Chemosphere 82 (2011) 329–339

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

# Ammonium, microcystins, and hypoxia of blooms in eutrophic water cause oxidative stress and C–N imbalance in submersed and floating-leaved aquatic plants in Lake Taihu, China

Meng Zhang [a,b], Zhengqi Wang [c], Jun Xu [a], Yaqin Liu [a], Leyi Ni [a,*], Te Cao [a,*], Ping Xie [a]

[a] Donghu Experimental Station of Lake Ecosystems, State Key Laboratory for Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China
[b] Jiangxi Academy of Environmental Sciences, Nanchang, China
[c] Shanghai Representative Office, American Hach Company, Shanghai, China

ARTICLE INFO

*Article history:*
Received 19 April 2010
Received in revised form 1 October 2010
Accepted 3 October 2010
Available online 13 November 2010

*Keywords:*
$NH_4^+$
Microcystins
Hypoxia
Antioxidant activity
C–N balance
Aquatic macrophytes

ABSTRACT

The heavy bloom of cyanobacteria is a disastrous consequence of freshwater eutrophication, and the bloom is highly toxic due to its secondary metabolites called microcystins (MCs). The release of organic substances from dense blooms causes an increase in $NH_4^+$ and decrease in oxygen in lake water. In the present study, the dynamics of physio-biochemical responses of five aquatic macrophytes to MCs and $NH_4^+$ stresses in Meiliang Bay were evaluated. The bay is one of the most seriously eutrophized areas dominated by the toxic cyanobacteria of Lake Taihu, China. The results demonstrate that aquatic macrophytes in Meiliang Bay are subjected to successive external stresses. From January to May, they are subjected to high $NH_4^+$ stress (>0.56 mg $L^{-1}$), whereas from June to September or during dense blooms, the macrophytes experience both MC proliferation and moderate $NH_4^+$ toxicity (>0.3 mg $L^{-1}$). In August, high $NH_4^+$ stress occurs along with hypoxia stress, whereas from September to December, the macrophytes experience moderate $NH_4^+$ stress, causing a serious imbalance in C–N metabolism and oxidative stress. Between the two aquatic plant life forms, floating-leaved plants are more resistant to the stresses of eutrophication than are submersed plants. Elevated MCs in the water column can aggravate oxidative stress and suppress the soluble protein contents of aquatic plants. High $NH_4^+$ in the water causes severe C and N imbalance in submersed macrophytes because of considerable carbon consumption for free amino acid synthesis. The superoxide dismutase activities of submersed macrophytes are suppressed by low light penetrating the eutrophic water, which might impair the antioxidative function of the plants. The findings of this study provide mainly field evidence that reveals the physical, chemical, and biological stresses on aquatic plants in bloom-prevailed eutrophic lakes.



## 1. Introduction

The occurrence of heavy cyanobacterial blooms in eutrophic freshwater is a worldwide ecological problem (Carmichael, 1992). Lake Taihu in China is a well-known case of wide-ranging bloom occurrence, which causes serious problems in the drinking water quality of supply (Guo, 2007; Yang et al., 2008). The cyanobacterial blooms in Lake Taihu occurred since the early 1980s and then deteriorated in 2000 onward. The occurrence of cyanobacterial bloom has adverse effects on many aquatic animals, including zooplankton, zoobenthos, fish, amphibians, and water birds (e.g. Ghadouani et al., 2003; Gérard and Poullain, 2005; Buryskova et al., 2006; Qiu et al., 2007; Atencio et al., 2008; Leão et al., 2008; Chen et al., 2009), as well as on aquatic plants (Yamasaki, 1993); this also causes the degradation of shallow lake ecosystems (Harper et al., 1994).

The mechanisms of submersed plants inhibited by cyanobacterial blooms are characterized by a competition for resources, such as light (Casanova et al., 1999; Pokorný et al., 2002), total inorganic carbon (TIC) (Pokorný et al., 2002), and allelopathy of cyanobacterial toxins (Pflugmacher, 2002; LeBlanc et al., 2005). Apart from released toxins, cyanobacterial blooms can degrade water quality in nearby water sources when toxic cyanobacterial blooms decay; these harmful effects include the exhaustion of dissolved oxygen (DO) (Brownlee et al., 2005) and a sharp increase in nutrients (Buryskova et al., 2006). Low oxygen and $NH_4^+$ stresses during the

* Corresponding authors. Address: Donghu Experimental Station of Lake Ecosystems, State Key Laboratory for Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China. Tel.: +86 27 68780620 (o); fax: +86 27 68780622.
*E-mail addresses:* Nily@ihb.ac.cn (L. Ni), caote@ihb.ac.cn (T. Cao).

0045-6535/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2010.10.038

period of bloom accumulation and decay can aggravate the adverse effect of blooms on aquatic macrophytes (Cizková-Koncalová et al., 1992; Buryskova et al., 2006). Previous experimental studies suggested that the decline of *Potamogeton maackianus* in eutrophic waters can be attributed to multiple environmental stresses, such as increased $NH_4^+$ loading and low DO (Li et al., 2007). However, no field study on the responses of macrophytes to the abovementioned stresses has been reported thus far.

Major cyanotoxins produced by the dominant cyanobacteria *Microcystis* in Lake Taihu are microcystins (MCs), which are among the most dangerous groups of potent hepatotoxins (Codd, 1995; Dawson, 1998). The inhibition effects of MCs on growth, photosynthesis, and/or biochemical–physiological processes have been detected in many aquatic macrophytes, including emergent plants (Pflugmacher et al., 2001; Pflugmacher, 2002), floating-leaved and free-floating plants (Weiss et al., 2000; LeBlanc et al., 2005; Mitrovic et al., 2005; Saqrane et al., 2007), and submersed plants (Pflugmacher et al., 1998a, 1999; Pflugmacher, 2002, 2004; Casanova et al., 1999; Pietsch et al., 2001; Romanowska-Duda et al., 2002; Romanowska-Duda and Tarczynska, 2002; Wiegand et al., 2002; Mitrovic et al., 2005; Yin et al., 2005a). The possible mechanism of MCs toxicity to aquatic macrophytes is attributed to MCs inhibition of protein phosphatases 1 and 2A (PP1 and 2A, Mackintosh et al., 1990; Ding and Ong, 2003). Oxidative stress in macrophytes was observed under MCs exposure, which was attributed to another important mechanism of MCs toxicity through the formation of reactive oxygen species (ROS, Pflugmacher, 2004; Peuthert et al., 2008). However, all of these studies were performed in the laboratory, and little evidence from field studies has been reported.

In addition, $NH_4^+$, one of the major components of domestic wastewaters and agricultural runoff, substantially increases during the eutrophication process. Many studies have shown that elevated $NH_4^+$ concentration is a primary stress factor in aquatic macrophytes because macrophytes are highly sensitive to its toxicity (Mulligan et al., 1976; Best, 1980; Roelofs et al., 1984; Rudolph and Voigt, 1986; Van Katwijk et al., 1997; Cao et al., 2004, 2007; Nimptsch and Pflugmacher, 2007). $NH_4^+$ enrichment can directly cause the decline in aquatic plant populations in natural waters (Ni, 2001; Brun et al., 2002; Cao et al., 2007). The mechanisms include the formation of ROS induced by $NH_4^+$ during its metabolism by the plants (Nimptsch and Pflugmacher, 2007) and the imbalance of C–N reserves in plants stemming from the incorporation of $NH_4^+$ into free amino acids (FAA) at the expense of soluble carbohydrates (SC) consumption (Smolders et al., 1996; Kohl et al., 1998; Saarinen and Haansuu, 2000; Cao et al., 2004, 2007). However, whether the effects of the stresses of MCs and $NH_4^+$ on macrophytes found in laboratory studies apply to natural waters remains uncertain as environmental factors are highly complicated and may interact with each other in the field. This diminishes the function of any single factor.

Few studies that investigate the dynamic patterns of aquatic plants exposed to long-term and/or frequent toxic cyanobacteria and $NH_4^+$ have been conducted *in situ*. This study aims to determine the seasonal physiological and biochemical responses of the aquatic macrophytes of different life forms to toxic blooms and high-$NH_4^+$ water in a large eutrophic Chinese lake, Lake Taihu, meanwhile, compare the sensitivity among plant species and life forms to toxic blooms and high-$NH_4^+$ water and discuss the possible mechanisms. The potential impacts of low oxygen stress are also discussed.

## 2. Materials and methods

### 2.1. Study area and sample collection

Lake Taihu (30°56′–31°34′N, 119°54′–120°36′E) is the third largest freshwater lake in China (with a catchment area of about 36 500 $km^2$, lake area of 2338 $km^2$, average depth of 1.9 m, and maximum depth of 2.6 m). During the past decades, rapid economic development in China has resulted in increased industrial and agricultural pollution, causing the lake to undergo steadily increasing eutrophication (Chang, 1996). In the recent decade, heavy cyanobacterial blooms have occurred every year during warm seasons over wide areas, sometimes covering approximately 1000 $km^2$ (Pu et al., 1998); such occurrence heavily influences the aquatic ecosystem of the lake. Meiliang Bay is one of the hyper-eutrophic areas in Lake Taihu. It is located in the northern region of the shallow lake and has an average depth of 1.8 m (Cai et al., 1997; Dokulil et al., 2000). Dense cyanobacterial blooms always cover this area from June to September.

Samples were taken monthly from large enclosures constructed in Meiliang Bay (Fig. 1) over the period December 2004–November. The enclosures covered a total area of about 2 $km^2$. Sampling was carried out in four sampling sites within the enclosures (Fig. 1). Their positions were located using a GPS (A: 31°30′21.5″N, 120°13′37.3″E, B: 31°30′29.6″N, 120°13′22.9″E, C: 31°30′16.1″N, 120°13′24.0″E and D: 31°29′55.8″N, 120°13′33.5″E). Water samples were a mixture of the surface (0–1 m), middle, and bottom layer samples of every site, and were collected with Tygon tubing fitted with a one-way valve. Water temperature (*T*), Secchi depth (SD), pH, conductivity, dissolved oxygen (DO), and soluble solids (SS) were measured *in situ*. Subsamples of phytoplankton were preserved with 1% acidified Lugol's iodine solution and concentrated to 30 mL after sedimentation for 48 h. After mixing, 0.1 mL of the concentrated samples was directly counted using a microscope at 600× magnification. Colonial *Microcystis* spp. cells were separated using a high-speed blender (Ultra-Turrax) and then counted. Taxonomic identification was processed according to Hu et al. (1979), and biomass was estimated from approximate geometric volumes of each taxon, assuming that 1 $mm^3$ equals $10^{-6}$ μg fresh weight (Shei et al., 1993). Water and algae samples in all the four sampling sites were determined individually.

The five species of aquatic macrophytes found in three sampling sites (A, B and D) belong to two life forms: submersed plants, including *Myriophyllum spicatum* (M. s), *Potamogeton malaianus* (P. m), and *Vallisneria natans* (V. n); and floating-leaved plants, including *Trapa bispinosa* (T. b) and *Nymphoides peltata* (N. p). All these plants are native to Lake Taihu. Ten individual plants per species were randomly harvested from these three sampling sites using a sickle. Five plants for each species were pooled together and stored at −20 °C for the analysis of soluble protein content and SOD (EC 1.15.1.1) activity (Cao et al., 2004). The rest of the plants were dried at 80 °C for 48 h for SC and FAA analyses (Yemm and Willis, 1954; Yemm et al., 1955).

### 2.2. Analysis of environmental factors

Water samples were kept in the dark in a refrigerator before laboratory analysis. Samples were filtered through a membrane filter (GF/C, Whatman, UK) for analysis of ammonium ($NH_4^+$—N), total nitrogen (TN), total phosphorus (TP), and chlorophyll *a* (Chl_*a*) content in the lake water. Biochemical oxygen demand ($BOD_5$) and chemical oxygen demand ($COD_{Mn}$) were assayed using the collected water in appropriate dilution ratios.

The $NH_4^+$—N, TN, TP, $BOD_5$ and $COD_{Mn}$ were analyzed using standard methods (SEPA, 2002). The determination of $BOD_5$ followed the procedures of the standard methods for sample pretreatment, dilution, incubation, and determination of initial and final DO. Chl_*a* was determined using a spectrophotometer (Lorenzen, 1967) after 24 h extraction of the residue on the glass-fiber filter in 90% acetone. The residue on the glass-fiber filter and the filtrate of the 500 mL water sample were used to detect intracellular and extracellular MCs, respectively. MCs were