Marine Pollution Bulletin 135 (2018) 776–782

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Environmental triggers of a *Microcystis* (Cyanophyceae) bloom in an artificial lagoon of Hangzhou Bay, China

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ARTICLE INFO

Keywords:
Estuary
Lagoon
Microcystis
Cyanobacterial blooms
Harmful algae blooms
Eutrophication

ABSTRACT

The relationship between *Microcystis* abundance and environmental variables was studied during a *Microcystis* bloom in the summer months of 2016 in an artificial lagoon of Hangzhou Bay, China. It was determined that *Microcystis* abundance increased from 0.16×10^4 cell/L to 5.8×10^7 cell/L within 17 days from 28 July to 14 August, contributing to 96.84–99.56% of the total phytoplankton abundance. Then, *Microcystis* gradually disappeared 57 days afterwards. The results showed that the growth of *Microcystis*, including the stage of recovery, outbreak, subsidence and disappearance, was significantly correlated with water temperature, salinity, soluble reactive phosphorus (PO_4-P), dissolved inorganic nutrients (DIN), silicate (SiO_4-Si), the ratio of DIN/SiO_4-Si and zooplankton abundance, and the key environmental triggers which promoted the outbreak of *Microcystis* were water temperature, PO_4-P concentration and zooplankton abundance in this artificial lagoon.

1. Introduction

Cyanobacterial blooms, which occur mainly during the summer months, are primarily caused by freshwater cyanobacteria species (Scholz et al., 2017). Approximately 90% of cyanobacteria blooms are known to be caused by species within the *Microcystis* genus, such as *M. aeruginosa*, *M. flosaquae*, *M. ichthyoblabe*, *M. weisenbergii*, and *M. pherta* (Chen et al., 2003). It has been reported that *Microcystis* blooms mainly occurred in eutrophic freshwater bodies, such as Tai lake (Zhang et al., 2012) and Chao lake (Li et al., 2017) in China, Biwa lake in Japan (Yoshida et al., 1996) and Apopka lake in America (Torres et al., 2012). These *Microcystis* blooms are not only caused by the settlement, dormancy, and recovery of *Microcystis* species (Deng et al., 2014), but also by interactions between physical, chemical, biological, and other environmental factors (Wang et al., 2017). When the environmental conditions, such as sea water temperature, nutrients and light intensity, are suitable for the growth of *Microcystis* species, they tend to float from the middle or lower layers to the surface where they become the dominant species (Kong et al., 2010). *Microcystis* blooms therefore occur under specific meteorological and hydrological conditions (Kong et al., 2010).

Coastal lagoons are highly productive systems, and function as the transitional zones between the land and the sea (Schubert and Telesh, 2017). The capacity of a lagoon is influenced by the rate at which the lagoon loses or gains water through evaporation, precipitation, groundwater input, surface runoff, and exchanges with the ocean (Allen et al., 1981). Coastal lagoons connect with the sea through tidal inlets, which can be temporary or permanent (de Wit, 2011). Compared to marine ecosystems, coastal lagoons are more sensitive to eutrophication, because they tend to concentrate anthropogenic nutrient inputs (Litchman et al., 2010) due to limited exchanges with ocean waters and long water residence times (Glibert et al., 2011).

Microcystis blooms have been reported in lagoons worldwide. *M. aeruginosa*, contributing to 30%–92% of the cyanobacterial abundance, was detected in the Kucukcekmece Lagoon, Turkey from October 2000 to June 2003 (Albay et al., 2005). Based on a comparison of the conditions associated with cyanotoxin episodes in 2000, 2001 and 2002, it was found that increases in microcystin toxin were related to temperature, high concentrations of dissolved nutrients, and high levels of photosynthetically active radiation (PAR) (Albay et al., 2005). In the Patos Lagoon located in southern Brazil, toxic blooms of cyanobacteria were observed in late summer and autumn 1994, and early summer

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https://doi.org/10.1016/j.marpolbul.2018.08.005
Received 24 February 2018; Received in revised form 27 July 2018; Accepted 1 August 2018

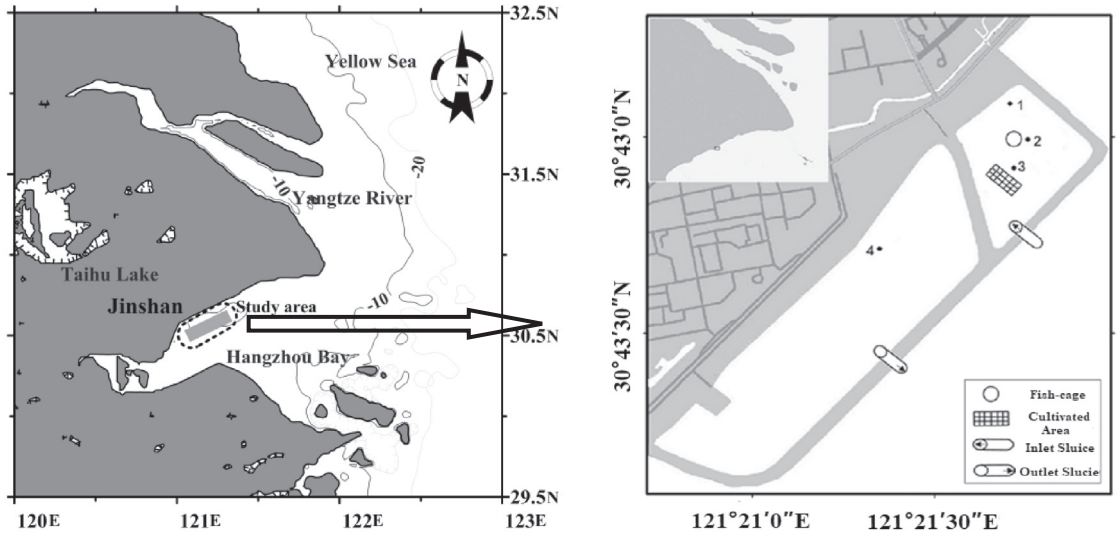

Fig. 1. A sketch map showing sampling sites within the artificial lagoon, Hangzhou Bay, China.

1995 with high abundances of *M. aeruginosa* detected (Yunes et al., 1996). The half lethal dose (LD50) of these toxic bloom samples on mice varied from 22 to 250 mg dry weight kg body weight^{-1},while the half lethal concentration (LC50) for brine-shrimp (*Anemia salina*) varied from 0.47–2.44 mg mL^{-1} (Yunes et al., 1996). There have many studies on the microcystin toxin of *Microcystis* blooms in lagoons; however, few have examined the relationship between *Microcystis* abundance and environmental variables.

This present study aimed to clarify the blooming process of a *Microcystis* bloom in an artificial lagoon in Hangzhou Bay, on the coast of the East China Sea, and to investigate the relationship between the bloom and environmental factors in order to determine which factors likely enhanced bloom conditions during this period.

2. Materials and methods

2.1. Study area

This study was conducted in an artificial lagoon, in the coastal zone of Hangzhou Bay, China (Fig. 1). The surveyed area was approximately 150 ha, and the average depth was 4.5 m. Four sampling sites were selected, as detailed in Fig. 1 and Table 1. Specifically, site 1 was located close to a dam, at the corner of the lagoon, site2 was close to a 0.125 ha aquaculture farm which stocked blackhead seabream (*Acanthopagrus schlegelii*) and yellow croaker (*Larimichthys crocea*), site 3 was within a 0.6 ha spiked water milfoil (*Myriophyllum spicatum*) aquaculture farm and site 4 was located near a beach area. The artificial lagoon was connected to the open water of Hangzhou Bay through an inflow gate and an outflow gate with seawater exchanges occurring when necessary. No seawater was exchanged during the experimental period of this study.

Table 1
Location of four sampling sites in the artificial lagoon within Hangzhou Bay, China.

Site	Longitude	Latitude	Water depth (m)
1	121°21′34.44″E	30°43′09.32″N	3.5
2	121°21′24.90″E	30°43′02.16″N	6
3	121°21′02.91″E	30°42′50.90″N	6
4	121°21′07.57″E	30°42′38.07″N	3

2.2. Water samples collection and analysis

During the experimental period, water temperature, pH, and salinity were measured simultaneously at 0.5 m depth using an YSI multi-parameter water quality meter (YSI, Yellow Springs, OH, USA). Water transparency was measured by a Secchi disc (SD20). Water samples were collected in triplicate using Niskin bottles between 10:00 and 12:00 bimonthly from July 15, 2016 to October 11, 2016. Water samples from each site were collected 0.5 m below the surface and were analyzed for dissolved inorganic nutrients (DIN), soluble reactive phosphorus (PO_4-P), ammonium (NH_4-N), nitrate nitrogen (NO_3-N), nitrite (NO_2-N), silicate (SiO_4-Si), and chemical oxygen demand (COD). Seawater samples for the measurement of DIN were filtered through cellulose membranes (0.45 μm), which were pre-immersed in 10% HCl for at least 10 h and rinsed with distilled water many times before use, and one to two drops of mercury (II) chloride was also added. The sample containers were labeled in the field, temporarily stored in ice-packed coolers, and transported to the laboratory, where they were then stored at approximately −30 °C until later analysis. Silicate concentrations were determined using silicon-molybdenum blue spectrophotometry (UV-1800 spectrophotometer, China). COD was determined by potassium permanganate titration (Tian and Wu, 1992), and the concentrations of PO_4-P, NH_4-N, NO_3-N, and NO_2-N were determined using a Skalar flow analyzer (Skalar San++, The Netherlands). The concentrations of DIN were determined as the sum of NH_4-N, NO_3-N, and NO_2-N values.

2.3. Collection and determination of plankton

Plankton samples were collected in triplicate using Niskin bottles immediately after water samples were collected at each sampling site. For phytoplankton, 1000 mL seawater was sampled at a depth of 0.5 m below the surface, and immediately preserved with neutralized formaldehyde at a final concentration of 4%. The samples were transported back to the laboratory under cool conditions where 250 mL of each sample was then placed in an Utermöhl counting chamber. Phytoplankton cells > 5 μm in diameter were identified and counted using an inverted microscope (Eclipse 100, Nikon, Tokyo, Japan) at 200× and 400× magnification. The density of phytoplankton was recorded as cells L^{-1} and was calculated based on the volume of seawater examined.

For zooplankton, samples were collected in triplicate using a plankton net (68 μm). The collected zooplankton samples were treated with carbonated water and preserved with 4% buffered formaldehyde