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EFFECTS OF PHOSPHORUS IN THE WASTEWATER FROM INTENSIVE CATFISH FARMING PONDS ON THE GROWTH AN PHOSPHORUS UPTAKE OF *Hymenachne acutigluma* (Stued.)

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ABSTRACT

Hymenachne aquatic grass, *Hymenachne acutigluma* was planted in the wastewater from intensive striped catfish (*Pangasianodon hypophthalmus)* cultivating ponds containing 2.1 mg N/L, which was enriched with a serious of inorganic phosphorus (P) concentrations. The experiment was arranged in a completely randomized design with three replications in the net house for 42 days. *The results* showed that P concentrations did not significantly affect the growth of *Hymenachne*. The presence of high P concentrations resulted in the increase of P content in plant tissues leading to higher P absorption at the P levels of 8 and 10 mg P/L. *H. acutigluma* removed 12.1–27.6% P from 88.3–95.9% P in the *w*astewater of striped catfish pond. This result indicated the low concentrations of N (2.1 mg N/L) and of 1–10 mg P/L were not optimal for the growth of *H. acutigluma*.

Keywords: *Hymenachne acutigluma*, biomass, nutrient uptake, phosphorus, striped catfish, wastewater.

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INTRODUCTION

Striped catfish farming is an important agriculture sector in the Mekong Delta in Vietnam. In 2016, catfish farming area was estimated at 5500 ha which provided 1.1 million tonnes of catfish annually (MARD 2016). To produce 1 tonne of catfish, 9133.3 m^3 of wastewater containing 9.1 kg phosphorus (P) was discharged into the water bodies (Anh et al., 2010) causing eutrophication. Therefore, P in catfish wastewater should be treated prior to discharging into water bodies for the sustainable aquaculture development.

Hymenachne acutigluma is an emergent and perennial aquatic plant. It adapts well to waterlogged areas (4 m in depth) and infertile acid soils. Moreover, it can be used as fodder with 0.16–0.20% P content in plant tissue (Cameron & Lemcke, 2003). *H. acutigluma* produces high biomass with about 4.86 tonnes/ha dry weigh in average after 90 days of planting and 45 days of regeneration (Nhan et al., 2014). In addition, *H. acutigluma* reduced 84.8–95.6 and 85.7–92.5% of TP (total phosphorus) and $PO_4^{3-}-P$ in wastewater from *s*triped catfish farming ponds with 5–40 mg N/L and 1.36 mg P/L, respectively

(Le Diem Kieu et al., 2015). Therefore, using *H. acutigluma* as a phytoextractor to remove nutrients in aquaculture wastewater is an environmentally friendly approach. However, information about P concentration affecting on the growth and nutrient uptake of *H. acutigluma* is limited. The aim of this paper is to assess the effects of P concentrations on the growth in *H. acutigluma* in the wastewater from intensive catfish cultivating ponds. For this purpose, the wastewater from intensive catfish cultivating pond was spiked with various concentrations of potassium phosphate (as a P source). We hypothesized that *H. acutigluma* grows better and accumulates higher P in its tissues at higher P concentrations in liquid media.

MATERIALS AND METHODS

Experimental set-up

The experiment was conducted at the net house in the campus of Dong Thap University, Cao Lanh city, Dong Thap province, Vietnam, from March to April, 2015. The basic growth solution was wastewater from striped catfish cultivation pond, which contains 2.1 mg N/L and 0.47 mg P/L. The liquid medium was supplemented with potassium phosphate at five concentrations (1, 2, 4, 8 and 10 mg P/L) and controls without addition of P. All treatments were arranged in a completely randomized design with three replications for 42 days. Initial concentrations of $PO_4^{3-}-P$, TP, NH_4^+-N, NO_2^--N, NO_3^--N and total Kjeldahl Nitrogen (TKN) in the catfish pond wastewater were 1.16 ± 0.03, 1.36 ± 0.07, 0.95 ± 0.04, 0.33 ± 0.023, 0.21 ± 0.03 and 1.51 ± 0.10 mg/L, respectively.

H. acutigluma was collected from a field in Hoa An Commune, Cao Lanh city and placed in tanks containing catfish-cultivated wastewater to adapt for 2 weeks. An initial 360 g fresh weight of total 12 individual plants of *H. acutigluma* was placed in a 45 L plastic pot (L × W × H: 60 × 40 × 24 cm).

Plant growth, biomass and phosphorus (P) concentration

The shoot height, root length and fresh weight were measured prior to transplant them into pots. After 42 days, the plants were harvested, rinsed thoroughly with deionized water, and then fractionated into shoots (stalks, leaves and flowers) and roots to determine the fresh and dry mass after drying at 60°C until the weight became constant. Water samples in the culture pots were collected every 14 days. P contents in plant tissues and in water samples were determined using the ascorbic acid method (APHA, 1998).

Data analysis

Relative growth rates (RGR) of biomass:

$$RGR = \frac{\ln W_1 - \ln W_2}{t_1 - t_2} \quad \text{(Coombs et al., 1985)} \qquad (1)$$

Where, W_1, W_2 were dry biomass of plants at the beginning (t_1) and at the end (t_2) of the experiment.

The amount of P accumulation in plants:

$$M_A = C_E \times W_E - C_I \times W_I \qquad (2)$$

Where, M_A was amount of P accumulation in plants; C_I, C_E were P content in plant tissues at the beginning and the end of the experiment, respectively; W_I, W_E were dry plant weight at the beginning and the end of the experiment, respectively.

Phosphorus use efficiency (PUE)

$$PUE = \frac{W}{M_A}(g\,DW / g\,P) \qquad (3)$$

(DW: dry weigh) (Steinbachová-Vojtíšková et al., 2006; Zhang et al., 2007; Rose & Wissuwa, 2012).

Two-way analysis of variance (ANOVA) using Type III sum of squares was used to determine the effects of P concentrations on plant growth and tissue P content. Post-hoc Tukey 5 for all statistical analyses. Pearson correlation and multivariate regression were also determined. The Sigmaplot software version 12.5 was used to plot figures.

RESULTS AND DISCUSSION

Plant growth and biomass

Although shoot height, root length, leave numbers of *H. acutigluma* were significantly different, statistically significant differences were not found among P levels enriched for all the growth parameters at the harvest time (Fig. 1). It was shown that the highest concentration of P (10 mg P/L) with 2.1 mg N/L did not increase the growth of Hymenachne grass after 42 days. This indicated that P was not a limiting factor for growth of *H. acutigluma*. According to Mao et al. (2015), only supplementation with 0, 1.2, 4.8 and 9.6 g P m^2/year to growth media, *Deyeuxia angustifolia* had lower aboveground biomass than that of initial plants.

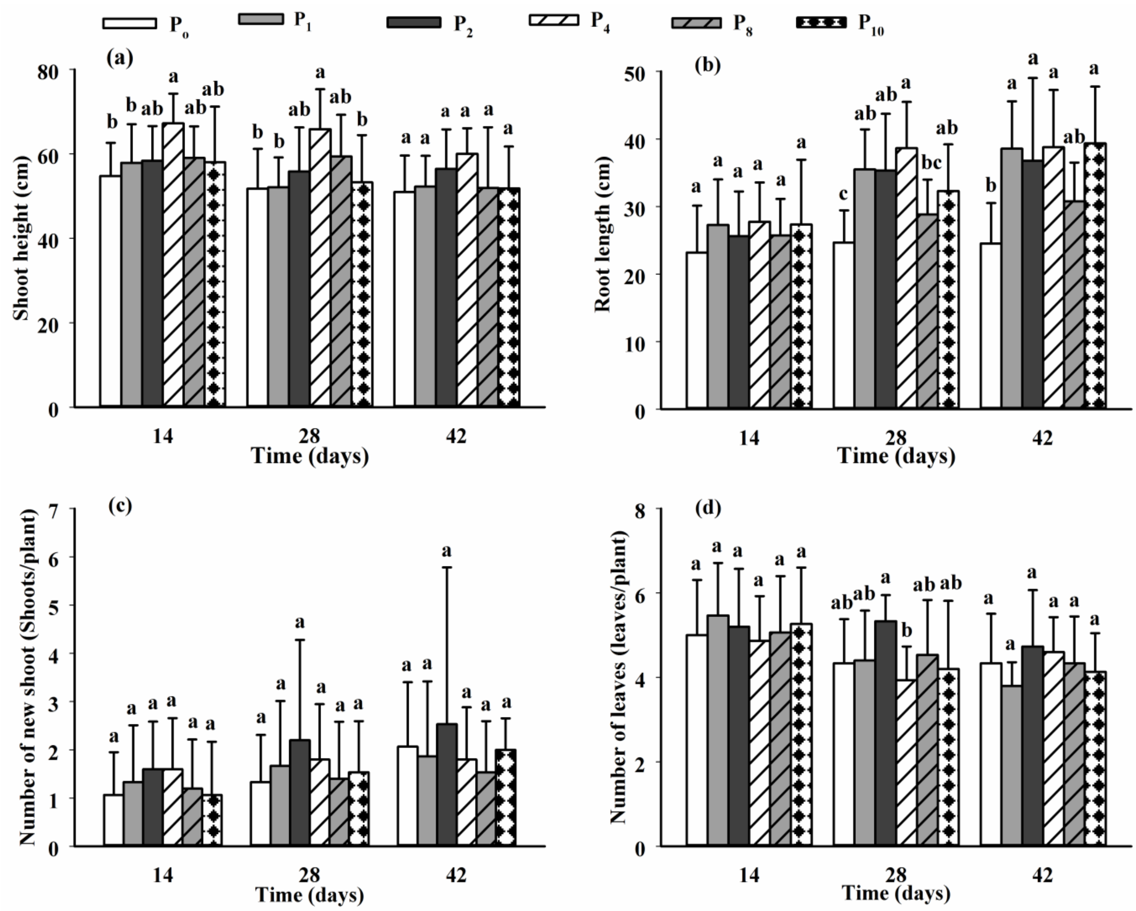

Figure 1. Effects of P levels on (a) shoot height, (b) root length, (c) new shoots numbers and (d) leave numbers of *H. Acutigluma*

Notes: Bars (Mean ± S.D., n = 15) with different letters (a, b and c) indicate signifcant differences among treatments in the same time ($p < 0.05$, Tukey test)

Similarly, fresh and dried biomass and RGR of biomass were not affected by P concentrations from 1 to 10 mg/L ($p > 0.05$, table 1), but these parameters in P8 treatment were higher than in the controls ($p < 0.05$, table 1). Biomass was not correlated with P concentrations in wastewater ($p > 0.05$, table 2). At the low (0.03 mg P/L) and the high (0.1 mg P/L) P concentrations, *Ludwigia peploides* and *Ludwigia grandiflora* cultivated on soil had RGR of biomass ranging from 13–21 and 23–32 mg/g/day, respectively (Gérard et al., 2014), which were higher than those of *H. acutigluma* in this study. These results indicated that 10 mg P/L might be not sufficient for the optimum growth of *H. acutigluma*. Biomass and RGR of biomass in this study (Table 2) were lower than those of *H. acutigluma* in catfish wastewater supplemented with various concentrations of N (5–40 mg N/L) with low P level (1.16 mg P/L) (Le Diem Kieu et al., 2015) and concentrations of N (30–120 mg N/L) with low P level (5–20 mg P/L) (Le Diem Kieu et al., 2018). These data suggest that N was the limiting factor for the growth of *H. acutigluma* rather than P. N concentration dependent growth and biomass of the plant was demonstrated by Elser et al. (2007) and Lewis & Wurtsbaugh (2008). Likewise & Romero et al. (1999) concluded that N concentration in water influenced the RGR of *Phragmites australis* while P concentration did not. Zhang et al. (2008) also confirmed that the aboveground biomass of *Canna indica* was not influenced by P concentration.

Table 1. Fresh and dry biomass and RGR of *Hymenachne grass cultivated with different P-levels*

Treatments	Fresh biomass (g/plant)	Dry biomass (g/plant)	RGR (mg/g/day)
P_o	32.2 ± 1.3^{b}	4.3 ± 0.1^{b}	4.4 ± 0.7^{b}
P_1	41.1 ± 1.9^{ab}	6.0 ± 0.5^{ab}	12.3 ± 1.9^{ab}
P_2	42.5 ± 9.6^{ab}	6.4 ± 1.3^{ab}	13.6 ± 4.9^{ab}
P_4	41.8 ± 8.2^{ab}	6.4 ± 1.6^{ab}	13.3 ± 6.0^{ab}
P_8	51.4 ± 8.0^{a}	7.8 ± 1.1^{a}	18.3 ± 3.3^{a}
P_{10}	41.9 ± 3.9^{ab}	6.2 ± 0.7^{ab}	13.1 ± 2.6^{ab}

*Different small superscript letters (a, b and c) indicate statistically significant differences ($p < 0.05$) in the same treatment groups (within a column). Data are means of the results from at least three individual experiments, and mean values and standard deviations are shown

Table 2. Pearson correlation coefficient

	P concentration (mg/L)	Biomass (g DW/plant)	P content in shoot (%)	P content in root (%)	P accumulation (mg/pot)
Biomass (g DW/plant)	0,254				
P content of shoot (%)	$0,927^{**}$	0,067			
P content of root (%)	$0,909^{**}$	0,401	$0,833^{**}$		
P accumulation (mg/pot)	$0,917^{**}$	$0,534^{*}$	$0,871^{**}$	$0,925^{**}$	
PUE (g DW/g P)	$-0,661^{**}$	0,122	$-0,784^{**}$	$-0,603^{*}$	$-0,608^{*}$

Notes: *Correlation was significant at the 0.05 level, **Correlation was significant at the 0.01 level (2-tailed)

Phosphorus (P) content and accumulation in the plant

Plants absorb and assimilate nutrients from water to produce their biomass which contributes to refresh water. Dry biomass of *H. acutigluma* was not affected by P concentrations in water (Table 2). However, the P contents in the shoot and root tissues increased in proportion to P concentrations ($r_p = 0.927$, $r_p = 0.909$; $p < 0.01$; table 2). P contents in the tissues of *Deyeuxia angustifolia* and *Glyceria spiculosa* increased with the addition of P in growth solution (Mao et al., 2015).

The amount of P accumulated in *H. acutigluma* was calculated by the regression equation (4).

$P_{accumulation}$ (mg/plant) = 0.497 × $P_{concentration}$ (mg/L) + 1,153 ($r^2 = 0.841$; $p < 0.05$) (4)

Although the P levels in cultivating water did not affect dry biomass of *H. acutigluma*, P was accumulated in their tissues in a dose-dependent manner with the highest P accumulation at the P8 and P10 treatments ($p < 0.05$; Fig. 2b and Table 2). The amount of P accumulated in the roots of *H. acutigluma* was influenced by the concentration of P (Le Diem Kieu et al., 2018). Chen et al. (2008) also reported dose-dependent P accumulation in the tissues of *Rhynchospora tracyi* cultivated at varying P concentrations in the growth media.

The phosphorus use efficiency (PUE) of *H. acutigluma* in the P1 treatment was significantly higher than that of the other treatments ($p < 0.05$, Fig. 2c) and was negatively correlated with P concentrations in water (Table 2). Lorenzen et al. (2001) presented that *Cladium jamaicense* and *Typha domingensis* also had a decrease of PUE when P concentrations in growth media was increased from 0.01 to 0.5 mg/L.

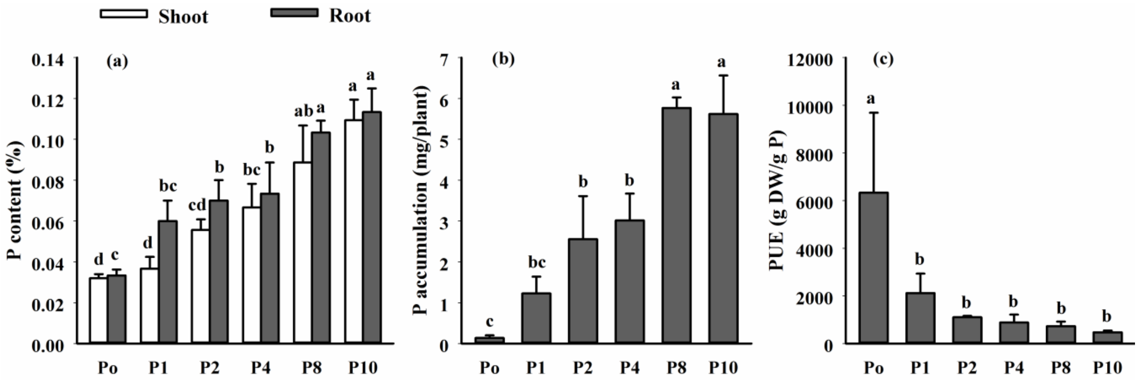

Figure 2. The mean of (a) P content, (b) P accumulation and (c) PUE of *H. acutigluma* planted in various P levels in water

Notes: Bars (Mean ± S.D., n = 3) with different letters (a, b and c) indicate signifcant differences among treatments (p < 0.05, Tukey test)

Phosphorus (P) mass balance

The P removal efficiency (ratios of P in the effluent to P in the influent) was from 88.3 to 95.9% after 42 days. *H. acutigluma* reduced 12.1–27.6% P in the water by absorption and accumulation in plant biomass (Table 3). P lost was probably due to P accumulated in microorganisms, sedimentation and lost during water sampling.

Table 3. Mass balance of P in water and *H. acutigluma* after 42 days

	Input (mg/pot)			Output (mg/pot)			Unaccounted
Treatments	**Water$^{(1)}$**	**Plant$^{(2)}$**	**Total**	**Water$^{(3)}$**	**Plant$^{(4)}$**	**Total**	**(mg/pot)**
P_0	26.1	15.1	41.2	6.3 ± 1.4	16.7 ± 0.8	23.0 ± 2.2	18.2 ± 2.2
P_1	55.5	15.1	70.6	6.5 ± 5.1	29.8 ± 4.9	36.3 ± 3.9	34.3 ± 3.9
P_2	111.0	15.1	126.1	4.6 ± 0.8	45.7 ± 12.7	50.3 ± 13.5	75.8 ± 13.5
P_4	222.0	15.1	237.1	17.3 ± 9.1	51.2 ± 7.9	68.6 ± 17.0	168.5 ± 17.0
P_8	444.0	15.1	459.1	43.3 ± 6.4	84.3 ± 3.1	127.5 ± 5.7	331.5 ± 5.7
P_{10}	555.0	15.1	570.1	110.9 ± 11.4	82.5 ± 11.3	193.4 ± 15.8	376.7 ± 15.8

Notes: (^{1}) Sum of P concentrations in water at the beginning; (^{2}) P content of the initial plants; (^{3}) Sum of P concentrations in water at harvest; (^{4}) P content of harvested plant biomass. Mean ± S.D., n=3

CONCLUSION

The low concentration of N of 2.1 mg N/L and varying concentrations of P (1–10 mg P/L) in the catfish pond wastewater were not optimal for the growth and biomass of *H. acutigluma*. P content and accumulation in plant tissues increased in a dose dependente manner. The plant of *H. acutigluma* resulted in the P removal in the wastewater from intensive striped catfish cultivating ponds.

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