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Original Research Article

Phytoremediation of Aquacultures Effluent by Pandan and Kesum Plants

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Abstract: Phytoremediation treatment is gaining more popularity in treating aquaculture effluent. The ability of herb plants such as pandan (*Pandanus amaryllifolius*) and kesum (*Polygonum minus*) in wastewater treatment was rarely studied by previous researchers. In this study, the aquaculture effluent was treated by phytoremediation employing these two herbs plants. The experiment was conducted in the hydroponic pot systems for four weeks. The aquaculture's effluent was analysed for biological oxygen demand (BOD_5), total suspended solids (TSS), ammonia and nitrate concentration. From the result, the pandan plant was able to remove 65.24% BOD_5, 94.99% TSS, 98.93% ammonia, and 84.29% nitrate, while kesum removed 82.78% BOD_5, 94.51% TSS, 99.78% ammonia, and 47.32 nitrate. Both plants are similarly able to remove about 94% TSS and 98% ammonia. Significantly, pandan was more efficient in reducing nitrate, while kesum performed better in reducing BOD_5. The combination of both plants can improve the quality of aquaculture effluent in a very cost-effective way.

Keywords: phytoremediation; aquacultures effluent; pandan; kesum

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1. Introduction

The fishery sector has contributed a significant resource of animal protein to Malaysians for decades (FAO, 2022). Besides catching and rearing fish from the river and offshore, fish farming has developed and played a vital role in fishery production. However, the effluent released by the aquaculture sector has created many environmental and ecosystem problems. Cyclical input of fish feeds, antibiotics, and chemicals into the aquaculture system in order to maintain the health and growth of aquatic life becomes the main factor in producing effluent that contains high concentrations of nutrients such as nitrogen and phosphorus (Wang *et al*., 2020). Many scientists have recently investigated and developed some treatment technologies for aquaculture effluent.

Phytoremediation is one of the techniques that scientists and engineers are focusing on. Phytoremediation removes contaminants and organic substances from air, soil, and water using living plants (Mustafa & Hayder, 2021). Phytoremediation uses the intrinsic capabilities of living plants to absorb, translocate, and transform contaminants or organic pollutants. The mechanism of phytoremediation can be divided into seven types which are phytoextraction, rhizofiltration, phytovolatilization, phytostabilization, phytodegradation, hydraulic control and rhizodegradation (Etim, 2012).

The plants employed in the phytoremediation treatment should have a rapid growth rate, high biomass production, large extension in the root system, and high tolerance to the toxic metal compounds, and the plants should be easily cultivated, regulated and harvested (Hu *et al*., 2020). Many plants have discovered their abilities in the phytoremediation treatment of aquaculture wastewater. The aquatic plants that have been utilized in the aquaculture effluent treatment include water hyacinth, water spinach, water lettuce, morning glory and microalgae (Daud *et al*., 2015; Kiridi & Ogunlela, 2020; Nizam *et al*., 2020).

Pandan plants, scientifically known as *Pandanus amaryllifolius*, a typically tropical plant well-known for its aroma, are cultivated mainly in Malaysia. *Pandamus amaryllifolius* is a palm-like shrub that has blade-like leaves. The average height of the plants is not more than 2 m, and the average length and width of the leaves are 30 cm and 6 cm, respectively (Rayaguru & Routray, 2010). Kesum plants, also known as *Polygonum minus*, are an annual herb that can be cultivated in tropical climate conditions. The height of the plants can grow in the range from 0.2 m to 1.4 m. Both plants are very commonly found in Malaysia and are easy to cultivate. They also can potentially be involved in the phytoremediation treatment of sewage and wastewater.

Phytoremediation is applicable in aquaculture effluent treatment by employing floating aquatic plants or setting up hydroponic or aquaponic systems. The application of phytoremediation treatment for aquaculture effluent needs to consider several practical issues, including pre-harvest and post-harvest strategies. Pre-harvest strategies include selecting the right plants and designing, operating, and maintaining the phytoremediation treatment system; post-harvest strategies involve disposing of plants and contaminants residuals (Zhao *et al*., 2016). Scientists are devoted to researching the contribution of different plant varieties to the phytoremediation treatment of environmental contamination.

Han *et al*. (2014) previously used pandan plants in phytoremediation, and the nitrate achieved 100 % removal and 64 % phosphate removal, obstructing the algae bloom and reducing the water turbidity and pH. The pandan plant extract was used to remediate the heavy metal (Ngadi *et al*., 2014). While the *Polygonum spp.* Plants were used for heavy metal remediation (Al-kubaisi & Mohammed, 2019). The potential of the kesum plant as phytoremediation should be further investigated (Gor *et al*., 2011). Thus, this project investigates and analyses the suitability of employing pandan and kesum in aquaculture effluent phytoremediation systems.

2. Materials and Methods

Five hydroponic pot systems were set up with two systems containing three plants of pandan around 8 to 12 months of age, two systems containing three plants of one month-age of kesum and one system that served as control without plants. A plastic wash basin with a capacity of 8 litres acted as the reservoir tank of the system. A net pot inserted polystyrene board formed a simple non-flow hydroponic system. Plants were planted in each net pot filled with the growing medium of leca ball for the roots to attach to it. Figure 1 shows the setup for pandan and kesum plants.

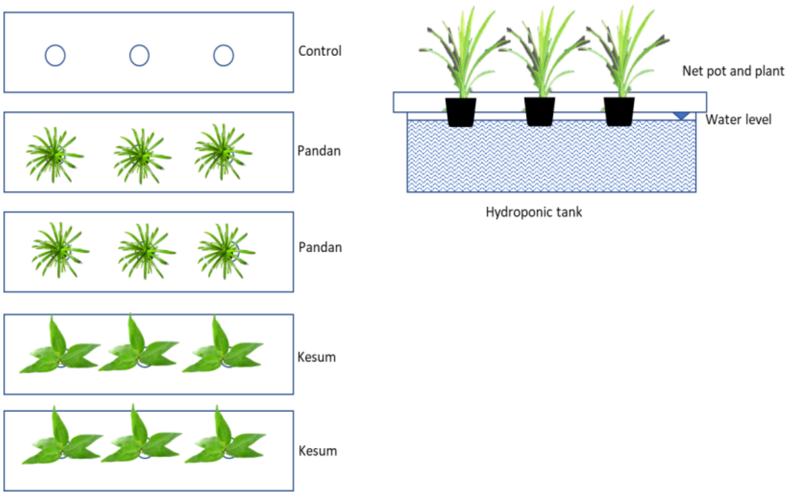


Figure 1. Set up hydroponic pot for pandan and kesum plants.

Aquaculture effluent filled the reservoir tank at 8 litres as the water source for the plant growth. The aquaculture effluent was collected from Sanctuary Ikan Air Tawar Timah Tasoh Perlis and acted as the water source for plant uptake. The experiment was conducted for four weeks.

The samples were measured for pH value, salinity, EC level, TDS, and DO using a multiparameter probe (YSI, ProQuatro). The BOD_5 test, total suspended solids (TSS), ammonia (NH_3-N) and nitrate (NO_3-N) concentration tests were also conducted in the Environmental Lab, Faculty of Civil Engineering Technology, UniMAP. All tests were conducted according to the Standard Methods for Examining Water and Wastewater (APHA, AWWA, WEF, 2012). The data on the physicochemical parameters of the effluent were taken weekly and statistically analysed.

The height of the plants was measured from the top of the plant until the shoot every week, and root height was not included. The weight of the plant was measured at the initial and end of the treatment period by using an electronic balance to determine the biomass weight.

The data was statistically analysed using the variance analysis (ANOVA). The data in percentage first underwent arcsine transformation before the ANOVA statistical analysis. One-way ANOVA was used to analyse the difference in the physicochemical parameters, nutrient removal and plant growth between two plant species.

3. Results and Discussions

3.1. Aquacultures Effluent Characterization

The physicochemical properties of aquaculture effluent were expressed in average ± standard deviation. The pH value of aquaculture effluent is 8.99±0.08 (Table 1), which was slightly alkaline. The cause of the high pH in the initial ee systems was due. The aquaculture effluent was observed to contain plenty of green algae during collection. During the daytime, algae uptake carbon dioxide and water for photosynthesis. Removing carbon dioxide causes the slow rise of pH in the aquaculture effluent.

From Table 1, it can seen that the salinity of aquaculture effluent was 0.05±0.01 ppt. Salinity is the concentration of dissolved salts and is commonly expressed in parts per thousand (ppt). The salinity of aquaculture water is usually less than 0.5 ppt as it is the optimum salinity so that their growth rate is at its maximum value. Electrical conductivity (EC) is attributed to the free ions flow in water to conduct electricity. Therefore, EC is influenced by the salinity (Boyd, 2000). The EC of aquaculture effluent was 83.28±25.79 µs/cm. Another parameter that can contribute to the high EC is the total dissolved solids (TDS). The TDS of the studied aquaculture effluent was relatively high, which was 64.22±20.17 mg/L. The studied aquaculture effluent's total suspended solids (TSS) was 100.86±26.06 mg/L.

Table 1. Initial physicochemical properties of aquaculture effluent.

Parameters	Value	Water Quality Standard*	Unit
pH	8.99±0.08	6-9	-
Salinity	0.05±0.01	1	ppt
EC	83.28±25.79	1000	µs/cm
TDS	64.22±20.17	1000	mg/L
TSS	8.49±0.22	50	mg/L
DO	100.86±26.06	5-7	mg/L
BOD	6.25±0.89	3	mg/L
NH3-N	2.28±0.16	0.3	mg/L
NO3-N	1.12±0.42	7	mg/L

*Based on Class II Interim National Water Quality Standard

Dissolved oxygen (DO) is the total oxygen concentration dissolved in the water, which is considered a vital parameter. The DO of the aquaculture effluent was 8.49±0.22 mg/L. At the same time, biological oxygen demand (BOD) measures the amount of oxygen required by microorganisms to biologically oxidized organic compounds (Rao *et al.*, 2019). The BOD of the aquaculture effluent was 6.25±0.89 mg/L. The studied aquaculture effluent's ammonia (NH_3-N) concentration was 2.28±0.16 mg/L. The fish excrete ammonia after the digestion of protein. Fish feed through their gills and in their feaces, so the ammonia concentration in aquaculture effluent is high. The nitrate (NO_3-N) concentration of the studied aquaculture effluent samples was 1.12±0.42 mg/L.

As compared to the Interim National Water Quality Standard (INWQS) by the Department of Environment (DOE) Malaysia, DO, BOD, and NH_3-N concentration in samples are unable to achieve the permissible limit of Class II of Water Quality Standard (Department of Environment Malaysia, 2010). The aquaculture's effluent samples were currently Class III, unsuitable for drinking water and needed extensive treatment, but they were still suitable for aquaculture activities for tolerance species. Therefore, the effluent must be treated to ensure the water quality is safe for recreational activities using phytoremediation treatment alternatives.

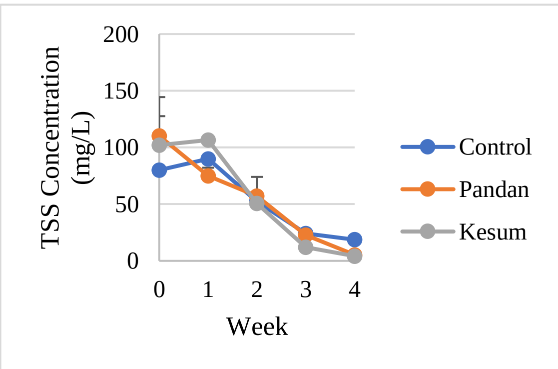
3.2 Phytoremediation Treatment Performance

Figure 2(a) showed that the control, pandan, and kesum efficiently decreased the TSS of treated aquaculture effluent. The removal of TSS in pandan system gradually decreased from 110.14±17.48 mg/L to 74.85±7.28 mg/L, 57.11±16.95 mg/L, 22.71±3.55 mg/L and 5.38±0.88 mg/L from initial to fourth week. The TSS concentration of aquaculture effluent in control and kesum systems had slightly increased, which were from 80.00 mg/L to 90 mg/L and from 102.00±42.43 mg/L to 106.56±0.16 mg/L, respectively, from initial to first week. The TSS concentration of treated aquaculture effluent in control system was then decreased to 52.38 mg/L, 24.17 mg/L and 18.67 mg/L, while concentration of TSS of treated aquaculture effluent in kesum system was reduce to 50.60±9.50 mg/L, 12.14±2.32 mg/L and 4.23±4.28 mg/L during the following second week to fourth week. Removal of TSS was due to the plant root system. The Pandan plant has a fibrous root system that can catch more TSS than the kesum plant with taproot (Aziz *et al.*, 2020).

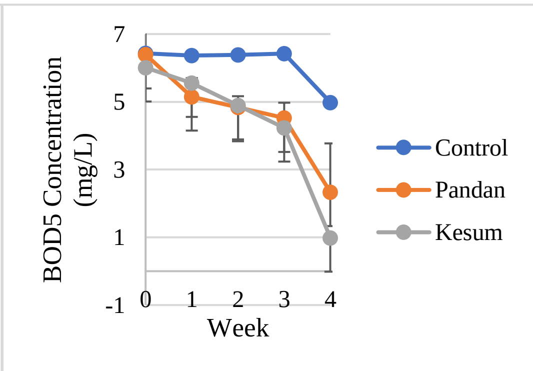
From Figure 2(b), The results showed that the BOD_5 of treated aquaculture effluent in all systems had decreased slowly from the initial to the third week and experienced a sudden drop from the third week to the fourth week. Pandan system had reduced BOD_5 from 6.40±1.46 mg/L at the initial to 2.33±1.44 mg/L in the fourth week, and the kesum system had removed BOD_5 of treated aquaculture effluent from 6.01±0.93 mg/L at initial to 0.99±0.47 mg/L at the fourth week. In contrast, the control system only removed 1.45 mg/L of BOD_5, from 6.43 mg/L at the initial to 4.98 mg/L in the fourth week. The reduction of BOD_5 is related to the nutrient removal performed by the pandan and kesum systems. The BOD_5 of a water sample is caused by the oxidation of organic matter by bacteria, oxidation of ammonia-nitrogen to nitrate by nitrifying microorganisms or respiration of plankton organisms in the sample (Aziz *et al*., 2020).

Figure 2(c) showed that the ammonia (NH_3-N) concentration of aquaculture effluent in control has a different shape from the treated aquaculture effluent in pandan and kesum systems, where they showed a sharp drop after four weeks of treatments. Pandan had successfully removed ammonia-nitrogen from 2.32±0.19 mg/L to 2.19±0.16 mg/L, 0.34±0.08 mg/L, 0.07±0.08 mg/L and 0.03±0.01 mg/L. Meanwhile, kesum had removed ammonia from 2.17±0.13 mg/L to 2.12±0.13 mg/L, 0.95±0.28 mg/L, 0.03±0.01 mg/L and 0.01±0.01 mg/L. Absorption of ammonia-nitrogen by plants through the root system reduces ammonia-nitrogen concentration, as nitrogen is the major nutrient required in plant growth (Ng & Chan, 2017). For control, the concentration reduction was caused by organic nitrogen degradation to ammonium and a decrement in bacteria uptake for its growth and nitrification mechanism.

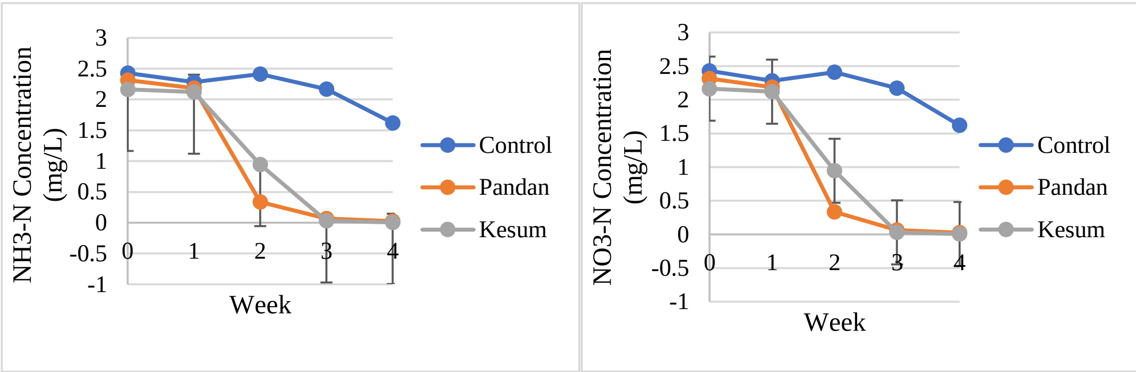
Although the nitrate concentration (NO_3-N) in aquaculture effluents of all three systems showed small fluctuations during the four weeks of treatment, there were small nitrate concentration reductions in all three systems, as in Figure 2(d). The concentration of nitrate in the aquaculture effluent fluctuated from 1.70 mg/L to 1.00 mg/L, 1.60 mg/L, 1.70 mg/L and 1.50 mg/L in the control system, from 1.20±0.28 mg/L to 0.60±0.28 mg/L, 1.00±0.00 mg/L, 0.70±0.28 mg/L and 0.20±0.14 in pandan system, and from 0.75±0.07 mg/L to 0.55±0.07 mg/L, 0.85±0.07 mg/L, 0.70±0.00 mg/L and 0.40±0.14 mg/L in kesum system. The nitrate concentration increases were attributed to the nitrification of ammonia to nitrate by nitrifying fixing bacteria. Nitrification is a biological process that converts ammonia (NH_3) to nitrite (NO_2) and then to nitrate (NO_3). The denitrification and plant and microbial uptake also reduce nitrate concentration (Ng & Chan, 2017; Zhai *et al*., 2016). The combination of these processes enhanced the concentration decrement.



(a)



(b)



(c) (d)

Figure 2. Pollutant concentration reduction (a) TSS; (b) BOD_5; (c) NH_3-N; (d) NO_3-N.

3.3. Pollutant Removal Efficiencies

Figure 3 shows the removal percentage of TSS, BOD_5, NH_3-N and NO_3-N in aquaculture effluent by control, pandan and kesum plant. The percentage of TSS removal of control, pandan and kesum were 76.7%, 94.99±1.60% and 94.51±6.48% respectively. One-way ANOVA tests were conducted, and it showed that pandan has a significant difference with the control system ($p<0.05$), while kesum does not show a significant value ($p>0.05$). However, there is no significant difference between the pandan and kesum plants' abilities in reducing TSS concentration ($p>0.05$).

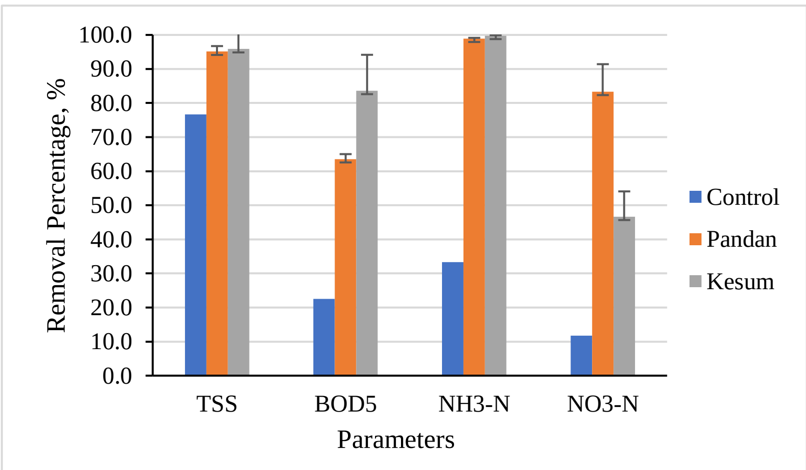


Figure 3. Removal percentage of the TSS, BOD, ammonia and nitrate.

The control system only removed 22.55% of BOD_5, while both pandan and kesum had achieved high efficiency in removing BOD_5. Pandan showed 65.24±14.6% of BOD_5 removal, while the kesum system showed 82.79±10.55% of BOD_5 removal. Both plants significantly removed the BOD_5 of aquaculture effluent ($p\leq0.05$). Since the BOD removal percentage of both plants was similar, there was no difference in removal efficiency between the two plants ($p>0.05$).

The percentage removal of NH_3-N by pandan was 98.93±0.21%, while kesum achieved 99.78±0.31% of removal. It showed a noticeable difference from the control system, which only achieved 33.33% of NH_3-N removal. Both plants show they were highly significant in reducing ammonia concentration of aquaculture effluent ($p<0.05$).

The removal of NO_3-N concentration in the aquaculture effluent by pandan, kesum and control was recorded to achieve 84.29±8.08%, 47.32±13.89% and 11.76%, respectively. Both plants significantly removed NO_3-N concentration in aquaculture effluent ($p\leq0.05$). Although the percentage of nitrate concentration removal performed by both plants was recognizably different, the ANOVA one-way test showed an opposite result, in which it was concluded that the mean percentage of nitrate concentration removal of these two plants was not significant ($p>0.05$)

3.3 Plant Growth Analysis

The plant growth analysis was taken by measuring the plants' weight and height initially and every week until four weeks of treatment. There are three plants in a system, and a total of 6 plants were considered for plant growth analysis for each plant species. Pandan plant had gained an average weight of 3.50±3.02 g, while kesum gained an average weight of 0.19±0.06 g. The growth rate of pandan is moderate, and there is no noticeable change in weight within a short period. The pandan plants were reported to generate leaves and stalks to an average of 3 to 5 kg per hill in a growing period of 6 months (Han *et al*., 2014).

Figure 4 illustrates the plant height changes for pandan and kesum plants over four weeks of experiments. Both plants show a positive growth rate percentage, whereby pandan and kesum have achieved 43% and 37% increases in plant height, respectively.

The length of pandan slowly rose over the weeks of growth, which developed a positive linear relationship. There was a slight decrease in the length in the first week. After the plants were transplanted to a hydroponic system, some time was required to adapt to a new environment. During adaptation, pandans experienced water and nutrient losses that caused some parts of the plants to wilt.

Kesum plants are fast-growing and can achieve physical maturity between 90 and 140 days from sowing to harvesting. The growth rate of kesum was abnormal as the age of plants used in the phytoremediation treatment had reached one month at the initial treatment. It should have grown to a pre-mature state, about an average length of 10 to 15 cm.

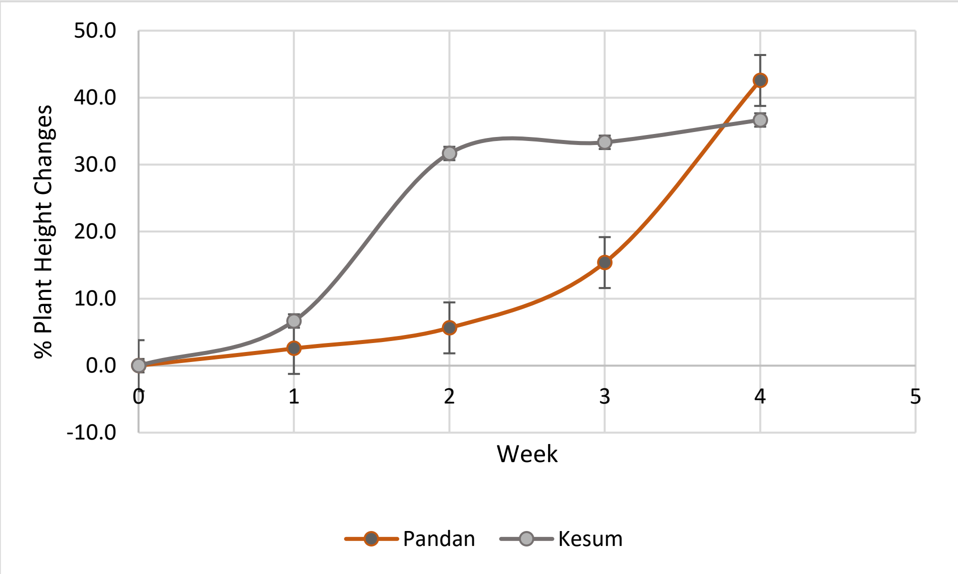


Figure 4. Plant height growth percentage.

4. Conclusions

The percentage of TSS, BOD, ammonia, and nitrate concentrations removed from aquaculture effluent by pandan and kesum were evaluated. Pandan plants had higher effectiveness in reducing TSS and nitrate concentration, where the percentage of its removal of TSS and nitrate concentration were 94.99% and 84.29%, respectively. Meanwhile, the removal of BOD and ammonia concentration were 65.24% and 98.93% respectively. Kesum plants were more effective in removing BOD and ammonia concentration than pandan, which removed 82.79% of BOD and 99.78% of ammonia. TSS and nitrate concentration reduction from aquaculture effluent were 94.51% and 47.32% respectively. Both pandan and kesum plants effectively removed BOD, NH_3-N and NO_3-N concentrations. There was no difference in the phytoremediation abilities between these two plants. The growth of pandan and kesum plants used in phytoremediation treatment was analysed. Overall, the plants gained some weight and grew in stem length. The length of the stem of Pandan and Kesum developed a positive linear relationship.

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