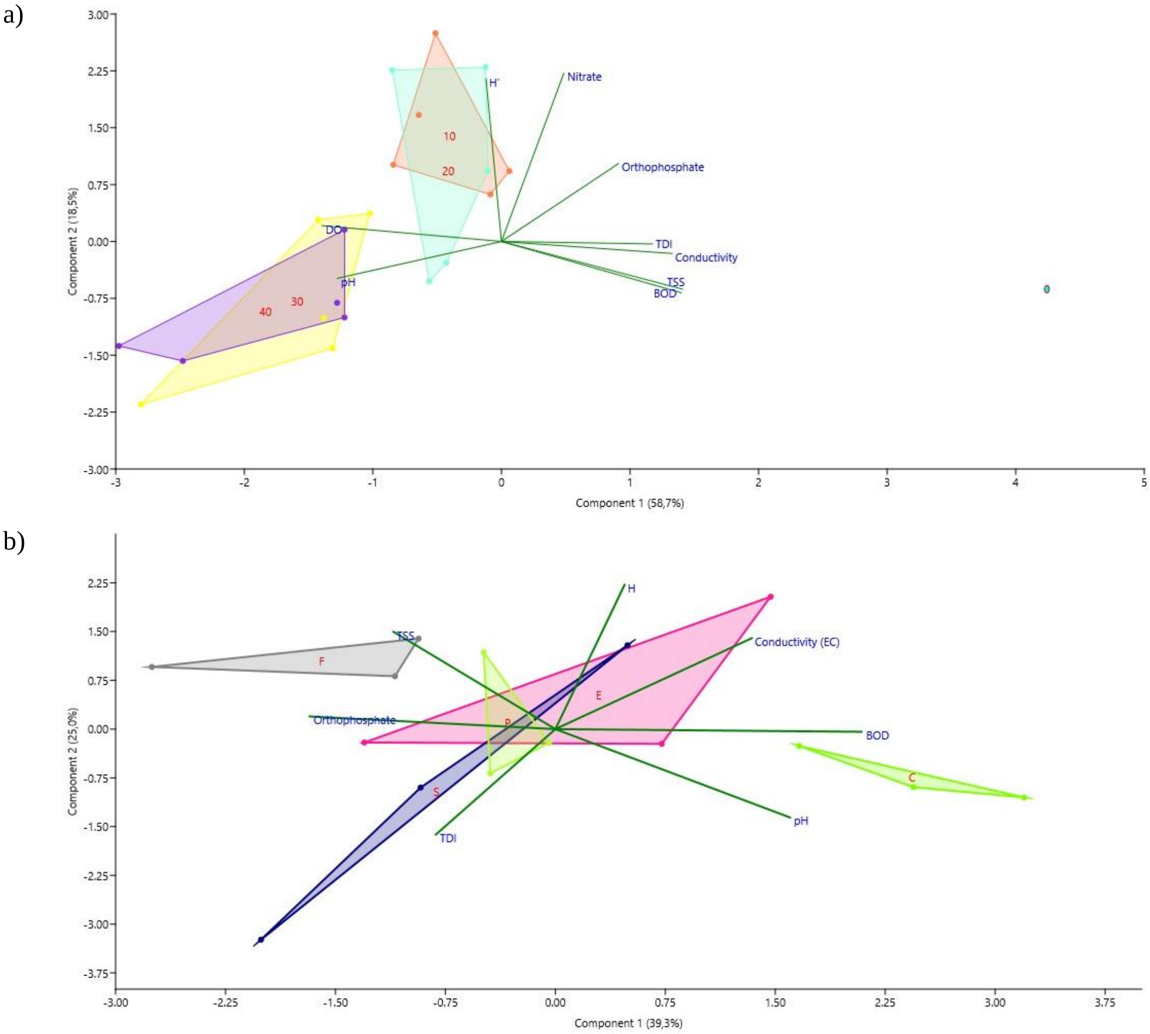

Figure 5. Principal Component Analisys (PCA) to evaluate the effectiveness of phytoremediation process in the post-harvest catfish pond using emergent, floating, submerged, polyculture and without hydromacrophytes on days 0 (immediately after acclimatization), 10, 20, 30, 40 after incubation. (a) PCA graph for each incubation time (b) PCA graph for each treatment on day 40. (Note: n=3; C = control; E = emergent; S = submerged; F = floating leaf; P = polyculture).

The success of the phytoremediation process of post-harvest catfish pond wastewater with local variations of hydromacrophytes in this study has been analyzed physically and chemically and based on the structure of the phytoplankton community, which is used as a bioindicator with an observation period of up to 40 days. All data were collected and analyzed using Principal Component Analysis (PCA) (Figure 5). The results of this analysis are expected to determine the treatment model that effectively reduces the levels of pollutants from catfish pond wastewater.

Based on the PCA (biplot) on the phytoremediation process from post-harvest catfish wastewater, it can be seen that the worst quality was observed on day 0 at any time after acclimatization for all treatments. This quality is indicated by high TDI, conductivity, and BOD values, and then water quality increases with increasing time of collection (shifted left from the graph) (Figure 5a). The high levels of nitrate and orthophosphate positively correlate with the treatment on days 10 and 20; this indicates that the treatment cannot be said to be influential in the success of the phytoremediation model. This result is also in line with %PTV which is also high at observations on days 10 and 20. Success in reducing BOD levels, conductivity, TDI, %PTV, and DO and pH levels were seen in all treatments on days 30 and 40 post-combination. The results can also support that the longer the incubation time, the better the water quality. The treatment of hydromacrophytes on day 40 reduced the water content in post-harvest catfish ponds with a lower variation compared to day 30.

PCA results on day 40 were used to determine which hydromacrophyte treatment was the most effective for this phytoremediation process (Figure 5b). All treatments have their respective roles in improving water quality through phytoremediation. The control treatment (without hydromacrophytes) was not able to reduce BOD but was able to increase pH and decrease orthophosphate. In contrast, floating leaf hydromacrophytes could reduce BOD, but orthophosphate was still high. For emergent hydromacrophytes, the variation in values is still very high, so the results are not recommended even though the H' value is high and TDI is low, while submerged hydromacrophytes have high TDI values, although BOD is low, orthophosphate is also low. Combination or polyculture of hydromacrophytes (P) is the most recommended treatment based on the results of this study which is characterized by low BOD, orthophosphate, conductivity, and TDI.

Even so, the reduction is still not optimal due to the high content of organic matter in wastewater. The sensitivity of plants often limits phytoremediation to contaminants, which inhibits plant growth and efficient contaminant uptake. There is a need for other phytoremediation methods, for example, with a continuous culture system or by utilizing the interaction between hydromacrophytes and microbes.

Conclusion

The phytoremediation process of post-harvest catfish pond wastewater with various types of local hydromacrophytes with a batch culture system has been proven to improve water quality. Phytoremediation treatment is proven to improve water's physical and chemical quality, which is indicated by increasing pH and DO levels, and decreasing conductivity, TSS, and BOD. Based on the biotic index, the phytoremediation process increased the diversity index (H') and decreased the total density, TDI, and %PTV. However, not all treatments can meet the water quality standards imposed by Indonesian government regulations. The most effective treatment is phytoremediation at an incubation time of 40 days with a polyculture of the three types of hydromacrophytes. However, water quality parameters are still unsuitable, so research and other systems, such as continuous culture, are needed for optimal results.

Acknowledgment

This research was supported by Universitas Brawijaya's "Hibah Doktor Lektor Kepala 2021" research grant. The authors thank the Dean of the Faculty of Mathematics and Natural Sciences and the Rector of Universitas Brawijaya for funding this study.

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