UPTAKE KINETICS OF NH_4^+, NO_3^- AND $H_2PO_4^-$ BY SUBMERGED MACROPHYTES *ELODEA NUTTALLII* (ST. JOHN, 1920) AND *VALLISNERIA NATANS* (JUSSIEU, 1826)

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Abstract. The source of lake eutrophication comes from the excessive input of nitrogen and phosphorus and their uncoordinated proportion, while nitrogen and phosphorus are essential elements for the growth of aquatic plants. Using aquatic plants to treat sewage has incomparable advantages compared with chemical and physical methods. It not only makes the whole process is derived for natural behavior, but also improves landscape and avoids new environmental problems. Studying the uptake kinetics of NH_4^+, NO_3^- and $H_2PO_4^-$ of aquatic plants can help us to select the proper aquatic macrophyte according to the status of eutrophic water. In June 2017, the absorption kinetics of NH_4^+, NO_3^- and $H_2PO_4^-$ by Elodea nuttallii and Vallisneria natans under the eutrophication stress were studied in indoor simulation experiments at the plant nutrition laboratory of Huazhong Agricultural University of China. The results showed that the V_{max} absorbed by NH_4^+ of *Elodea nuttallii* and *Vallisneria natans* at three phosphorus levels decreased with the increase of P concentration. The Km value increased with the increase of P concentration. On three phosphorus levels, in two plants the V_{max} of NO_3^- decreased with the increase of P concentration. The Km value of NO_3^- absorbed by *Elodea nuttallii* at different levels of phosphorus decreased with the increase of P concentration. The Km of NO_3^- on *Vallisneria natans* increased with the increase of P concentration. The V_{max} of $H_2PO_4^-$ of *Elodea nuttallii* and *Vallisneria natans* at three nitrogen levels decreased with the increase of N concentration. The Km value of *Elodea nuttallii* and *Vallisneria natans* at three nitrogen levels increased with the increase of N concentration. In eutrophication environment, the stress of submerged plants will affect their nutrient absorption. The nutritive stress of low N,P increased the absorption potential of N,P. High NP nutrition stress reduced the absorption rate and affinity.
Keywords: *aquatic macrophyte, stress, eutrophication, nitrogen and phosphorus, uptake kinetics*

Introduction

Lake eutrophication is one of the hot topics in the world today. Nitrogen and phosphorus are two important factors for the eutrophication of freshwater lakes, and they are also the main nutrient elements of plants. The high concentration of nitrogen and phosphorus in water can also cause the imbalance of physiological functions of submerged macrophytes, inhibit their growth and even lead to decline (Cao and Ni, 2004; Been, 2001; Arts, 2002). The latest research shows that the high concentration of nitrogen in lake water is closely related to the degeneration of submerged macrophytes in shallow lakes (Sagrario et al., 2005). High concentration of ammonium nitrogen has attracted much attention due to its physiological stress on aquatic plants (Carr et al., 1997; Xiong et al., 2010; Farnsworth-Lee and Baker, 2000). The direct absorption of nitrogen and phosphorus by plants in water is one of the main mechanisms of eutrophication. At present, the eutrophication management with aquatic plants as the core has become a research hotspot (Huang et al., 2010), especially the restoration of submerged plants, as an important measure to restore the aquatic ecosystem and purify the water eutrophic substances, has attracted more and moreattention (Jiang et al., 2004;

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Wang et al., 2008). Kinetic method is an effective way to study the characteristics of plant nutrient absorption. The study of nutrient absorption kinetics began in the early 1950s. Epstein and Hagen (1952) first used the kinetic equation of enzymatic reaction to study the absorption of ions by plants. In 1978, Nielsen and Barber (1978) revised the Michaelis-Menten equation and put forward the concept of the lowest equilibrium concentration, so that the equation can quantitatively describe the characteristics of root absorption of nutrients. In terms of research methods, Classen and Barber (1974) first established the exhaustion method in 1974 to obtain various parameters of the kinetic equation, i.e. after hydroponics of plants, the dynamic changes of ion concentration in the culture solution that decrease with time were measured.

On the basis of the kinetic theory of enzymatic reaction, the absorption kinetics of nitrogen and phosphorus in a variety of submerged plants was studied. The mechanism of removing nitrogen and phosphorus from water bodies was further clarified, and the theoretical and practical significance of selecting aquatic plants in the ecological restoration project for the eutrophic water bodies with different nutritional characteristics was carried out (Chang et al., 2008; Han and Yu, 2017).

At present, there are many studies on the absorption kinetics of nitrogen, phosphorus and other nutrient pollutants in submerged plants. The effects of temperature, light and pH on the uptake of nitrogen and phosphorus in aquatic plants were mostly concentrated (Tang et al., 2011; Shen et al., 2006; Jampeetong and Brix, 2009). Liu et al. (2009) have studied the *Potamogeton malaianus* on the absorption kinetics of ammonia nitrogen. Chang and other studies have focused on the absorption kinetics of ammonia nitrogen and nitrate nitrogen by *Jussiaea stipulaceaohwe*, *Elodea nuttallii* and *Eichhornia crassipes* of hyacinth (Chang et al., 2008). Chen et al. (2012) have studied the pollution absorption dynamicsof *Hydrilla verticillata* and *Vallisneria asiatica* on nitrogen and phosphorus. Zhang et al. (2013) studied the absorption kinetics of ammonia by *Vallisneria natans* and *Myriophyllum spicatum* under light and dark conditions. The interaction between nitrogen and phosphorus, two important factors of eutrophication in freshwater lakes, has been neglected.

Vallisneria asiatica and *Elodea nuttallii* are common submerged macrophytes in lakes and rivers in the middle and lower reaches of Yangtze River. Their ecological adaptability is wide, their ability to adsorb pollutants is strong, and their community is easy to rebuild. Therefore, in this study, *Elodea nuttallii* and *Vallisneria asiatica* are selected as research object. Studying the effects of nitrogen and phosphorus absorption kinetics under different eutrophic conditions is to provide a theoretical basis for selecting suitable plants for the remediation of water bodies with different pollution levels.

Materials and methods

Experimental materials

Vallisneria asiatica and *Elodea nuttallii* were taken from Liangzi Lake in China (*Fig. 1*). Each plant was taken about 1000 g. Simulation experiment was completed at the plant nutrition laboratory of Huazhong Agricultural University of China in June 2017. The test was carried out in a glasshouse with a temperature of 22°C-26°C, light intensity of 5000 LX and a relative humidity of 70%-80%.The plants were washed with distilled water and then cultured in 1/8 Hoagland nutrient solution (pH 6.5) for 20 d. Replace the nutrient solutions per 3 d. After water culture for 20 d, the plants with good

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growth were selected for nutrient absorption test. Remove plants from the nutrient solution, rinse the plants with deionized water and then transfer to 0.2 mmol.L^{-1} $CaS0_4$ solution. Pre-culture in a greenhouse for 24 h to make it hungry.

Figure 1. *Plant sampling point*

Absorption test

The experiment set up 3 levels of P, to determine kinetics parameters of NH_4^+ and NO_3^- in two plants under different P stress conditions. The experiment also set 3 levels of N, to determine the kinetic parameters of $H_2PO_4^-$ uptake by two plants under different N stress conditions. The design concentration of NH_4^+, NO_3^- and $H_2PO_4^-$ as shown in *Table 1*.

Table 1. *The concentration of NH_4^+, $N0_3^-$ and $H_2P0_4^-$*

	P1	P2	P3		N1	N2	N3
NH_4^+ 1mg.L^{-1}	0.04	0.08	0.5	**$H_2PO_4^-$** 0.01mg.L^{-1}	0.4	1.2	3.6
NO_3^- 1mg.L^{-1}	0.04	0.08	0.5				

The absorption kinetics parameters of NH_4^+, NO_3^- and $H_2PO_4^-$ were determined by conventional exhaustion. The 10 g plants after starvation were moved into the triangular bottle equipped with 250 mL curture solution for absorption experiments. 3 repetitions were set in each absorption test. The experiment was carried out in an artificial climate incubator with a temperature of 25°C, a light intensity of 5000 LX, and a relative humidity of 75%. The absorption liquid samples were taken at 0 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, respectively. After sampling for 1 ml, 1 ml of deionized water was added. The plant was taken out immediately after the sampling was completed. The moisture content on the surface of plants is dried up by filter paper , then measuring fresh weight. The absorption curve was drawn according to the concentration and absorption time of the culture solution. Then the kinetic parameters are obtained according to the absorption curve equation.

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Determination method

The concentration of NH_4^+ was determined by indolo blue colorimetry. The concentration of NO_3^- was determined by Ultraviolet spectrophotometry. $H_2PO_4^-$ concentration was determined by molybdate ammonium spectrophotometric method (State Environmental Protection Administration 2002 in Chinese).

Calculation of dynamic parameters

Firstly, the equation of ion consumption curve is obtained. The commonly used equation is univariate quadratic polynomial:

$$Y=a+bX+cX' \qquad \text{(Eq.1)}$$

In the equation, X is the absorption time and Y is the ion concentration. The first derivative of the equation is obtained to obtain a concentration change rate equation:

$$Y'=b+2cX \qquad \text{(Eq.2)}$$

Equation 2 is treated as follows: x → 0, at this time, y' = b, thus,the maximum rate of change of concentration is obtained. By considering the volume and root weight of the absorbent liquid and using the formula V_{max}=b×V/ fresh weight, the maximum absorption rate per unit weight can be obtained. V_{max} reflects the inherent potential of plants to absorb nutrients. Y' = 1 / 2b is substituted into *Equation 2* to find x, and x is replaced by *Equation 1* to find Y. Y is the Km value (concentration of medium at 1 / 2 V_{max}). Km represents the affinity of the absorption system. The small Km value indicates that the root absorption system has great affinity for this ion.

Method of data analysis

Data processing using double reciprocal transformation of Michaelis Menten dynamic equation (Epstein and Hagen, 1952). Then calculate the kinetic parameters Vmax and Km. The data are processed by double reciprocal transformation by using Michaelis-Menten dynamics equation. The dynamic parameters V_{max} and Km are obtained. All the data were processed and analyzed by Excel 2003 software.

Results

The effect of P on the kinetics of absorption of $NH_4^+$by twoaquatic macrophytes

NH_4^+ absorption concentration of *Elodea nuttallii* and *Vallisneria natans* changes with time, as show in *Figure 2*. Elodea has the fastest absorption rate in the first hour. After 3 h, the absorption rate tends to be stable. The rate of NH_4^+ uptake by 2 water plants decreased with the increase of P concentration. Through the absorption curve of two plants, the ion depletion equation of NH_4^+ was obtained after fitting (*Table 2*). The kinetic parameters of NH_4^+ in plant absorption were obtained according to the ion depletion equation (*Table 3*). From *Table 3*, we can see that the maximum absorption rates of NH_4^+ are 5.055 $ug^{-1}.g^{-1}.h^{-1}$, 5.022 $ug^{-1}.g^{-1}.h^{-1}$ and 4.902 $ug^{-1}.g^{-1}.h^{-1}$, respectively. The maximum absorption rates of NH_4^+ V_{max} of *Vallisneria natans* are 4.83 $ug^{-1}.g^{-1}.h^{-1}$, 4.57 $ug^{-1}.g^{-1}.h^{-1}$ and 4.33 $ug^{-1}.g^{-1}.h^{-1}$. V_{max} has a tendency to decrease with the increase of P concentration. The maximum absorption rate V_{max} of *Elodea nuttallii* to NH_4^+

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decreased slightly with the increase of P concentration. The maximum absorption rate V_{max} of *Vallisneria natans* to NH_4^+ decreases greatly with the increase of P concentration, which is about 5%. The Km values of NH_4^+ absorbed by *Elodea nuttallii* are 164.8688 ug.L^{-1}, 1206.3271 ug.L^{-1} and 240.0115 ug.L^{-1}, respectively. Km values of NH_4^+ uptake by *Elodea nuttallii* under P2 and P3 are 25.15% and 45.58%, which are higher than those under P1, respectively. Km value of NH_4^+uptake by *Elodea nuttallii* under P3 condition is 16.33%, which is higher than that under P2 condition. The absorption of NH_4^+ Km values of *Vallisneria natans* are 176.1859 ug.L^{-1}, 201.9644 ug.L^{-1} and 215.2731 ug.L^{-1}. Km values of NH_4^+ uptake by *Vallisneria natans* under P2 and P3 are 14.63% and 22.19%, which are higher than that under P1, respectively. Km value of NH_4^+ uptake by *Vallisneria natans* under P3 condition is 6.58%, which is higher than that under P2 condition. The Km value has a tendency to increase with the increase of P concentration.

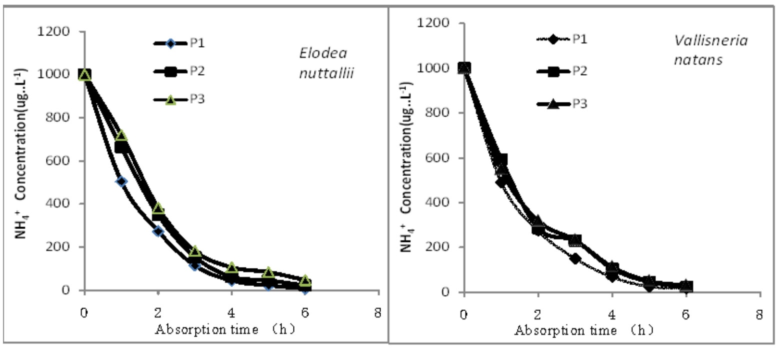

Figure 2. *Changes of NH_4^+ concentrations in culture solution for the two aquatic macrophytes P1-0.04 mg.L^{-1}, P2-0.08 mg.L^{-1}, P3-0.5 mg.L^{-1}, n=3*

Table 2. *Equations of NH_4^+ concentration versus uptake time for aquatic macrophytes*

	Elodea nuttallii	*Vallisneria natans*
P1	$y = 70.27x^2 - 504.5x + 844.0$	$y = 66.49x^2 - 483.0x + 834.3$
P2	$y = 68.15x^2 - 507.2x + 914.1$	$y = 60.02x^2 - 457.0x + 864.4$
P3	$y = 64.96x^2 - 490.2x + 933.6$	$y = 57.93x^2 - 443.5x + 851.9$

P1-0.04 mg.L^{-1}, P2-0.08 mg.L^{-1}, P3-0.5 mg.L^{-1}, n=3

Table 3. *Kinetic parameters of NH_4^+ absorption by the two aquatic macrophytes*

Plant species	Vmax (ug^{-1}.g^{-1}.h^{-1})				Km (ug.L^{-1})			
	P1	P2	P3	R^2	P1	P2	P3	R^2
Elodea nuttallii	5.045	5.022	4.902	0.9652	164.8688	206.3271	240.0115	0.9832
Vallisneria natans	4.83	4.57	4.33	0.9768	176.1859	201.9644	215.2731	0.9796

P1-0.04 mg.L^{-1}, P2-0.08 mg.L^{-1}, P3-0.5 mg.L^{-1}, n=3

The effect of P on the kinetics of absorption of $NO_3^-$by twoaquatic macrophytes

The NO_3^- concentration of *Elodea nuttallii* and *Vallisneria natans* varies with the liquid absorption time, as show in *Figure 3*. With the prolongation of absorption time, the concentration of NO_3^- in absorption solution decreases. The absorption of NO_3^- by

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Vallisneria natans in the first 2 hours 1s faster than that of *Elodea nuttallii*. Through the absorption curves of the two plants, the ion depletion equation for absorbing NO_3^- is obtained after fitting (*Table 4*). From *Table 5*, we know that V_{max} to NO_3^- of *Elodea nuttallii* are 2.45 mg.g^{-1}.h^{-1}, 2.26 mg.g^{-1}.h^{-1} and 1.97 mg.g^{-1}.h^{-1}, respectively. V_{max} of NO_3^- absorbed by *Elodea nuttallii* under P2 and P3 are 7.76% and 19.59%, which are lower than that under P1, respectively.

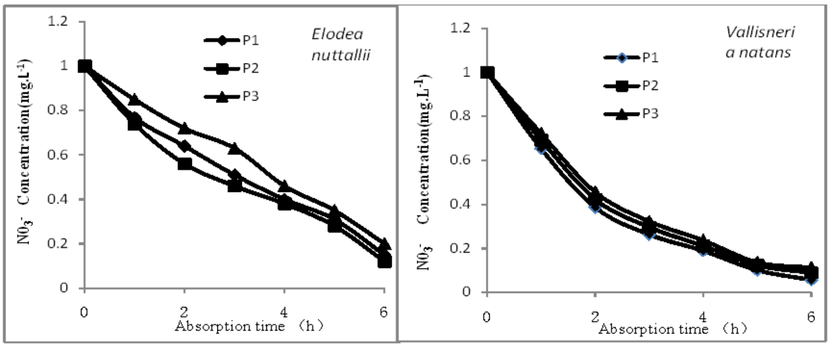

Figure 3. *Changes of NO_3^- concentrations in culture solution for the two aquatic macrophytes P1-0.04 mg.L^{-1}, P2-0.08 mg.L^{-1}, P3-0.5 mg.L^{-1}, n=3*

Table 4. *Equations of NO_3^- concentration versus uptake time for aquatic macrophytes*

	Elodea nuttallii	*Vallisneria natans*
P1	$y = 0.019x^2 - 0.245x + 0.929$	$y = 0.052x^2 - 0.417x + 0.890$
P2	$y = 0.023x^2 - 0.266x + 0.907$	$y = 0.050x^2 - 0.407x + 0.909$
P3	$y = 0.009x^2 - 0.197x + 0.962$	$y = 0.048x^2 - 0.396x + 0.923$

P1-0.04 mg.L^{-1}, P2-0.08 mg.L^{-1}, P3-0.5 mg.L^{-1}, n=3

Table 5. *Kinetic parameters of NO_3^-absorption by the two aquatic macrophytes*

Plant species	Vmax (mg^{-1}.g^{-1}.h^{-1})				Km (mg.L^{-1})			
	P1	P2	P3	R^2	P1	P2	P3	R^2
Elodea nuttallii	2.45	2.26	1.97	0.981	0.3366	0.3102	0.1534	0.9758
Vallisneria natans	4.17	4.01	3.86	0.969	2631	0.2878	0.3104	0.9699

P1-0.04 mg.L^{-1}, P2-0.08 mg.L^{-1}, P3-0.5 mg.L^{-1}, n=3

The V_{max} of NO_3^- absorbed by *Elodea nuttallii* under P3 condition is 12.83%, which is lower than that under P2 condition. The V_{max} of NO_3^- on *Vallisneria natans* are 4.17 mg.g^{-1}.h^{-1}, 4.01 mg.g^{-1}.h^{-1} and 3.86 mg.g^{-1}.h^{-1}. V_{max} values of NO_3^- absorbed by *Vallisneria natans* under P2 and P3 are 3.83% and 7.43%, which are lower than that under P1, respectively. The V_{max} value of NO_3^- absorbed by *Vallisneria natans* under P3 condition is 3.74%, which is lower than that under P2 condition. The V_{max} of the two plants decreased with the increase of P concentration. The Km values of NO_3^- absorbed by *Elodea nuttallii* are 0.3366 mg.L^{-1}, 0.3102 mg.L^{-1} and 0.1534 mg.L^{-1}, respectively. Km values of NO_3^- absorbed by *Elodea nuttallii* under P2 and P3 are 7.84% and 54.43%, which are lower than that under P1, respectively. The Km value of NO_3^- absorbed by *Elodea nuttallii* under P3 condition is 50.55%, which is lower than that

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under P2 condition. The Km value of *Elodea nuttallii* decreases with the increase of P concentration. The absorption of NO_3^- *Vallisneria natans* Km values are 0.2631 mg.L^{-1}, 0.2878 mg.L^{-1} and 0.3104 mg.L^{-1}. Km values of NO_3^- absorbed by *Vallisneria natans* under P2 and P3 conditions are 9.39% and 17.98%, which are higher than that under P1 conditions, respectively. The Km value of NO_3^- absorbed by *Vallisneria natans* under P3 condition is 7.85%, which is higher than that under P2 condition. The Km value of *Vallisneria natans* increases with the concentration of P and the rising trend.

The effect of N on the kinetics of absorption of $H_2PO_4^-$ by two aquatic macrophytes

Figure 4 shows that the ion concentration in solution decrease with the increase of absorption time. Because of plant starvation, the absorption rate of ions is the fastest within 2 h after the start of the experiment. According to *Figure 3*, the ion depletion equation of two plants is derived (*Table 6*). Then the kinetic parameters are obtained (*Table 7*). From *Table 7*, we know that the maximum absorption rates (V_{max}) of *Elodea nuttallii* to $H_2P0_4^-$ are 0.29 mg.g^{-1}.h^{-1}, 0.25 mg.g^{-1}.h^{-1}, and 0.23 mg.g^{-1}.h^{-1}, respectively. V_{max} values of $H_2P0_4^-$ absorbed by *Elodea nuttallii* under P2 and P3 conditions are 13.79% and 20.69%, which are higher than that under P1 conditions, respectively. V_{max} value of $H_2PO_4^-$ absorbed by *Elodea nuttallii* under P3 condition is 8.0%, which is higher than that under P2 condition. The maximum absorption rates (V_{max}) of *Vallisneria natans* to $H_2P0_4^-$ are 0.25 mg.g^{-1}.h^{-1}, 0.22 mg.g^{-1}.h^{-1}, 0.18 mg.g^{-1}.h^{-1}. V_{max} values of $H_2P0_4^-$ absorbed by *Vallisneria natans* under P2 and P3 conditions are12.0% and 28.00%, which are lower than that under P1 conditions, respectively. V_{max} value of $H_2P0_4^-$ absorbed by *Vallisneria anatans* under P3 condition is 18.18%, which is lower than that under P2 condition. The V_{max} of the two plants decreases with the increase of N concentration. The Km values of $H_2P0_4^-$ absorbed by *Elodea nuttallii* are 0.0132 mg.L^{-1}, 0.0158 mg.L^{-1} and 0.0172 mg.L^{-1}, respectively. Km values of $H_2P0_4^-$ absorbed by *Elodea nuttallii* under P2 and P3 are 19.70% and 30.30%, which are higher than that under P1, respectively. Km value of $H_2P0_4^-$ absorbed by *Elodea nuttallii* under P3 condition is 8.86%, which is higher than that under P2 condition. The Km values of the absorption of $H_2PO_4^-$ on *Vallisneria natans* are 0.0242 mg.L^{-1}, 0.0380 mg.L^{-1} and 0.0441 mg.L^{-1}, respectively. The Km values of $H_2P0_4^-$ absorbed by *Vallisneria natans* under P2 and P3 conditions are 57.02% and 82.23%, which are higher than that under P1 conditions, respectively. The Km value of $H_2PO_4^-$ absorbed by *Vallisneria natans* under P3 condition is 16.05%, which is higher than that under P2 condition. The Km value has a tendency to increase with the increase of N concentration.

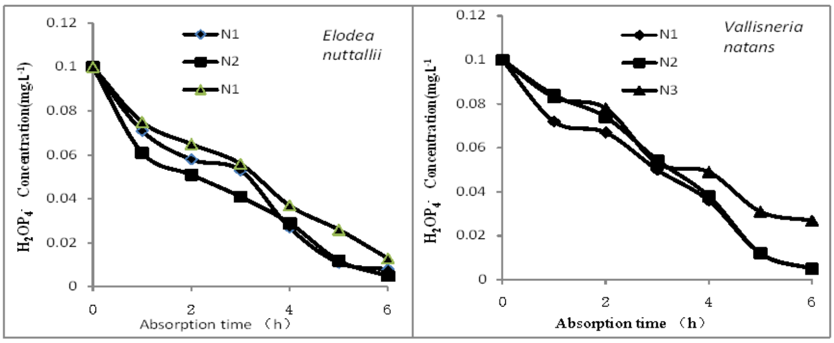

Figure 4. *Changes of $H_2PO_4^-$ concentrations in culture solution for the two aquatic macrophytes, N1-0.4 mg.L^{-1}, N2-1.2 mg.L^{-1}, N3-3.6 mg.L^{-1}*

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Table 6. *Equations of $H_2PO_4^-$ concentration versus uptake time for aquatic macrophytes*

	Elodea nuttallii	*Vallisneria natans*
N1	$y = 0.002x^2 - 0.029x + 0.092$	$y = 0.001x^2 - 0.025x + 0.093$
N2	$y = 0.002x^2 - 0.030x + 0.087$	$y = 0.001x^2 - 0.028x + 0.099$
N3	$y = 0.001x^2 - 0.023x + 0.092$	$y = 0.002x^2 - 0.024x + 0.098$

N1-0.4 mg.L^{-1}, N2-1.2 mg.L^{-1}, N3-3.6 mg.L^{-1}

Table 7. *Kinetic parameters of $H_2PO_4^-$ absorption by the two aquatic macrophytes*

Plant species	Vmax (mg^{-1}.g^{-1}.h^{-1})				Km (mg.L^{-1})			
	P1	P2	P3	R^2	P1	P2	P3	R^2
Elodea nuttallii	0.29	0.25	0.23	0.955	0.0132	0.0158	0.0172	0.9812
Vallisneria natans	0.25	0.22	0.18	0.969	0.0242	0.038	0.044	0.9798

N1-0.4 mg.L^{-1}, N2-1.2 mg.L^{-1}, N3-3.6 mg.L^{-1}

Discussion

Dynamic parameters Km and V_{max} can represent the characteristics of aquatic plants absorbing nutrients. The kinetic parameters Km and V_{max} of nitrogen and phosphorus absorption of submerged plants differ greatly (Chen et al., 2012) and are influenced by environment. Zhang (2012) research found that the V_{max} and affinity of the *Vallisneria natans* to absorb NH_4^+ were affected by light, temperature and pH. When the temperature is 12°C, the V_{max} was 0.037 mmol. (g.h)$^{-1}$ and Km was 0.21 mol.L^{-1}. When the temperature is 12°C, the V_{max} was 0.208 mmol. (g.h)$^{-1}$ and Km was 0.61 mol.L^{-1}. Zhang et al. (2013) found that the light intensity had a significant effect on V_{max} of the *Vallisneria natans* to absorb NH_4^+. Under the light conditions, the V_{max} of the *Vallisneria natans* to absorb NH_4^+ was 1.44 times in the dark conditions. Experimental studies have found that the V_{max} and Km of Elodea and Vallisnerium for NH_4^+ are affected by the P concentration in the environment. The V_{max} and affinity of the absorption of NH_4^+ by Elodea and Vallisneria tend to decrease with the increase of P concentration at the three phosphorus levels. The experimental results showed that the maximum absorption rate and affinity to NH_4^+ of *Elodea nuttallii* and *Vallisneria natans* on the three phosphorus levels decreased with the increase of P concentration. That is to say, with the increase of high P stress, both absorption rate and affinity decrease. The hydrolysate of NH_4^+ is directly passed through cells by free through the way of mountain diffusion, and there are little energy consumption (Runcie et al., 2003; Mehrer and Mohr, 1989). Under severe stress, the cell membrane of plants is destroyed (Xiong et al., 2010). It affects the absorption of NH_4^+. Plant uptake of P is active transportation, so plants generally don't overeat P. Ma et al. (2008) thinks that the injury of plants under high P stress is mainly manifested in two aspects. One is that high concentration of salt causes excessive osmotic pressure, which leads to water uptake and even loss of water in plant cells. The other one is that excessive salt ions enter the cells, resulting in cell metabolic disorder.

This study found that the V_{max} of *Elodea nuttallii* and *Vallisneria natans* for NO_3^- decreased with increasing P concentration. The affinity to NO_3^- of *Elodea nuttallii* is decreased with the increase of P concentration under three levels of phosphorus. *Vallisneria natans* affinity for NO_3^- increased with the increase of P concentration.

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Zhang (2012) research found that high temperatures severely limit the affinity of *Vallisneria natans* to NO_3^-. Under too low temperature conditions, *Vallisneria natans* showed some inhibition on NO_3^- affinity. The V_{max} gradually decreases with the increase of illuminance, showing the phenomenon of strong light suppression. NO_3^- uptake by plants is also an active process of absorption. Glutamine synthetase is the first enzyme that has been found to be related to NO_3^- absorption. It is found that under dark conditions, glutamic phthalamine synthetase has higher activity in the roots of large leaf algae (Pregnall, 2004). The uptake of NO_3^- by plants depends on the continuous synthesis of proteins. There may be a transporter between them (Jackson et al., 1973). Ma et al. (2008) studied that when P is high (0.8 $mg.L^{-1}$), could hardly inhibit or even promote the growth of the three plants within 3 days. It indicates that the tolerance of submerged plants to phosphorus is relatively strong. Under high concentration of phosphorus ribs, there will be no obvious damage to submerged plants in the short term.

Nitrogen and phosphorus are two important essential nutrients for plant growth. Studies (Qian et al., 2005) show that when the nitrogen concentration in the medium is constant, the N/P ratio has a significant influence on the absorption of seaweed P. Similar phenomena have been found in studies on submerged species (Li and Yuan, 2000). This study found that the V_{max} and affinity to $H_2PO_4^-$ of *Elodea nuttallii* and *Vallisneria natans* decreased with the increase of N concentration. Zhang (2012) research found that the V_{max} and affinity of the *Vallisneria natans* to absorb $H_2PO_4^-$ were affected by light, temperature and pH. the V_{max} and affinity of $H_2PO_4^-$ were the strongest under 22°C and pH=7 condition. NP are involved in plant metabolism process, N can promote the absorption of $H_2PO_4^-$ in a certain concentration range. With the increase of N concentration in the solution, the degree of plant stress increased. Thus affecting the absorption of $H_2P0_4^-$ Yan et al. (2007) found that in eutrophic conditions, the increase of ammonia nitrogen concentration would affect the physiological function of *Hydrilla verticillata*. High ammonia nitrogen concentration on *Hydrilla verticillata* is a kind of stress, which can inhibit the growth of plants and even lead to death. Xu et al. (2006) found that when the concentration of ammonia nitrogen is 4 mg, it had a strong stress effect on chlorophyll. Litav and Lehrer (1978) thought that ammonia could cause toxicity to uncoupling of photosynthesis and phosphorylation in chloroplast envelope. Cao and Ni (2007) found that excessive ammonia nitrogen (> 0.56 $mg.L^{-1}$) could lead to the degradation of the species of the herb and disrupt the metabolic balance of soluble carbohydrates and free amino acids in plants.

Conclusion

The V_{max} absorbed by NH_4^+ of *Elodea nuttallii* and *Vallisneria natans* at three phosphorus levels decreased with the increase of P concentration. The Km value increased with the increase of P concentration. Under the three phosphorus levels, the V_{max} of NO_3^- in two plants decreased with the increase of P concentration. The Km value of NO_3^- absorbed by *Elodea nuttallii* at different levels of phosphorus decreased with the increase of P concentration. The Km of NO_3^- on *Vallisneria natans* increased with the increase of P concentration. The V_{max} of $H_2PO_4^-$ decreased with the increase of N concentration. The Km value increased with the increase of N concentration. In eutrophication environment, the stress of submerged plants will affect their nutrient

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absorption. The nutritive stress of low N,P increased the absorption potential of N,P. High NP nutrition stress reduced the absorption rate and affinity. This method can be used to screen plants for water ecological restoration in engineering practice, and can also be used as an indicator of plant stress response.

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