POTENTIAL USE OF *Scirpus littoralis* AND *Typha orientalis* FOR NITROGEN AND PHOSPHORUS REMOVAL FROM INTENSIVE WHITELEG SHRIMP WASTEWATER

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ABSTRACT

The rapid development of whiteleg shrimp farming in the Vietnamese Mekong Delta has an adverse impact on the environment due to large amount of nitrogen and phosphorus content in wastewater and pond sludge/sediment. Phytoremediation is a promising technique to use plant for mitimigating environmental impacts from intensively whiteleg shrimp culture. Selection of potential plant species having fast growth, high biomass, high adaptive and nutrient uptake capacity in wetlands condition is one of the steps for the success of this technique. Growth responses, nitrogen and phosphorus removal process were investigated at three water levels of +15 cm, +30 cm and +45 cm with plants (*Typha orientalis* and *Scirpus littoralis*) and without plants (as control treatment). They were arranged in a completely randomized design with three replications. The plants were supplied wastewater from intensive whiteleg shrimp tanks once every two weeks. Waterlogged assessment was conducted for 71 days. Water levels significantly affected plant growth rate and nutrient removal capacity. *S. littoralis* grew well with a lower survival rate and had no statistical reduction of biomass compared to *T. orientalis* at the highest water level of +45 cm. *T. orientalis* had the best perfromance in growth and biomass responses to waterlogged at water levels of +15 cm and +30 cm. The results indicated that *S. littoralis* was the best of choice to grow in waterlogged condition of the shrimp pond for maintaining water quality.

Keywords: *Aquaculture effluent, plant biomass, Litopenaeus vannamei, water levels, waterlogged.*

Received: 12 May 2022; revised: 12 July 2022; accepted: 19 August 2022.

1. INTRODUCTION

Total area and production of shrimp farming in Vietnam in 2021 was 752,900 ha and 996,269 tonnes, in which the Vietnames Mekong Delta (VMD), South of Vietnam, accounted for over 92% and 84% of Vietnamese shrimp farming area and production [1]. Specifically, whiteleg shrimp (*Litopenaeus vannamei*) has increased rapidly in production in recent years, accounting for 67% of total shrimp production from 16% of the total farming area of the country. Anh *et al.* (2010) [2] reported that to produce 1 ton of shrimp 5,345-7,151 m^3 wastewater, 259 kg BOD, 769 kg COD, 1170 kg TSS, 30 kg N, 3.7 kg P and 4.8 kg $N\text{-}NH_3$ discharged into the environment. Shrimp aquaculture growth in Vietnam has suffered many problems which similar to Asia' situation in recent years. The major factors contributing to the problem in sustaining shrimp aquaculture are disease outbreaks, environmental degradation and poor management practice [3]. Poor water quality from the river that is one of the causes in failure of 3,081 ha whiteleg shrimp [1]. Therefore, there is an urgent need to develop a more sustainable aquaculture industry that uses less water, and that

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does not deteriorate the water quality of the rivers. Phytoremediation is defined as the use of plants and their associated microbes for environmental clean-up [4]. One of the phytoremediation processes, in which plants are used to remove contaminants from soils or water into harvestable plant biomass, is called phytoextraction. Phytoextraction is used mainly for extracting heavy metals from polluted soils and water [5], but the use of plant uptake and plant-mediated conversions also has great potential for the removal of nutrients from nutrient enriched waters.

In the present study, *Scirpus littoralis* Schrab and *Typha orientalis* C. Presl were chose to study waterlogged stress. In fact, *T. orientalis* and *S. littoralis* are cultivated in the paddy field and/or in the shrimp ponds in the MD for human food and providing habitat for shrimp, respectively. That is the reason to evaluate flooded level is suitable for plant growth. According to farmers, the purpose of planting sedge is to improve the pond environment and to increase sources of organic feed materials as well as density of natural food in the pond. In addition, the sedge grass helps regulate pond temperature in hot weather. Trang *et al.* (2018) [6] reported that between two studied plants, *T. orientalis* accumulated a higher Na^+ and Cl^- concentration in the roots and shoots especially at the high levels of NaCl of 15-30‰ and *T. orientalis* has lower salinity tolerant capacity compared to S. *littoralis*. They also suggested that both plants are able to withstand salt stress and can be considered the best bio-filter candidate in the integration of constructed wetlands and marine shrimp culture towards sustainable aquaculture in the Mekong delta, Vietnam. To understand the potential effects of increased water levels on these aquatic macrophytes, information is needed regarding the duration and water regime exposure in relation to the response of the plant species in term of growth, biomass and nutrient removal. Therefore, the present study was carried out to identify waterlogged-tolerant species through survival, growth, biomass and nitrogen (N) and phosphorus (P) removal.

2. MATERIALS AND METHODS

2.1. Experimental setup

A completely randomized design consisting of two plant species (*Scirpus littoralis* and *Typha orientalis*) and three water levels (+15, +30 and +45 cm) was set up in triplicates. The corresponding unplanted containers were filled with three water levels of +15, +30 and +45 cm which were considered as control treatments. The experiment was conducted in the greenhouse at the College of Environment and Natural Resources of Can Tho University, Vietnam (10.03° N latitude and 105.76° E longitude).

2.2. Plant materials and growth conditions

Rhizomes and young plants of the two studied species were collected from the fields. Twenty-seven plastic containers of each size of 50-L, 45-L and 120-L were used. These corresponding containers were filled with a 25-L, 30-L and 80-L of wastewater that made up water levels at +15, +30 and +45 cm above sediment. Sixteen kg of sediment (76.2% dry weight) which was collected from the intensive whiteleg shrimp pond in Soc Trang province were filled in the growth containers to mimic the bottom pond condition.

A valve connected with Ø 34 mm PVC tube was fitted through a hole in the side of the containers at the middle of water level to sample water (Figure 1). The individual young and similar size plants for each species were placed in each container for acclimation of two months before flooding. Then plants were thinned out to keep a density of 15 plants/m^2 [7]. Intensive whiteleg shrimp wastewater collected from the culture tanks, which were stocked at 400 shrimps per one m^3 water were used as growth solution for plants.

2.3. Plant growth and biomass measurement

At the initiation of the flooding treatments, the representative specimens of each container were sampled to determine their initial shoot height, root length, number of shoots, fresh and dry weight. At harvest, plants were carefully removed from the sediment and the roots were carefully washed to remove particle and sediment. The fractions were weighed for fresh biomass and dried at 60°C until constant weight to determine for dry weight.

The shoot and root growth rate were calculated from the difference of shoot height and root length measurements at the beginning and at the end of the study divided by time (a total of waterlogged perios was 71 days). Relative growth rate of biomass was calculated from the difference of natural logarithm of harvested biomass and beginning biomass divided by time.

2.4. Water analysis

Water samples were taken every two weeks at 6:00 - 7:00 AM. Temperature (°C), dissolved oxygen (DO, mg/L), pH, salinity (‰), electrical conductivity (EC, mS/cm), redox potential (E_h, mV) of the water in the containers were measured at the experimental site using respective portable meters. Water samples were collected and transferred immediately to the laboratory for analysis of alkalinity ($CaCO_3$), Ammonium nitrogen (NH_4-N), Nitrite nitrogen (NO_2-N), Nitrate nitrogen (NO_3-N), total Kjeldahl nitrogen (TKN), orthophosphate (PO_4-P) and total phosphorus (TP). All the analytical measurements were carried out according to Standard Methods [8].

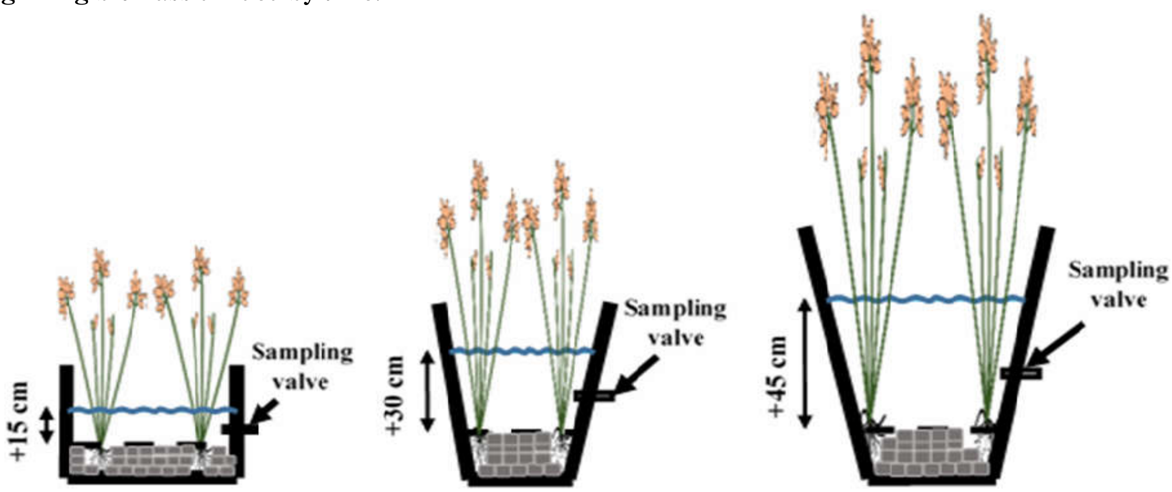

Figure 1. An overview of treatment plots which represented one plant species for three water levels of +15, +30 and +45 cm above sediment

2.5. Statistical analysis

Data were tested for normal distribution, variance homogeneity (Levene's test) and logarithmically transformed if necessary. Differences in water quality were identified by two-way repeated measures ANOVA (General Linear Models) with factor A was plant types (two plants and one unplanted treatment); factor B was water levels (3 levels) which was nested in factor A and factor C was sampling times (6 times) using Type III sum of squares. Differences in plant growth and biomass were identified by one-way ANOVA. Tukey Honestly Significant Differences (HSD) was used to compare significant differences between treatments at the 5% probability level. The software

Statgraphics Centurion XV (StatPoint, Inc., USA) was used for all statistical analyses.

3. RESULTS AND DISCUSSION

3.1. Plant growth and biomass allocation

Water levels significantly affected plant survival rate, number of new shoot and root length ($p<0.05$; Figure 2A, 2B, 2C), but did not affect plant height and growth rate of shoot and root ($p>0.05$; Figure 2). The same fingdings in *Typha domingensis* when the plants were flooded at 40-137 cm depth for six weeks [9]. *T. orientalis* in the water level of +45 cm showed stress symptom of leaf rolling and wilting at the 6th week of waterlogged, and the major part of shoots eventually dried out at the 10th week of waterlogged. Therefore, at the harvest we counted the number of survival plant for *T. orientalis* in the water level of +45 cm which was zero due to no green leaves, but number of new shoot and biomass of those plants was collected and measured. *S. littoralis* had an average of 21% mortality at the water level of +45 cm (Figure 2A). Although two tested species have been known as wetlands plants and they were grown in the commercial extensive shrimp pond in the VMD, they both showed stress of waterlogged at the water level of +45 cm. It can be explained that the growth condition with sallow sediment layer with low nutrient concentration in the sediment and in the wastewater, which might cause unfavourable condition for plant growth [10]. Number of new shoots of *S. littoralis* was higher than that of *T. orientalis* in all water levels and it was also reduced at the highest water level of +45 cm ($p<0.05$). The root length of both species tended to be shortened at the highest water level of +45 cm ($p<0.05$). Between two tested species *S. littoralis* showed more tolerant to waterlogged condition in term of survival. Grace (1989) [11] and Newman *et al.* (1996) [10] reported that nutrient enrichment and shallow water depths (i.e., 20 - 30 cm) were usually favor the dominance of *Typha* species in wetlands. *Typha latifolia* and *T. domingensis* can grow at a wide range of water depths (0 - 115 cm), these species have high shoot density and flowering incidence only within a narrow range of water depths [11]. As water depth increases, *T. domingensis* increases its shoot height, produces fewer but larger ramets, and decreases the incidence of flowering. Increasing water depth reduces the anchorage capacity of plants by decreasing biomass allocation to rhizomes and roots [11].

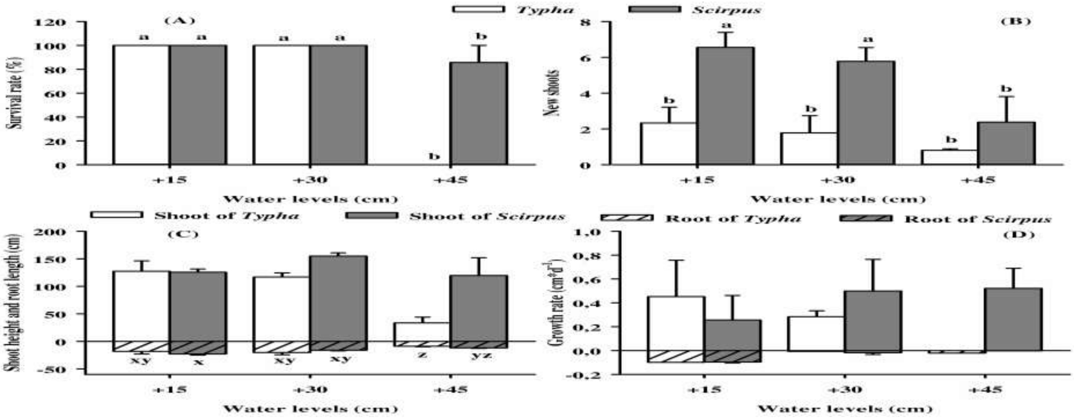

Figure 2. Survival rate (A), number of new shoot (B), shoot height and root length (C) and growth rate of shoot and root (D) of *Typha orientalis* and *Scirpus littoralis* grown at different water levels

Notes: Values are the means of 3 replicates ± S.D. Different letter ^{a,b,x,y} *indicates significant difference based on a Tukey HSD test (p<0.05).*

Water levels significantly affected shoot and root dry and fresh weight at the harvest of the two species ($p<0.05$; Figure 3A & 3B) but did not affect relative growth rate (RGR) of their biomass ($p>0.05$; Figure. 3C & 3D). The species also influenced fresh and dry weight of the shoots and roots. Although the biomass was reduced in both species in response to water levels ($p<0.05$; Figure. 3), it nevertheless was significantly greater in *T. orientalis* than *S. littoralis* under +30 cm water levels ($p<0.05$; Figure 3A & 3B). *S. littoralis* showed no statistical reduction in fresh and dry weight of the shoot and root's fractions across water levels, while *T. orientalis* had a reduction of these parameters at the highest water level of +45 cm.

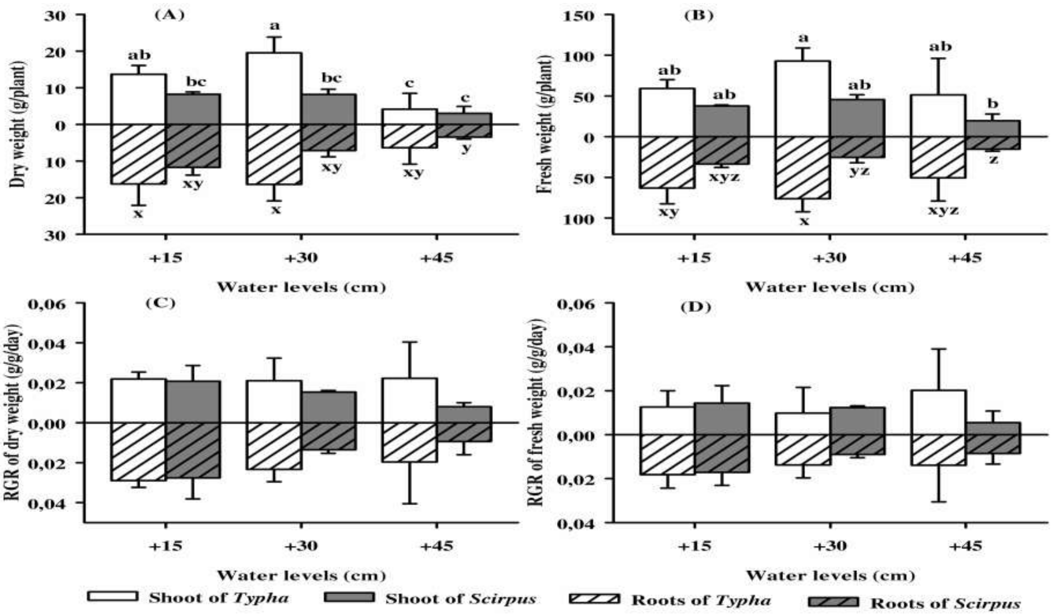

Figure 3. Dry weight (A), fresh weight (B), relative growth rate (RGR) of dry weight (C) and fresh weight (D) of *T. orientalis* and *S. littoralis* grown at different water levels.

Notes: Values are the means of 3 replicates ± S.D. Different letter [a,b] indicates significant difference between treatment in the shoot fractions based on a Tukey HSD test (p<0.05). Different letter [x,y] indicates significant difference between treatment in the root fractions based on a Tukey HSD test (p<0.05).

3.2. Water quality

3.2.1. Values of dissolved oxygen, redox potential, temperature and chemical oxygen demand in the water

There were significant differences for dissolved oxygen (DO), redox potential (E_h), temperature and chemical oxygen demand (COD) in the water between treatments and between sampling times ($p<0.05$; Figure 4). Dissolved oxygen is the most critical water quality for shrimp growth. The DO concentrations tended to increase over time ($p<0.05$; Figure 4A) and was at the lowest value in the containers planted *T. orientalis* at the water level of +45 cm ($p<0.05$; Figure 4A). Although the water DO concentration was the lowest in the treatment of *T. orientalis* at +45 cm water level but it was higher than that of the permitted limit (≥3.5) for requirement on shrimp rearing water quality regarding to Appendix 1 of the Vietnamese standards No. 02-19/2014/BNNPTNT (issued on July 29, 2014) [12]. Redox potential is inextricably linked to oxygen supply and the processes of consumption thereof by microorganisms and plant roots. Therefore, the redox potential is used as an indicator of the oxygenation status and the content of biogenic forms and toxins in the soil environment and sediment. In the case of submerged sediment in our study, penetration of atmospheric oxygen into the sediment was limited due to low rates of oxygen diffusion and, hence, low redox potential, which inhibits plant growth through inhibition of respiration and production of toxins in reducing conditions. Therefore, the reported water redox potential values had similar trend to DO concentration, it increased over time and had the highest values at the end of the study ($p<0.05$; Figure 4B). The average values of E_h in the water of the treatments were in the range of -101 to -75 mV and were classified as reduced environment [13]. The average water temparature of the treatments were in the range of 30.3 - 31.4°C (Figure 4C) which was in the permitted limit (18.0-33.0°C) for requirement on shrimp rearing water quality [12]. Chemical oxygen demand (COD) was not listed in the former Circular No. 45/2010/TT-BNNPTNT [14] for permitted limit in water quality for shrimp growth or in discharged water; however, in the updated Vietnamese standards No. 02-19/2014/BNNPTNT [12] COD parameter was placed in the permitted limit for discharge effluent after treatment of wastewater (≤150 mg/L). In our study, COD concentrations in all the treatments fluctuated and tended to increase at the end of the study, but they were 6-times lower than that of the permitted limit. In sum, DO, temperature and COD concentrations in the water across the treatments meet the Vietnamese standards requirement on shrimp rearing water quality. The water in the treatments was qualified and can be reused for rearing shrimp.

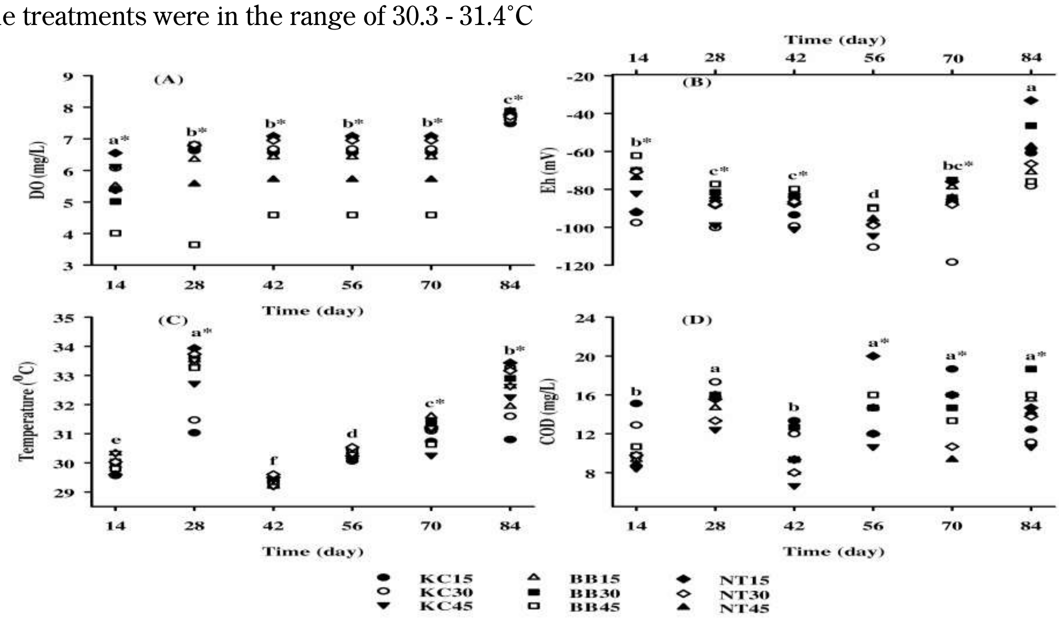

Figure 4. Dissolved oxygen (A), redox potential (B), temperature (C) and chemical oxygen demand (D) in the water over time of *T. orientalis* (BB), *S. littoralis* (NT) and unplanted (KC) at +15, +30 and +45 cm water levels

Notes: Values are the means of 3 replicates. Asterisk (*) indicates significant difference between treatments within sampling time. Different letter ^{a,b,c} indicates significant difference between sampling times based on a Tukey HSD test (p<0.05).

3.2.2. Values of pH, electrical conductivity, salinity and alkalinity in the water

The pH values can affect to the physicaly, chemically, biological elements of the environment and shrimp health. In particular, an increase of pH and temperature values is the cause of increase of NH_3-N concentration that can toxic to the aquatic organisms. During the experiment, the average of water pH in the treatments maintained between 7.9 and 8.4 (Figure 5A) that was in the optimum level for shrimp growth regarding to Appendix 1 of the Circular No. 45/2010/TT-BNNPTNT (7.5-8.5) [14] and in the permitted limit (7.0-9.0) for requirement on shrimp rearing water quality [12]. The pH values had similar trend with DO concentrations in the treatment without plants which was higher than that of the other planted treatments ($p<0.05$; Figure 5A). Similar to DO, pH values were also related to algae photosynthesis because algae bloomed (via visual observation) in the unplanted treatments.

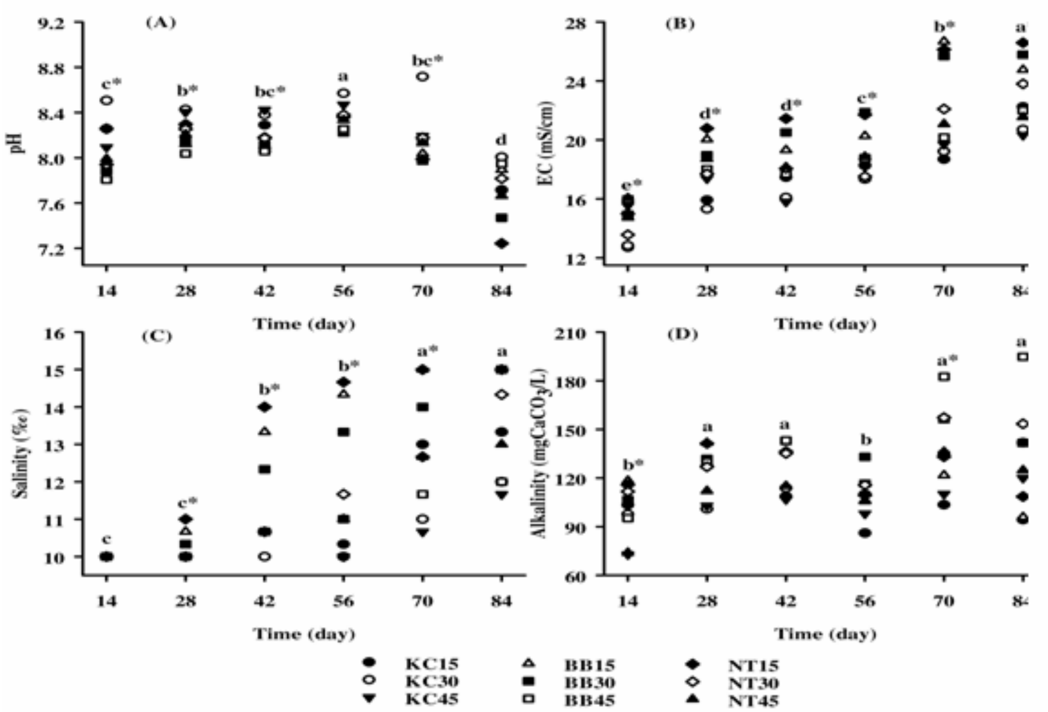

Figure 5. Values of pH (A), electrical conductivity (B), salinity (C) and alkalinity (D) in the water over time of *T. orientalis* (BB), *S. littoralis* (NT) and unplanted (KC) at +15, +30 and +45 cm water levels.

Notes: Values are the means of 3 replicates. Asterisk (*) indicates significant difference between treatments within sampling time. Different letter [a,b,c] indicates significant difference between sampling times based on a Tukey HSD test ($p<0.05$).

Electrical conductivity (EC) did not directly affect shrimp growth but it might cause effect on plant growth because EC was strongly related to salinity. The water EC had the same trend of salinity and reached the highest values at the end of the study ($p<0.05$; Figure 5B). The salinity stress inhibited plant growth and had the same symptom with water-stress. Water salinity level in this study was controlled at 10‰ in the added water during the study; however, the concentrations of salinity in all treatments tended to increase over 10‰ and reached to 15‰ at the end of the study (Figure 5C). It can be explained that at the sampling point salinity in the water increased due to water loss via evapotranspiration and salinity even accumulated more at the end of the study ($p<0.05$; Figure 5C). However, they were in the permitted limit (5.0-35.0‰) for requirement on shrimp rearing water quality [12]. Konisky and Burdick (2004) [15] documented that only 50% of narrowleaf cattail (*Typha angustifolia*) transplants grown at the salinity of 10‰ in the field experiment were survived. Therefore, waterlogged and low nutrient concentration in water and sediment was not only the cause of growth and survival rate reduction in *Typha orientalis* [10], but salinity also might be the cause. The average of alkalinity in all the treatments was in the range of 101.8-143.7 $mgCaCO_3/L$ ($p<0.05$; Figure. 5D) which were in the permitted limit (60.0-180 $mgCaCO_3/L$) for requirement on shrimp rearing water quality [12].

3.2.3. Nitrogen and phosphorus concentrations in the water

Nitrite nitrogen (NO_2-N) is toxic to shrimp and exposure to high concentrations may cause retarded growth and mortalities [16]. The average NO_2-N concentrations in the treatments tended to reduce over time and increase again at the last sampling time ($p<0.05$; Figure 6A). Similar trend was observed in nitrate nitrogen (NO_4-N), ammonium nitrogen (NH_4-N) and total kjeldahl nitrogen (TKN) concentrations ($p<0.05$; Figure 6B, 6C & 6D) that reflected debris of plants and microorganism body degraded causing water pollution again. The treatments with +45 cm water level had the highest average NO_2-N concentrations. In general, water NO_2-N concentrations in the treatments were in the range of 0.03-0.3 mg/L, which was in the permitted range for normal shrimp growth [14]. However, NO_2-N concentration the in the unplanted treatments was slightly higher which was the same finding reported by Doan *et al.* (2016) [7] who using constructed wetlands planted *Typha orientalis* to purify intensive whiteleg shrimp wastewater. Gross *et al.* (2004) [16] suggested a safe concentration for whiteleg shrimp production in the ponds to be less than 0.45 mg/L NO_2-N.

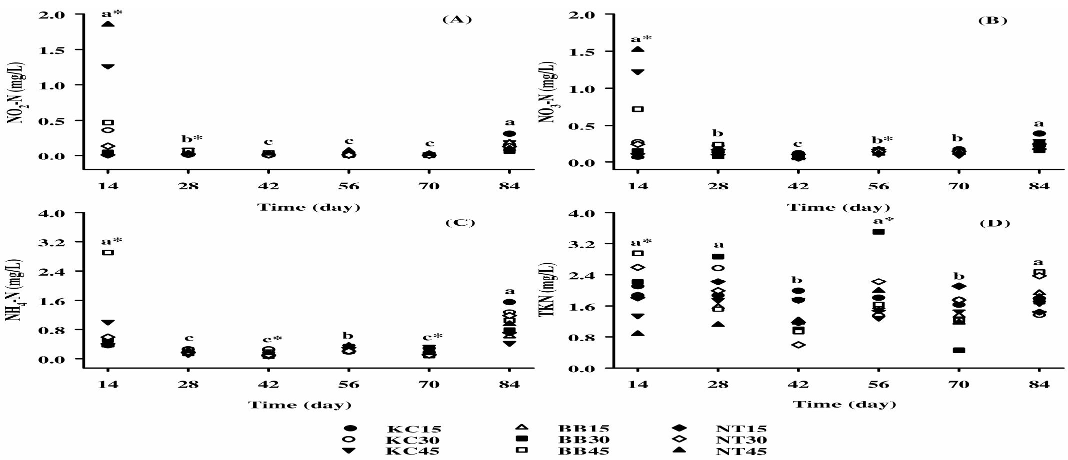

Figure 6. Nitrite nitrogen (A), nitrate nitrogen (B), ammonum nitrogen (C) and total kjeldahl nitrogen (D) in the water over time of *T. orientalis* (BB), *S. littoralis* (NT) and unplanted (KC) at +15, +30 and +45 cm water levels

Notes: Values are the means of 3 replicates. Asterisk (*) indicates significant difference between treatments within sampling time. Different letter [a,b,c] indicates significant difference between sampling times based on a Tukey HSD test ($p<0.05$).

The NO_3-N concentration is not harmful for shrimp growth, however, higher accumulation of NO_3-N might be toxic to shrimp [17]. In this study, NO_3-N concentration likely reduced overtime, except for the last sampling ($p<0.05$; Figure 6B). The NO_3-N concentrations in the treatments were negligible ranging from 0.14 to 0.37 mg/L. The NH_4-N concentrations in the treatments were in the range of 0.3-0.8 mg/L and the planted treatments had lower NH_4-N concentrations compared to the unplanted treatments ($p<0.05$; Figure 6C). The planted treatments with +15 and +30 cm water levels had lower NH_4-N concentrations because the plants grew well in these treatments resulting in a better NH_4-N uptake rate by the plants. This led to the lower TKN concentrations in those treatments too. However, TKN concentrations across treatments highly fluctuated and increased at the end of the study ($p<0.05$; Figure 6D) that might be due to algae and the death plant tissues returned nitrogen back into the water environment.

The concentrations of orthophosphate (PO_4-P) and total phosphorus (TP) of all the treatments had the same trend of reduction over time but increased at the last sampling point ($p<0.05$; Figure 7A & 7B). There were significant differences between the treatments within sampling time. Although P is not harmful to aquatic animals, P supports algae and aquatic plants growth. The increase in water level from +15 to +45 cm P concentrations increased. Rubio *et al.* (1997) [18] found that waterlogged soils increased the ability of plants to uptake phosphorus and could increase the soil phosphorus availability. However, repeated flooding can result in a phosphorus release from soils, which introduces additional phosphorus to soils and waterways [19, 20].

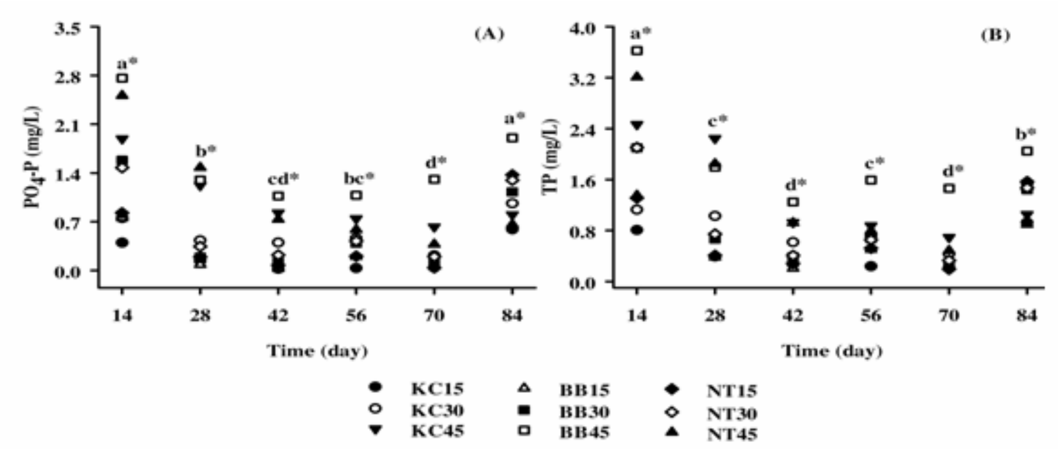

Figure 7. Orthophosphate (A) and total phosphorus (B) in the water over time of *T. orientalis* (BB), *S. littoralis* (NT) and unplanted (KC) at +15, +30 and +45 cm water levels

Notes: Values are the means of 3 replicates. Asterisk (*) indicates significant difference between treatments within sampling time. Different letter [a,b,c] indicates significant difference between sampling times based on a Tukey HSD test (p<0.05).

In sum, the concentrations of the tested water quality parameters across the treatments meet the Vietnamese standards requirement on shrimp rearing water quality. The treatments with plants helped to maintain better quality than that of the unplanted treatments. Water quality was relevant to plant growth response to the water levels. *Typha orientalis* grew better in the water levels of +15 and +30 cm that led to the lower concentrations of nitrogen and phosphorus in the water.

4. CONCLUSION

In conclusion, the growth and biomass production responses of *T. orientalis* and *S. littoralis* was different at the water levels ranging from +15 to +45 cm. *Scirpus littoralis* grew well with lower survival rate and had no statistical reduction of biomass compared to *T. orientalis* at the highest water level of +45 cm while *T. orientalis* had the best perfromance in growth and biomass at water levels of +15 cm and +30 cm. The concentrations of the tested water quality parameters across the treatments meet the Vietnamese standards requirement on shrimp rearing water quality that can be reused for shrimp ponds. The treatments with plants helped to maintain better quality than that of the unplanted treatments. The results indicated that *S. littoralis* was the best of choice to grow in waterlogged condition of the shrimp pond for maintaining water quality. However, further studies must be conducted using a commercial shrimp pond or a settlement pond to grow *S. littoralis* together and/or separate to further assess the feasibility of using this species.

ACKNOWLEDGEMENTS

This work was financially supported by the project grant B2016-TCT-10ĐT from Ministry of Education and Training of Vietnam. The authors thank to the Department of Environmental Sciences for providing laboratories which enable us to complete our study.

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