Bull Environ Contam Toxicol (2009) 82:296–299
DOI 10.1007/s00128-008-9531-5

# Physiological Stress of High $NH_4^+$ Concentration in Water Column on the Submersed Macrophyte *Vallisneria Natans* L.

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Received: 4 January 2008 / Accepted: 22 August 2008 / Published online: 22 October 2008


**Abstract** The submersed macrophyte, *Vallisneria natans* L., was cultured in laboratory with $NH_4^+$-enriched tap water (1 mg $L^{-1}$ $NH_4$-N) for 2 months and the stressful effects of high ammonium ($NH_4^+$) concentrations in the water column on this species was evaluated. The plant growth was severely inhibited by the $NH_4^+$ supplement in the water column. The plant carbon and nitrogen metabolisms were disturbed by the $NH_4^+$ supplement as indicated by the accumulation of free amino acids and the depletion of soluble carbohydrates in the plant tissues. The results suggested that high $NH_4^+$ concentrations in the water column may hamper the restoration of submersed vegetation in eutrophic lakes.

**Keywords** $NH_4^+$ stress · *Vallisneria natans* · Eutrophication · Macrophyte restoration

Although ammonium ($NH_4^+$) is an important nitrogen source for plant growth, it is also a toxicant at high concentrations. Many higher plants exhibit toxic syndromes in exclusive $NH_4^+$ environments (Britto and Kronzucker 2002). High $NH_4^+$ concentrations inhibit photosynthesis, trigger oxidative stress and cause internal carbon-nitrogen imbalance of submersed macrophytes (Rudolph and Voigt 1986; Cao et al. 2004). In some eutrophic Chinese lakes, the concentration of $NH_4^+$ is very high in the water column and the restoration of submersed vegetation is far from success. *Vallisneria natans* L. is a common rosette submersed macrophyte in China. In the program of the restoration of submersed vegetation, it is frequently selected as a pioneer species to be introduced into some eutrophic Chinese lakes. However, there is little information on the effects of high $NH_4^+$ concentrations on the plant. In this study, *V. natans* was cultured in laboratory with $NH_4^+$-enriched tap water for two months. The aim was to examine the stressful effects of high $NH_4^+$ concentration on the plant by monitoring the plant growth and carbon and nitrogen metabolisms.

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## Materials and Methods

In May 2003, eighty *V. natans* seedlings of 15 cm height were collected from Lake Liangzi at the outskirt of Wuhan city, China. The seedlings were separately transplanted into 80 cups (7 cm in diameter, 10 cm high) that was filled with sediment collected from the mesotrophic part of Lake Donghu. The concentrations of $NO_3$-N, $NH_4$-N and $PO_4$-P in the sediment pore-water were 0.22 mg $L^{-1}$, 1.59 mg $L^{-1}$ and 0.04 mg $L^{-1}$, separately. The seedlings were cultured outdoor in four tanks ($1 \times 1 \times 0.8$ m), among which two tanks were filled with tap water ($NO_3$-N: 1.06 mg $L^{-1}$, $NH_4$-N: undetectable, $PO_4$-P: undetectable) and regarded as the control and the other two tanks were filled with $NH_4^+$-enriched tap water by adding ammonium carbonate to achieve 1 mg $L^{-1}$ $NH_4$-N in the water column.

The water in the tanks was refreshed in every 5 days and ammonium carbonate was appropriately added to the water. The experiment lasted for 2 months, during which the concentration of $NH_4$-N in the water column ranged from 0.44 to 1.03 mg $L^{-1}$ in the treated tanks, with an average of 0.75 mg $L^{-1}$.

During the experiment, three plants were collected from each tank in every 10 days. The plants biomass and leaf length were measured. A small part of the plants leaves was stored at −18°C for enzyme analysis. The other part was oven-dried at 80°C to constant weight and grounded into powder for the analysis of free amino acids (FAA), soluble carbohydrate (SC) and starch. About 100 mg of each powder was extracted by 10 mL 80% ethanol at 80°C for 20 min and centrifuged at 10,000*g* for 15 min. The supernatant was used for the examination of FAA and SC contents with alanine and glucose as standards (Yemm and Willis 1954; Yemm and Cocking 1955). The residue was used for the analysis of starch contents following the method of Dirk et al. (1999). For the analysis of soluble protein contents and peroxidase (POD) activities, the frozen leaf was ground into fine powder in liquid nitrogen with a mortar and a pestle. About 0.5 g powder of each sample was extracted by 2.5 mL 50 mM phosphate buffer at 4°C for 20 min, and centrifuged at 15,000*g* for 10 min. The contents of soluble protein in the supernatant were measured following the method of Bradford (1976). The activities of POD in the supernatant were measured by monitoring the oxidation of guaiacol at 470 nm with a spectrophotometer (Chance and Maehly 1995).

The mean and standard deviation (SD) were calculated from the measurements of six replicative samples by Microsoft Excel programs. The effects of $NH_4^+$ supplement on the growth and physiology of *V. natans* were analyzed by the *T*-test program of the Statistica (Version 6.0) software.

## Results and Discussion

The growth of *V. natans* was severely inhibited by the $NH_4^+$ supplement in the water column. The biomass of the $NH_4^+$-treated plants was 1.36 g per cup at the end of the experiment, which was significantly lower than the control plants (3.06 g per cup) (Fig. 1). The $NH_4^+$-treated plants sharply accumulated FAA (from 2.1 to 13.9 mg $g^{-1}$ DW) in the tissues during the experiment, and showed higher $NH_4^+$ contents and FAA/SC ratios than the control plants at the end of the experiment (Fig. 2). The contents of $NH_4^+$, FAA and the ratio of FAA/SC were relatively stable for the control plants during the experiment (Fig. 2). The contents of starch decreased gradually (from about 200 to 50 mg $g^{-1}$ DW) in both the $NH_4^+$-treated and the control

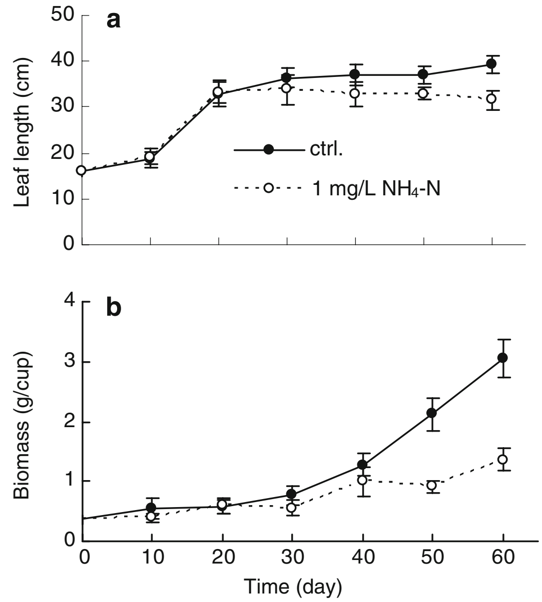


**Fig. 1** Temporal changes in leaf length (**a**) and biomass (**b**) of *V. natans* in $NH_4^+$-enriched (1 mg $L^{-1}$ $NH_4$-N) and control (ctrl) treatments. Vertical bar was the SD of six replicates

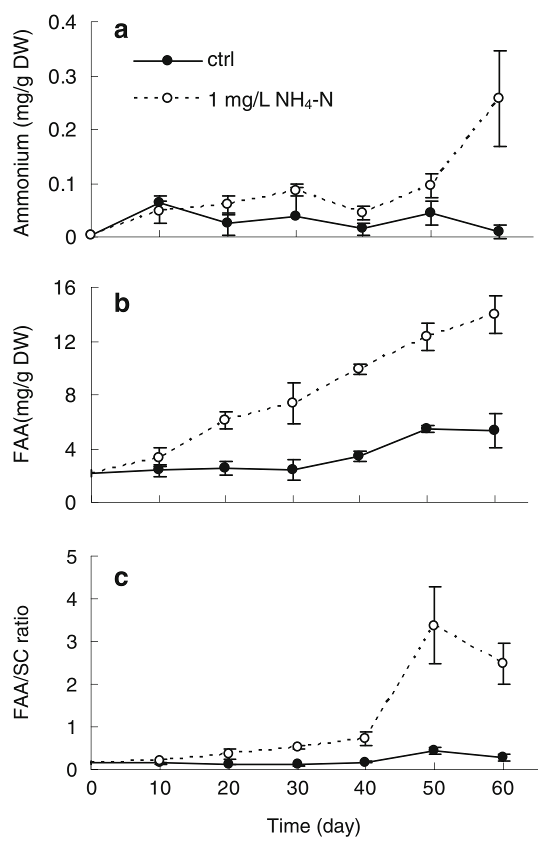


**Fig. 2** Temporal changes in contents of $NH_4^+$ (**a**) and FAA (**b**), and the FAA/SC ratios in the tissues of *V. natans* in $NH_4^+$-enriched (1 mg $L^{-1}$ $NH_4$-N) and control (ctrl) treatments. Vertical bar was the SD of six replicates

plants. The $NH_4^+$-treated plants showed lower SC contents, higher protein contents and higher POD activities than the control plants in the majority time of the experiment (Figs. 3, 4).

Ammonium toxicity has been reported for many macrophytes. In experimental studies, 0.42, 0.5 and 3.7 mg $L^{-1}$ $NH_4$-N inhibited the growth of *Zostera marina*, *Potamogeton maackianus* and *P. densus*, respectively (Mattes and Kreeb 1974; van Katwijk et al. 1997; Li et al. 2007). In the present study, *V. natans* in the $NH_4^+$-enriched water showed strong physiological responses as indicated by the FAA accumulation, SC depletion, high FAA/SC ratios, and increased POD activities in the plants tissues. These results supported that the concentration of $NH_4$-N at 0.56 mg $L^{-1}$ in the water column might be the upper limit for *V. natans* (Cao et al. 2007). In a field survey, Chambers (1987) found rosette macrophytes (e.g. *Valisneria*) grows at an advantage on sediments of intermediate fertility. *V. natans* also prefers to grow in mesotrophic lakes in the Yangtze River basin (Cao et al. 2007). Therefore, it is possible that the release of $NH_4^+$ from the fertile sediments into the water column would inhibit the growth of the *Valisneria* genus in eutrophic lakes.

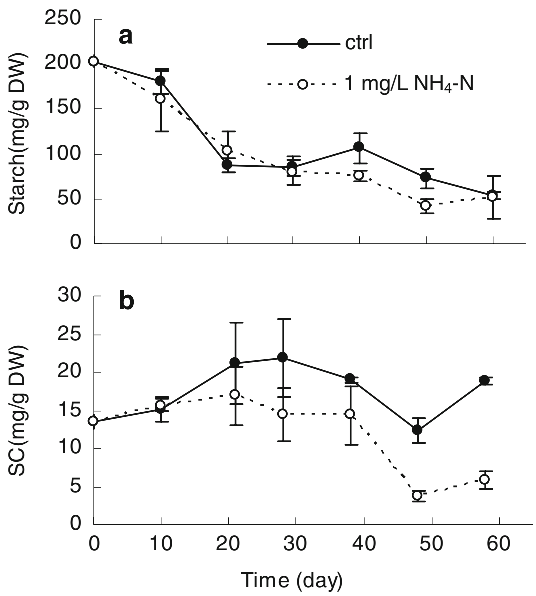


**Fig. 3** Temporal changes in the contents of starch (**a**) and SC (**b**) in the tissues of *V. natans* in $NH_4^+$-enriched (1 mg $L^{-1}$ $NH_4$-N) and control (ctrl) treatments. Vertical bar was the SD of six replicates

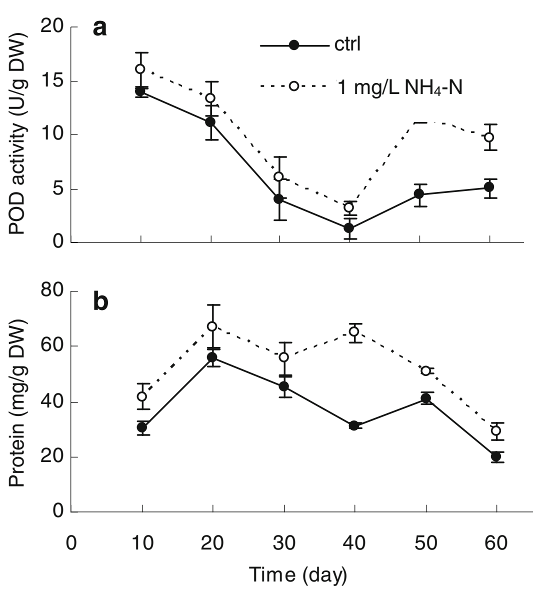


**Fig. 4** Temporal changes in the activity of POD (**a**) and the contents of soluble protein (**b**) in the tissues of *V. natans* in $NH_4^+$-enriched (1 mg $L^{-1}$ $NH_4$-N) and control (ctrl) treatments. Vertical bar was the SD of six replicates

Generally, higher plants passively uptake $NH_4^+$ at high external $NH_4^+$ concentrations (Kronzucker et al. 1996). To prevent $NH_4^+$ accumulation in the tissues, plants actively transport $NH_4^+$ out of the plant cells and expend energy (Britto and Kronzucker 2002). In addition, the synthesis of FAA can diminish the accumulation of $NH_4^+$ in the plant cell (Rabe 1990). In the present study, the extended $NH_4^+$ supplement in the water column led to the depletion of SC and accumulation of FAA in the tissues of *V. natans* and the consequent high FAA/SC ratios indicated that the plant carbon and nitrogen metabolisms were severely disturbed by the $NH_4^+$ supplement. The sharp increase of the contents of $NH_4^+$ in the tissues of *V. natans* at the end of the experiment indicated the inefficient incorporation of $NH_4^+$ into FAA in the situation of SC shortage. In a 6-day experiment, *P. maakianus* that grown in $NH_4^+$-enriched water showed constant SC levels with a decrease in the starch contents, indicating that the degradation of starch may help the plant capable to tolerate $NH_4^+$ stress (Li et al. 2007). *V. natans* under $NH_4^+$ stress showed lower SC contents, instead of lower starch contents, than the control plants, suggesting that the plant may not tolerate extended $NH_4^+$ stress. Our results, together with previous studies (Cao et al. 2007), demonstrated that *V. natans* was vulnerable to high $NH_4^+$ concentrations in the water column. Because the concentrations of $NH_4$-N in the water column is generally at the magnitude of milligram per liter in many eutrophic Chinese lakes (Jin 2003), caution should be used when selecting *V. natans* as a pioneer species in the restoration of submersed vegetation in these lakes.

**Acknowledgements** This research was funded by State Key Technologies Research and Development Programme "Study on Eco-techniques for Improving Water Quality of Drinking Water Source in Meiliang Bay, Lake Taihu (grant no. 2002AA601011), and a NSFC project (grant no: 30570280).

## References

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein dye-binding. Anal Biochem 72:248–254. doi:10.1016/0003-2697(76)90527-3

Britto DT, Kronzucker HJ (2002) $NH_4^+$ toxicity in higher plants: a critical review. J Plant Physiol 159:567–584. doi:10.1078/0176-1617-0774

Cao T, Ni LY, Xie P (2004) Acute biochemical responses of a submersed macrophyte, *Potamoteton crispus* L., to high ammonium in an aquarium experiment. J Freshw Ecol 19:279–284

Cao T, Xie P, Ni LY, Wu AP, Zhang M, Wu SK, Smolders AJP (2007) The role of $NH_4^+$ toxicity in the decline of the submersed macrophyte *Vallisneria natans* in lakes of the Yangtzeriver basin, China. Mar Freshw Res 58:581–587. doi:10.1071/MF06090

Chance B, Maehly A (1995) Assay of catalase and peroxidase. In: Colowick SP, Kaplan NO (eds) Methods in enzymology. Academic Press, New York, pp 764–775

Chambers PA (1987) Light and nutrients in the control of aquatic plant community structure. II. In situ observation. J Ecol 75:621–628. doi:10.2307/2260194

Dirk LMA, Alexander RVK, Dick V, Henk WMH, Derek JB (1999) Galactomannan, soluble sugar and starch mobilization following germination of *Trigone Ua foenum-graecum* seeds. Plant Physiol Biochem 37:41–50. doi:10.1016/S0981-9428(99)80065-5

Jin XC (2003) Analysis of eutrophication state and trend for lakes in China. J Limnol 62:60–66

Kronzucker HJ, Siddiqi MY, Glass ADM (1996) Kinetics of $NH_4^+$ influx in spruce. Plant Physiol 110:773–779

Li HJ, Cao T, Ni LY (2007) Effects of ammonium on growth, nitrogen and carbohydrate metabolism of *Potamogeton maackianus* A. Benn. Fundam Appl Limnol 170:141–148. doi:10.1127/1863-9135/2007/0170-0141

Mattes H, Kreeb K (1974) Die nettophotosynthese von wasserpflanzen, insbesondere *Potamogeton densus*, als indicator für die verunreinigung von gewässern. Angew Bot 48:287–297

Rabe E (1990) Stress physiology: the function significance of the accumulation of nitrogen-containing compounds. J Hortic Sci 65:231–243

Rudolph H, Voigt JU (1986) Effects of $NH_4^+$-N and $NO_3^-$-N on growth and metabolism of *Sphagnum magellanicum*. Physiol Plant 66:339–343. doi:10.1111/j.1399-3054.1986.tb02429.x

Van Katwijk MM, Vergeer LHT, Schmitz GHW, Roelofs JGM (1997) Ammonium toxicity in eelgrass *Zostera marina*. Mar Ecol Prog Ser 157:159–173. doi:10.3354/meps157159

Yemm EW, Cocking EC (1955) The determination of amino acids with ninhydrin. Analyst 80:209–213. doi:10.1039/an9558000209

Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57:508–514