

*Full Length Research Paper*

# The effect of hydrodynamics on nitrogen accumulation and physiological characteristics of *Vallisneria spiralis* L in eutrophicated water

Pei-Fang Wang<sup>1,3</sup>, Chao Wang<sup>2</sup>, Xiao-rong Wang<sup>1\*</sup>, Jun Hou<sup>2</sup> and Song-He Zhang<sup>2</sup>

<sup>1</sup>State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210093, China.

<sup>2</sup>Key Laboratory of Intergrated Regulation and Resource Development on Shallow Lakes of Ministry of Education, College of Environmental Science and Engineering, Hohai University, Nanjing 210098, China.

<sup>3</sup>State Key Laboratory of Hydrology-Water Resources and Hydraulic Engineering, Nanjing, 210098, China.

Accepted 6 June, 2008

**Natural water hydrodynamic conditions play an important role in the nutrients transport among water, soil and plants. Meanwhile, aquatic plants affect the water flow characters and pollutants purification capability. However, there are limited studies on how these conditions affects the nutrient uptake and physiological response of aquatic plants. From May 18 to June 28, 2007, a kind of submerged plant, *Vallisneria spiralis* L, was selected to observe the difference in nitrogen accumulation and antioxidant system and their changes in both shoots and roots of the plant between hydrodynamic condition (D flume) and relative hydrostatic condition (S flume). The results showed that under hydrodynamic conditions, the nitrogen accumulation in shoots increased rapidly, and maintained a higher level of nitrogen accumulation than that of the hydrostatic flume. Furthermore, in the initial stage of the experiment, the total chlorophyll of *V. spiralis* in the D flume decreased because of the hydrodynamic stress inhibition, while Chl a and b appeared to be a little synthesized in the S flume. Moreover, rapid induction in antioxidant enzyme activity occurred in shoots of the plant in the D flume, and the activity levels of POD and GSH were significantly higher than those of the S flume.**

**Key words:** running water, *Vallisneria spiralis* L, nitrogen accumulation, chlorophyll, antioxidant defense.

## INTRODUCTION

Aquatic plants play an important role in maintaining the purification capability of water and the entire aquatic ecosystem. They can significantly impact the assimilation of pollutants such as nitrogen and phosphorus from water by biological absorption or physicochemical adsorption (Eriksson et al., 1999; Tong et al., 2004; Haseeb et al., 2004). Submerged aquatic plants can increase the transparency of water through withholding suspended solids, thereby improving water quality of shallow lakes and ecosystem stability (Hou et al., 2006). However, the impact of human activities, such as agricultural and industrial

wastewater discharges, has accelerated the process of eutrophication of water bodies, and led to the degradation of aquatic plants (Cao et al., 2004). At the same time, various water projects aiming to the governance of anti-eutrophication, such as water transfer projects, also influence the growth of aquatic plants through water flows and its shearing forces. Thus, concerns have been raised on the ecological stability and structural integrity of water eco-system.

Plants can often self-induce reactive oxygen species such as O<sup>2</sup>·, O·, and OH under stresses including cold, heat, salt, pH, etc. (Mittler et al., 2004; Pflugmacher et al., 2004). Over-producing reactive oxygen species in plant can cause oxidative damage to proteins, DNA and lipids (Apel et al., 2004). Meantime, reactive oxygen species can also induce the production of antioxidant enzymes

\*Corresponding author. E-mail: [ekxr@nju.edu.cn](mailto:ekxr@nju.edu.cn). Tel: +86-(25)-83786696.

and antioxidants in the plant to mitigate and reduce oxidative damage (Blokhina et al., 2003). Therefore, scientists measure the content and activity of those enzymes to monitor changes in the stress level. Yan et al. (1998) investigated the changes in glutathione (GSH) level at different nitrogen concentrations in *Beta vulgaris* L. Similarly, Cao et al. (2004) examined the effect of increased inorganic nitrogen level on the antioxidant enzymes in *Ceratophyllum demersum*. Under different concentrations of nitrogen and phosphorus, the growth of *Elodea nuttallii* and *Myriophyllum spicatum* L. and their responses to antioxidant enzymes (CAT, SOD, and POD) were also studied by Fan (2007). Wang et al. (2005) compared the changes in contents of protein, chlorophyll, and activities of peroxidase (POD) and super-oxide (SOD) in *C. demersum* under different nutritional levels (medium nutrition, high nutrition, extremely high nutrition, and Hoagland-nutrition media), and found that serious impact on the growth appeared at high nutrient concentrations, which reduced its self-defensibility. In addition, Fan et al. (2005) characterized the nutrient accumulation and distribution in *M. hyllum spicatum* in varying degrees of water eutrophication. These studies have greatly advanced the understanding of oxidative stress and nutrient accumulation of aquatic plants in water eutrophication. However, these results are mostly from static indoor simulation. Few researches have been done on nitrogen and phosphorus absorption and physiological responses of aquatic plants under hydrodynamic conditions.

In fact, the characteristics of many eutrophicated shallow lakes are greatly affected by wind, wave, and the flow velocity of water entering the lake. In running waters, macrophytes can affect nutrient retention by increasing the water residence time and by acting as a filter for suspended particulate matter (Schulz et al., 2003). While aquatic plants purify water by filtering out suspended particulates, they also retain particle-adsorbed pollutants and enhance the deposition of particulates (Sand, 1998; Madsen et al., 2001). Schulz et al. (2003) postulated a good estimate of nutrient retention due to sedimentation, and found that deposition retained up to 12% of total phosphorus load. The hydrodynamic conditions may change the growth environment of aquatic plants, affect the growth of plants, and therefore impact the absorption of nitrogen and phosphorus (Xu et al., 2004). This may also alter the adaptability and reactivity of the plants. On the other hand, bottom shear stress is the major force regulating the deposition of particles. Its effect highly depends on the flow velocity (Kozerski et al., 1999; Wheel et al., 1980), and can be significantly reduced when water is flowing through stocks of submerged plants. In this situation, particles preferably settle within the area, and high retention may occur in vicinity of macrophytes stands. This also enhances the settling of suspended matter (Sand, 1998). Few studies have attempted to quantify the influence of macrophytes on sedimentation or re-suspension in running waters. *Vallis-*

*neria spiralis* L is a kind of predominant aquatic submerged plants distributed in Taihu Lake and river courses in the downstream of Yangtze River (Li, 2006). It is a perennial wet plant, and has several slim shoots with 10 to 18 cm long and 0.5 to 1.0 cm wide while it is grown up since the mid-day of May. Wang and Bao (Wang et al. 2008; Bao, 2007) found that *V. spiralis* can remove the nutrients from static water through plants uptake, and the growth of *Vallisneria* was affected by the level of nutrients and its environment factors (Wen et al., 2008; Ma, 2007). However, few studies show how running water affects the nutrients uptake in *V. spiralis* and how it bears this stress.

In the present study, we designed a comprehensive approach using two comparative simulation flumes to evaluate the physiological characters of *V. spiralis* under running water. We intended to investigate the effect of plant physiological changes and nitrogen accumulation under varying water flow velocities.

## MATERIALS AND METHODS

### Experiment facility

Two simulation water flumes, a conditioning water tank, a high water tank and two pumps were employed in the experiment. The water flumes were made of polyethylene plates. The size of the small flume (D) was 2000 × 300 × 500 mm, and the size of the large flume (S) was 2000 × 2000 × 500 mm. Water in flume D was circulated and regulated by a water pump (a) and a regulating water tank (b), while water in flume S was circulated and controlled by a water pump (c), a high water tank (d) and a regulating water tank (b). The water flow velocities and water quality in flumes S and D were continuously regulated by the regulating water tank b. Figure 1 shows the specific structural setup for the experiment.

### Treatment conditions and plant material

Because of the difference in cross-section size of the two flumes, the flow velocities were significantly different, which was measured by an acoustic doppler velocimeter (ADV). The depth-mean velocity at the middle section of the flume water was 4.81 cm s<sup>-1</sup> for the D flume and 0.16 cm s<sup>-1</sup> for the S flume. Therefore, the water flow velocity of S flume was 3.3% of that of D flume, and the difference was significant ( $p < 0.05$ ). The flow in the S flume was supposed as static condition, and served as a control for the study. Thus, flume D was considered as a moving flume, while flume S was considered as static water tank.

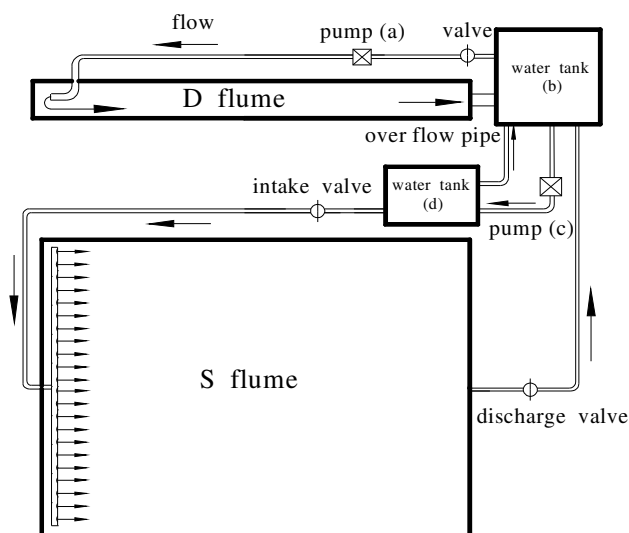
Because the pollution loading, water temperature, pH, dissolved oxygen saturation, and other conditions were same in the two flumes, they were considered as having the same external environment conditions. The test results of various parameters during the experiments were listed in Table 1. Among them, water temperature, pH, the percent saturation of oxygen (ODO%), NH<sub>4</sub><sup>+</sup>-N, and NO<sub>x</sub><sup>-</sup>-N were concurrently determined using multi-parameter water quality monitors (Model: YSI 6600).

Samples of *V. spiralis* used in the experiment were from the lakeside zone of Meiliang Bay of the Taihu Lake. The samples were cultured with soil in laboratories of Hohai University for two weeks. Plants of healthy growth, uniform in size, and length of 15 to 18 cm were selected, cleaned, cultured in 1:10 Hogland nutrition medium for 3 d. *Vallisneria* samples were fixed by a layer (8 cm thick) of gravel (gravel diameter 0.5 to 1.0 cm), which had been brushed and

**Table 1.** Physicochemical properties of water samples collected from the flume D and S

Date	Temp (°C)		pH		ODO (%)		Nitrate N (mg/L)		NH <sub>4</sub> <sup>+</sup> -N (mg/L)	
	D	S	D	S	D	S	D	S	D	S
05-18-07	24.9	24.6	7.67	7.55	101.2	98.4	30.00	30.49	30.92	29.99
05-19-07	32.9	32.7	7.71	7.61	104.9	103.5	28.14	28.32	26.64	26.10
05-21-07	30.1	29.6	7.24	7.24	103.2	99.9	31.6	32.47	23.73	23.43
05-24-07	27.4	27.1	7.03	7.02	91.1	85.7	33.33	33.20	23.55	23.50
05-31-07	27.8	27.4	6.37	6.43	104.5	103.9	29.74	29.61	12.94	12.79
06-07-07	27.7	27.4	6.56	6.49	107.6	101.8	30.36	31.77	6.50	6.42
06-14-07	27.7	27.4	6.31	6.30	102.2	95.5	31.63	31.49	3.35	3.31
06-21-07	28.2	27.9	5.96	5.95	105.6	98.5	36.52	37.39	1.90	1.88
06-28-07	26.3	26.2	6.41	6.49	104.2	103.2	35.80	37.66	1.09	1.12

Data are the means of three values (n=3).



**Figure 1.** Experimental design for hydrodynamic and hydrostatic flumes.

cleaned with tap water, soaked in pure water for 3 d. Water nitrogen was prepared using inorganic nitrogen KNO<sub>3</sub>-N and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-N at the ratio of 1:1, and the initial TN concentration was 60 mg L<sup>-1</sup> as a similar study suggested (Wang, 2008). According to the size and volume of the two water flumes at the same chosen depth (h = 35 cm), the total water volume was calculated to be 1000 L.

#### Plant growth and nitrogen accumulation

Three subgroups of plants from each group were collected, and immediately washed with pure water. Shoot and root samples were then extracted. Plant growth (fresh weight FW) was measured using twelve plants for each cultivar, and recorded according to their initial serial numbers. After weighing, external and internal shoots were removed, and two typical shoots were used for determining nitrogen accumulation. Samples were then oven-dried at 80°C for 48 h to constant weight (dry weight, DW), and were brought to a standard volume with H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>. The content of nitrogen was determined by the sodium colorimetric method (Turner, 1976).

#### Chlorophyll a fluorescence measurements

Prior to the extraction, fresh shoot samples were cleaned with deionized water to remove surface contaminants. Chlorophyll extraction was carried out on fresh and fully expanded shoot materials. One g shoot sample was ground using a pestle and mortar, and then extracted by 90% acetone under the dark condition. The absorbance at 663 and 645 nm was measured using a UV/Visible spectrophotometer (Shimadzu, UV-2450, Japan). Chlorophyll concentrations were calculated using the equation proposed by Inskeep (1985).

$$\text{Chl. a (mg ml}^{-1}\text{)} = 12.72(A_{663}) - 2.59(A_{645})$$

$$\text{Chl. a (mg ml}^{-1}\text{)} = 22.88(A_{645}) - 4.67(A_{663})$$

A663 and A645 represent absorbance readings at 663 and 645 nm wavelengths, respectively.

#### Tissue and enzyme extraction

Fresh shoot or root samples of 1.0 g were extracted in 3 ml of 50 mM sodium phosphate buffer (pH 7.8) containing 1.0 mmol l<sup>-1</sup> EDTA and 2% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 5,000 g for 15 min at 4°C, and the supernatant was used for the enzyme assays (Zhang et al., 2007).

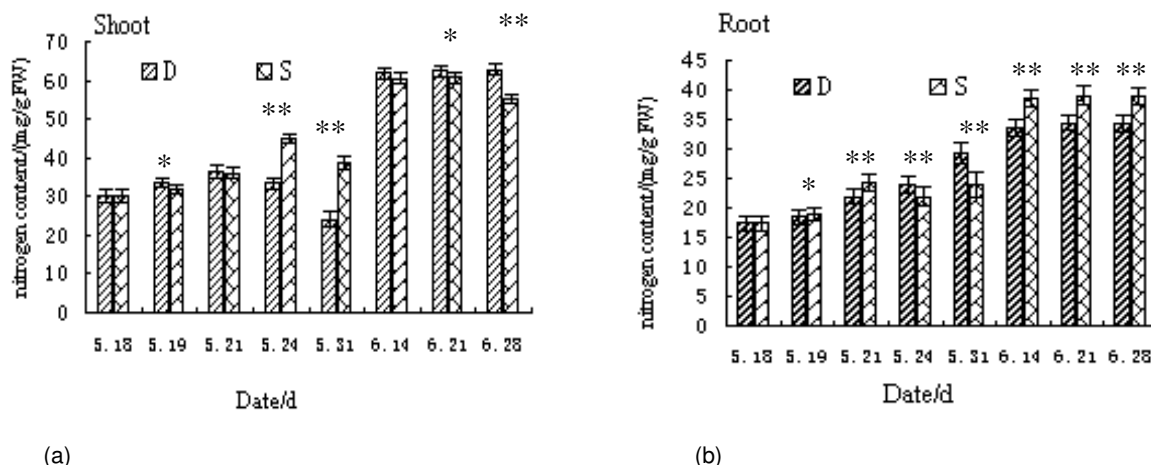
#### Assay of antioxidant enzymes

##### POD

POD activity was assayed in a reaction mixture containing 10 mM potassium phosphate buffer (pH 7.0), 10 mM H<sub>2</sub>O<sub>2</sub> solution, 20 mM guaiacol and 10 μl of crude extract (Chance et al., 1955). The reaction started by simultaneously adding H<sub>2</sub>O<sub>2</sub> and guaiacol solution, and the activity was determined by monitoring the increase of absorbance at 470 nm. One unit of POD activity was defined as the amount required to decompose 1 μmol guaiacol min<sup>-1</sup> mg<sup>-1</sup> FW.

##### GSH

Glutathione reductase activity was assayed according to method of Smith et al. (1988) by following the increase in absorbance at 412



**Figure 2.** Nitrogen accumulation in shoots and roots of *Vallisneria spiralis* L. All the values are mean of triplicates  $\pm$  S.D. ANOVA significant at  $p < 0.05$ . \* and \*\* indicate significant difference between D flume and S flume at  $p < 0.05$  and  $p < 0.01$ , respectively.

nm due to 5,5'-dithiobis-2-nitro benzoic acid (DTNB) reduction by glutathione reduced form (GSH) generated from glutathione oxidized form (GSSG) (Smith et al., 1988). The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 7.5), 1 mM EDTA, 0.75 mM DTNB in 0.01 M sodium phosphate buffer (pH 7.5), 0.1 mM NADPH, and 1 mM GSSG. The reaction started by the addition of enzyme extract. The content of GSH was then calculated according to a glutathione reductase activity standard curve with the unite  $\mu\text{g g}^{-1}$  FW.

### Statistical analysis

The experiments were conducted with a randomized block design. Two-way analysis of variance (ANOVA) was performed with all the data to confirm the variability of data and validity of results. Independent samples T test was used to determine the significant difference among the treatments. Correlation analysis was performed for all the data of experiment duration with respect to change between hydrodynamic flume and hydrostatic flume.

## RESULTS

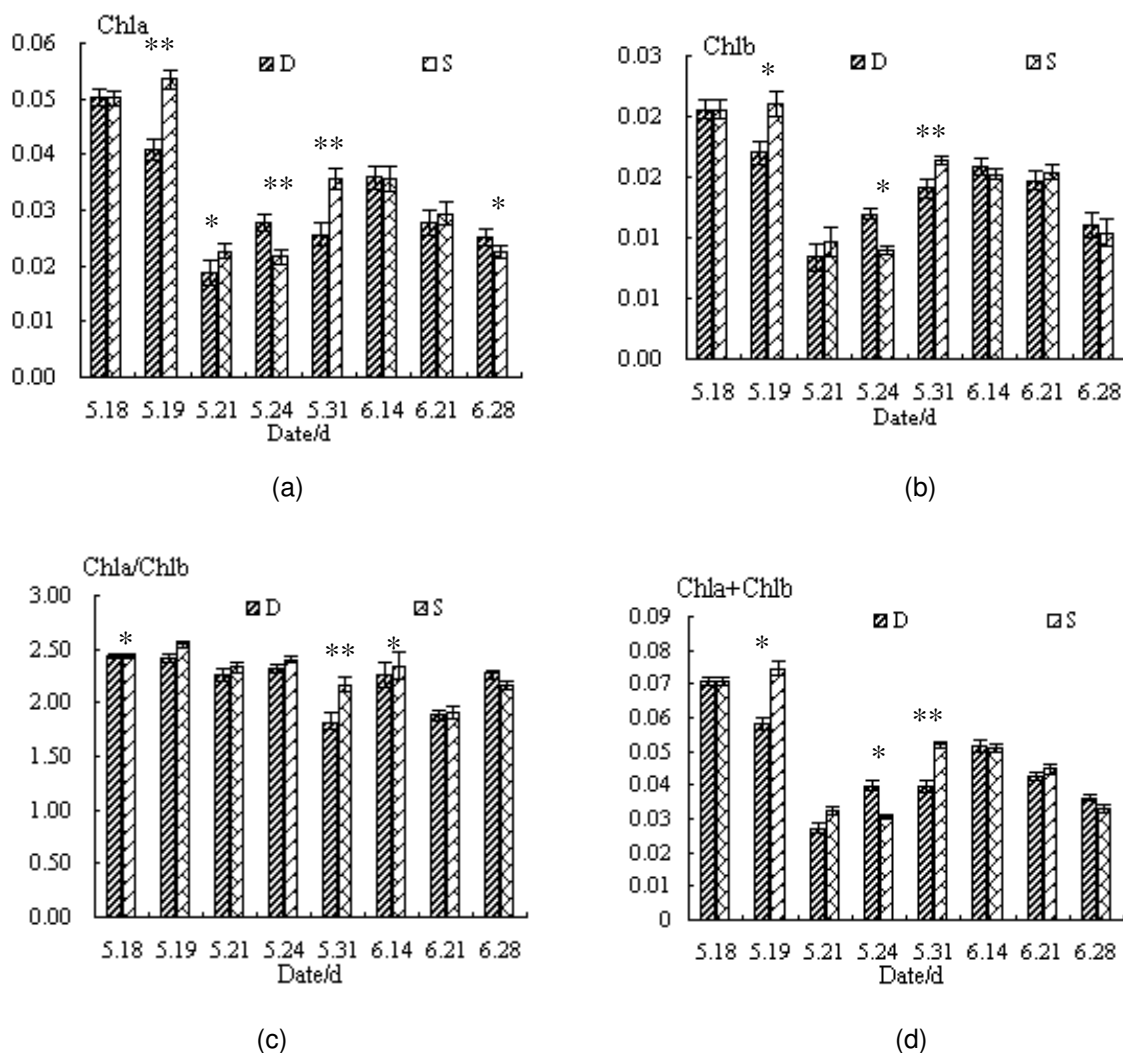
### The characteristics of nitrogen accumulation in *V. spiralis* at hydrodynamic conditions

Figure 2 shows the comparison of nitrogen accumulation in the shoots and roots of *V. spiralis* based on unit dry weight in the hydrodynamic flume and the hydrostatic flume. From Figure 2a, it is evident that there was characteristic difference in nitrogen accumulation in the shoot of *V. spiralis* in the hydrodynamic and hydrostatic flumes. Nitrogen accumulation in *V. spiralis* in D flume elevated with time, but exhibited a declining trend after the first 7 d until the minimum at the 13th d. After that, rapid increase in nitrogen accumulation appeared again, and this trend was continued until the end of the experiment. As a whole, the shoot of the plants maintained a

higher level of nitrogen content in the hydrodynamic flume. Compared to S flume, the nitrogen accumulation continually increased from 30.31 mg to 44.91 mg  $\text{N g}^{-1}$  (FW) in the shoots in the first week of the experiment, accounting for 48.1% increase. This trend was significantly slowed down in the second week, but accumulation was enhanced quickly after that, and stayed high until the last week of the experiment, when the enhancement was reduced again. Compared to those of hydrostatic control flume, the nitrogen accumulation in the shoots of *V. spiralis* in the hydrodynamic flume D became sensitive to the changes. The variation of nitrogen accumulation responded more quickly than that of S flume. About 27 d after the experiment, nitrogen accumulation in the shoot of *V. spiralis* was greater than that of S flume ( $p < 0.05$ ).

From Figure 2b, it was evident that the characteristics of nitrogen accumulation in the root of the *V. spiralis* in D and S flumes were also different. The nitrogen was gradually accumulated in the root of the *V. spiralis* in the D flume for the first 27 d, reaching 33.53 mg  $\text{N g}^{-1}$  (FW) on June 14, and this level was maintained to the end of the experiment. For the S flume, the nitrogen accumulation in the root varied in the second week (May, 24). It first appeared some decrease, and then became increasing, even reaching a higher level than D flume on June 14 ( $p < 0.01$ ).

It is evident that the shoot of *V. spiralis* is profoundly affected by the running water. The variation of nitrogen accumulation per unit dry weight in the hydrodynamic flume was greater than that of hydrostatic flume. Furthermore, the nitrogen accumulation was maintained at a higher level than the hydrostatic flume within 2 weeks of the experiment. The root of *V. spiralis* showed a strong resistance to the hydrodynamic condition, and did not show much stress inhibition during the experiment period,



**Figure 3.** Comparison of variations of chlorophyll in *Vallisneria spiralis* L shoots. All the values are mean of triplicates $\pm$ S.D. ANOVA significant at  $p < 0.05$ . \* and \*\* indicate significant difference between D flume and S flume at  $p < 0.05$  and  $p < 0.01$ , respectively.

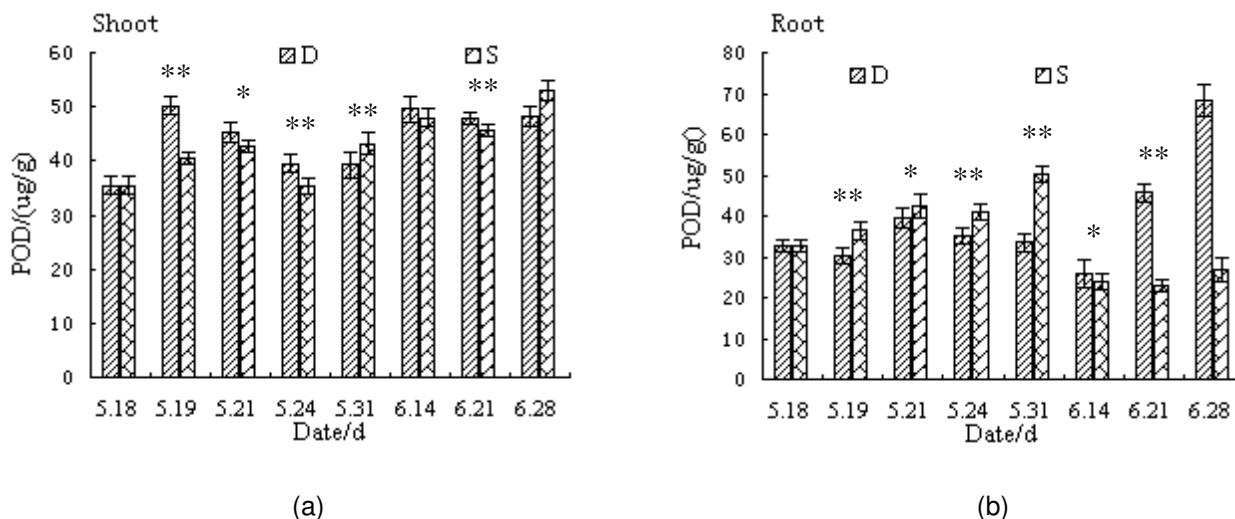
characterized by continuous increase of nitrogen accumulation.

### The variation of chlorophyll

The variation of chlorophyll a, b, and a + b in D and S flumes exhibited significant decrease in the first 3 d. That loss was quickly recovered, and the contents of chlorophylls continually advanced before they were again lowered at the end of the experiment. The obvious difference of chlorophyll changes in *V. spiralis* shoots between the hydrostatic flume and hydrodynamic flume was that, in the static flume, the contents of Chl a, Chl b, Chl (a + b) at the second day were a little greater than the initial value ( $p > 0.05$ ), while those of hydrodynamic flume were declined rapidly in 2 d, suggesting that the flow of

water inhibited the synthesis of chlorophyll in *V. spiralis* L. From Figure 3, Chl a content in shoots of *V. spiralis* in D flume 3 d after in the beginning of the trial was decreased by 62.4%, followed by an upward trend on May 24 reaching 0.036 mg/g on June 14. On the 31st d, the content of Chl a began to decline. At the end of the experiment, however, the content of Chl a in the hydrodynamic flume was greater than that in the static flume ( $p < 0.05$ ). The variation Chl b and Chl (a + b) of *V. spiralis* shoots in the of D and S flumes was similar to the variation of Chl a ( $r = 0.972$  and  $r = 0.998$ ).

In general, the ratio of Chl a / Chl b in both D and S flumes slightly declined with a few fluctuations on May 31 and June 21 (Figure 3c). Compared to the S flume, the chlorophyll ratio Chl a/b of *V. spiralis* shoots in the D flume was slightly lower than that of S flume at the first 27 d, and the difference was minimized after that, except for



**Figure 4.** Variation of POD in shoots and roots of *Vallisneria spiralis* L. All the values are mean of triplicates  $\pm$  S.D. ANOVA significant at  $p < 0.05$ . \* and \*\* indicate significant difference between D flume and S flume at  $p < 0.05$  and  $p < 0.01$ , respectively.

the significant difference on June 28 when the experiment was complete ( $p < 0.05$ ). This further demonstrated that *V. spiralis* shoots were sensitive to the dynamics of water, characterized by initial growth inhibition and then recovery at the later part of the experiment. This behavior was consistent with the characteristics of enhancement of nitrogen uptake and accumulation of *V. spiralis* shoots on June 14 (Figure 2).

As a result, changes in *V. spiralis* chlorophyll are general agreement between the hydrodynamic and hydrostatic flumes during the experiment period. However, for the second day in the S flume, Chl a, and b showed a phenomenon of assimilation, and their values were little higher than the initial values. Besides these, the other values were evidently lower, indicating that *V. spiralis* shoots were significantly affected by excessive nitrogen and water flow.

### Response of POD activity

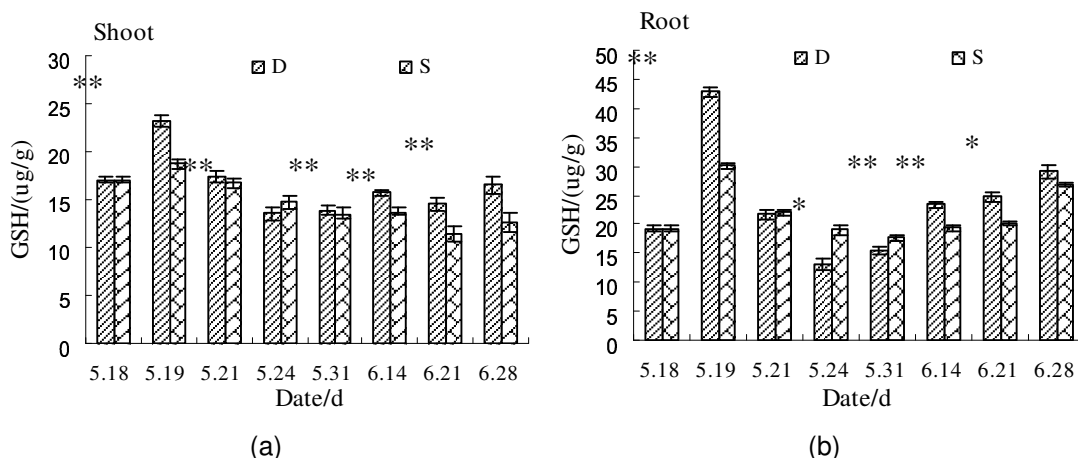
Figure 4 shows the variation of *V. spiralis* POD with time. The variation of POD antioxidant enzyme activity in the shoot of *V. spiralis* within the period of 40 d in both D and S flumes can be summarized the initial inductive enhancement, and then inhibitive decline, followed by an overall restoration to increase. In the early stage, the POD activity in the D flume was significantly higher than hydrostatic S flume. For example, the POD activity in *V. spiralis* shoots in the D flume was rapidly increased in the first day, while the POD activity of the S flume was enhanced gradually over the period of 3 d. This implies that, under running water condition, the shoot of the *V. spiralis* can urgently adjust its peroxidase systems in order to reduce lipid peroxidation, and release membrane damage.

Figure 4b shows the variation of POD in the root of the *V. spiralis*. For the D flume, POD activity in the underground part of *V. spiralis* was not affected in the first day, but it rapidly increased in the third day, reaching  $39.6 \text{ units mg}^{-1} \text{ protein}$ . After that, the activity was inhibited, and gradually declined. On June 21 (33th d), however, the activity increased again. For the control S flume, the POD activity in the root of *V. spiralis* stayed enhancing in the first 13 d, followed by inhibition and a rapid decline. This status was maintained for two weeks until some recovery occurred. Up to June 28, the POD activity was slightly elevated.

Comparing Figure 4a to 4b, it can be concluded that the variation of POD activity in shoot and root was quite different. 1) The activity levels in the shoot were consistently higher than those of the root in the whole duration. 2) POD activity in the shoot of the D flume rose rapidly to the maximum value on the first day, but that of the root reached the highest value on the third day. Furthermore, a recovery increase appeared in the shoot on June 14 (27th d), while such increase for the root occurred on June 21 (34th d). The root seemed to be relatively less responsive than the shoot, but the overall variation was basically same. 3) For the S flume, significant difference was observed between the shoots and roots. The shoot was basically under activity induction, and demonstrated a trend of gradual increase during the experiment, while the underground part exhibited stress-induced inhibition in the later stage of the experiment.

### Response of GSH

From Figure 5, glutathione in both D and S flumes was induced to varying degrees. The GSH contents in the



**Figure 5.** Variation of GSH contents in shoots and roots of *Vallisneria spiralis* L. All the values are mean of triplicates $\pm$ S.D. ANOVA significant at  $p < 0.05$ . \* and \*\* indicate significant difference between D flume and S flume at  $p < 0.05$  and  $p < 0.01$ , respectively.

shoots of *V. spiralis* in D flume were inductively enhanced. After 3 d, GSH content was decreased, and formed stress-related inhibition. This status was maintained until June 14 (27th d) when the GSH contents resumed the rising trend (Figure 5). Moreover, compared to those of the D flume, the GSH content in shoots in the hydrostatic flume S were significantly low, especially in the first testing day, and 27 d later ( $p < 0.01$ ). However, the overall trend of the changes was similar ( $r = 0.753$ ). In general, the GSH contents in the roots of *V. spiralis* were greater than those of shoots (Figure 5b). The GSH contents in the root of the *V. spiralis* in the D flume varied much more extensively than those in the S flume in the first 6 d, and stayed in a relatively high level after 27 d with little variation.

Results show that changes in antioxidant enzyme activities in the plants were significantly influenced by the running water. Active hydrodynamic conditions resulted in rapid increase of POD and GSH activities in the shoot of *V. spiralis*. This level was maintained by the plant through self-regulation to retain a level higher than that of the hydrostatic system, in order to reduce the cell membrane damage in plants.

## DISCUSSION

Nitrogen is a necessary nutrient for plant growth. However, plant's nitrogen absorption and accumulation was often affected by the environmental conditions, nutrient concentrations, hydrodynamic characteristics etc. (Ye et al., 2007). The results of this study indicated that, under the same external environment conditions, the difference in the hydrodynamic characteristics had an obvious impact on the nitrogen uptake and accumulation process in *V. spiralis* shoots and its roots. For example,

the nitrogen accumulation in *V. spiralis* shoots in the hydrodynamic D flume increased much more than that of the control S flume in the initial days, and the difference kept throughout the later stage of the experiment. The reason may be that larger numbers of oxygen bubbles produced by shoot photosynthesis adhered to the surface of shoots, and baffled the direct contact between shoots and the nitrogen and thus decreased nutrient absorbing process in S flume. While, in D flume, the running water flow destroyed these bubbles and reduced this impact. Similarly, Xu et al. (2004) found that the nitrogen uptake of asparagus under water flow rate of 160-175 L h<sup>-1</sup> within 12 h was 18.3-24.3% higher than that in static water. This conclusion was consistent with ours and also indicated that the flow velocity had an important influence on the nutrient absorption and plant growth.

In the later stage of our experiment, the nitrogen accumulation in *V. spiralis* shoots in both D and S flumes maintained a relatively high level, suggesting that plants had a limit to absorb and accumulate external sources of nitrogen. It is because that more energy is needed to keep higher nitrogen level in plants, and the excessive nitrogen perhaps induces larger quantity of active oxygen, damaging to the plants themselves. Moreover, using 1-105 mg L<sup>-1</sup> nitrate for *C. demersum* for 4 weeks, Best (1980) found that its nitrogen content was stabilized around 4%. This result was similar to our results.

During the experiment period, the nitrogen accumulation in the root of *V. spiralis* was kept an increasing trend. This trend was particular evident for the hydrodynamic flume D. It implied that the hydrodynamics did not produce significant effects on the growth of its root, and therefore it did not significantly affect the nitrogen accumulation in the root of the plant. It may attribute to the roots all buried in the gravel layer of flume bottom, thus decrease the effects of hydrodynamics of running

water. Ye et al. (2007) studied *Vallisneria natans* for 45 d, and found that the plant biomass was continuously enhanced over time. In our study, however, the nitrogen accumulation in the root of the *V. spiralis* exhibited accumulation suppression on the 6th to 13th d in the hydrostatic S flume, with the increase trend of the shoot in this period, correspondingly. This phenomenon implies that there is some degree of inhibition in the shoot of *V. spiralis* in the hydrodynamic D flume, but had no significant impact on the root from the 6 to 13th d. However, the mechanism of this behavior needs to be further investigated.

Normally, the present study demonstrated significant loss of pigments along with the whole period of this experiment, except in S flume of the second day. The significant decrease of Chl a and b during the experiment indicated that there were severe suppression on the shoot tissues of *V. spiralis*, witnessed by the chlorosis and the reduction of biomass of the shoots in D flume. The water flow was due to existence in two readily interconvertible oxidation states that made it highly reactive, which could catalyze the formation of free radicals that damage photosynthetic apparatus (Davi et al., 1998; Vajpayee et al., 2005). It may also catalyze the degradation of proteins through oxidation modification and increased proteolytic activity (Romero et al., 2002). While, the contents of Chl a, b in shoots of D flume decreased remarkably, but increased a little in S flume in the second day, indicating strong sensitivity of the shoots to hydrodynamic conditions. The reason of this may be that the flowing conditions stimulate the shoot tissues of the plant by water shear stress, and thus interfere in the normal photosynthesis process. Meanwhile, strong water flow may make the shoots rollback or even overlapped, then reduced the synthesis of Chl a and b in D flume. While, in S flume, the most of oxygen bubbles adhered with shoots protect them from outside water conditions and provide them normal growing process at the second day. However, Wheel et al. (1980) examine the growth of *Macrocystis pyrifera* and found that the photosynthesis of *M. pyrifera* leave was expected to increase by over 300% as the flow velocity was increased from 0 to 4 cm s<sup>-1</sup>. So, the detailed mechanism reasons of these phenomena need to be further researched. At the end of our experiment, all the contents of Chl a, b, Chl (a+b), and Chl a / b in *V. spiralis* in the D flume were greater than those in the S flume. This was consistent with the nitrogen accumulation in shoots. It can be explained by certain degree of recovery of *V. spiralis* shoots in the D flume, which enhanced the nitrogen uptake.

Aquatic plants can activate its defense system under external stresses, induced by various antioxidant enzymes. Excessive nutrients, such as nitrogen, phosphorus, etc. can induce plants' antioxidant system to remove excess free oxidant radicals (Wang, et al., 2006). Wang et al. (2002) found that, as water ammonium salt proportion increased the glutamine synthetase (GS) and (POD)

activity of *Hydrilla verticillata* rose. Zhu et al. (2005) explored the effect of different nitrogen forms and concentrations on *V. spiralis* physiology, and revealed that stress could induce *Vallisneria* POD activity. The results of this study show that the level of POD and GSH activities were all increased at the initial experiment days, indicating the excessive nitrogen of 30.0 mg · L<sup>-1</sup> ammonia and 30.0 mg · L<sup>-1</sup> nitrate performing physiological stress on *V. spiralis*. Furthermore, under the same level of nutrition, light, and pH conditions, the POD activity of *V. spiralis* shoots in flowing water was induced to a higher level on the second day, and this relatively higher level was maintained until 45 days of the experiment, as compared to those in the hydrostatic flume. It suggested that flowing water could stimulate POD activity to a high level, and therefore reduce lipid peroxidation and membrane damage.

It has been reported that the existence of high level of ammonia in water can cause acidification of water bodies (Körner et al., 2001, 2003). In the present study, it has been observed that the pH of water declined as decreasing NH<sub>3</sub> concentration (the correlation coefficient was 0.94). At the same time, excessive ammonia can produce toxic effects to plants. Nimptsch (2007) claimed that ammonia stress could induce increases in glutathione peroxidase content and catalase activity. Recently, Wang et al. (2008) reported that water ammonia content higher than 5.6 mg L<sup>-1</sup> significantly inhibited *Vallisneria* chlorophyll, inducing the anti-oxidation system. Our results showed that, at high ammonia concentrations, chlorophyll content of *V. spiralis* shoots decreased, while the activity of antioxidant enzyme POD and GSH content increased, indicating that *V. spiralis* had ammonia stress under both hydrodynamic and hydrostatic conditions. Moreover, the great variations of water POD and GSH contents in the early stage of the experiment might be possibly because of the urgent stress responses from the running water stress. The small difference between hydrodynamic and hydrostatic flumes during the late stage of experiment might reflect plant's ecological adaptability.

Glutathione (GSH) is a part of antioxidation system of plants. They protect cell membranes through GSH peroxidase system and GSH reductase to inhibit lipid peroxidation. They can greatly enhance the response under a major stress (Giblin, 2007). In the present study, GSH in shoots and roots of *V. spiralis* was induced, characterized by a higher level of GSH in the hydrodynamic flume than the hydrostatic flume. This indicated that under the same conditions, the hydrodynamic water was far more effective in inducing *V. spiralis* antioxidant system than the hydrostatic water. A long-term high nutritional stress might inhibit plants' growth and suppress their resistance (Yan et al., 2007). Therefore, in the 5th and 13th d of the beginning of the experiment, GSH levels for both hydrodynamic and hydrostatic flumes were significantly decreased and stayed in a low level. After that, GSH has emerged to increase, especially in the root

systems, of which GSH level in both hydrodynamic and hydrostatic conditions demonstrated a higher level in the later stage of the experiment. This may be because of a higher activity of glutathione reductase in roots, which can restore oxidized glutathione to rapidly regenerate GSH and thus protect the plants.

## ACKNOWLEDGEMENTS

We are grateful for grants from the National Natural Science Foundation of China (NO. 50709009), the Key Project of Chinese Ministry of Education (NO. 106088) and the basic research project of Jiangsu province (BK2007526). And we thank Ai Yang, Ping Ou Yang and Bo Liu for their technical assistance.

## REFERENCES

- Apel K, Hirt H (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55: 373-399.
- Bao Xian-ning, Chen Kai-ning, Fan C-X (2006). Effects on nitrogen and phosphorus distribution in interstitial water and sediment-water nitrogen and phosphorus release with growing of submerged macrophytes. *J. Lake Sci.*, 18(5): 515-522.
- Best EPH (1980). Effects of nitrogen on the growth and nitrogenous compounds of *Ceratophyllum demersum*. *J. Aquat. Bot.* 8: 197-206.
- Blokhina, O, Virolainen, E, Fagerstedt, K.V (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. *J. Ann. Bot.* London 91: 179-194.
- Cao T, Ni LY (2004). Responses of Antioxidases of *Ceratophyllum Demersum* to the Increase of Inorganic Nitrogen in Water Column. *Acta Hydrobiol. Sinica*, 28(3): 299-303.
- Cao T, Ni LY, Xie P (2004). Acute biochemical responses of a submerged macrophyte, *Potamogeton crispus* L., to high ammonium in an aquarium experiment. *J. Freshwater Ecol.* 19: 279-284.
- Chance B, Maehly C (1955). Assay of catalase and peroxidases. *Methods Enzymol.* 11: 764-775.
- Davi SR, Prasad MNV (1998). Copper toxicity in *Ceratophyllum demersum* L. (Coontail), a free floating macrophyte: response of antioxidant enzymes and antioxidants. *Plant Physiol.* 98: 853-858.
- Eriksson PG, Weisner SWB (1999). An experimental study of effects of submerged macrophytes on nitrification and denitrification in ammonia-rich aquatic systems. *Limnol. Oceanogr.* 44:1993-1999.
- Fan GL, Li W (2005). Response of Nutrient Accumulation Characteristics and Nutrient Strategy of *Myriophyllum spicatum* L. under Different Eutrophication Conditions. *J. Wuhan Bot. Res.*, 23(3): 267-271.
- Fan YY (2007). The Influences of N, P on Growth and Physiology of Submerged Macrophytes in Eutrophic Waters. Master's Thesis of Central China Normal University.
- Giblin FJ (2000). Glutathione: a vital lens antioxidant. *Ocul. Pharmacol. Ther.*, 16: 121-135.
- Haseeb Md. Irfanullah, B M (2004). Factors influencing the return of submerged plants to a clear-water, shallow temperate lake. *Aquat. Bot.* 80: 177-191.
- Körner S, Das SK, Veenstra S (2001). The effect of pH variation at the ammonium/ammonia equilibrium in wastewater and its toxicity to *Lemna gibba*. *Aquat. Bot.* 71: 71-78.
- Körner S, Vermaat JE, Veenstra S (2003). The capacity of duckweed to treat wastewater: Ecological considerations for a sound design. *J. Environ. Qual.* 32: 1583-1590.
- Kozerski HP, Leuschner K (1999). Plate sediment traps for slowly running waters. *Water Res.* 33: 2913-2922.
- Li E H (2006). Effects of Aquatic Macrophytes on Nutrient Cycling in Shallow Freshwater Lake Ecosystems. Ph.D thesis of Wuhan Botanical Garden, the Chinese Academy of Science, Wuhan.
- Ma JM, Jin TX, Jin P, Chen SP, He F, Wu J, Wu ZB (2007). Responses of *Elodeanuttallii* and *Vallisnerianatans* to the Stress of Nitrate. *J. Henan Normal University (Natural Science)*, 35(3): 115-118.
- Madsen JD, Chambers PA., James WF, Koch EW, Westlake DF (2001). The interaction between water movement, sediment dynamics and submerged macrophytes. *Hydrobiologia*, 444: 71-84.
- Mittler R, Vanderauwera S, Gollery M, Van breusegem F (2004). Reactive oxygen gene network of plants. *Trends Plant Sci.* 9: 490-498.
- Nimptsch J, Pflugmacher S (2007). Ammonia triggers the promotion of oxidative stress in the aquatic macrophyte *Myriophyllum mattogrossense*. *Chemosphere* 66: 708-714.
- Pflugmacher S (2004). Promotion of oxidative stress in the aquatic macrophyte *Ceratophyllum demersum* during biotransformation of the cyanobacterial toxin microcystin-LR. *Aquat. Toxicol.* 70(3): 169-178.
- Romero-Puertas MC, Palma JM, Gómez M, del Río A, Sandalio LM (2002). Cadmium caused the oxidative modification of proteins in pea plants. *Plant Cell Environ.* 25: 677-686.
- Sand-Jensen K (1998). Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams. *Freshwater Biol.*, 39(6): 63-79.
- Schulz M, Kozerski HP, Pluntke K (2003). The influence of macrophytes on sedimentation and nutrient retention in the lower River Spree (Germany). *Water Res.*, 37: 569-578.
- Schulz M, Rinke K, Köhler J (2003). A combined approach of photogrammetrical methods and field studies to determine nutrient retention by submerged macrophytes in running waters. *Aquat. Bot.* 76: 17-29.
- Smith IK, Smith TL, Thorne CA (1988). Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis-(2-nitrobenzoic acid). *Anal. Biochem.*, 175: 408-413.
- Vajpayee P, Rai UN, Ali, MB, Tripathi RD, Kumar A, Singh SN (2005). Possible involvement of oxidative stress in copper-induced inhibition of nitrate reductase activity in *Vallisneria spiralis* L. *Bull. Environ. Contam. Toxicol.*, 74: 745-754.
- Wang B, Li W (2002). Physiological reactions of *Potamogeton malaianus* to different N and P concentrations in the growth medium. *Acta Ecol. Sinica*, 22(10): 16-20.
- Wang C, Zhang SH, Wang PF, Hou J, Li W, Zhang WJ (2008). Metabolic adaptations to ammonia-induced oxidative stress in shoots of the submerged macrophyte *Vallisneria natans* (Lour.) Hara. *Aquat. Toxicol.*, 87: 88-98.
- Wang J, Gu Y F, Zhu ZY, Wu B, Yin DQ (2005). Physiological responses of *Ceratophyllum demersum* under different nutritional conditions. *Chinese J. Appl. Ecol.*, 16(2): 337-340.
- Wang J, Gu YF, Ji DC, Yin DQ (2006). Effects of Different Form of Nitrogen on *Hydrilla verticillata* under Eutrophic Nutrient Condition. *J. Res. Environ. Sci.* 19(1): 71-76.
- Wang PF, Wang C, Wang XR, Xue Y, Yang A (2008). Purification Effects on Nitrogen under Different Concentration and Nitrogen Conformation Transform Principles by *Vallisneria Spiralis* L. *Environ. Sci.*, 29(4): 890-896.
- Wen MZ, Li KY, Wang CH (2008). Effects of Nutrient Level on Growth of *Vallisneriaatans* in Water. *Res. Environ. Sci.*, 21(1): 74-77.
- Wheel ERWN (1980). Effect of boundary layer transport on the fixation of carbon by the giant kelp *Macrocystis pyrifera*. *Mar. Biol.*, 56: 103-110.
- Xu YJ, Qian LM (2004). Effects of water movement on nitrogen uptake by *Gracilaria lemaneiformis* (*Rhodophyta*). *Marine Environ. Sci.*, 23(2): 32-35.
- Yan CZ, Zeng AY, Jin XC, Zhao JZ, Xiu QJ, Wang XM (2007). Physiological effects of ammonia-nitrogen concentrations on *Hydrilla verticillata*. *Acta Ecol. Sinica*, 27(1): 1050-105.
- Yan GP, Ma FM, Li WH, Gao JG (1998). Research on Glutamate Synthase Activity in Sugar Beet (*Beta vulgaris* L.) under Different Levels of Nitrogen. *J. Northeast Agric. University*, 5(1): 15-11.
- Ye C, Zou GY, Fu ZS (2007). Responses of three submerged macrophytes to total nitrogen supply. *J. Environ. Sci.*, 27(5): 739-746.
- Zhang FQ, Wang YS, Lou ZP (2007). Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in shoots and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). *Chemosphere*, 67: 44-50.

Zhu ZY, Wang J, Yin DQ, Chen C (2005). Physiological Effects of Different Ratios of  $\text{NO}_3^-$ -N to Urea-N on *Vallisneria spiralis*. J. Nanjing University (Natural Sciences), 4(6): 627-633.