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Phytoremediation potential of *Azolla pinnata* on water quality and nutrients from agricultural water

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ABSTRACT

The area of polluted water expands day by day due to the rapid increase in agricultural activities. Application and the excessive amount of fertilizer in the agricultural activity can reduce the water quality. One of the alternative way to remove pollutants from polluted water is phytoremediation technique. This paper attempt to evaluate the potential of *Azolla pinnata* act as a phytoremediation to treat the agricultural water. Three types of media with different dosage which are tap water, water added with organic fertilizer (chicken manure: dosage range of 10–30 g), and water added with inorganic fertilizer (growing fertilizer: dosage range of 10–30 g) were employed. Media B10 (water added with 10 g organic fertilizer) had the highest removal efficiencies for NH_3–N, PO_4^{3-}, and NO_3^- with 25%, 38.5% and 30%. In addition, turbidity and pH value diminished to 15.65% and 18.6%, respectively. However, it indicated that *Azolla pinnata* has the potential as an agent of phytoremediation especially for water polluted with organic fertilizers.

Keywords: Water quality; Water pollution; Phytoremediation; Nutrient; Treatment

1. Introduction

Agriculture plays as a significant industry in Malaysia. In 2019, the agricultural sector covered about 7.1% or RM 101.5 billion to the gross domestic product (GDP) of Malaysia. Moreover, approximately 1541100 of public in Malaysia are rely on agricultural activities as a source of income [1,2]. Million tonnes of heavy metal, agrochemical,

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organic matter, sediments and other toxic substances from agricultural activities are disposed into the water bodies [2–4]. These pollutants were generated by the extensive use of anthropogenic substances like fertilizer, pesticides and herbicides [5]. Accumulation of these substances lead water contamination which have negative impact on marine ecosystem, human health, and economic activities [6]. High concentration of substances, such as ammoniacal nitrogen, phosphorus and nitrate, can jeopardise the physical, biological and chemical characteristic of water quality [7–11]. Farmer, plantation company and government agencies utilize these substances for chemical fertilizer, sludge and manure.

Eutrophication is one of the phenomena that occur due to excessive level of substances in water [12,13]. Nitrates and phosphorus are the primary factor of eutrophication [14]. Both substances enhance the proliferation of aquatic plants and algae to cover the entire water body, resulting in the mortality of aquatic living organism such as fish due to the depletion of dissolved oxygen [15–21]. Moreover, high concentration of nitrates in water can lead blue baby syndrome. Entering human body, nitrates convert into nitrites which inhibit haemoglobin's normal oxygen-capacity. Thus, the substances are needed to be eliminated from water.

To reduce these substances, the suitable technique is urgently required. An affordable, simple, and eco-friendly technique as phytoremediation can be applied cover water from these substances [22]. Phytoremediation employed living organism, especially plant, to remove contaminant in soil or water as well as bioremediation [23]. The technique is able to treat various contaminants, for instance organic and inorganic pollutant, heavy metal, pesticides, crude oil, polycyclic aromatic hydrocarbons (PAHs) and herbicides contamination in a wide range of environment [24].

Sarma [25] reported that over 500 plant species have a potential in phytoremediation, for instance *Azolla pinnata*. Several studies showed that *Azolla pinnata* has an excellent ability to remediate the water pollutants [26–28]. It is an aquatic macrophyte that comes from the order of Pteridophyta and family of *Azolla pinatace*. According to Azab and Soror [29], *Azolla pinnata* has a high content of protein, low carbohydrate and low oil content. By dry weight, *Azolla pinnata* contain 25%–35% protein content, 10%–15% mineral content and 7%–10% a combination of amino acids, biopolymer and bio-active substances. Utilization and effectiveness of *Azolla pinnata* in phytoremediation method was confirmed to treat aquaculture wastewater, textile wastewater, municipal wastewater, oil and petroleum wastewater [30,31]. Based on these outcomes, it revealed that *Azolla pinnata* was applicable in phytoremediation technique [32]. However, the effectivity of *Azolla pinnata* to treat agriculture wastewater is still unsubstantiated and need to be investigated.

Aforementioned above, the objective of the study is to evaluate the effect of organic and inorganic fertilizer usage on the water quality as well as its' influence on the physiological behaviors of *Azolla pinnata*. Moreover, this study assesses the potential of *Azolla pinnata* as an agent of phytoremediation to remediate agricultural waste which was polluted by excessive fertilizer. In addition, this study highlights the best applicable of *Azolla pinnata* in reducing pollutants from agricultural wastewater that depends on the type of fertilizer and the amount of *Azolla pinnata*.

2. Materials and methods

2.1. Preparation of material and media

In this study, 7 boxes with different media were prepared. Three types of media were chosen, such as tap water, tap water added with chicken manure and tap water with growing fertilizer. Chicken manure represented as organic fertilizer, whilst growing fertilizer was represented by inorganic fertilizer. Both fertilizers were purchased from local nursery in granular form and crushed into a smaller particle to ensure the fertilizer mixing with the water and to facilitate the absorption of nutrients in plants. The organic and inorganic pulverized fertilizer were weighted for 10, 20 and 30 g and put in each box. It was then filled with 7,000 mL of tap water and set aside for 1 d. Each box was labeled A for tap water, B for organic fertilizer media mixed with tap water, and C for inorganic fertilizer media mixed with tap water.

In this study, *Azolla pinnata* was collected at Faculty of Civil Engineering Technology. The *Azolla pinnata* was filtered for 2 h to remove the excessing water and to ensure that it is not affecting the analytical measurement. After 2 h, 5 g of *Azolla pinnata* was weighted and placed on each media. Then, 10, 20 and 30 g of organic and inorganic fertilizer were transferred to each media, such as organic fertilizer (B10, B20 and B30) and inorganic fertilizer (C10, C20 and C30). Control media contained tap water only. The media that contained organic and inorganic fertilizer were stirred using spatula for 10 min to obtain homogenous solvent. Finally, 5 g of *Azolla pinnata* was transferred to each media. 120 mL of water sample were collected from each plastic box once in 3 d interval time for further analysis.

2.2. Observation on Azolla pinnata

The effect of media on the *Azolla pinnata* were visually investigated to understand the population of the *Azolla pinnata* at the starting (day-0) and at the end of experiment (day-9), including leaves width and root's length. The leaves colour were also recorded. On day 9, the final weight of *Azolla pinnata* in media was measured using analytical.

2.3. Water quality parameter tested

Five parameters of water quality were tested for this study to understand the quality before and after treatment. The parameters are pH, turbidity, ammoniacal nitrogen, phosphorus, and nitrate. All procedures were according to APHA [20] and HACH [21].

2.4. Data analysis

The ability of *Azolla pinnata* in uptake the pollutants from the media were expressed by percentage removal efficiency (RE) and is calculated according to Eq. (1).

$$\%\text{RE} = \frac{\left(C_o - C_t\right)}{C_o} \times 100 \tag{1}$$

where C_o represents initial nutrient concentration in water and C_t represent the nutrient concentration on *t*-day.

3. Results and discussion

3.1. Effect of media on Azolla pinnata

Visual assessment of *Azolla pinnata* for different type of media were monitored throughout the 9 d of the experiment. Table 1 indicates the characteristics of *Azolla pinnata* in each media throughout the experiment study along with Fig. 1 show the growth of *Azolla pinnata* on day 9 for different type of media.

As seen in Table 1, *Azolla pinnata* initially had yellow colour at some parts on day-0 for each type of media. It was also identified the initial average width of the leaves and average root's length were between 0.5–0.7 cm and 0.5–1.0 cm. After 9 d, the result indicated that there was a characteristic increase. Generally, most of the *Azolla pinnata* in the media were able to growth and reproduced. The average number of leaf widths and root lengths had increased, from 0.5–0.7 cm to 0.3–1.0 cm for leaves and from 0.5–1.0 cm to 0.2–1.3 cm for roots. Furthermore, the colour of *Azolla pinnata* also had changed from yellow to green, except media A.

Previous study described that *Azolla pinnata* had the diameter of 1.0–2.5 cm in triangular or polygonal shape for small size [33]. In addition, the roots of *Azolla pinnata* can only grow 5 cm in length [34]. However, according to the result, it was accepted that the observation on *Azolla pinnata* was established only for 9 d experiment.

Table 1
Characteristics of *Azolla pinnata* obtained during the experiment

Media type	Day	Leaves' width (cm)	Roots' length (cm)	Leaves' colour	Initial *Azolla pinnata* weight (g)	Final *Azolla pinnata* weight (g)
Media A	0	0.5–0.7	0.5–1.0	Yellow at some part	5.0	23.7
	9	0.3–0.5	0.2–0.8	Yellow at some part		
Media B10	0	0.5–0.7	0.5–1.0	Yellow at some part	5.0	29.8
	9	0.3–1.0	0.2–1.3	Green		
Media B20	0	0.5–0.7	0.5–1.0	Yellow at some part	5.0	22.2
	9	0.3–0.8	0.2–1.1	Green		
Media B30	0	0.5–0.7	0.5–1.0	Yellow at some part	5.0	15.9
	9	0.3–0.7	0.2–1.0	Green		
Media C10	0	0.5–0.7	0.5–1.0	Yellow at some part	5.0	28.7
	9	0.3–0.8	0.2–1.3	Green		
Media C20	0	0.5–0.7	0.5–1.0	Yellow at some part	5.0	32.6
	9	0.3–0.7	0.2–1.2	Green		
Media C30	0	0.5–0.7	0.5–1.0	Yellow at some part	5.0	37.1
	9	0.3–0.7	0.2–1.2	Green		

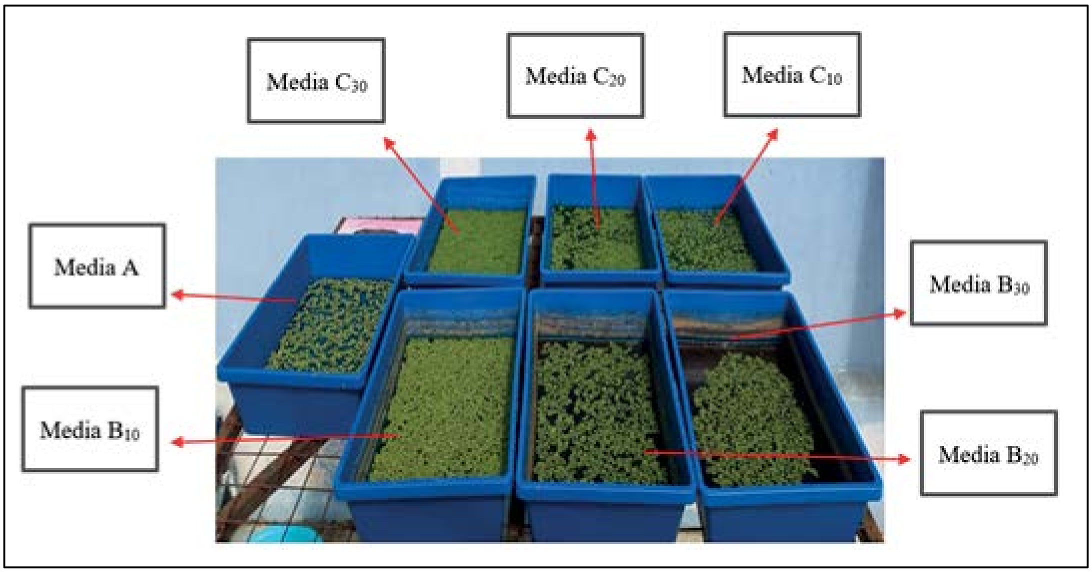

Fig. 1. Growth of *Azolla pinnata* on day 9 in different type of media.

Moreover, throughout the study period, there was no plant necrosis was found. The result provided that *Azolla pinnata* grown in media A had a low growth rate as the total dry mass on day 9. In addition, it had no significant increase after day 9. Azab and Soror [29] mentioned that although *Azolla pinnata* does not require nitrogen in its growing medium, the level of nitrogen in the water affects its growth and nitrogen-fixation rates because the nitrogen is assimilated by anabaena and *Azolla pinnata* from the atmosphere and from the water, respectively. Anabaena was symbiotically associated with *Azolla pinnata* for fixing nitrogen substances. The final average leaves width and roots length is between 0.3–0.5 cm and 0.2–0.8 cm. It indicated that the leaves became smaller. In addition, the leaves colour also turned yellowish. This condition was arisen due to development of anthocyanin pigment under phosphorus and potassium deficiency [35].

This study also found that *Azolla pinnata* growth in organic fertilizer (B10, B20 and B30) obstruct the eutrophication. It was reasonable since the concentration of nutrients in organic media did not stimulate the algal bloom. In this cases, high dosage of organic fertilizer only decelerated the *Azolla pinnata* growth rate without generated eutrophication. According to Othman [36–38], eutrophication was led by the excess primary nutrients concentration like nitrogen and phosphorus which came from anthropogenic source like fertilizer, detergent and other organic debris. In this study, media B10 was identified as the most suitable media for *Azolla pinnata* growth compared to B20 and B30. Instead of forbid eutrophication, this condition was proven as the total dry mass were increased continuously indicating a normal growth rate. In addition, there were also an increment on the final average leaves width and roots length from 0.5–0.7 cm to 0.3–0.8 cm for leaves width and from 0.5–1.0 cm to 0.2–1.2 cm for roots length. This condition revealed that *Azolla pinnata* began to reproduce and double its biomass constantly. Similar observation was also reported that the *Azolla pinnata* has increased in biomass after being treated with organic and inorganic fertilizer [29].

There was an unexpected investigation regarding the growth of *Azolla pinnata* in inorganic fertilizer (C10, C20 and C30) because of the eutrophication. Initially, the growth rate of *Azolla pinnata* is slow. However due to high concentration of NH_3–N, PO_4^{3-} and NO_3^- in the media, they encourage *Azolla pinnata* to rapidly reproduce making the water surface crowded. Media C30 was distinguished as the fastest media that stimulate to this phenomenon, as seen in Fig. 2. The rapid growth rate of media C30 was confirmed on day 3 together with the algal bloom. The total dry mass were increased dramatically on day 9 with final average leave's width and roots length are between 0.3 to 0.7 cm and 0.2 to 1.2 cm. Similar observation also found in media C20 and C10 which started to have the eutrophication phenomena on day 6 onwards.

3.2. Phytoremediation of water quality parameters by Azolla pinnata

Instead of observation on *Azolla pinnata* behaviour, the water quality also is being monitored. In this study, the changes in pH as well as turbidity of the media has been determined. Fig. 3 and Table 2 show the value of pH and turbidity for each water media, respectively.

There were variations of pH according to media observed in this study. The results showed that the initial pH of organic media, for instance A, B10, B20 and B30, are slightly alkaline (7.4–8.7). For inorganic media, such as C10, C20 and C30, the initial pH was slightly acidic (5.9–5.5). However, on day 3, there was a reduction in pH for organic media. pH in media B10 decreased up to 15.65% compared

Table 2
Turbidity value (NTU) obtained during the experiment study

Type of media	Day 0	Day 3	Day 6	Day 9	% Removal
Media A	0.317	0.300	0.283	0.279	12.00
Media B10	215	200	195	175	18.60
Media B20	430	424	415	396	8.00
Media B30	728	725	719	695	4.50
Media C10	0.615	0.632	0.654	0.750	–21.95
Media C20	0.753	0.757	0.921	1.070	–42.10
Media C30	0.841	0.943	1.330	1.370	–62.90

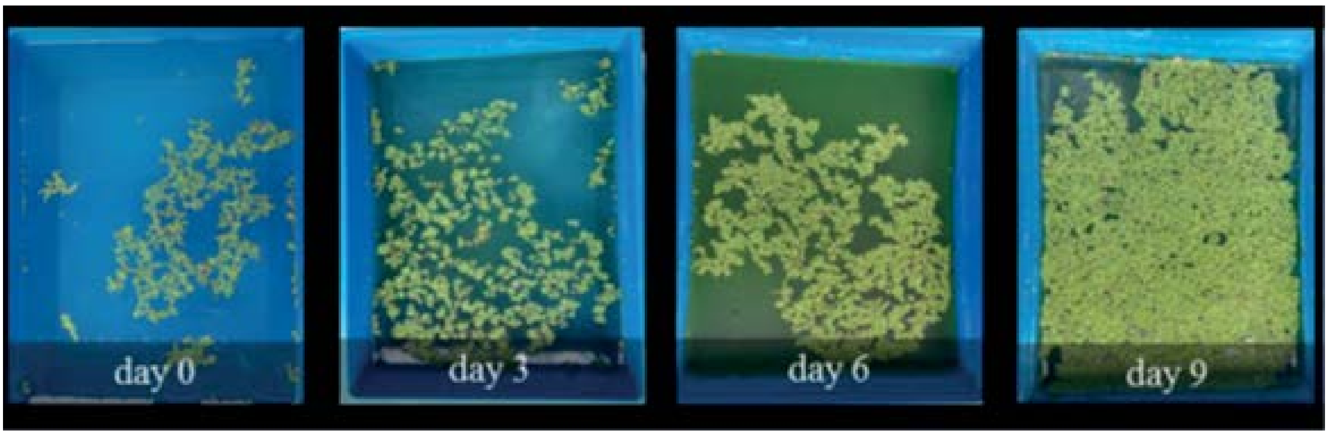

Fig. 2. Eutrophication was detected on media C30 starting on day-3 of experiment.

to media A, media B20, media B30 with the removal percentage of 2.7%, 12.24% and 11.25%, respectively.

Ugya [39] mentioned that the reduction of pH in water could be associated to a decrease in ammonia and nitrate. It is reasonable as absorption of ammonia and other nitrogen compounds typically inspired the biological reaction that produced hydrogen ion and indirectly decreased the pH of water. Previous study mentioned that *Azolla pinnata* can survive with a water pH range from 3.5–10, but it has optimum growth between pH 4.5–7 [40,41]. Hence, it can be confirmed that the pH value ranging from (5.5–8.7) were able to support *Azolla pinnata* growth.

For inorganic media, Table 2 reveals that the pH value increased in media containing inorganic fertilizer because of eutrophication. During eutrophication, photosynthesizing algae will cover the lake or pond surface, competing for light [42]. The chemical by-products of this photosynthesis process increase the pH of the water, making it more basic. According to Othman et al. [36], there are several terms used in eutrophication, representing the steps in the process from clear water to algal bloom. The first stage was oligotrophic. At this stage, only a few developments of either macrophyte or phytoplankton due to poor status of nutrient and primary productivity. Next, mesotrophic stage is started when nutrients are added into the water bodies. It lead phytoplankton biomass to increase. Then, the final eutrophic state occurs when primary and phytoplankton productivity is greatly increased, causing water quality to decline.

Thus, based on this study, it can conclude that the reduction of pH in water containing organic fertilizer was due to the absorption of ammonia and nitrate compound. The increment of pH in water containing inorganic fertilizer was due to the high concentration of nutrient which triggered the growth rate of algal bloom.

Turbidity was another parameter tested in this study. It was a measurement of water clarity where solids in the water obstruct the transmittance of light through the sample. The results show that the initial turbidity of media A was 0.317 NTU turbidity of organic media (B10, B20 and B30) and inorganic fertilizers (C10, C20 and C30) are ranging 215–728 NTU and 0.615–0.841 NTU, respectively.

Based on the result, it was clearly projected that the turbidity of organic media were higher compare to water added with inorganic media. It is well understood owing to the sizes of organic compounds and the accumulation of these compounds in the media. From visual observation throughout the experiment, the size of organic fertilizer compound was bigger compared to inorganic fertilizer compound. Particle size influence the rate of solubility [43]. The bigger the particles size, the slower the rate of solubility as only small surface area were exposed in a solvent. Thus, this condition will cause accumulation of undissolved compound that indirectly will lead to high turbidity value in water containing organic media. Furthermore, the dissolved coloured compound in chicken manure also are one of the factors that contributed to high turbidity value. Materials that cause water to be turbid include clay, silt very tiny inorganic and organic matter, and dissolved coloured compounds. The higher the intensity of scattered light, the higher the turbidity [44].

Furthermore, the result also indicated that there was a reduction of turbidity in media A and organic media. Table 2 highlighted the removal of turbidity only found in media A, and organic media, for instance B10, B20 and B10 with 12%, 18.6%, 8% and 4.5% of removal efficiency, respectively. This could be the result of the *Azolla pinnata* condition in which the plants were placed in the pots during the experiment. Their roots absorbed the pollutants. The roots of *Azolla pinnata* had increased at the end of experiments that helps in accumulate the matters at the roots. According to Mohd Nizam et al. [45], the devaluation in turbidity by plants fundamentally depends on the plant root system. Plant with adventitious fibrous roots like *Azolla pinnata* can accumulate more suspended solid compared to those with taproot. As stated by Austin et al. [46], the presence of

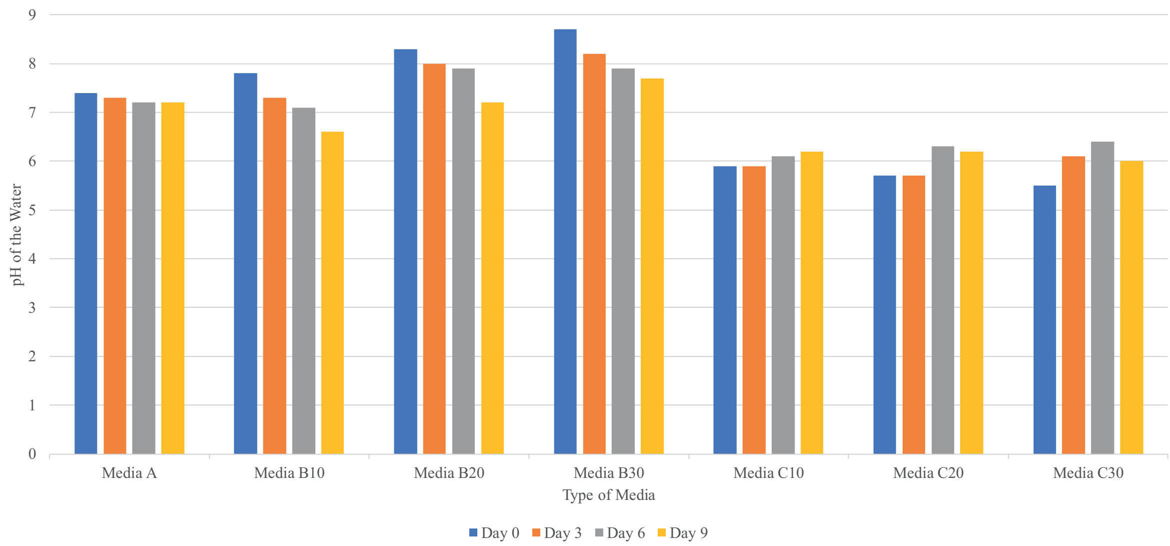

Fig. 3. pH of different media type in this study.

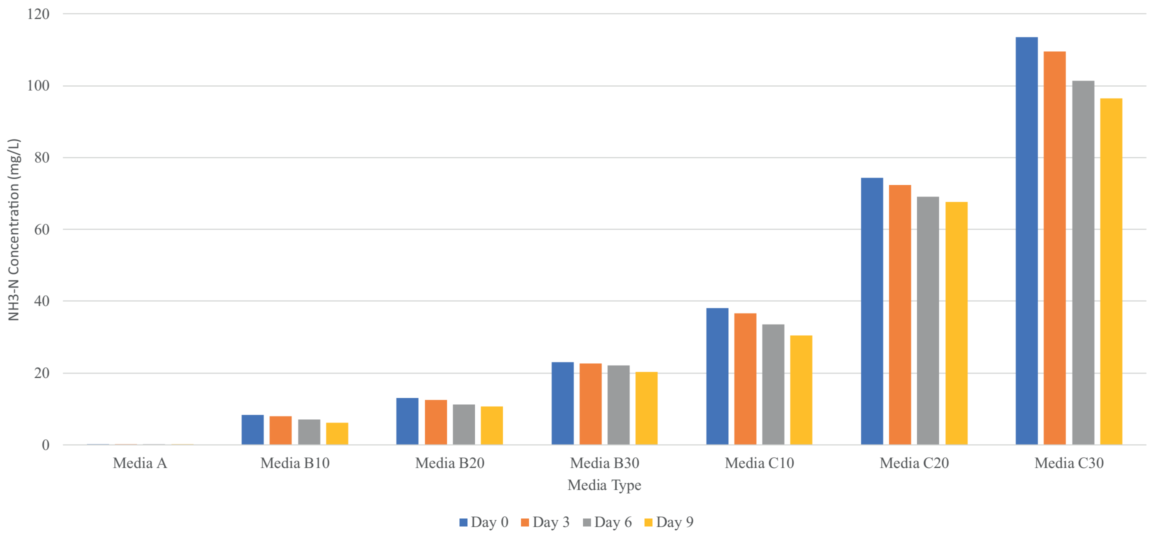

Fig. 4. Concentration of ammonium nitrate (mg/L) in different type of media.

suspended particles in water will cut down the amount of light that can reach aquatic plants. It will cause aquatic plant to die due to insufficient amount of sunlight.

Throughout this study, there was an increment in turbidity value when it came to inorganic media (C10, C20 and C30) because of eutrophication. The primary environmental implications of eutrophication include increased suspended particulate matter that is caused by large macroalgal blooms, loss of water clarity, and increased precipitation rates, resulting in loss of benthic habitat due to submerged plant shading [47]. Davis and Shaw [48] also confirmed that the negative effect of eutrophication include high in turbidity, offensive of odor, taste and release of toxins that lead to environmental problems and human health.

3.3. Nutrients uptake by Azolla pinnata

Nitrogen in agricultural wastewater is usually presented in organic form such as ammonia (NH_4^+, NH_3–N) and inorganic forms such as nitrate (NO_3^-) and nitrite (NO_2^-). Ammonia nitrogen is the principal form of a toxic ammonia [49]. Toxic concentration of ammonia in humans may cause loss of equilibrium, convulsions, coma and death. Besides that, ammonia levels on excess of the recommended limits may harm aquatic life [50]. Fig. 4 shows the concentration of ammonium nitrate from day 0 up to day 9 in different type of media. Based on the result, media C30 recorded the highest initial concentration of NH_3–N by 113.5 mg/L followed by media C20 (74.4 mg/L), C10 (38 mg/L), B30 (23.1 mg/L), B20 (13.1 mg/L), B10 (8.28 mg/L) and media A (0.20 mg/L).

In this study, *Azolla pinnata* has been placed in the prepared pot containing organic and inorganic media in order to determine the efficiency of *Azolla pinnata* as phytoremediation. Results showed that there were a decline trend recorded during the experiment study. Previous study also confirmed that *Azolla pinnata* has an ability to remove NH_3–N from wastewater [51]. NH_3–N reduced was best in media B10 by 25% removal efficiency followed by media C10 (20%), B20 (17%), C30 (15%), B30 (12%), C20 (9%) and media A (5%) respectively. Naima et al. [19] declared that NH_3–N was removed due to the biological mechanism in nutrient uptake and the existence of ammonia uptake. Additionally, it also represent the consequence growth and accumulation of high nitrification bacteria. Plant uptake the inorganic nitrogen as NH_3^- or NH_4^+ ions that were assisted by bacteria. Hence, this study affirmed that *Azolla pinnata* was able to uptake NH_3–N in all type of media.

Phosphorus is the most essential nutrient for plant after nitrogen and commonly are taken up as a orthophosphate ion, PO_4^{3-} [52]. High concentration of phosphorus are not only caused eutrophication but it also disturb the inhibition of photosynthesis and alteration of water balance. Fig. 5 shows the concentration of phosphorus in different type of media in this study

According to the result, media C30 had the highest initial concentration of PO_4^{3-} by 132.1 mg/L followed by media C20 (95.3 mg/L), C10 (52.8 mg/L), B30 (76.5 mg/L), B20 (43.2 mg/L), B10 (26.5 mg/L) and media A (0.27 mg/L). Previous finding projected that the natural level of phosphorus in water usually range between 0.005 to 0.05 mg/L [53]. Thus, this study indicated that both organic media and inorganic had high phosphorus concentration which exceeded the natural phosphorus level in the water. Meanwhile, control media was slightly exceeded the concentration range.

This study also revealed that media B10 had the highest removal efficiency for this study. Media B10 reduce (38.5%) of phosphorus followed by media C10 (26.1%), C30 (20.1%), B20 (17.3%), C20 (13.1%), B30 (11%) and media A (5%). The results indicated that *Azolla pinnata* had a good potential in reducing phosphorus concentration in the water thru phytoremediation. Wang et al. [54] stated that the *Azolla pinnata* had a good potential to adsorb phosphorus, while Rezooqi et al. [55] showed that the *Azolla pinnata* had reduced the phosphorus concentration in wastewater. Moreover, based on the percentage removal, it indicated that low dosage of fertilizer can affects the value of removal efficiency in polluted water. Kamaruddin et al. [56] declared that the devaluation of phosphate in water

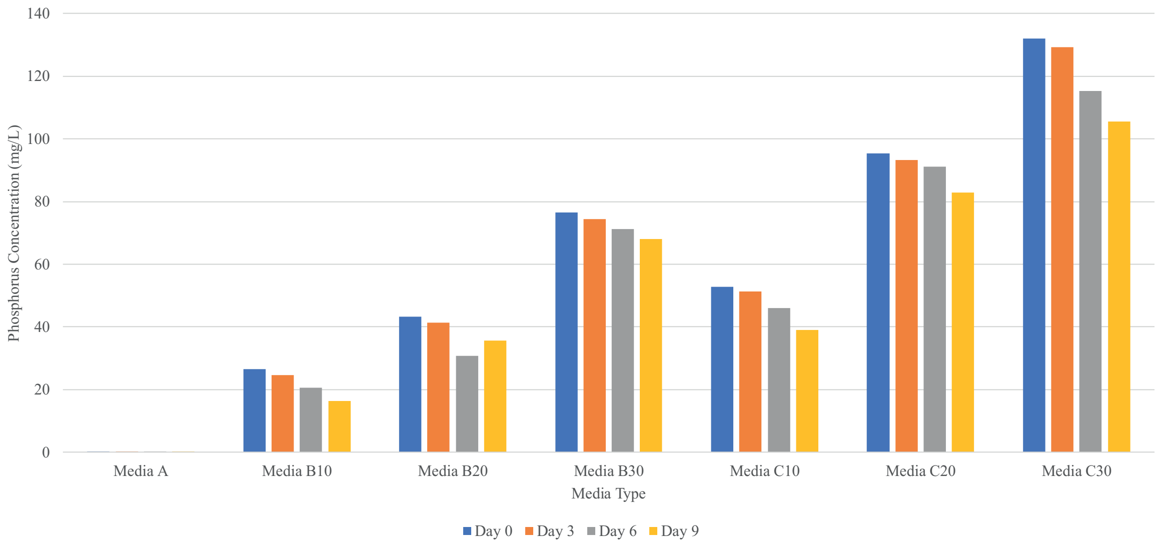

Fig. 5. Concentration of phosphorus (mg/L) in different type of media.

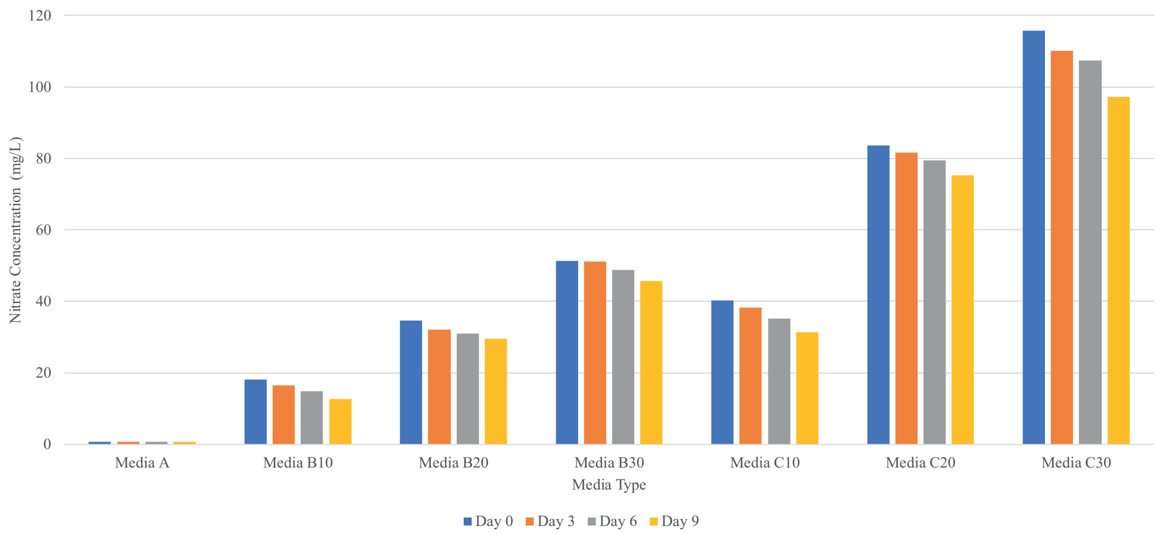

Fig. 6. Concentration of nitrate (mg/L) in different type of media.

was due to the uptake by plant and due to the substrate diversity in the media.

Nitrate is typically to be ions in nature and is a part of nitrogen cycle [57]. Redundant amount of nitrate in water not only poses eutrophication, but also will be resulted in health issue like methemoglobinemia, stomach cancer, thyroiditis and several other to human health [58]. Fertilizer, animal waste and septic tank are the main source of nitrate contamination [59]. Thus, nitrate contamination was need to be treated and monitored.

As seen in Fig. 6, nitrate concentration was monitored in different type of media. Media C30 obtained the highest initial concentration of NO_3^- (115.7 mg/L) followed by media C20 (83.6 mg/L), C10 (40.2 mg/L), B30 (51.4 mg/L), B20 (34.7 mg/L), B10 (18.2 mg/L) and media A (0.80 mg/L). Strong evidence of nitrate concentration was found that the highest removal efficiency of nitrate was in media B10, and the lowest removal was in media A. The percentage removal for media B10 was 30% followed by C10 (22%), C30 (16%), B20 (15%), B30 (11%), C20 (10%) and lastly media A (7%). These results indicated that *Azolla pinnata* were able to remediate the nitrate from agricultural wastewater drawn. Similar observations mentioned that which the *Azolla pinnata* can remove the nitrate contained in the water [55,60,61].

4. Conclusions

This study was conducted to evaluate the water quality and nutrient uptake of *Azolla pinnata* production in different media. There were seven type of media, such as control media (A), organic media (B10, B20, B30) and inorganic media (C10, C20, C30) with different dosage of fertilizer that can determine the potential of *Azolla pinnata* as an agent of phytoremediation. Throughout this study, the *Azolla pinnata* has been observed in term of leaves size, leaves colour, root length and *Azolla pinnata* weight. Result disclosed that the range of leaves size and root length for control media was between 0.3–0.5 cm and 0.2–0.8 cm. For organic media, leaves size and roots length were 0.3–1.0 cm and 0.2–1.3 cm. For inorganic media, the range of leaves size and root length were 0.3–0.8 cm and 0.2–1.3 cm. Next, green colour of *Azolla pinnata* was shown in all media except for media A which maintained yellowish. In term of *Azolla pinnata* weight, media C30 was recorded as the heaviest media followed by media C20, media B10, media C10, media A, media B20 and media B30 with 37.1, 32.6, 29.8, 28.7, 23.7, 22.2 and 15.9 g. It was proven that *Azolla pinnata* were capable in treating the polluted water by phytoremediation method. Media B10 was evaluated as the best media. The result shows that the pH and turbidity value were reduced to 15.65% and 18.6%. For nutrient uptake, the concentration of NH_3–N, PO_4^{3-} and NO_3^- in media B10 was reduced up to 25%, 38.5% and 30%, respectively. This revealed that *Azolla pinnata* has shown as a remarkable effectiveness in treating the polluted water. The effluent after phytoremediation is suitable for reuse and recycle along with a yield of *Azolla pinnata*. This yield can be used to increase productivity. as *Azolla pinnata* can be used as cattle feed for high milk production and it can also be used as a bio-fertilizer for agricultural purpose. It is recommended that more parameters such as heavy metals, COD and BOD will be evaluated for phytoremediation treatment by *Azolla pinnata* in both organic and inorganic media in future study.

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Conflicts of interest

The authors declare no conflict of interest.

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