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Full Length Research Paper

Interactive Influence of N and P on their uptake by four different hydrophytes

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The uptake kinetics of nitrogen (N) and phosphorus (P) by hydrophytes can be influenced by the interaction between N and P. In this study, *Pistia stratiotes* (a floating plant), *Eichhornia crassipes* (a floating plant), *Vallisneria spiralis* (a submerged plant), and *Cyperus papyrus* (an emergent plant) were selected to measure the uptake kinetics of N and P. The results indicated that the values of V_{max} and K_m of P were between 0.29 and 0.88 μmolg^{-1}fwh^{-1} and 12 and 5.7 μmol L^{-1}, respectively, and that they varied with different plant species. *V. spiralis* had the greatest V_{max} and the smallest $K_{m.}$ As for *P. stratiotes*, N and P uptake kinetics were influenced by interaction of N and P. Moreover, it was also found that peroxidase (POD) activity and nitrate reductase activity (NRA) were influenced. The NRA of *P. stratiotes* was found to increase first and then decrease with increasing P content, while the P content affected peroxidase (POD) activity in *P. stratiotes.* Both NRA and POD could indicate the rates and abilities of nutrient removal of aquatic plants.

Key words: aquatic plants, kinetics, V_{max}, $K_{m,}$ interaction, peroxidase, nitrate reductase, mechanisms.

INTRODUCTION

Nutrient enrichment of water leads to deterioration of the environment and sometimes even to eutrophication, especially in densely populated areas (Vollenweider et al., 1969; Hosper, 1984). Ecologists have made great efforts to prevent the decline in environmental Project supported by the Foundation for Key Program of Ministry of Education, China (Grant No. 106088 and The Six Top Talents of Jiangsu Province) quality by controlling exacerbation in different ways, including physical dredging, ecological projects, etc. (Schnoor et al., 1995; Zhang et al., 1998). Since the 1970s, many researchers have emphasized biological function and proposed biomanipulation, which stresses management of the entire ecosystem to a dominant eutrophication form of nutrition (Reddy and DeBusk, 1985; Gersberg et al., 1986; Hammer, 1989; Shaver and Melillo, 1984). The high productivity and nutrient removal capability of aquatic plants have created substantial interest for wastewater treatment (Bastviken et al., 2005; Huett et al., 2005; Nahlik and Mitsch, 2006). There are three types of aquatic plants: submerged plants, emergent plants and floating plants. Previous stu-dies have shown that all of them are very efficient in absorbing nutrients excreted into water from various sources (Carpenter and Lodge, 1986; Chiang et al., 2000; Janjit et al., 2007).

However, estimates of the nutrient removal by the plants differ. For example, Mars et al. (1999) found that *Triglochin huegelii,* a submerged plant found in Western Australia, had consistently higher concentrations of nitrogen and phosphorus in wastewater treatment experiments than *Schoenoplectus validus,* an emergent plant commonly used for wastewater nutrient stripping. For another example, in Janjit's study (2007), among the experimental plants, the mean N removal rates ranged from 45.5 to 134.11 mgNm$_{ap}^{-2}$day^{-1} or 6.4-68.3 mgNkg$_{ap}^{-1}$day^{-1}, and P removal rates ranged from 40.6 to 1190.6 mgPm$_{ap}^{-2}$day^{-1} or 5.4-59.9 mgPkg$_{ap}^{-1}$day^{-1}. Those values suggest that nutrient uptake rates and abilities of aquatic plants vary considerably with different kinds of aquatic plants. To further detect the variety, Michaelis–Menten kinetics of nutrient removal was determined for 4 plant

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species of the three aquatic plants in this study. The four plants were *Pistia stratiotes* (a floating plant), *Eichhornia crassipes* (a floating plant), *Vallisneria spiralis* (a submerged plant), and *Cyperus papyrus* (an emergent plant).

Nitrate reductase (NR) (EC 1.6.6.1) is an inducible enzyme, and there is a close relationship between NR activity and nitrate concentration in plants (Skrdleta et al., 1979; Chen et al., 2004). Determining the nitrate reductase activity (NRA) is a useful technique to assess the degree of utilization of nitrate by a plant. NRA values vary with plant species (Alexandre et al., 2004). NRA has been extensively studied in terrestrial plants (Huber et al., 1992; Kaiser et al., 1999; Fan et al., 2002; de la Haba et al., 2001; Chen et al., 2004) and in aquatic plants (Cedergreen et al., 2003). Peroxidase (POD) (EC 1.11.1.7) is ubiquitous in the plant kingdom (Duarte et al., 2002; Robinson, 1991). It was found that peroxidase activity is strongly related to the tolerance capabilities of aquatic macrophytes (Roy et al., 1992). According to Rout et al. (2001), increases in POD activity in response to NaCl were positively correlated with the salt tolerance of a plant. To the best of our knowledge, the study of NRA and POD have been conducted on the four plants to detect possible mechanisms of varieties of kinetics in interaction substrate between N and P by *P. stratiotes* and of difference in the uptake kinetics of nitrogen and phosphorus by the four hydrophytes.

In spite of the knowledge on the individual hydrophytes in the uptake kinetics of phosphorus (Perez-Liorens et al., 1995; Amy et al., 2003; Shen et al., 2006), very little is known about the difference of the uptake kinetics on phosphorus by different types of hydrophytes affected by nitrogen. In this study, Michaelis-Menten kinetics of N and P by *P. stratiotes* in interaction substrate of the two elements and of P by *P. stratiotes* (a floating plant), *E. crassipes* (a floating plant), *V. spiralis* (a submerged plant), and *C. papyrus* (an emergent plant) were determined. The objective of this study was to provide quantitative data that could help offer an understanding of the differences in V_{max} and K_m among different types of aquatic plants so that the most effective aquatic plants can be utilized to remove nutrients from wastewater or natural water. Meanwhile, the kinetics of *P. stratiotes* was investigated more deeply than the other three in interaction substrate between N and P to know the removal of N and P influenced mutually by the two elements. Moreover, NRA and POD activity were determined to detect the possible mechanisms of difference in the uptake kinetics of phosphorus by the four hydrophytes.

MATERIALS AND METHODS

Sample collection and preparation

The plants, *P. stratiotes*, *E. crassipes*, *V. spiralis*, and *C. papyrus* were collected in June, 2006 from Yileen Garden in Nanjing, China (118°E, 32°N). The plants were uprooted from water to prevent the roots and rhizomes from damage, and were transported in a chamber full of water to the laboratory (Amy et al., 2003), where they were gently cultivated with intact roots and rhizomes into 490 x 350 x 260 mm boxes. These plants were refreshed for 14 days prior to the experiment in aerated one tenth Hoagland solution in a greenhouse with natural light and temperature. During the course of refreshment, the hydroponic culture was replaced every 3 days, and the pH was adjusted to about 5.6 with HCl or NaOH. Following refreshment, the plants were left for 3 days in hydroponic culture without nitrogen and phosphorus.

After this 3-day famine, the plants were soaked and watered three times with 0.2 mmol L^{-1} $CaSO_4$ solution, and then were placed into 500 ml plastic beakers containing 100 ml solutions of various treatments for 4 h absorption (Gras et al., 2003; Zhang et al., 2004) with 4000lux and 28°C. After the uptake experiment, the roots were harvested and wiped water to a constant weight, and the fresh weights were recorded. Each beaker was wrapped with opaque plastic membrane.

Treatments

Potassium dihydrogen phosphate (KH_2PO_4) was used as the source of phosphorus. Potassium nitrate and ammonium sulfate were used as the sources of NO_3^- and NH_4^+ to maintain a ratio of 1:1. The pH of the uptake solution was adjusted to 5.6 using 0.1 mmol/l HCl. For *P. stratiotes,* eight nitrogen concentrations (0, 0.1, 0.6, 6, 10, 20, 40, and 60 mmol/l) and three phosphorus concentrations (0.1, 0.6, 6 mmol/l) were used, along with a blank control. For *E. crassipes*, *V. spiralis,* and *C. papyrus*, the phosphorus concentrations were 0.1, 0.3, 0.6, 1, 3, 6, and 10 mmol/l and the nitrogen concentrations were 0.6, 6, and 60 mmol/l. These seven different treatments of phosphorus with excess nitrogen were tested on *E. crassipes*, *V. spiralis,* and *C. papyrus*, namely, phosphorus concentration: 0, 0.1, 0.3, 0.6, 1, 3, 6, 10 mmol/l with nitrogen concentration of 6 mmol/l. Each level was repeated three times.

P and N uptake

The amounts of P and N uptake by the plants were calculated from the decrease of P and N in solution. The uptake amounts of P and N per plant (P_iP and N_iP) were calculated using the following equation (Wu et al., 2006):

$$P_iP = (CPi_b - CPi_f) \times V_u/T$$

Where V_u is the volume of the solution at the beginning of experiment, T is the absorption time and CP_{ib} and CP_{if} are the P concentrations at the beginning and end of the experiment, respectively.

P uptake per unit root fresh weight (P_iR) was calculated using this equation (Wu et al., 2006):

$$P_iR = P_iP/ RFW$$

Where RFW is the root fresh weight per plant. The P concentration in the solution was determined using the ammonium molybdate spectrophotometric method (Wei, 1997). The calculation for P_iR was also applied to N_iR (N uptake per unit root fresh weight). The concentrations of NO_3^- and NH_4^+ in the solution were determined using the spectrophotometric method with phenol disul-fonic acid and Nessler's reagent colorimetric method, respectively (Wei, 1997).

The interactions of P and N in the four species of aquatic plants were described according to Michaelis-Menten kinetics. To obtain values of K_m and V_{max} from straight lines on the graph, the Michaelis-Menten equation can be transformed (Yu et al., article in press) into the most commonly used form, double-reciprocal plot (Henderson, 1992):

Table 1. Michaelis–Menten kinetic parameters of phosphorus: V_{max} and K_m, with asymptotic standard errors of the parameters in parentheses.

Nitrogen concentration (mmol/L)	V_{max} (µmol.g^{-1}.fw.h^{-1})	K_m (µmol/L)	R^2	*P*-level
0	0.33(0.05)	12.5(2.1)	0.97	0.01
0.6	0.39(0.07)	10.4(1.6)	0.92	0.01
6	0.43(0.12)	8.5 (1.7)	0.97	0.01
60	0.39(0.03)	7.7 (0.9)	0.96	0.01

$$\frac{1}{I} = \frac{k_m}{V_{\max}}\left(\frac{1}{C}\right) + \frac{1}{V_{\max}}$$

The slope of this linear equation is K_m/V_{max}, the intercept on the 1/ coordinate is $1/V_{max}$, and the deduced 1/C intercept is $-1/K_m$. In most conditions, the drawback of the Lineweaver-Burk plot is that experimental measurements of C and are not distributed evenly on the graph (Yu et al., article in press).

Study of protein

Frozen material (0.5 g) of nitrate reductase (NR) was ground in a mortar and homogenized with 4 mL 50 mmol/l HEPES KOH (pH7.5) buffer, containing 0.5 mmol/l EDTA, 5.5 mmol/l $MgCl_2$, 14 mmol/l β-mercaptoethanol, 0.1% Triton X100 (v/v), 10% glycerol (v/v), 10% polyvinyl-pyrrollidone (w/v), 50 µM leupeptin, and 0.5 mmol/l PMSF. After the mixture was centrifuged at 5000×*g* for 20 min at 4℃, the supernatant of the sample was assayed for NRA (Leleu et al., 2000). The NRA was expressed as µmol NO_2^-·min^{-1}·mg^{-1} DW.

Peroxidase (POD) was extracted from 1.0 g shoot (fresh weight) after the shoot sample was ground in a mortar and homogenized with 5 ml 50 mmol/l phosphate buffer (pH 7.0). The extract was then centrifuged at 10,000 g for 10 min, and the supernatant was used to determine POD activity. For the measurement of guaiacol-dependent peroxidase activity, the reaction mixture contained 50 mmol/l phosphate buffer (pH 7.0), 50 mmol/l guaiacol, 10 mmol/l H_2O_2, and enzyme. The activity was determined by measuring the increase in absorbance at 470 nm due to guaiacol oxidation (Nakano and Asada, 1981; Tao et al., 2007).

Statistical analysis

The data were subjected to one-way analysis of variance to compare the results from different types of aquatic plants. Differences between individual means were tested using Least significance Difference (LSD) tests at 0.05 significance level.

RESULTS

Nitrogen and phosphorus uptake kinetics

Effect of interaction between N and P on the P uptake kinetics of the floating plant (*P. stratiotes*)

Phosphorus uptake in *P. stratiotes* increased with time at all levels of P and under various N concentrations. However, as the N concentration changed, the values of V_{max} and K_m varied (Figure 1 and Table 1). When the N concentration was 6 mmol L^{-1}, the V_{max} of *P. stratiotes* was approximately 10 - 30% higher than at other N concentrations, which were higher than the control. Half saturation constants (K_m) for the control and N concentration of 0.6 mmol L^{-1}, however, were generally higher than those at 6 and 60 mmol L^{-1} N levels, indicating a low P affinity under low N concentrations (Table 1).

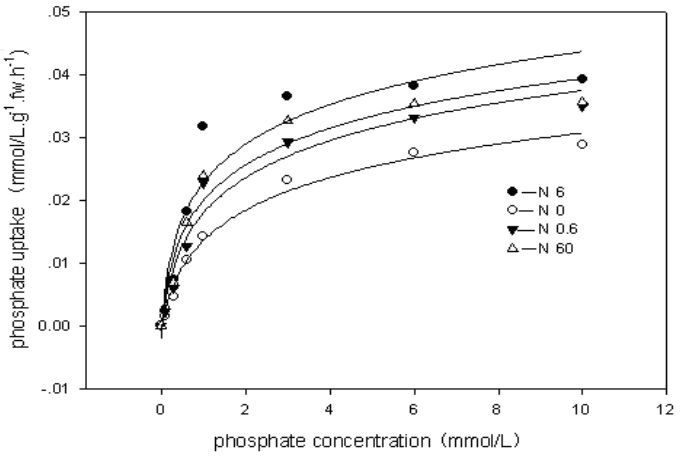

Figure 1. P_i uptake rates (µmol g^{-1} fw h^{-1}) by *P. stratiotes* root. Curves represent best fit using Michaelis-Menten kinetics. N0, N0.6, N6 and N60 refer to different N levels.

Effect of interaction between N and P on the N uptake kinetics of the floating plant (*P. stratiotes)*

Nitrogen uptake in *P. stratiotes* increased with time at all eight levels of N concentrations and various P concentrations. However, the V_{max} and the K_m values for NO_3^- and NH_4^+ varied with different P concentrations (Figures 2 and 3, and Table 2). When the P concentration was at 0.6 mmol L^{-1}, the N uptake of *P. stratiotes* demonstrated a higher V_{max} value for NO_3^- and NH_4^+ than that at other P concentrations. At 0.6 mmol L^{-1}, the V_{max} value for NO_3^- was approximately 5 - 33% greater than at other P concentrations, while the V_{max} value of NH_4^+ was approximately 7 - 16% greater than at other P concentrations, indicating that the V_{max} value for NO_3^- was more influenced by P concentration than that for NH_4^+ in the plant. The V_{max} values for NO_3^- were similar to each other

Table 2. Michaelis–Menten kinetic parameters of NO_3^- and NH_4^+. V_{max} and K_m, with asymptotic standard errors of the parameters in parentheses.

Phosphate concentration (mmol/L)	V_{max} (μmol.g^{-1}.fw.h^{-1})		K_m (μmol/L)		R^2	
	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
0	4.6(0.12)	5.6(0.36)	3.99(0.81)	2.91(0.13)	0.96	0.98
0.1	5.8(0.31)	6.1(0.12)	3.49(0.23)	2.27(0.09)	0.97	0.95
0.6	6.1(0.55)	6.5(0.25)	3.20(0.12)	1.98(0.07)	0.97	0.94
6	5.9(0.16)	6.1(0.34)	3.28(0.13)	1.93(0.09)	0.94	0.93

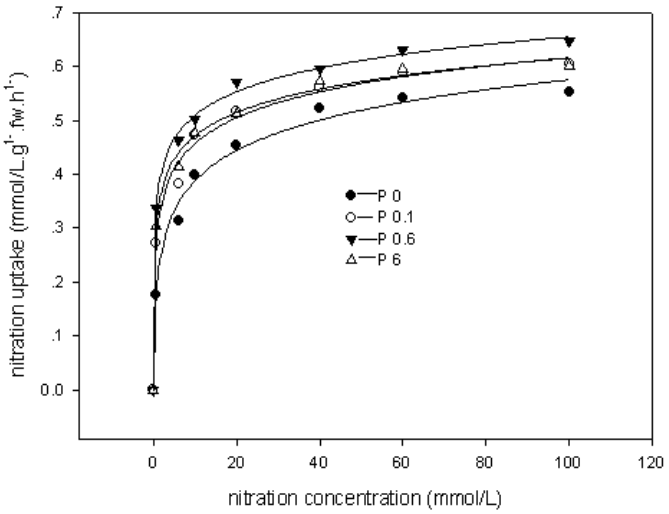

Figure 2. NH_4^+ uptake rates (μmol g^{-1} fw h^{-1}) by *P. stratiotes* root. Curves represent best fit using Michaelis-Menten kinetic. P0, P0.1, P0.6 and P6 refer to different N levels.

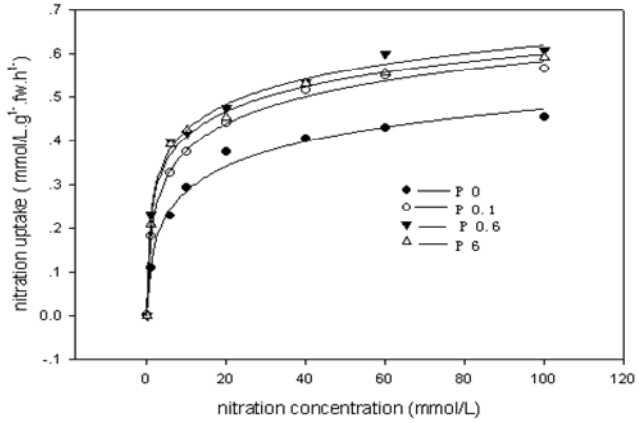

Figure 3. NO^{3-} uptake rates (μmol g^{-1} fw h^{-1}) by *P. stratiotes* root. Curves represent best fit using Michaelis-Menten kinetic. P0, P0.1, P0.6 and P6 refer to different N levels.

under both 0.1 and 6 mmol L^{-1} P, while the V_{max} value was the lowest when there was no P applied. The trend was also applicable to the pattern of V_{max} for NH_4^+. Half saturation constants (K_m) for both NO_3^- and NH_4^+ in the 0 - 0.1 mmol L^{-1} P range were greater than those at the 0.6 – 6 mmol L^{-1} P range.

Effect of N on the P uptake kinetics of all three types of hydrophytes

The existence of nitrogen in the substrate affected P-uptake of different types of hydrophytes roots. Phosphorus uptake by the roots of these hydrophytes was enhanced with increasing P concentration (Figure 4). However, the different types of hydrophytes demonstrated different V_{max} and K_m values. For example, the V_{max} for *V. spiralis* was significantly different from the V_{max} of *Cyperus papyrus* ($P < 0.05$). For the other types, there was no remarkable difference in V_{max}, between *P. stratiotes* and *E. crassipes*. The K_m values of the plants showed a similar trend to those of V_{max} (Table 3).

Effects of N and P interaction on nitrate reductase and peroxidase in hydrophytes

Nitrate reductase activity in different types of hydrophytes

High nitrate reductase activity (NRA) was observed in all the experimental plants, although levels of NRA were different in different plants and at different P concentrations (Figure 5). The NRA level of *V. spiralis* was 1.8 times greater than those of other plants when N concentration was 6 mmol L^{-1} without P applied. Similarly, the NRA of *V. spiralis* increased from 0 to 6 mmol L^{-1} P, while it decreased from 6 to 10 mmol L^{-1} P. The NRA levels in *E. crassipes* and *P. stratiotes* showed similar patterns with increasing P concentrations. However, the highest NRA levels of *E. crassipes* and *P. stratiotes* were slightly significantly lower ($P < 0.05$) than that of *V. spiralis*. Interestingly, NRA in *C. papyrus* increased steadily regardless of P concentration.

Peroxidase in different types of hydrophytes

Significantly higher peroxidase (POD) activity levels were found in *V. spiralis* than in all the other plants ($P < 0.05$)

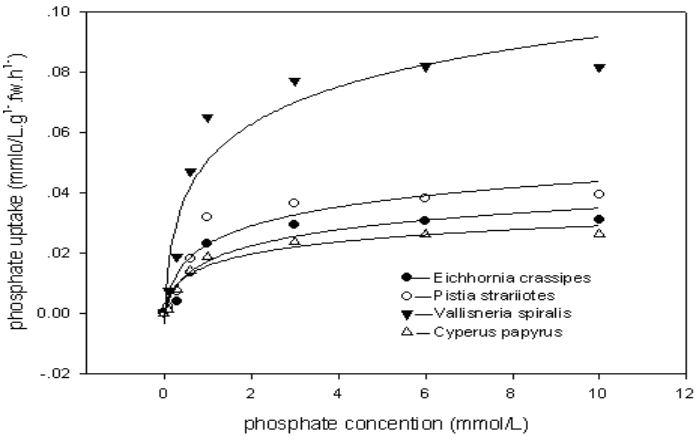

Figure 4. P uptake rates (µmol $g^{-1}fwh^{-1}$) of different types of hydrophytes roots affected by N in substrate. Curves represent best fit using Michaelis-Menten kinetic.

Table 3. Michaelis–Menten kinetic parameters of phosphorus: *V*max and *K*m, with asymptotic standard errors of the parameters in parentheses.

Aquatic species	V_{max} (µmol.g^{-1}.fw.h^{-1})	K_m (µmol/L)	R^2
E. crassipes	0.36(0.12)	12(3.2)	0.94
P. stratiotes	0.43(0.12)	8.5(1.7)	0.94
V. spiralis	0.88(0.21)	5.7(1.1)	0.91
C. papyrus	0.29(0.09)	8.4(0.9)	0.96

(Figure 6). The lowest level of enzyme activity was detected in *C. papyrus*. However, no significant difference in peroxidase activity was observed between *E. crassipes* and *P. stratiotes*, which belong to the same hygrophyte type. With increasing P concentration, peroxidase activity in the four plants increased at 0 – 0.6 mmolL^{-1} P and then decreased at 0.6 – 10 mmol L^{-1} P (Figure 6). For example, the peroxidase activity of *V. spiralis* at 0.6 mmol L^{-1} was approximately 2.4 times greater than that at 0 mmol L^{-1} P. Meanwhile, the POD at 10 mmol L^{-1} P was 71% lower than that at 0.6 mmol L^{-1} P (Figure 6).

Nitrate reductase activity in P. stratiotes

Figure 7 shows that N level affected NRA in *P. stratiotes* significantly. When there was no N in the solution, NRA stayed at a low level regardless of P concentration. However, NRA at 6 mmol L^{-1} N was greater than at other nitrogen concentrations (Figure 7). NRA demonstrated smooth change at low N levels and remarkable change at high N levels. At 0 mmol L^{-1}N, NRA was approximately 1.12 µmol/l.NO_2^-.h^{-1}.g^{-1}.FW, as compared to 2.41 µmol/l.NO_2^-.h^{-1}.g^{-1}.FW at 0 mmol L^{-1} and 3.62 µmol/l.NO_2^-.h^{-1}.g^{-1}.FW at 0.6 mmol L^{-1} P when N was 60 mmol L^{-1}.

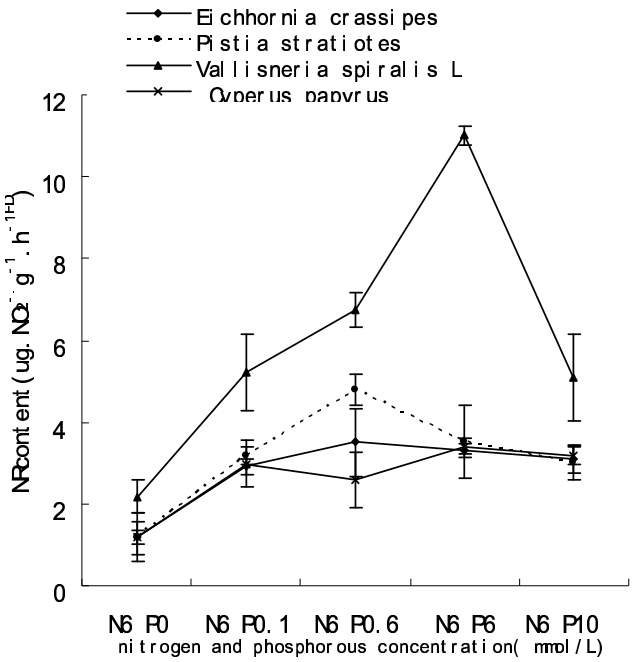

Figure 5. Effects of nitrogen and phosphorous interaction concentrations on nitrogen reductase activity in different kinds of hydrophytes. The error bars are SD.

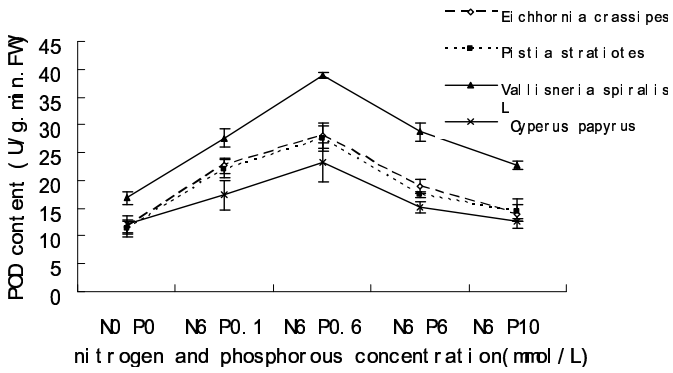

Figure 6. Effects of nitrogen and phosphorous interaction concentrations on peroxidase of different kinds of hydrophytes .The error bars are SD.

Peroxidase in P. stratiotes

Significantly higher peroxidase activity was found at 0.6 mmol L^{-1} N than at any other N concentration ($P < 0.05$). The lowest enzyme activity was detected at the 0 mmol L^{-1} N level. For example, when P concentration was 0.6 mmol L^{-1}, the POD content was 30.55 $U.min^{-1}$.FW at 0.6 mmol L^{-1}N, 1.9 times greater than that at 0 mmol L^{-1} N and 1.6 times greater than that at 60 mmolL^{-1} N (Figure 8). There was no significant difference in POD observed between 0 and 60 mmol L^{-1} N at 0 - 0.6 mmol L^{-1} P levels and between 0.6 and 6 mmol L^{-1} N at 6 – 10 mmol L^{-1} P levels.

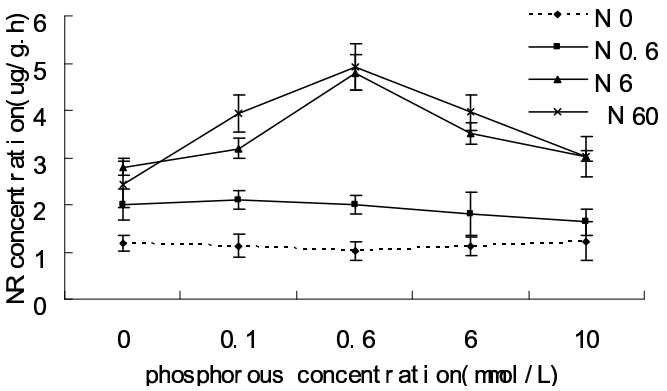

Figure 7. Effects of nitrogen and phosphorous interaction concentrations on nitrogen reductase activity of *Pistia stratiotes*. The error bars are SD.

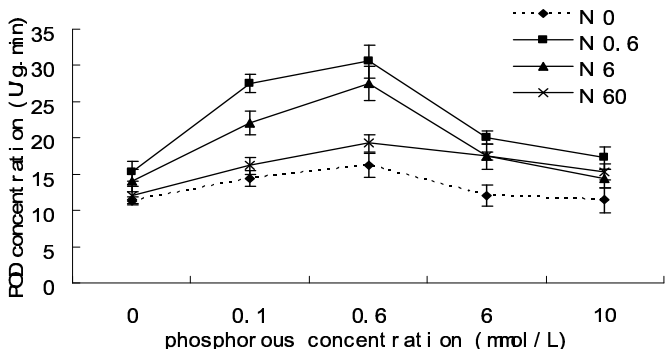

Figure 8. Effects of nitrogen and phosphorous interaction concentrations on peroxidase of *Pistia stratiotes*. The error bars are SD.

DISCUSSION

Shen (2006) used a similar technology to determine K_m and V_{max} for duckweed (*Spirodela oligorrhiza*). The values of half saturation constant, K_m, were approximately 5.890 mmol L^{-1} for NH_4^+ and 7.123 mmol/l for NO_3^-; and the maximum absorption capacity, V_{max}, was about 0.00712 $mmolg^{-1}FWh^{-1}$ for NH_4^+ and 0.01255 $mmolg^{-1}FWh^{-1}$ for NO_3^-. In a similar study, Gras et al. (2003) determined K_m as 12.4 - 1.05 µmol/l of P. In this study, the Michaelis-Menten kinetics of P and N removal were determined. The value of V_{max} for P was between 0.29 and 0.88 $\mu molg^{-1}FWh^{-1}$, and the value of K_m was between 12 and 5.7 µmol L^{-1}. These values varied with plant species (Figure 4 and Table 3).

The value of V_{max} represents the plant's ability to absorb a nutrient. The higher the V_{max} value, the more could be absorbed. The value of K_m corresponds to the plant's affinity for a certain element, and the lower the K_m value, the greater the affinity. In our study, the V_{max} value of *V. spiralis* (the submerged plant) was 0.88 $\mu molg^{-1}$ FW h^{-1}, the highest V_{max} (*P < 0.05) among the experimental plants (Table 3), indicating that *V. spiralis* absorbs more phosphorus than the other experimental plants. Furthermore, the K_m value of *V. spiralis* was 5.7 µmol L^{-1}, the smallest among the plants, confirming that *V. spiralis* has a greater affinity for P than the other plants. The significant difference between these two K_m values indicates that different types of hydrophytes have different P affinity (Figure 4 and Table 3). Yu et al. (2006) found that different cultivars had different Michaelis-Menten kinetics in the same substrate. In this study, there was same conclusion that the V_{max} and K_m values of *P. stratiotes* and *E. crassipes,* both floating plants, varied even at the same N treatment concentration. For example, the V_{max} values of *P. stratiotes* and *E. crassipes* were 0.36 and 0.43 $\mu molg^{-1}FWh^{-1}$, respectively.

In a eutrophic water body, N and P coexist simultaneously. In Tsutomu's research (1988), it was found that the removal efficiency of P was influenced by N concentration in the solution. Our results indicate that the uptake kinetics of *P. stratiotes* is affected by the interaction between N and P. It was found that different P levels resulted in different V_{max} and K_m values for both NO_3^- and NH_4^+ (Figures 2 and 3, and Table 2) and vice versa (Figure 1 and Table 1), indicating that the removal of N and P were mutually influenced.

Nitrate reductase activity might decrease with decreasing nitrate-N content in solution. Our results showed that the nitrate-N level significantly affected the NRA level in *P. stratiotes* (Figure 7). At 60 mmol L^{-1} N, NRA was greater than at all other N concentrations. Moreover, NRA first increased and then decreased as P content increased, indicating that excessive P could degrade the plant's ability to absorb N. In our study, the highest NRA was observed in *V. spiralis* regardless of P content (Figure 5), although NRA increased between 0 and 6 mmol L^{-1}P, and then decreased between 6 and 10 mmol L^{-1}P. However, for *C. papyrus*, NRA was almost steady regardless of P content. It was concluded that *V. spiralis* was the strongest plant among the hydrophytes to absorb NO_3^- and to withstand adversity, consistent with its V_{max} and K_m, i.e. the NRA value could reflect the rates and abilities of nutrient removal of aquatic plants.

Peroxidase is an important protective enzyme and belongs to enzymatic antioxidant systems. Many studies have shown that adversity can induce POD activity (Tao et al., 2007; Shu and Chen, 1999). The highest enzyme activity in *V. spiralis* was found when the ratio of carbamide and total N was 1/3 in solution, which protected the structure and function of the plant's membrane to combat the adversity (Zhu et al., 2005). However, increasing carba-mide in solution resulted in a low peroxidase activity, suggesting that *V. spiralis* had a certain ability to withstand the adversity, but the ability was restrained by heavy adversity. In our study, the peroxidase activity in *V. spiralis* was the highest among the four plants (Figure 6), implying that *V. spiralis* was most able to resist adversity, consistent with its V_{max} and K_m. That is, as with NRA, the

value of the POD also could indicate the rates and abilities of nutrient removal of aquatic plants. Meanwhile, under different N treatments, the highest peroxidase activity was found at 0.6 mmol L^{-1} N ($P < 0.05$), indicating that a proper N content might alleviate the toxicity of superfluous P and improve the removal efficiency of P on *P. stratiotes.*

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