Efficiency of Five Selected Aquatic Plants in Phytoremediation of Aquaculture Wastewater

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Abstract: The lack of clean water sources, due to the presence of pollutants in water, is a major issue in many countries, including Malaysia. To overcome this problem, various methods have been introduced, including phytoremediation treatment. Therefore, this phytoremediation study examined the ability of five aquatic plants—*Centella asiatica*, *Ipomoea aquatica*, *Salvinia molesta*, *Eichhornia crassipes*, and *Pistia stratiotes*—to remove three pollutants—total suspended solids (TSS), ammoniacal nitrogen (NH_3-N), and phosphate—from aquaculture wastewater. Using wastewater samples, each containing 50 g of one of the plants, the pollutant levels were measured every two days for 14 days. The results showed a drastic decline in the concentration of pollutants, where *C. asiatica* was able to remove 98% of NH_3-N, 90% of TSS, and 64% of phosphate, while *I. aquatica* showed the potential to eliminate up to 73% of TSS and NH_3-N, and 50% of phosphate. *E. crassipes* drastically removed 98% of phosphate, 96% of TSS, and 74% of NH_3-N, while *P. stratiotes* was able to eliminate 98% of TSS, 78% of NH_3-N, and 89% of phosphate. *S. molesta* was efficient in removing 89.3% of TSS and 88.6% of phosphate, but only removed 63.9% of NH_3-N.

Keywords: aquaculture; aquatic plants; phytoremediation; wastewater treatment

1. Introduction

Aquaculture is the breeding, rearing, and harvesting of aquatic life, such as fish, shellfish, prawns, algae, and other organisms living in an aquatic ecosystem. Aquaculture is one of the fastest-growing economies in the world food economy. Global aquaculture production has doubled in the past decade in terms of value and volume, and now supplies one-third of the seafood consumed worldwide [1]. Asia dominates this production, accounting for 88.91% by volume in 2014 [2]. There is no doubt that the contribution of aquaculture to the food supply will increase in the future due to the growing world population.

However, unsustainable aquaculture can give rise to new problems, such as water pollution, if the effluent and wastewater are not meticulously maintained. Water pollution caused by effluent from ponds is one of the main issues related to aquaculture [3,4]. Aquaculture has become large enough to have significant impacts on the environment, and some environmental concerns have been expressed by environmental activists and scientists [5]. In most freshwater aquaculture ponds, wastewater is generated before harvesting the stock, in which all of the particles and nutrients are

present. Potential pollutants come from fertilizers, food spills, and the waste of reared animals. Some of the major sources of wastewater pollution are residual food waste, feces, metabolic by-products, and residual biocides and biostats [6]. These products contain organic carbon, nitrogen, ammonia, urea, bicarbonate, phosphate, vitamins, and therapeutics, which, in high concentrations, are harmful to the aquatic ecosystem and eventually disrupt the natural food chain [7,8]. According to Hanafiah et al. [9], wastewater containing these substances will have major negative effects on human health as well as the environment. This will cause major disturbance to our ecosystem balance eventually, making the Millennium Development Goals (MDGs) hard to achieve [9,10].

There are a lot of conventional methods that help to remove pollutants from the aquatic environment such as adsorption, biological treatments, disinfection, ultraviolet irradiation, and advanced treatment technologies like advanced oxidation processes (AOPs). Although these methods can significantly improve the removal of pollutants, the capital, operational, and maintenance costs for these treatments are high, especially for advanced treatments [11,12]. Phytoremediation, however, is one of the environmentally friendly and low-cost methods of removing pollutants from water and soil. This treatment is a remediation method that uses various plants to reduce, extract, and hold excessive nutrients and pollutants from a contaminated medium [13]. Phytoremediation methods can be categorized based on how the pollutants are removed from the environment, such as through degradation, extraction, containment or a combination of two or more techniques. Plant species considered to be ideal phytoremediation agents have a high biomass, a good root system, and are fast-growing and easy to grow [14,15].

Therefore, this study aims to determine the efficiency of five different plant species—*Centella asiatica, Ipomoea aquatica, Salvinia molesta, Eichhornia crassipes*, and *Pistia stratiotes*—in removing pollutants from catfish aquaculture wastewater. These five plants are believed to have a high potential for reducing pollutants from wastewater. Plant selection in phytoremediation treatment is crucial, as the plant is the main tool in removing the pollutants [16].

2. Materials and Methods

2.1. Aquatic Plants

C. asiatica, I. aquatica, S. molesta, P. stratiotes, and *E. crassipes* were used to determine the efficiency of each plant in absorbing and removing total suspended solids (TSS), ammoniacal nitrogen (NH_3-N), and phosphate from aquaculture wastewater. These aquatic plants were filled in tanks containing distilled water for a week as an acclimatization period, before being exposed to contaminants from the aquaculture wastewater.

2.2. Analysis of Wastewater Quality

The initial water quality measurements were taken to determine the reduction rate after phytoremediation treatment. The dissolved oxygen (DO) level was determined using the YSI 5000 multiparameter instrument. Biochemical oxygen demand (BOD) was determined using the BOD_5 method [17]; the first DO reading was recorded on day one, and then the water samples were kept in a 20 °C incubator for five days. On the fifth day, the DO reading was taken again. The calculation for the determination of BOD_5 is as follows:

$$BOD_5 = DO \text{ (reading on the first day)} - DO \text{ (reading on the fifth day)} \tag{1}$$

TSS was determined using the gravimetric method, wherein a 0.45 μm filter paper was used to trap TSS. The filter paper was initially filtered through distilled water to make sure all salts and foreign substances were removed, and then it was dried in the oven at a temperature of 103–105 °C and weighed. The dried filter paper was then used to filter the wastewater sample, and then dried again at 80 °C to weigh the residual substances. The calculation for the determination of TSS is as follows:

$$\text{Total suspended solids (mg/L)} = (A\text{-}B)/V \times 1000 \text{ mL} \tag{2}$$

where,

A = Weight of filter paper after filtration (weight of filter paper + dried residue), mg
B = Weight of filter paper before filtration (weight of filter paper), mg
V = Volume of filtered water sample, mL

For the phosphate parameter, the ascorbic acid method (reactive orthophosphate) was used, and a packet of PhosVer® (ascorbic acid) reagent pillows was needed for each sample analysis. The NH_3-N parameter was determined and recorded using the Nessler method and the HACH DR/2010 spectrophotometer [15]. All values were recorded in the form of ± standard deviation average and mean values. The parameters and methods involved are shown in Table 1.

Table 1. Standard methods and instrumentation used for water quality parameters.

Parameter	Standard Method (APHA 2007)	Equipment
BOD_5	BOD_5 (APHA 2007)	YSI multiparameter 5000
TSS	Gravimetric (APHA 2007)	Vacuum pump
NH_3-N	Nessler Method (Method 8038)	HACH DR 2800 Spectrophotometer
Phosphate	Ascorbic Acid Method (Method 8048)	HACH DR 2800 Spectrophotometer

2.3. Set-up of the Phytoremediation Laboratory Experiment

Experiments were performed in rectangular tanks with a volume of 0.012 m³ (40 cm length × 20 cm width × 15 cm depth). All the plants were washed, dried, and left in a tank filled with 15 L distilled water for a week. This step was necessary to make sure the plants were neutralized prior to the next step. Excess water was allowed to drain off and was dried with a dry cloth; then, 50 g of each plant was weighed before being placed in the aquaculture wastewater sample.

The wastewater sample was measured for 5 L for each tank where 50 g of plants per species were used to treat the 5 L wastewater. The first tank was filled with the measured wastewater along with *C. asiatica*, then another tank was filled with the wastewater sample without plants, as the control, and these steps were repeated with the other four plant species. Three replications for each species were prepared for an average reading and to assess the role of the plants in removing organic and inorganic pollutants. No new wastewater was added to the tanks throughout the experiment to allow a constant pattern of pollution reduction [18].

The levels of TSS, NH_3-N, and phosphate in the wastewater samples were measured every two days, starting from day 0, to evaluate the changes in water quality and the reduction rate achieved by the phytoremediation process. Figure 1 shows a schematic diagram timeline of how the phytoremediation treatment was conducted in the laboratory under fluorescent tubes.
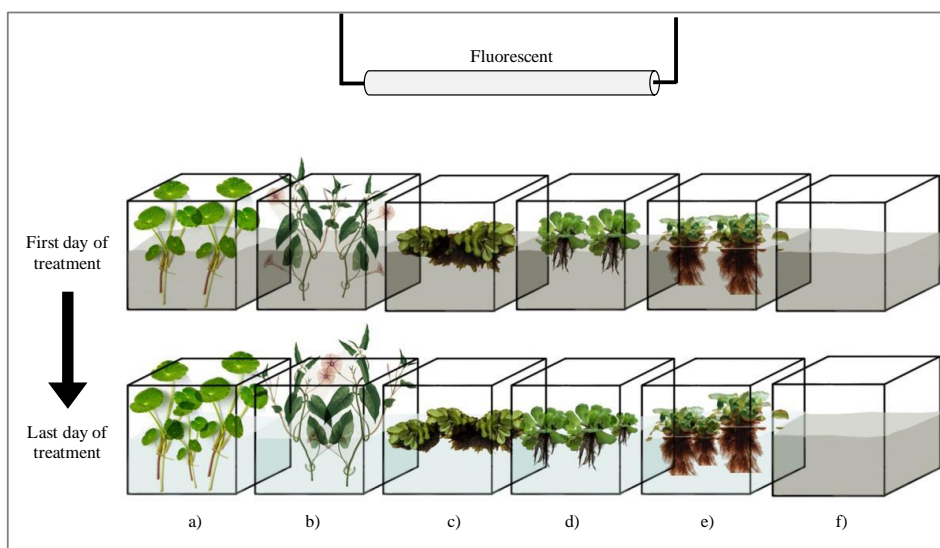
Figure 1. Schematic diagram of the phytoremediation treatment in the laboratory, which (**a**) *C. asiatica*; (**b**) *I. aquatica*; (**c**) *S. molesta*; (**d**) *P. Stratiotes*; (**e**) *E. crassipes*; (**f**) Control.

2.4. Statistical Analysis

Mean values were recorded for the three replicates of each plant. Statistical analysis was performed using Statistical Package for the Social Sciences 23 (IBM SPSS 23) software, where a one-way ANOVA and an independent t-test were conducted. All data collected in this research were used to determine the significant differences between the plants in removing pollutants and excess nutrients. The comparisons of the means for each parameter after the 14-day phytoremediation treatment were calculated for p-values, and the significant level was determined at $p < 0.05$.

3. Results and Discussion

3.1. Initial Parameters of Aquaculture Wastewater before Phytoremediation

Based on the Environmental Quality (Sewage and Industrial Effluent) Regulations (2009), the initial physico-chemical parameters, including the temperature, pH, DO, TSS, and NH_3-N, of the aquaculture complied with the required a and b standards. The same goes for the other parameters, including phosphate, turbidity, conductivity, total dissolved solids (TDS), and BOD_5, which were taken during water sampling, were all at permissible levels. Table 2 shows the initial reading of the aquaculture wastewater parameters prior to phytoremediation process.

Table 2. Initial reading of aquaculture wastewater parameters before phytoremediation.

Parameter	Unit	Average Value	Class/Standard
Temperature	°C	27.77 ± 0.06	Standard A
pH	-	8.29 ± 0.02	Standard A
DO	mg/L	4.63 ± 0.04	Standard A
Conductivity	µs/cm	95.73 ± 0.15	-
Turbidity	NTU	205.00 ± 1.00	-
TSS	mg/L	45.67 ± 0.60	Standard A
TDS	mg/L	59.49 ± 0.45	-
BOD_5	mg/L	1.06 ± 0.03	-
NH_3-N	mg/L	4.20 ± 0.10	Standard A
Phosphate	mg/L	0.35 ± 0.10	-
Temperature	°C	27.77 ± 0.06	Standard A
pH	-	8.29 ± 0.02	Standard A
DO	mg/L	4.63 ± 0.04	Standard A

3.2. Phytoremediation of Aquaculture Wastewater

The removal rates of TSS, NH$_3$-N, and phosphate were determined by using five selected aquatic plants as phytoremediation agents. These plants were chosen due to their great ability in removing pollutants from various wastewater. As stated by Manan et al., *C. asiatica* leaves, roots, and stems are good pollutant accumulation agents. Since it needs extra nutrients, such as zinc and lead, for biochemical processes, especially photosynthesis, the roots actively translocate nutrients to the leaves, making *C. asiatica* an efficient phytoremediation agent [19]. *I. aquatica* stems and leaves are also very efficient in absorbing nutrients and heavy metals, and use the pollutants as food in order to grow, which is why this species is known as a fast-growing plant [20]. Meanwhile, the roots of *S. molesta* are very fibrous, and the same goes for *P. stratiotes*, allowing it to easily and efficiently trap suspended solids in the water body. It is also a fast-growing plant; therefore, it needs a high amount of nutrients to stay healthy, alive, and grow. According to Ebel et al. [21], *E. crassipes* is one of the best macrophytes to be used as a phytoremediation agent because it removes pollutants from wastewater efficiently while having a high tolerance to the polluted environment [21].

According to Sa'at et al. [22], *I. aquatica* can reduce 92.6% of total suspended solids (TSS) and 82.7% of ammoniacal nitrogen (NH$_3$-N) from palm oil mill wastewater. *C. asiatica* is able to trap 100% of TSS from water body and *S. molesta*'s leaves and roots are highly efficient in accumulating and removing pollutants, especially heavy metals [22–24]. Akhtar et al. [25] stated that *E. crassipes* and *P. stratiotes* efficiently removed heavy metals without showing any kind of damage or bad reactions to the metals.

3.3. Reduction Rate of TSS

TSS are solid in a water body which includes a wide variety of materials, such as silt, decaying plants, animal matters, and other solid wastes [26]. Figure 2 shows the reduction rate of TSS using 50 g of *C. asiatica, I. aquatica, S. molesta, P. stratiotes*, and *E. crassipes* plotted against the days of treatment. It was found that *P. stratiotes* reduced TSS the most, with up to 98% reduction rate and a total of 43.3 mg/L of TSS by the 14th day of treatment.
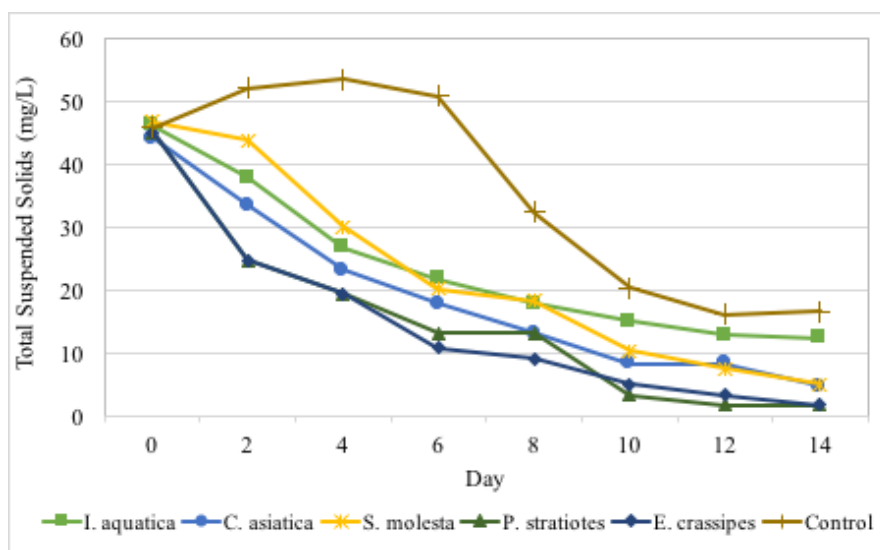

Figure 2. Reduction rate of TSS using five plant species after 14 days of phytoremediation.

Umar et al. [27] stated that the charges in the root systems of aquatic plants or macrophytes improve their ability to trap and attract particles, such as TSS, in a medium. Hence, the reduction of TSS by plants primarily depends on the plants' root systems, and plants with fibrous roots can accumulate more TSS than those with taproots. In the present study, 98% of the TSS was reduced by *P. stratiotes*, which has fibrous and long roots [27]. This result is also supported by the previous

studies, which showed that *P. stratiotes* was able to increase water clarity by trapping suspended solids in the roots [28]. It is believed that this species is also able to remove heavy metals magnificently. Based on the index of bio-concentrating factors, *P. stratiotes* have been reported by Lu et al. to be hyperconcentrated in the roots area for heavy metals like Cr, Cu, Fe, Mn, Ni, Zn and Pb [29]. According to Akhtar et al. [25], 77.3% of Cr and 91.29% of Cu were removed during one month of treatment.

Moreover, in *E. crassipes*, *C. asiatica*, *I. aquatica*, and *S. molesta* tanks, the TSS concentration levels were reduced to 96%, 90%, 73%, and 89.3%, respectively. *I. aquatica* possesses taproots; hence, it trapped the lowest amount of TSS among the five species. *E. crassipes* has fibrous roots, which explains why the concentration of TSS on the last day of treatment was significantly reduced. However, *C. asiatica* does not possess fibrous roots but effectively reduced TSS because it can be physically and biologically removed through filtration and sedimentation. TSS are trapped and accumulated when passing through the plant roots and eventually sink to the bottom by gravitational force or metabolized by microorganism [23]. The sedimentation process also helps to reduce TSS concentration by 60% in the control tank after 14 days, however, there was an increased TSS concentration for four days due to some disturbance to the tank on day 1 of experiment.

The graph plot of TSS reduction in Figure 2 shows that TSS consistently decreased with the help of phytoremediation agents and the reduction was very inconsistent without the aid of any plants. The plots of *P. stratiotes* and *E. crassipes* showed the most significant decreasing patterns, followed by those of *C. asiatica*, *S. molesta* and *I. aquatica*. However, starting from day 10, the concentration of TSS by most species tested in this experiment started to slowly decrease or stayed constant, possibly because it had already reached the maximum removal concentration.

A one-way ANOVA was conducted and showed a significant value ($p < 0.05$) for the reduction rate of TSS, from the first day of treatment until the end, by all five species. However, a statistical independent t-test showed no significant value ($p < 0.05$) between the five plants, since the difference between the highest and lowest TSS concentrations removed was only 9.6 mg/L. This proves that all five species practically have almost the same potential to reduce TSS.

3.4. Reduction Rate of NH_3-N

The concentration of NH_3-N in wastewater can be unpredictable due to the decomposition of organic waste matter and nitrogen fixation processes [30]. Figure 3 shows the reduction rate of NH_3-N using *C. asiatica*, *I. aquatica*, *S. molesta*, *P. stratiotes*, and *E. crassipes* plotted against the days of the treatment. *C. asiatica* reduced the most NH_3-N concentration by the end of the experiment compared to the other four species, reaching 98% reduction. *C. asiatica* is known as a medicinal plant for its high nutritional value and because of its ability to take in organic substances and nutrition from the environment, which explains why it serves as an excellent phytoremediation agent [31].

This study shows *E. crassipes* and *P. stratiotes* serve more effectively as phytoremediation agents in catfish aquaculture wastewater than in tilapia fish aquaculture wastewater. The reduction rates of NH_3-N by *E. crassipes* and *P. stratiotes* in catfish aquaculture wastewater reached until 74% and 78% respectively, compared to a reduction rate of only 69% and 65% respectively, in tilapia fish aquaculture wastewater [32].

The total average removed concentrations of NH_3-N in the wastewater sample after 14 days of treatment with *C. asiatica*, *I. aquatica*, *S. molesta*, *P. stratiotes*, and *E. crassipes* were 4.07 mg/L, 2.87 mg/L, 2.65 mg/L, 3.3 mg/L, and 3.1 mg/L, respectively. Although the percentage of removal and the total average concentration showed some distinct differences, statistical independent t-tests showed the opposite, and the value was not significant ($p < 0.05$). However, these species are believed to have high oxygen transfer abilities, providing suitable environments for bacterial nitrification while enhancing the removal of NH_3-N [33]. In addition, all species reached a constant concentration of NH_3-N after the 12th day of treatment because they had reached their removal capacity. The concentration in the control tank was higher than those obtained in the tanks with plants at the end of the experiment.
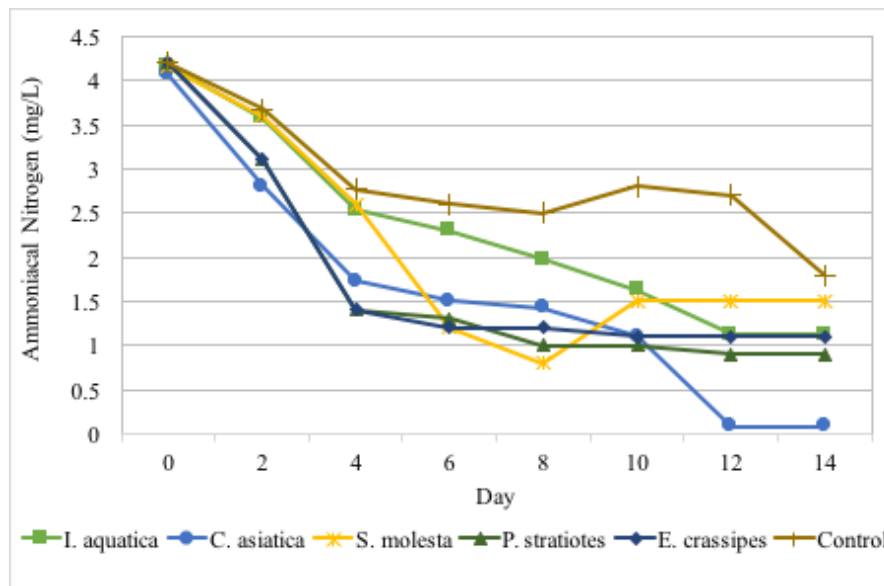
Figure 3. Reduction rate of NH_3-N using five plant species after 14 days of phytoremediation.

The increasing concentrations of NH_3-N at day 10 by *S. molesta* were caused by the death of the plants and their debris. Plant decomposition is a major cause of increased ammonia content in wastewater because the nitrogen compounds in the organic matter re-enter the water and are broken down by microorganisms, known as decomposers. The decomposition process produces ammonia, which undergoes nitrification. The decomposition of ammonia, however, yields hydrogen and nitrogen, and is an important process in nature [30]. The removal of NH_3-N was also caused by the biological mechanisms in nutrient intake and ammonia absorption [34]. The other possible reason for the NH_3-N removal was the high growth of denitrifying bacteria [35,36].

3.5. Reduction Rate of Phosphate

In wastewater, phosphates are transformed into orthophosphate and are available for plant uptake as a source of nutrients; however, this may cause a nutrient imbalance and an imbalance in the material cycling processes of the natural phosphorus cycle in the ecosystem [37]. Controlling excessive phosphorus content in wastewater is a key factor in deterring the eutrophication of surface water. Its excessive presence may cause losses of livestock and introduce potentially lethal algal toxins into drinking water, which are very bad for aquaculture industries and humans alike [38].

E. crassipes is the best plant among the five species to treat phosphate in aquaculture wastewater, as shown in Figure 4. Since organic phosphate acts as a nutrient for plants, *E. crassipes* was highly efficient in absorbing phosphate for its nutritional needs, with a reduction rate almost reaching 100% and a total of 0.34 mg/L phosphate reduced after 14 days of phytoremediation treatment. This species has long fibrous roots capable of providing extensive surface area for the plant to carry out physical, chemical and microbial processes for nutrient absorption and nitrification processes [18]. In addition, [39] reported *E. crassipes* reduced 50.04% of phosphate from an aquarium with a population of *Sarotherodon melanotheron* (blackchin tilapia) within 12 days of treatment.
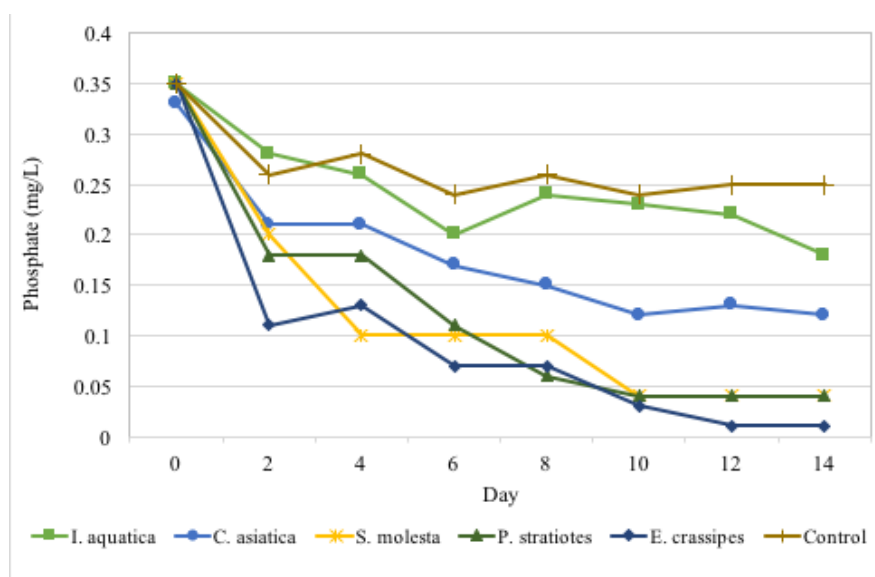
Figure 4. Reduction rate of phosphate using five plant species after 14 days of phytoremediation.

Based on the graph of the phosphate concentrations, *I. aquatica* was very inconsistent and removed the least amount of the pollutant. However, the concentration of phosphate decreased at days 4, 6, and 12. *S. molesta* had the most consistent phosphate removal rate, but the readings reached a maximum removal concentration on the 10th day and stayed constant until day 14. The concentration of phosphate at the end of the experiment in the control tank was the highest compared to tanks with plants, because phosphate is essential for plants' life-sustaining reactions, which include energy transfer, protein activations, and regulation of metabolic processes [40,41]. That could explain the difference between the control tank with no plants for absorption, and the tanks with five macrophytes species.

An independent t-test analysis showed no significant value ($p < 0.05$) between the phosphate reductions by all five species after 14 days, except for *E. crassipes* and *I. aquatica*, since the highest average removal value was 0.34 mg/L by *E. crassipes*, with I. *aquatica* being the lowest—0.17 mg/L. The efficient reduction of phosphate from the wastewater comes from plant uptake and the presence of diverse substrates [42]. This pollutant is the major constituent in adenosine diphosphate (ADP) and adenosine triphosphate (ATP), which acts as a building block for nucleic acids, sugar phosphates, nucleotides, and many more, especially during the active growth of plants. Phosphates are crucial for energy storage and transfer in photosynthesis and respiration, essential for normal growth, maturity, and root system development, but too much phosphate in a plant's system may be lethal [40–43]. In addition, some macrophytes need phosphate more than other macrophytes, depending on the plants' type, requirements and its surrounding environment.

The results found in the present study were in line with other previous studies [9,44–48]. A similar pattern of results was obtained to indicate the ability of phytoremediation in removing different pollutants. Phytoremediation for treating wastewater remains popular until now due to it being cost-effective, environmentally friendly, and sustainable with low maintenance.

4. Conclusions

In this study, the effectiveness of five aquatic plants—*C. asiatica, I. aquatica, S. molesta, P. stratiotes*, and *E. crassipes*—in removing nutrients from aquaculture wastewater was investigated. Through phytoremediation, the reduction rates of three pollutants—TSS, NH_3-N, and phosphate—were studied. These aquatic plants performed well towards the reduction and removal of excessive nutrients from the wastewater, without being physically affected by them. It was found that *P. stratiotes* is the best eliminator of TSS from aquaculture wastewater, with *C. asiatica* being the best to reduce NH_3-N,

and *E. crassipes* the most efficient for phosphate removal. Overall, the results indicate that all five species have their own effectiveness and are somewhat efficient as phytoremediation agents, since the reduction rates after 14 days of treatment were greater than 50%, for suspended solids content, ammoniacal nitrogen, and phosphate.

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