JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2024, 25(8), 237–252
https://doi.org/10.12911/22998993/188903
ISSN 2299–8993, License CC-BY 4.0

Received: 2024.05.09
Accepted: 2024.06.21
Published: 2024.07.01

Treatment of Domestic Wastewater Using Free Floating Constructed Wetlands Assisted by *Eichhornia crassipes* and *Pistia stratiotes*

S. Sayanthan[1,2], Hassimi Abu Hasan[1,3*], Junaidah Buhari[1], Siti Rozaimah Sheikh Abdullah[1,3]

[1] Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

[2] Department of Biosystems Technology, Faculty of Technology, University of Jaffna, Sri Lanka

[3] Research Centre for Sustainable Process Technology (CESPRO), Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

* Corresponding author's e-mail: hassimi@ukm.edu.my

ABSTRACT

Floating phytoremediation offers a feasible, ecofriendly, and economically viable method for treating wastewater. The objective of this study was to assess the effectiveness of *Eichhornia crassipes* and *Pistia stratiotes* in treating domestic wastewater with varying initial strength at different hydraulic retention time (HRT). This was to find out a best treatment combination of macrophyte, wastewater strength and HRT by optimizing those parameters. The growth parameters of the plants were assessed and linked to the removal of contaminants. The wastewater phytoremediation by *E. crassipes* and *P. stratiotes* conducted for twenty-two days in a green house. Result showed that *Eichhornia crassipes* able to treat 50% strength of wastewater (50 WH) efficiently with removal of ammonia by 97.4%, phosphate by 68.5%, and COD by 54.0%, respectively, at the HRT of three days. The corresponding initial concentrations of ammonia, phosphate, and COD were likely to be 6.62 mg/L, 2.54 mg/L, and 37 mg/L, respectively. The 50WH experimental units showed the lowest relative growth rate (RGR). The results proved the higher efficiency of *E. crassipes* in treating the domestic wastewater.

Keywords: phytoremediation, sewage wastewater, floating aquatic macrophytes, water hyacinth, effluent treatment.

INTRODUCTION

Domestic wastewater is one of the sources contributing to surface water pollution because of its contents which include ammonia nitrogen, organic matter, bacteria, biochemical oxygen demand (BOD) and other substances (Cheng et al., 2019; Othman et al., 2023). Due to the lack of sufficient treatment, the wastewater has resulted in the existence of increased concentrations of organic matter, nutrients, and detrimental microorganisms. The quality of the effluent water remains higher than the established criteria, namely in terms of nutrient levels such as ammonia, nitrite, nitrate, and phosphate. Hence, additional treatment is required to decrease the levels of pollutants. In recent years, phytoremediation has made tremendous advancements as a novel method for purifying polluted soil and water resources (Imron et al., 2023; Mirzaee et al., 2021; Said et al., 2020). According to Werkneh (2024), phytoremediation through constructed wetlands (CWs) are a decentralised sanitation solution that can be economically viable and well-suited for the environmental circumstances of developing nations. It is widely recognised as efficient, ecologically diversified, environmentally friendly, and naturally productive ecosystems that integrate physical, chemical, and biological treatment methods. Constructed wetlands have numerous benefits compared to traditional treatment systems. These advantages encompass reduced operating and maintenance expenses, much lower investment expenses in specific countries, the capability to treat low concentration wastewaters, and the lack of necessity for continuous feeding and operation (Vymazal, 2019). A floating wetland is a basic phytotechnology that may be effectively used to treat wastewater. It involves the use of aquatic plants as phytoremediators. Floating aquatic plants are frequently utilized due to their low maintenance requirements and cost-effectiveness (Zainuddin et al. 2022, Imron et al., 2023). In addition, when compared to native plants, invasive plants have significantly greater effectiveness in removing nutrients due to their high capacity for nutrient uptake. As a result, they contribute to the process of water purification (Prabakaran et al., 2019).

Aquatic macrophytes, like *E. crassipes* and *P. stratiotes*, are invasive and float freely. They can be utilised to remediate various pollutants from wastewater (Buhari et al., 2023). The robust and extensive root system of this plant is well-suited for the aerobic bacteria to effectively treat the pollutant. These plants exhibit exceptional growth rates, high efficiency in absorbing pollutants, cost-effectiveness, and they are also source of renewables (Selvaraj and Velvizhi, 2021). Both species occur inevitably in various regions worldwide and are known for their fast growth, high biomass production, and extensive root system, which enhances their ability to come into contact with organic pollutants (Correa dos Santos et al., 2022). According to Qin et al., (2020), *E. crassipes* shown significant capabilities in removing nitrogen (N) and phosphorus (P), even in challenging circumstances characterised by low levels of dissolved oxygen (DO < 1 mg/L) and high amounts of ammonium (NH_4^+-N > 7 mg/L). Previous study utilized *P. stratiotes* and *E. crassipes*, which effectively decreased the concentration of heavy metals, specifically Fe by 89% and Mn by 74%. Both plants effectively decrease other pollutants, including BOD, COD, phosphate, ammonia, nitrite, and nitrate within five weeks of time period (Wibowo et al., 2023).

Though the phytoremediation shows a series of advantages over other treatment methods they have their own constraints. Out of all the barriers of phytoremediation technology, the longer time taken for the treatment process and the possibility of phyto plants being affected by the intolerable concentrations of pollutants are to be focused more (Khandare and Govindwar, 2015). Therefore, with the intension of corelating the possible damages of different strengths of wastewater in growth of phyto plants and to find out a shorter HRT using diluted wastewater, an experiment was conducted. The prime objective of this study was to determine the response of *E. crassipes* and *P. stratiotes* to different loading strengths of domestic wastewater at different HRT by examining the removal performances and their growth parameters. The removal performances were measured by water quality parameter studies and plant growth performances were represented by fresh weight gain and RGR. The scope of the study is limited to the treatment of different loading strengths of domestic wastewater using *E. crassipes* and *P. stratiotes* for twenty-two days. The concentration of ammonia was focused as the deciding parameter of HRT.

MATERIALS AND METHODOLOGY

Collection and acclimatization of *E. crassipes* and *P. stratiotes*

Pistia stratiotes of uniform size were acquired from a plant nursery in Bangi, Malaysia, while *E. crassipes* plants were gathered from the pond located in the Universiti Kebangsaan Malaysia (UKM). The plants had a thorough rinsing with a gentle stream of water to effectively eliminate any presence of insect pests, weeds, soil and dirt. Subsequently, the plants were placed in sizable containers and exposed to tap water within a greenhouse for a duration of seven days to get accustomed to the new environment.

Establishment of experimental setup

The wastewater was initially obtained from the sewage treatment plant of Kolej Keris Mas, which is one of the hostels of UKM. The sampling was done after the primary sedimentation. The temperature inside the greenhouse fluctuated between 26 °C and 38 °C. Following the collection, the wastewater was evenly diluted with tap water to maintain its strengths at 25%, 50%, 75%, and 100%. The water quality parameters of different strengths of diluted wastewater is shown in the Table 1. The reactors utilised were cylindrical containers with a capacity of 3 litres, and they were filled with diluted and raw wastewater up to the designated mark at the top according to the requirement. Each wastewater sample was subjected to testing using *P. stratiotes* and *E. crassipes*. Additionally, a plant control experiment was

Table 1. Initial water quality parameters of different strengths of wastewater

Water quality parameters	Strength of wastewater			
	25	50	75	100
Ammonia (mg/L)	2.71	6.67	11.24	15.72
Nitrate (mg/L)	0.7	1.1	1.6	2.3
Nitrite (mg/L)	0.006	0.011	0.027	0.04
Phosphate (mg/L)	1.87	2.23	3.92	5.77
COD (mg/L)	17	37	70	116
pH	7.6	7.5	7.6	7.81

conducted in each concentration of wastewater, which held only the wastewater without any macrophytes. Each experiment was conducted with triplicates as shown in Figure 1. The experiment was conducted under greenhouse conditions and it was presumed that all other variables remained constant other than macrophytes, wastewater strength and HRT.

Sampling of treated wastewater

The treated wastewater from each reactor was collected in a centrifugal tube using a micropipette. The pipette facilitated precise positioning of the sample at the required depth without causing any disruption to the reactor. The sampling was conducted across a span of 22 days during

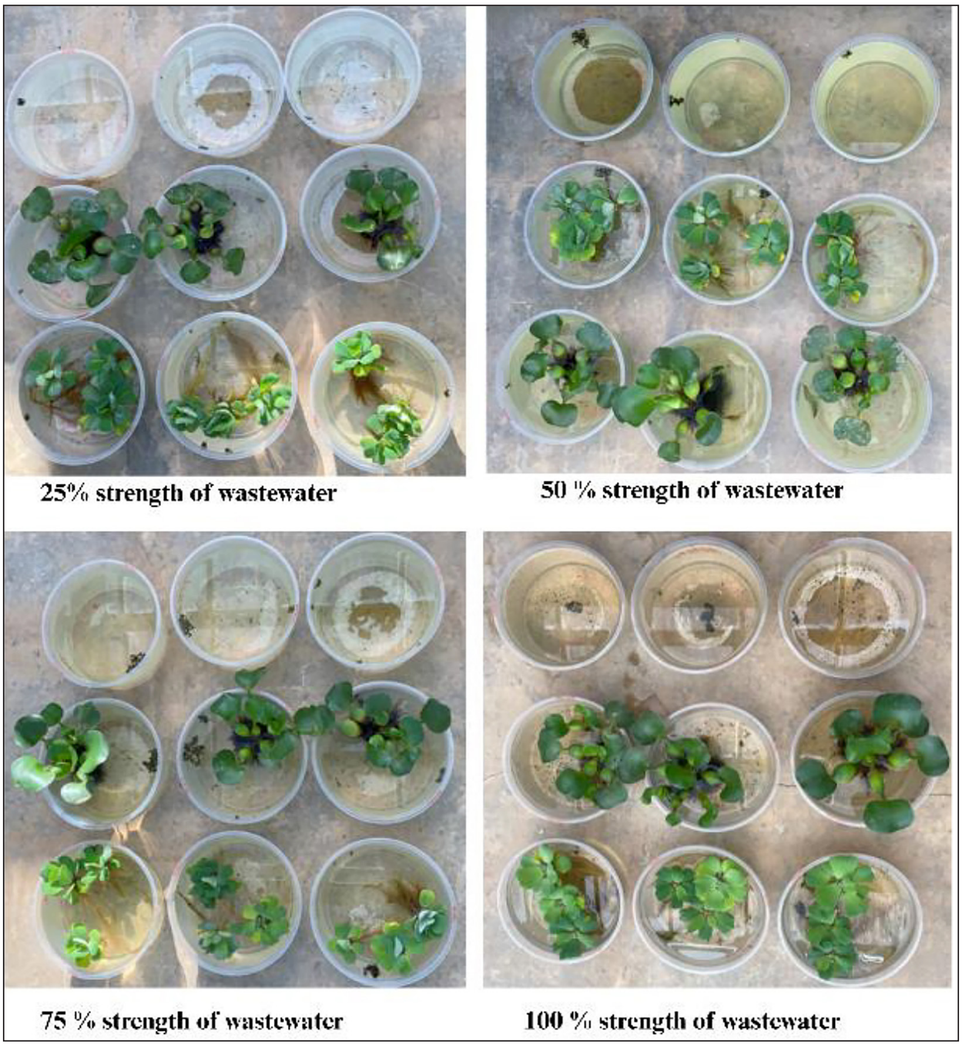


Figure 1. Established triplicates of experimental and control units

the experimental period, at certain time intervals on the 0th, 1st, 2nd, 3rd, 5th, 10th, 14th, 18th, and 21st days. Before collection, the water level in the storage tank was ensured to remain at the starting level that was marked. If the process of evaporation resulted in a decrease in the water level in the storage tank below the predetermined level, tap water was introduced to maintain the balance.

Water quality analysis

The samples collected from triplicates were measured to enhance the precision of the experiment. Following the collecting process, all samples underwent vacuum filtration using 0.45 – micron Whatman filter paper to prevent the presence of particles in the samples from affecting the testing procedure. Subsequently, the samples underwent analysis for ammonia, nitrate, nitrite, phosphate, COD, and pH.

The Nessler method (HACH method 8038) was employed to measure ammonia levels. The COD analysis was conducted using the reactor digestion method, namely the HACH method 8000. Low-range (3–150 mg/L) COD vials were utilised due to the initial low COD levels. Dilution was performed as necessary.

Nitrate, nitrite, and phosphate were measured by HACH DR 6000 spectrophotometry using corresponding powder pillows and the dilution was carried out when ever needed. Nitrate measurement was conducted in Cadmium reduction method (HACH method 8039) with the usage of NitraVer® 5 reagent powder pillow. USEPA Diazotization Method[1] was utilized to measure the nitrite level with the help of NitriVer® 3 reagent powder pillow. Reactive phosphorous was measured using ascorbic acid method (HACH method 8048) and PhosVer 3® powder pillow as reagent. All the water quality parameters were measured by HACH DR6000 spectrophotometer.

Determination of plant fresh weight and pollutant removal efficiency

Even though the dry basis measurements are reliable for aquatic plants to get accurate biomass, the wet basis measurement also can be effective whenever the destructive method is not applicable. The method of fresh weigh measurement in this study was influenced by a study carried out by Li et al. (2015). According to the surface covering area of plant leaves, number of plants per reactor was defined. All the utilized plants were washed thoroughly and left in a blotting paper for five minutes and confirmed the removal of water droplets from the surface of the plants and weighed using laboratory scale before the water loss from the plant tissue. On the basis of final and initial plant/plants weight of a reactor the weight gained percentage was calculated. Further to study the relative growth rate (RGR) of the plant/plants in reactors the following Equation 1 was used according to the guideline of (Mustafa and Hayder, 2021).

$$RGR = \frac{\ln W_2 - W_1}{t_2 - t_1} \quad (1)$$

where: W_1 and W_2 are plant dry weights at time t_1 and t_2, respectively To calculate the pollutant removal efficiency of macrophytes, the following Equation 2 was applied (Seroja et al., 2018).

$$Percentage\ of\ removal\ efficiency = \frac{C_i - C_f}{C_i} \times 100 \quad (2)$$

where: C_i – the initial measurement of water quality parameter, C_f – the last measurement of water quality parameter.

Statistical analysis

The experiment was intentionally planned as a two-factor factorial. The triplicates of the water quality parameters and plant growth characteristics were analysed statistically using two-way ANNOVA. Prior to the study, the data underwent examination to ensure normality and homogeneity of variance. The paired comparison was conducted with the Tukey post hoc test. The statistical analysis was conducted with the IBM SPSS Statistics version 21 software programme.

RESULT AND DISCUSSION

Visual observations of plants growth

At first, the wastewater appeared gloomy, but it quickly began to clarify once the reactors were installed. From the very beginning, the sediment settled in the bottom of the reactors and around the root hairs of plants. The experimental units in all four wastewater strengths had a conspicuous green coloration starting from day two. The plant growth became evident on day 5 in all of the experiment units, which consist *E. crassipes* and *P. stratiotes*. Additionally, on day 5, the wilting and the natural shedding of plant leaves also observed. As shown in Table 2, by the 18th day, the surface of the reactors containing aquatic plants were nearly completely enveloped by their respective plant species. During 21st day the leaves of the plants were densely arranged and increased in size and yellowing of some leaves were able observed predominantly.

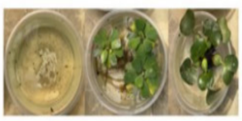
Nutrient reduction

In order to assess the effectiveness of and *E. crassipes* and *P. stratiotes* in removing nutrients from domestic wastewater, samples of treated wastewater were collected at various time intervals (0th, 1st, 2nd, 3rd, 5th, 10th, 14th, 18th, and 21st day). These samples were then analysed for levels of ammonia, nitrate, nitrite, phosphate, COD, and pH. Figure 2 displays the levels of ammonia and the related rates of removal by *E. crassipes*, *P. stratiotes*, and the control units containing plants, under different amounts of domestic wastewater with strength of 100%, 75%, 50%, and 25%.

The experimental units consisting of *E. crassipes* (100 WH), *P. stratiotes* (100 PI), and a plant control group (100 PC), effectively decreased the concentration of ammonia in wastewater with a strength of 100%. The concentration decreased by 99.2%, 98.7%, and 95.1% within a period of 21 days. Within the experimental units with 75% wastewater strength, *E. crassipes* (75 WH), *P. stratiotes* (75 PI), and the plant control (75 PC) achieved reductions in ammonia levels of 98.5%, 98.3%, and 93.4%, respectively, over the trial period. The experimental units, including *E. crassipes* (50 WH), *P. stratiotes* (50 PI), and the plant control (50 PC), achieved average ammonia removal rates of 96.4%, 97.4%, and 90.4%, respectively, within the wastewater samples containing 50% strength. The experimental units consisted of *E. crassipes* (25 WH), *P. stratiotes* (25 PI), and a plant control (25 PC), resulted a reduction in ammonium levels by 96.2%, 96.4%, and 96.7%, respectively, over the course of the study.

The ammonia concentration and decrease rate within the experimental units of various wastewater strengths exhibited a distinct pattern, which may be attributed to variations in the decomposition rates of organic matter and nitrogen fixation (Le and Boyd, 2012). The ammonia concentration in each reactor was affected by many processes, including nitrogen fixation,

Table 2. Observations of experimental and control units throughout the study period

Reactors / Days	25% Strength PC PI WH	50% Strength PC PI WH	75% Strength PC PI WH	100% Strength PC PI WH
Day 0				
Day 3				
Day 10				
Day 18				
Day 21				

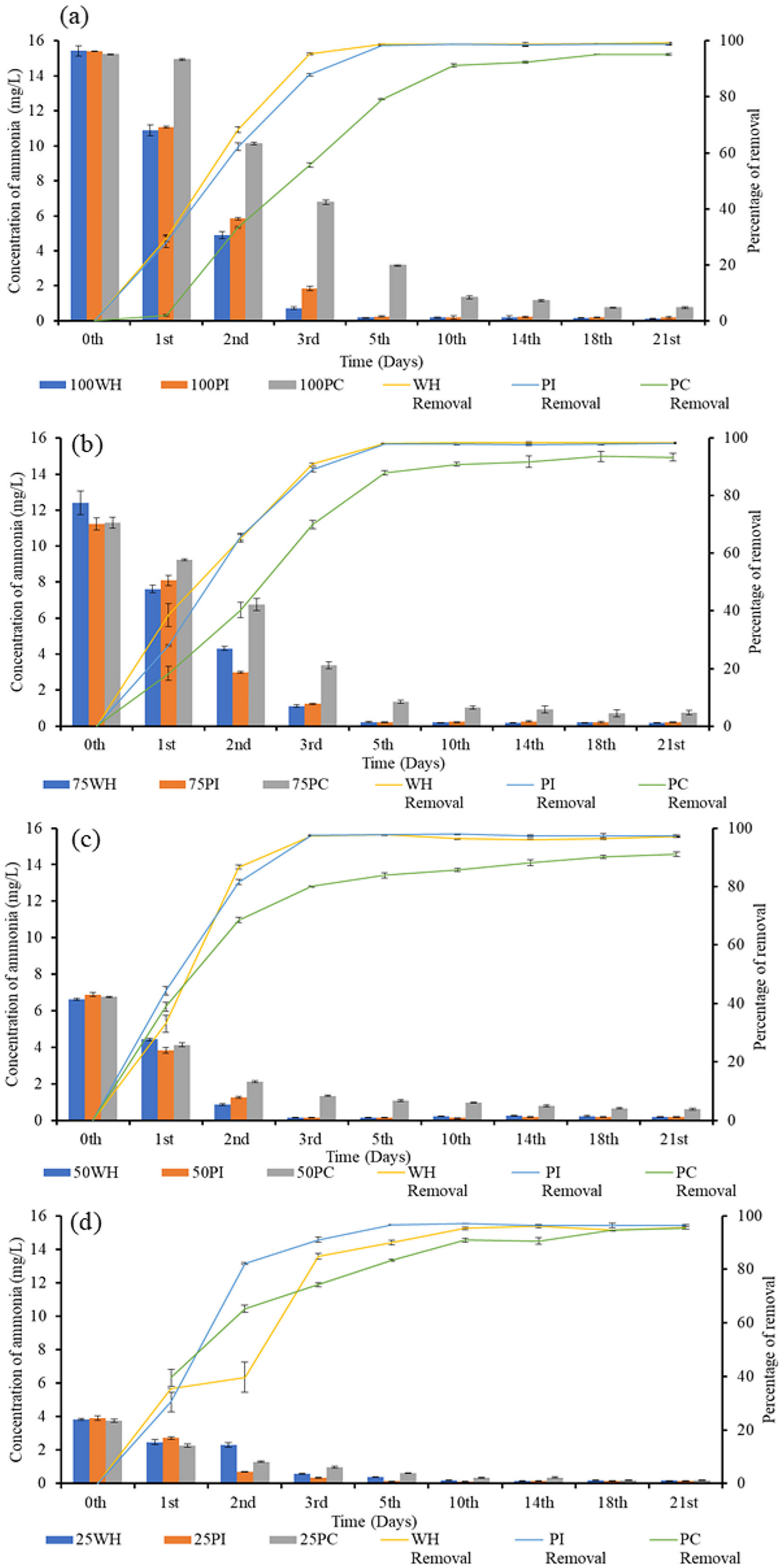


Figure 2. Temporal variation of ammonia concentration and removal percentage of all three experimental units of (a) 25% strength of wastewater (b) 50% strength of wastewater (c) 75% strength of wastewater (d) 100% strength of wastewater

nitrification, plant absorption, and mineralization. The former and latter raise the ammonium concentration in an ecosystem, while the remaining two decrease it (Le and Boyd, 2012). The study conducted by Mukherjee et al. (2015) demonstrated the successful application of *P. stratiotes* in reducing the ammonia concentration by 97% in a 50% diluted oil mill effluent, utilising tap water. The study cited above closely resembles the range of ammonia removal achieved in this research. Moreover, the current findings can be supported by another empirical study that utilises *E. crassipes* and *P. stratiotes* to remove ammonia from aquaculture wastewater. They removed 96.1% and 91.8% of ammonia, respectively. This scenario exhibits the superiority of *E. crassipes* over *P. stratiotes* in ammonia removal (Akinbile and Yusoff, 2012). Based on the study conducted by Qin et al. (2020) in an open pond, with the initial ammonia concentration of 6.55 mg/L and with the water hyacinth stocking density of 4 kg/m^2, the ammonia removal after 24 hrs of HRT was 70.2%. This study performed superior to the current study in ammonia removal within 24 hrs of HRT. Such lacking in performance may attributed to the maintained low stock density (maintained stock density in the current study was 0.45 kg/m^2) and the differed climatic conditions.

The removal rate of experimental units containing *E. crassipes* and *P. stratiotes* in wastewater with strengths of 100% and 75% (100 WH, 100 PI, 75 WH, and 75 PI) reached its highest point on the second day, except for 75 WH, which reached its peak on the first day. The decrease rate of ammonia concentration remained significant until day five for the stated reactors and exceeded the Malaysian criterion for Class III on day five. Exceptionally 100 WH, achieved this level on the third day due to superior removal performance. Following a significant decrease until the fifth day, the ammonia content exhibited fluctuations within a small numerical range. Both the plant control units for the 100% and 75% strengths of wastewater exhibited a significant decrease in percentage till day 18, meeting the Malaysian standard requirement for Class III of Water Quality Index.

Regarding the ammonia removal rate in wastewater with strengths of 50% and 25%, all three experimental units (50 WH, 50 PI, 50 PC, 25 WH, 25 PI, and 25 PC) exhibited the highest ammonia removal on day one other than 50 WH and 25 PI, which were able to showed a peak removal at the 2nd day. From day zero, the reduction was significant until the fifth day for 50 WH and 50 PI, the eighteenth day for 50 PC, the tenth day for 25 WH and 25 PI, and the twenty-first day for 25 PC. The duration needed to achieve the Malaysian Class III criteria also differed among the reactors. On the 3rd, 5th, and 18th days, 50 WH, 50 PI, and 50 PC reactors achieved a concentration below 0.9 mg/L. At a concentration of 25% in wastewater, both 25 WH and 25 PI achieved the recommended level on the third day, however the 25 PC reactors took ten days to reach it. The ammonia removal rates seemed to be peaked in different days for different reactors within a short range of period. The ammonia removal in a floating constructed wetland can be influenced by the concentrations of ammonia, the decomposition, microbial conversion, plant uptake, volatilization, sedimentation, and adsorption-fixation reactions (Kartohardjono et al., 2015, Ezaz et al., 2020). Based on this study the significant removal rates were observed until the previous ammonia concentrations were at least at the level of 0.6 mg/L. Therefore, it can be said that until the ammonia concentrations of 0.6 mg/L the microbial activity and plant uptake were prominent.

The rise in ammonia concentration can be predominantly attributed to the breakdown of organic materials present in wastewater or plant debris (Ng and Chan, 2017). The decomposition process yields ammonia in the reactors, which is subsequently transformed into nitrate and assimilated by plants and microorganisms, particularly the filamentous algae in both forms (nitrate and ammonia) (Ariffin et al., 2019). If the rate of nitrification, absorption by plants and microorganisms, and volatilization is slower than the rate of decomposition, an increase in ammonia concentration can be detected (Ng and Chan, 2017), which occurred in all reactors at some point.

With the intension of selecting the best reactor with appropriate HRT, the level of ammonia concentration was taken as the deciding factor. The initial ammonia concentration significantly varied ($p < 0.05$) among all the experimental units. 100 WH, 50 WH, 50 PI, and 25 WH showed a significant difference ($p < 0.05$) between the consecutive days until day three. The 100 PI, 75 WH, 75 PI, 75 PC, 50 PC, and 25 PI reactors significantly lowered ($p < 0.05$) the ammonia concentration in consecutive days until day five. The 100 PC and 25 PC showed a significant variation ($p < 0.05$) in ammonia concentration until day 10.

The water quality index for Malaysia (Class III) was taken as the benchmark for acceptable release of treated wastewater. Accordingly, 100 WH, 50 WH, 25 PI, and 25 WH reached the recommended level in day three; 100 PI, 75 WH, 75 PI, and 50 PI reached the recommended level in day five; 25 PC attained the recommended level in day ten; and 100 PC, 75 PC, and 50 PC reached the recommended level in day 18. The day three was taken as the treatment ceasing point as it was the least HRT period registered to release the wastewater with the recommended ammonia level.

Experimental units with *E. crassipes*, and plant control showed a significant difference ($p < 0.05$) between each on day three. Therefore, it can be concluded that reactors with *E. crassipes* are the best out of the three mentioned. Among the reactors occupied by *E. crassipes*, 100 WH, 50 WH, and 25 WH reached the recommended level on day three. On the basis of the statistical analysis between 100 WH, 50 WH, and 25 WH on day three, all three were statistically significant ($p < 0.05$) between them and 50 WH experienced the least mean of 0.170, and at the same time, 50 WH showed the highest reduction percentage of ammonia in day three at the level of 97.4%, whereas 100 WH and 25 WH reduced the ammonia by 95.3% and 84.9%, respectively. In addition, the COD and phosphate concentrations were also supportive for 50 WH on day three according to Malaysian Water Quality Standards of Class III, which are 17 mg/L and 0.8 mg/L respectively. Therefore, it was identified that among all established reactors, 50 WH reactors effectively treated the domestic wastewater in three days of HRT.

The nitrate measurements of all four distinct intensities of wastewater in all three experimental units exhibited a non-rhythmic fluctuation pattern as showed in Figure 3. The nitrate measurements reached their highest levels on day five in reactors with capacities of 100 PI, 100 WH, 75 PI, 75 WH, 50 PI, 50 WH, 25 PI, and 25 WH. During this period, the corresponding ammonium levels were lower, confirming the occurrence of nitrification. The subsequent substantial reduction in nitrate levels can be attributed to the denitrification and absorption of nitrate by the biomass present in the reactors (Wang and Chu, 2016, Hachiya and Sakakibara, 2017). The plant control units in all experimental units reached their highest eruption on day 10, while maintaining consistently low ammonium levels.

Phosphate

According to Figure 4, the phosphate concentration in all experimental units exhibited an immediate increase on the first day. This scenario suggests that phosphate solubilization has occurred in all units other than the process of phosphate absorption. Among all the tests done, the reduction efficiency over the study period was highest for 100WH, achieving a level of 96.34%. The reduction levels for the other trials were as follows: 100 PI – 94.0%, 75 WH – 91.5%, 75 PI – 90.9%, 50 WH – 87.8%, 50 PI – 88.9%, 25 WH – 90.4%, and 25 PI – 83.6%. Victor et al., (2016) also examined the supremacy of *E. crassipes* over *P. stratiotes* in phosphate reduction at a concentration of 30.1 mg/L. The abundant fibrous roots of *E. crassipes* enable a larger surface area, which promotes the growth of a diverse microbial population and enhances the effectiveness of physical and chemical processes in removing pollutants (Nizam et al., 2020). The findings of investigations conducted by many researchers were consistent with the current study. The consistent pattern observed in the results demonstrates the capacity and dependability of utilising *E. crassipes* and *P. stratiotes* for wastewater treatment (Akinbile and Yusoff, 2012; Nizam et al., 2020).

The plant control experimental units exhibited a lack of efficiency in comparison to the other two studies across all four wastewater intensities. The plant control units achieved a maximum efficiency of 33.3%, which was observed in 25 PC reactors. Other reactors, such as those operating at 100 PC, 75 PC, and 50 PC, were able to reduce phosphate levels by 0%, 10.7%, and 11.4% respectively. The trend indicates that as the wastewater strength increases, the phosphate reduction efficiency decreases in plant control units. Conversely, in reactors containing *E. crassipes* and *P. stratiotes*, the phosphate reduction efficiency reaches its highest point at the highest wastewater strength. The above-mentioned situation strongly indicates that the uptake of phosphate from domestic wastewater was greater in cases where the macrophytes and their rhizosphere interacted with the wastewater, compared to situations where only microorganisms were present in the control units. Phosphorous is an essential

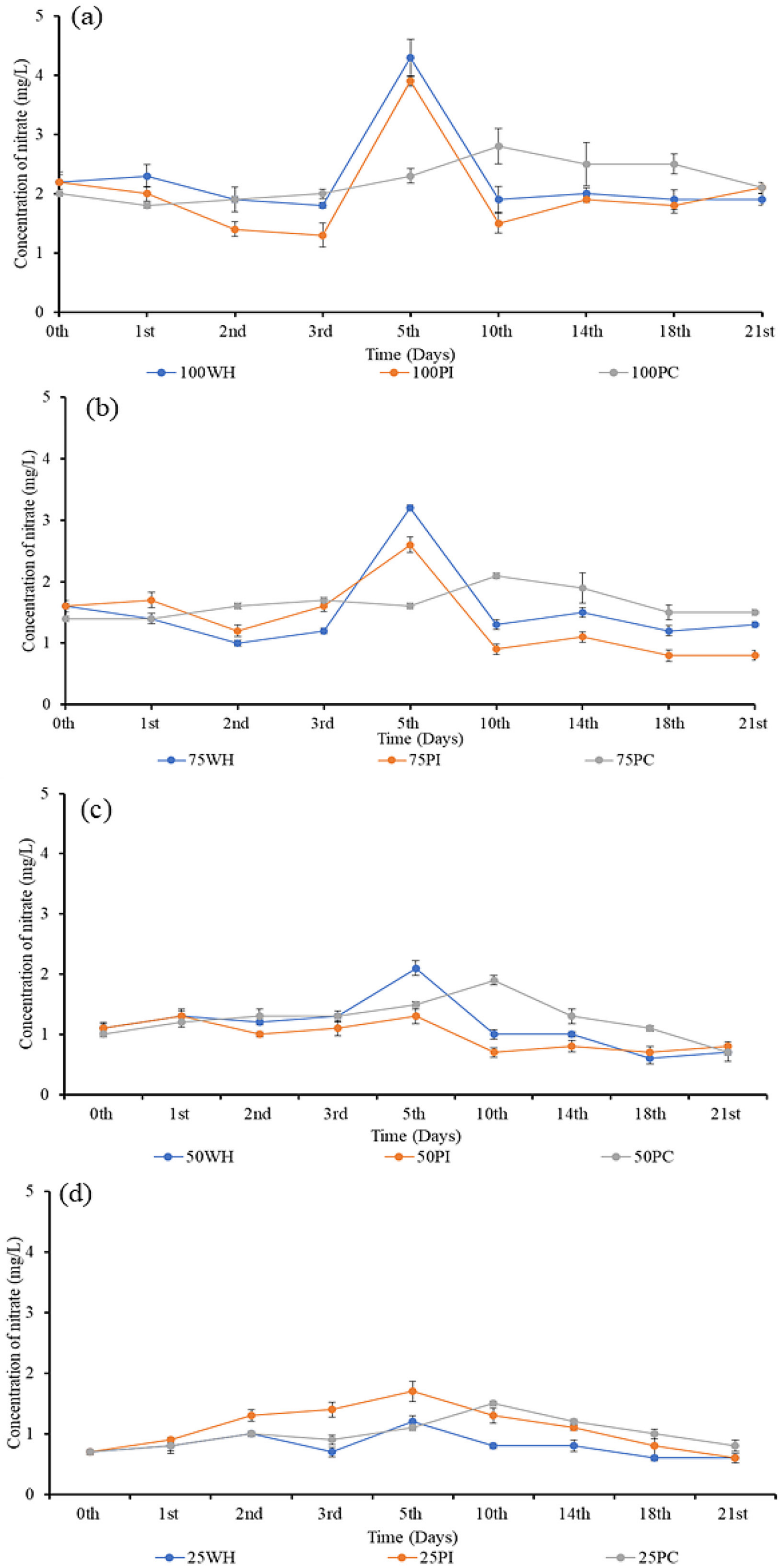


Figure 3. Temporal variation of nitrate concentration in all three experimental units of (a) 25% strength of wastewater (b) 50% strength of wastewater (c) 75% strength of wastewater (d) 100% strength of wastewater

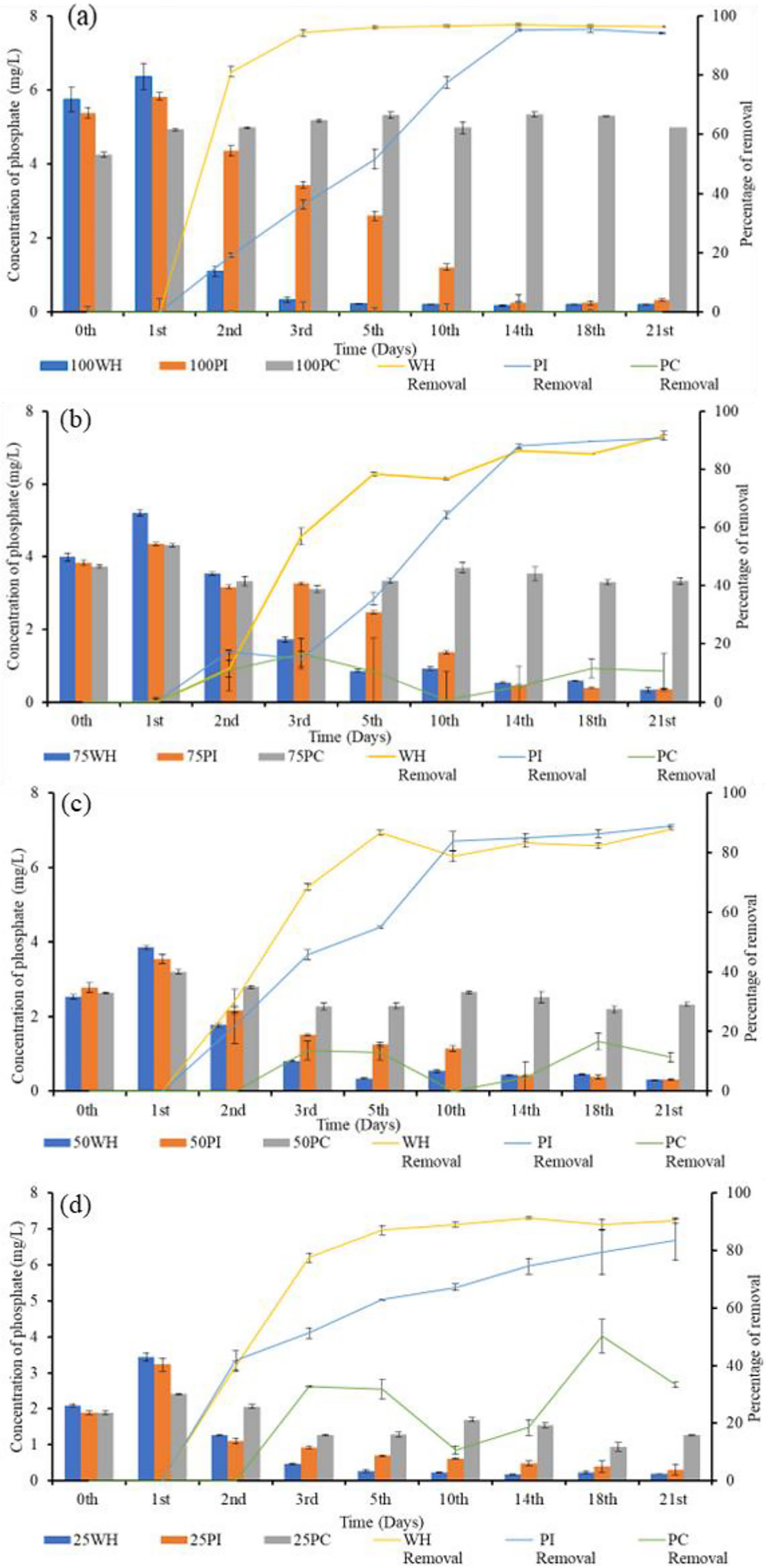


Figure 4. Temporal variations of phosphate concentration and removal percentage of experimental units and control units of (a) 25% strength of wastewater (b) 50% strength of wastewater (c) 75% strength of wastewater (d) 100% strength of wastewater

macronutrient for plants, playing a crucial role in their growth and development. Specifically, it is a vital part of adenosine triphosphate (ATP) and adenosine diphosphate (ADP), which are essential for the respiratory and photosynthetic processes in plants (Pandey 2018). Hence, the plant will assimilate the phosphate, specifically in the form of unbound orthophosphate.

COD

The highest efficiency of COD removal was achieved in the experimental units with 100 PI, with a removal rate of 89.91%. This was followed by units with 100 WH, 25 PI, 75 WH, 25 WH, 75 PI, 25PC, 50 WH, 50 PI, 100 PC, 75 PC, and 50 PC, with removal rates of 89.1%, 88.1%, 88.4%, 88.2%, 84.7%, 78.4%, 76.2%, 72.5%, 68.1%, and 64.3% respectively. As shown in Figure 5, The experimental units, composed of *E. crassipes* and *P. stratiotes*, exhibited a consistent decrease on most consecutive days, with the exception of 100 WH on the 18th day, 100 PI on the 10th day, 50 WH on the 14th day, 50 PI on the 21st day, and 25 PI on the 18th and 21st days. All experimental units designed with plant control exhibited fluctuation after three consecutive reductions. Nevertheless, all the studies effectively decreased the COD value to a level below the Malaysian Water Quality Standard of Class III.

The primary cause for the decrease in COD during phytoremediation is the complete breakdown of organic substances. Simultaneously, incomplete degradation results in the formation of certain intermediates, including amino acids and sugars. These intermediates can maintain an elevated COD value in plant control units. But in units containing *E. crassipes* and *P. stratiotes*, there is a potential for direct absorption of specific sugars and amino acids by the macrophyte plants. Root mats exhibit significant development during the growth period of plants and possess a remarkable ability to efficiently filter suspended solids at a high rate (Cao et al., 2015; Grant and Beevers, 1964). Thus, the plant units can consistently achieve ongoing decrease in comparison to the plant control units, which solely rely on the complete breakdown of organic matter for COD removal. Several studies, including Rezania et al., (2015) and Wickramasinghe and Jayawardana, (2018) have found evidence that supports the effectiveness of specific macrophytes in reducing COD levels. These findings further enhance the credibility and acceptance of the current work.

Plant growth analysis

At first, the leaf area was planned to be equal for both macrophytes, resulting in varying beginning weights for each macrophyte. Simultaneously, the fresh weight of the macrophytes between the reactors carrying the same macrophytes was more or less the same. During the latter phase of the investigation, a substantial boost in wet weight between the initial and final measurements was seen, regardless of the type of macrophyte. Figure 6, demonstrates that *P. stratiotes* exhibited a significantly greater growth rate than *E. crassipes* at various levels of wastewater strength. Notably, the plants in 75 PI reactors displayed the highest percentage of weight gain, reaching 218.8%. Among the reactors containing *E. crassipes*, the reactor with 100 WH exhibited a greater percentage of weight gain (155.4%), while the reactor with *E. crassipes* with 50% strength wastewater showed the lowest weight gain. However, the majority of the reactors with *E. crassipes* exhibited superior performance in removing ammonia, phosphate, and COD compared to those with *P. stratiotes*, even though the latter one showed a higher wet weight gain percentage. Malcolm J. Hawkesford and Kok, (2014) and Adhikari et al., (2022) assert that nutrition use efficiency (NUE) and subsequent yield are complex processes that encompass not only the absorption of nutrients but also their transportation, storage, mobilization, and utilization within the plant. Thus, it is justifiable that the percentage of increment in biomass production of *P. stratiotes* is higher than that of *E. crassipes*, despite the fact that nutrient absorption is high.

According to Lowry and Smith, (2018), the RGR of a plant is its competitiveness of accumulating additional biomass. Hence, RGR is another measure of plant growth. As shown in Figure 7, the RGR was higher for *P. stratiotes* compare to *E. crassipes* cultivated in the same strength of wastewater. Further the highest RGR of *P. stratiotes* and *E. crassipes* was obtained in the maximum strength of wastewater. Such condition was explained by Heard and Winterton, (2000) as "growth rates increase with in water nitrogen amount". While 50 WH performed very low among all the experimental units in terms of RGR and among the reactors of *P. stratiotes*, 25 PI performed lower.

The statistical analysis of the initial wet weight of the plant resulted in a significant

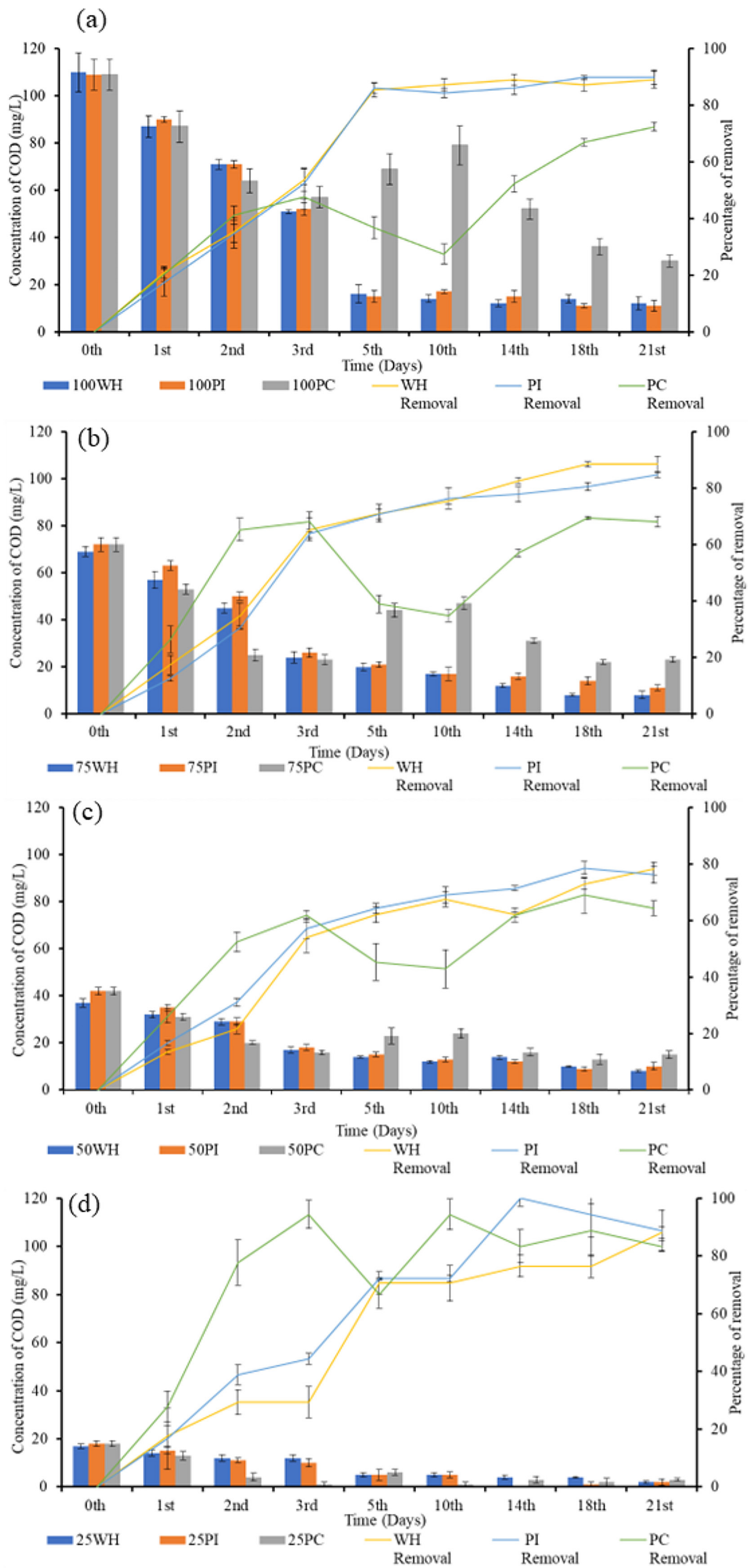


Figure 5. Temporal variation of COD concentration and removal percentage experimental and control units of (a) 25% strength of wastewater (b) 50% strength of wastewater (c) 75% strength of wastewater (d) 100% strength of wastewater

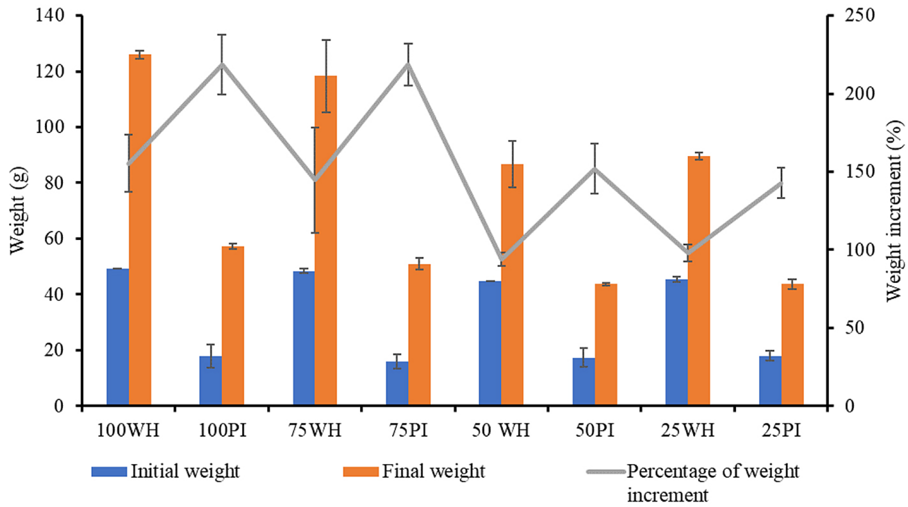


Figure 6. The average Initial and final weights and weight gained percentage of plants in different experimental units

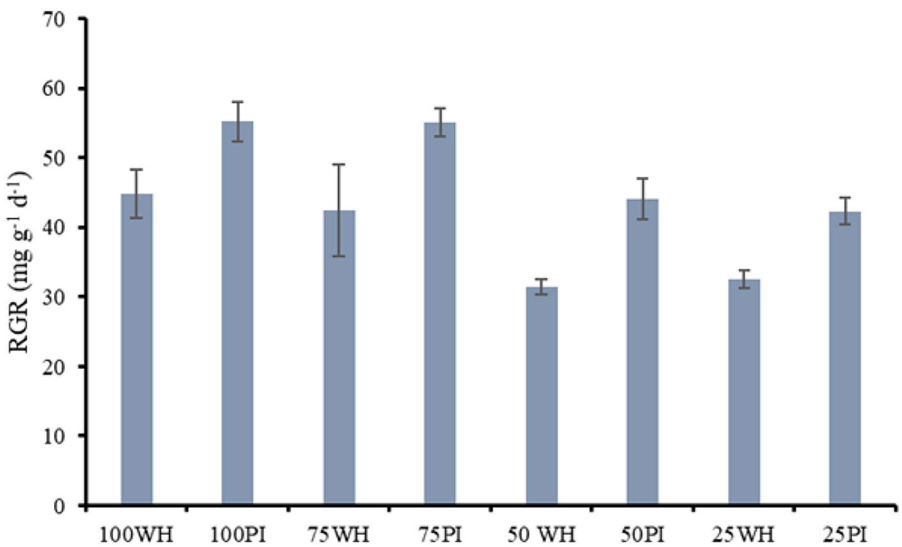


Figure 7. The average relative growth rates of plants in different experimental units

variation ($p < 0.05$) between *P. stratiotes* and *E. crassipes*, where the mean wet weight was lower for *P. stratiotes*. The growth of the plants in different strengths of wastewater showed statistical significance ($p < 0.05$) between two sets, where 100% and 75% in one set and 50% and 25% in the other set, had a lower mean growth rate. Based on the statistical analysis of RGR, 100 PI showed the highest mean of RGR, and an insignificant ($p > 0.05$) RGR was observed with 100 WH, 75 PI, and 50 PI, while other experimental units showed a significant variation ($p < 0.05$) with 100 PI. Based on the RGR study and nutrient removal study, the result showed an inverse corelation. According to Bashan and de-Bashan (2005) plant growth can be affected by several factors such as genetic factor, phonotypic characters and environmental factors. Here, this inversed trend of plant weight gain and pollutant removal cannot be attributed to certain factors as it can be an outcome of combined effect. If the current study is peculiarized it can be said that the microbes associated with the rhizosphere of water hyacinth may diverse in microbial strains and high in count. The corresponding removal of nutrients will be attributed to microbes than that of water hyacinth uptake or the nutrient use efficiency of water hyacinth may be lower than that of *P. stratiotes* even they absorbed more nutrients (Ezaz et al., 2020, Hawkesford et al., 2016).

The disparity between the outcomes achieved in the greenhouse and those observed in the real field study is significant. Under controlled conditions, plant growth is effectively aided. However, in natural environments, plant growth can be influenced by abiotic factors such as climate, fluctuating nutrient levels, and biotic ones such as pests and diseases. Additionally, in contrast to the greenhouse experiment, the actual field experiment might encounter shifting water quality metrics; hence, expected pollutant reductions may not occur. The variation in dissolved oxygen content in the pond, influenced by environmental circumstances and pond structure, can affect microbial populations and lead to altered pollutant removal capabilities. Hence, it is crucial to determine the precise efficacy of pollutant removal in real-life circumstances in order to endorse and advance the laboratory-scale procedure towards the establishment of a wastewater treatment facility.

CONCLUSIONS

Phytoremediation using floating aquatic macrophytes namely *E. crassipes* and *P. stratiotes* showed a great performance in removing ammonia, phosphate and COD. Both macrophytes thrived and survived in 25%, 50%, 75% and 100% of domestic wastewater. At the end of 21 days of treatment the final water quality parameters varied insignificantly between the experimental and control units. The third day was the shortest HRT to pass the Malaysian Water Quality Index (Class III) in terms of ammonia concentration. According to the statistical analysis 50 WH experimental units showed the better performance in removing ammonia than other reactors on day three. Further the concentrations of COD also maintained below the recommended level by the third day. When considering the RGR, 50 WH performed as the experimental unit possessed lowest RGR, where 100 WH showed an insignificance with the experimental unit holding highest RGR (100 PI). Therefore, it can be concluded that the 50WH units still have the capacity to absorb more nutrients and convert them in to plant biomass. Hence it is recommended to conduct future studies with different dilution of wastewater above 50% up to highly concentrated wastewater more than 100% strength of used wastewater with more options. This is to find out the accurate point of wastewater dilutions of different sources of domestic wastewater with maximum removal at minimal HRT and optimum plant growth.

Acknowledgement

The authors would like to acknowledge the Universiti Kebangsaan Malaysia for funding this research project through the Geran Universiti Penyelidikan with grant no. GUP-2022-028.

REFERENCES

1. Adhikari, S., Anuragi, H., Chandra, K., Tarte, S.H., Dhaka, S.R., Jatav, H.S., Hingonia, K. 2022. Molecular basis of plant nutrient use efficiency - concepts and challenges for its improvement, in: Sustainable Plant Nutrition: Molecular Interventions and Advancements for Crop Improvement. https://doi.org/10.1016/B978-0-443-18675-2.00001-8
2. Akinbile, C.O., Yusoff, M.S. 2012. Assessing water hyacinth (*Eichhornia crassopes*) and lettuce (*Pistia stratiotes*) effectiveness in aquaculture wastewater treatment. Int. J. Phytoremediation.https://doi.org/10.1080/15226514.2011.587482
3. Ansari, A.J., Hai, F.I., Price, W.E., Drewes, J.E., Nghiem, L.D. 2017. Forward osmosis as a platform for resource recovery from municipal wastewater - A critical assessment of the literature. J. Memb. Sci. https://doi.org/10.1016/j.memsci.2017.01.054
4. Ariffin, F.D., Halim, A.A., Hanafiah, M.M., Ramlee, N.A. 2019. Phytoremediation capability by azolla pinnata in aquaculture wastewater treatment. Sains Malaysiana. https://doi.org/10.17576/jsm-2019-4802-03
5. Bashan, Y. and De-Bashan, L.E. 2005. Fresh-weight measurements of roots provide inaccurate estimates of the effects of plant growth-promoting bacteria on root growth : a critical examination. Soil Biology & Biochemistry, 37**,** 1795–1804.
6. Buhari, J., Hasan, H.A., Kurniawan, S.B., Abdullah, S.R.S., Othman, A.R. 2023. Future and challenges of co-biofilm treatment on ammonia and Bisphenol A removal from wastewater. J. Water Process Eng. https://doi.org/10.1016/j.jwpe.2023.103969
7. Cao, X.C., Wu, L.H., Ma, Q.X., Jin, Q.Y. 2015. Advances in studies of absorption and utilization of amino acids by plants: A review. Chinese J. Appl. Ecol.
8. Cheng, H., Zhu, Q., Xing, Z. 2019. Adsorption of ammonia nitrogen in low temperature domestic wastewater by modification bentonite. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2019.06.079
9. Correa dos Santos, N.M., Monteiro, P.G., Ferreira, E.A., Alencar, B.T.B., Cabral, C.M., dos Santos, J.B. 2022. Use of Eichhornia crassipes and Pistia stratiotes for environmental services: Decontamination of aquatic environments with atrazine residues. Aquat. Bot. https://doi.org/10.1016/j.aquabot.2021.103470

10. Ezaz, Z., Azhar, R., Rana, A., Ashraf, S., Farid, M., Mansha, A., Naqvi, S.A.R., Zahoor, A.F., Rasool, N. 2020. Current trends of phytoremediation in wetlands: mechanisms and applications. Plant Ecophysiology and Adaptation under Climate Change: Mechanisms and Perspectives II: Mechanisms of Adaptation and Stress Amelioration**,** 747–765.
11. Grant, B.R., Beevers, H. 1964. Absorption of sugars by plant tissues. Plant Physiol. https://doi.org/10.1104/pp.39.1.78
12. Wibowo, G.Y., Nugraha, T.A., Rohman, A. 2023. Phytoremediation of several wastewater sources using Pistia stratiotes and Eichhornia crassipes in Indonesia. Environ. Nanotechnology, Monit. Manag. https://doi.org/10.1016/j.enmm.2023.100781
13. Hachiya, T. and Sakakibara, H. 2017. Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. Journal of Experimental Botany, 68**,** 2501–2512.
14. Hawkesford, M.J., Kopriva, S., De Kok, L.J. 2016. Nutrient use efficiency in plants.
15. Heard, T.A., Winterton, S.L. 2000. Interactions between nutrient status and weevil herbivory in the biological control of water hyacinth. J. Appl. Ecol. https://doi.org/10.1046/j.1365-2664.2000.00480.x
16. Imron, M.F., Firdaus, A.A.F., Flowerainsyah, Z.O., Rosyidah, D., Fitriani, N., Kurniawan, S.B., Abdullah, S.R.S., Hasan, H.A., Wibowo, Y.G. 2023. Phytotechnology for domestic wastewater treatment: Performance of Pistia stratiotes in eradicating pollutants and future prospects. J. Water Process Eng. 51, 103429. https://doi.org/10.1016/j.jwpe.2022.103429
17. Kartohardjono, S., Elkardiana, K., Sangaji, A., Ramadhan, A., Fermi, M. 2015. The removal of dissolved ammonia from wastewater through a polypropylene hollow fiber membrane contactor. International Journal Of Technology, 6.
18. Khandare, R.V. and Govindwar, S.P. 2015. Phytoremediation of textile dyes and effluents: current scenario and future prospects. Biotechnology Advances, 33**,** 1697–1714.
19. Le, P.T.T. and Boyd, C. 2012. Comparison of phenate and salicylate methods for determination of total ammonia nitrogen in freshwater and saline water. Journal Of The World Aquaculture Society, 43, 885–889.
20. Li, J., Yang, X., Wang, Z., Shan, Y., Zheng, Z. 2015. Comparison of four aquatic plant treatment systems for nutrient removal from eutrophied water. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2014.11.053
21. Lowry, C.J., Smith, R.G. 2018. Weed control through crop plant manipulations, in: Non-Chemical Weed Control. https://doi.org/10.1016/B978-0-12-809881-3.00005-X
22. Malcolm J. Hawkesford, S.K., Kok, L.J. De. 2014. Nutrient use efficiency in plants. Springer Cham. https://doi.org/https://doi.org/10.1007/978-3-319-10635-9
23. Metcalf, E., Eddy, H. 2003. Wastewater engineering: treatment and reuse, Wastewater Engineering, Treatment, Disposal and Reuse. Techobanoglous G, Burton FL, Stensel HD (eds). Tata McGraw-Hill Publishing Company Limited, 4th edition. New Delhi, India.
24. Mirzaee, M.M., ZakeriNia, M., Farasati, M. 2021. The effects of phytoremediation of treated urban wastewater on the discharge of surface and subsurface drippers (Case study: Gorgan wastewater treatment plant in northern Iran). Clean. Eng. Technol. https://doi.org/10.1016/j.clet.2021.100210
25. Mukherjee, B., Majumdar, M., Gangopadhyay, A., Chakraborty, S., Chaterjee, D. 2015. Phytoremediation of parboiled rice mill wastewater using water lettuce (*Pistia Stratiotes*). Int. J. Phytoremediation. https://doi.org/10.1080/15226514.2014.950415
26. Mustafa, H.M., Hayder, G. 2021. Cultivation of S. molesta plants for phytoremediation of secondary treated domestic wastewater. Ain Shams Eng. J. https://doi.org/10.1016/j.asej.2020.11.028
27. Ng, Y.S., Chan, D.J.C. 2017. Wastewater phytoremediation by Salvinia molesta. J. Water Process Eng. https://doi.org/10.1016/j.jwpe.2016.08.006
28. Nizam, N.U.M., Hanafiah, M.M., Noor, I.M., Karim, H.I.A. 2020. Efficiency of five selected aquatic plants in phytoremediation of aquaculture wastewater. Appl. Sci. https://doi.org/10.3390/APP10082712
29. Othman, M.F., Hasan, H.A., Muhamad, M.H., Babaqi, B.S. 2023. Biopolishing of domestic wastewater using polyvinyl alcohol – supported biofilm of bacterial strain *bacillus velezensis* isolate JB7. J. Ecol. Eng. https://doi.org/10.12911/22998993/165780
30. Prabakaran, K., Li, J., Anandkumar, A., Leng, Z., Zou, C.B., Du, D. 2019. Managing environmental contamination through phytoremediation by invasive plants: A review. Ecol. Eng. https://doi.org/10.1016/j.ecoleng.2019.07.002
31. Qin, H., Diao, M., Zhang, Z., Visser, P.M., Zhang, Y., Wang, Y., Yan, S. 2020. Responses of phytoremediation in urban wastewater with water hyacinths to extreme precipitation. J. Environ. Manage. https://doi.org/10.1016/j.jenvman.2020.110948
32. Radzali, M.H., Kamal, N.A.M., Diah, N.M. 2016. Measuring leaf area using otsu segmentation method (LAMOS). Indian J. Sci. Technol. https://doi.org/10.17485/ijst/2016/v9i48/109307
33. Rezania, S., Ponraj, M., Talaiekhozani, A., Mohamad, S.E., Md Din, M.F., Taib, S.M., Sabbagh, F., Sairan, F.M. 2015. Perspectives of phytoremediation using water hyacinth for removal of heavy metals, organic and inorganic pollutants in wastewater. J. Environ. Manage. https://doi.org/10.1016/j.jenvman.2015.08.018

34. Rice, E.W., Bridgewater, L., Association, A.P.H. 2012. Standard methods for the examination of water and wastewater, American public health association Washington, DC.
35. Said, N.S.M., Abdullah, S.R.S., Ismail, N. Izzati, Hasan, H.A., Othman, A.R. 2020. Phytoremediation of real coffee industry effluent through a continuous two-stage constructed wetland system. Environ. Technol. Innov. https://doi.org/10.1016/j.eti.2019.100502
36. Selvaraj, D., Velvizhi, G. 2021. Sustainable ecological engineering systems for the treatment of domestic wastewater using emerging, floating and submerged macrophytes. J. Environ. Manage. https://doi.org/10.1016/j.jenvman.2021.112253
37. Seroja, R., Effendi, H., Hariyadi, S. 2018. Tofu wastewater treatment using vetiver grass (Vetiveria zizanioides) and zeliac. Appl. Water Sci. https://doi.org/10.1007/s13201-018-0640-y
38. Shah, M., Hashmi, H.N., Ali, A., Ghumman, A.R. 2014. Performance assessment of aquatic macrophytes for treatment of municipal wastewater. J. Environ. Heal. Sci. Eng. https://doi.org/10.1186/2052-336X-12-106
39. Van Nes, E.H., Scheffer, M., Van den Berg, M.S., Coops, H. 2002. Aquatic macrophytes: Restore, eradicate or is there a compromise? Aquat. Bot. https://doi.org/10.1016/S0304-3770(01)00212-1
40. Victor, K.K., Séka, Y., Norbert, K.K., Sanogo, T.A., Celestin, A.B. 2016. Phytoremediation of wastewater toxicity using water hyacinth (Eichhornia crassipes) and water lettuce (Pistia stratiotes). Int. J. Phytoremediation. https://doi.org/10.1080/15226514.2016.1183567
41. Vymazal, J. 2019. Constructed wetlands for wastewater treatment, in: encyclopedia of ecology: 1–4, Second Edition. https://doi.org/10.1016/B978-0-12-409548-9.11238-2
42. Wang, J. and Chu, L. 2016. Biological nitrate removal from water and wastewater by solid-phase denitrification process. Biotechnology advances, 34**,** 1103–1112.
43. Werkneh, A.A. 2024. Decentralized constructed wetlands for domestic wastewater treatment in developing countries: Field-scale case studies, overall performance and removal mechanisms. J. Water Process Eng. 57. https://doi.org/https://doi.org/10.1016/j.jwpe.2023.104710
44. Wickramasinghe, S., Jayawardana, C.K. 2018. Potential of aquatic macrophytes *Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia molesta* in phytoremediation of textile wastewater. J. Water Secur. https://doi.org/10.15544/jws.2018.001
45. Zainuddin, N.A., Md Din, M.F., Nuid, M., Abdul Halim, K., Abdul Salim, N.A., Elias, S.H., Mat Lazim, Z. 2022. The phytoremediation using water hyacinth and water lettuce : correlation between sugar content, biomass growth rate, and nutrients. J. Kejuruteraan 34, 915–924.