An ecological study on dynamics of toxic *Microcystis* blooms in a eutrophic park pond, Wuhan, China*

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Abstract The results of ecological investigation of *Microcystis* blooms and their toxicity in a eutrophic park pond (where the annual average of total nitrogen and total phosphorus was 6.1 $mg \cdot L^{-1}$ and 1.79 $mg \cdot L^{-1}$, respectively) are presented. The blooms were mainly contributed by *M. aeruginosa* occurring in the period from May to October when water temperature ranged from 16 to 33℃. Three remarkable growth peaks of *Microcystis* during the period were observed with chlorophyll *a* level of 0.73 $mg \cdot L^{-1}$, 1.44 $mg \cdot L^{-1}$, and 1.30 $mg \cdot L^{-1}$, respectively. The blooms were independent of phosphorus, but highly dependent on ammonium. A level of ammonium of 9.5 $mg \cdot L^{-1}$ could trigger the outbreak of the bloom, while that below 0.89 $mg \cdot L^{-1}$ could inhibit its formation. In other words, ammonium in higher concentrations could promote blooming, while that in lower concentrations could be inhibitory. *Microcystis* toxicity tended to increase with the blooming process, but the toxic peaks lagged behind their corresponding growth peaks.

Keywords: ***Microcystis* bloom, microcystin, environmental factors.**

With the development of global eutrophication, the increasing of freshwater blooms and ocean red tides have become a significant threat to environments and fishery resources. Cyanobacteria (blue-green algae) have already accounted for 78.56% yearly of overall phytoplankton in contaminated freshwaters, but only for 28.68% in uncontaminated waters[1]. *Microcystis* is the most common genus of cyanobacterial blooms in China and other countries. Many big lakes in China, such as the East Lake of Wuhan City, the Taihu Lake of Jiangsu Province, and the Dianchi Lake of Yunnan Province have been damaged by *Microcystis* blooms and related toxins[2]. Most ponds and reservoirs in mid-southern China have been impacted by *Microcystis* blooms which last as long as 7 ~8 months in a year. Most *Microcystis* blooms are capable of producing a hepatotoxin named microcystin (MCYST, MC), which is a 7-amino-acid cyclic polypeptide and highly stable in structure and function. The release of microcystins to water after the bloom cells decay may result in serious problems to drinking water and fisheries. Up to date, a number of studies have been conducted on algal blooms and toxins under both field and laboratory conditions, including bloom monitoring, toxin analysis and pathologic approaches[3], and many hypotheses have been put forth on incidence and collapse of blooms. However, little is known about the blooming mechanism. There is no clear understanding of ecological functions of toxins produced. In particular, the dynamics of *Microcystis* blooming in relation to its toxicity have not been characterized. In order to further understand the characteristics of *Microcystis* blooming together with its toxicity in environments and the "promoter"[4] for the outbreak of *Microcystis* bloom, we report here the results of a year-round investigation on a eutrophic park pond.

1 Investigation location and methods

1.1 Investigation location

The pond in the Liberation Park in Wuhan City, mid-southern China, is a highly eutrophicated water body, about 20000 m^2 in area, an average of 1.5 m in depth, and is little affected by wind. There is relatively less water movement and no aquatic breeding. *Microcystis* blooms have been frequently registered in recent 3 years. Due to the above reasons, the pond

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was considered as a good model for the present study.

1.2 Methods

A 3L cylindric water collector made of organic glass was employed for sampling. The samples were collected weekly from early January to mid-November in 2000. Three collections were marked as samples A, B, and C, respectively. Samples A and B were collected at the fixed sites, chloroform was added to A for fixation[5] whereas B remained original. Sample C was prepared by mixing the samples with equal volume collected from 5 separate fixed sites without any treatment. Samples A and B were stored at low temperature (2～4℃) and sample C at room temperature. Water temperature, electric conductance, and pH were measured simultaneously for the samples.

Chemical analysis of nitrogen and phosphorus was performed within 24 h of sampling. An HANNA C200 Polyparameter Ionmeter was used for measuring ammonium, nitrite, dissolved inorganic phosphate, and total phosphorus (TP). Chemical titration method was adopted for analyzing nitrate[6]. Sample A was analyzed for the level of ammonium, nitrite, and phosphate, while sample B for that of nitrate and TP.

Sample C was used for algal identification under a microscope and for biomass analysis of the phytoplankton within 12 h of sampling. Chlorophyll *a* (Chla) level was measured according to Eley's method[7] which reflects biomass variation.

The toxicity of the blooms was estimated by mouse intraperitoneal injection (i.p.), and half lethal dose or LD_{50} (milligrams of dry algae used for per kg mouse body weight) was adopted to represent the toxicity according to the method of Dow et al.[8]. Also, the signs of poisoning and liver damage symptom were observed to determine the property of the toxins (hepatotoxin/microcystin or neurotoxin).

Data obtained were analyzed statistically using a software of SPSS10.0.

2 Results and discussion

2.1 Relationship between the blooms and environmental factors

2.1.1 General description of the plankton change during the year Table 1 shows the changes of the planktons during the period in the pond. The biomass pattern of *Microcystis* blooms is presented in Fig. 1, which shows the 3 remarkable *Microcystis* blooms occurring separately in June, July, and August, and a small recurrence in mid-October. The cyanobacterial blooms were mainly attributed to *Microcystis*, especially to *M. aeruginosa*, since it represented up to 91.1% on the average.

Table 1. Year-round changes of the planktons in the pond

Period	Description of plankton
Early Jan. ~ mid-Feb.	Lack of planktons
Mid-Feb. ~ mid-Mar.	*Chlamydomonas* bloom
Mid-Mar. ~ early Apr.	Growth of a few green algae and duckweed
Early Apr. ~ end-Apr.	Increased green algae but no bloom forming
End-Apr. ~ early-May	Emergence of hornwort, rotifer
Mid-May ~ mid-Oct.	*M. aeruginosa* blooms
Mid-Oct. ~ early-Nov.	little algae, disappearance of duckweed

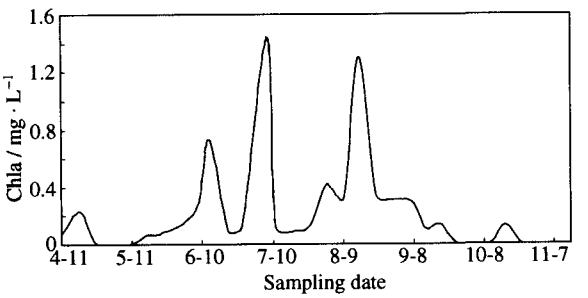

Fig. 1. Changes in biomass of the blooms.

2.1.2 Relationship between the blooms and temperature, phosphorus and ammonium Table 2 shows a significantly positive correlation between Chla and water temperature with a significance level of 95.6%, suggesting that high temperature was likely to be critical to blooming. The average total nitrogen (TN), TP and phosphate contents throughout the blooms were 6.1 $mg \cdot L^{-1}$, 1.79 $mg \cdot L^{-1}$ and 0.76 $mg \cdot L^{-1}$, respectively. Both phosphate and TP during the blooming period were present in excess for cyanobacterial growth, ranging from 0.23 to 14.38 and averaging 4.25 annually. The ratio of TN: TP was much less than 16, which was considered as the optimum for the growth of *M. aeruginosa*[9]. The above data indicate that despite the high concentrations of both nitrogen and phosphorus in the pond, the considerably low N: P ratio "forced" the cyanobacteria taking nitrogen to blooming. Thus, we suggest that blooming is unlikely to be limited by

phosphorus, and which was confirmed by the SPSS correlation analysis.

Table 2. Measurements of some physical and chemical parameters

Date	Water temperature (℃)	Dissolved phosphate ($mg \cdot L^{-1}$)	TP ($mg \cdot L^{-1}$)	Ammonium ($mg \cdot L^{-1}$)	TN ($mg \cdot L^{-1}$)
04-11	19	0.77	—	5.25	5.58
04-19	22	0.54	1.00	0.98	1.18
04-26	21	0.69	1.00	1.13	1.34
05-10	25	1.07	2.25	6.01	6.15
05-17	16	1.26	2.03	0.65	0.84
05-23	28	1.14	1.65	10.80	10.89
06-07	28	0.74	1.70	0.25	0.40
06-13	27	0.75	1.80	0.50	0.55
06-21	28	0.13	0.99	8.00	8.10
06-27	29	0.42	1.33	9.51	9.55
07-08	29	0.65	1.45	8.25	8.39
07-11	30	0.64	1.09	7.00	7.09
07-20	29	1.15	2.17	5.00	5.10
07-26	32	0.28	1.10	4.85	5.01
08-02	30	0.60	1.07	0.89	1.00
08-09	30	0.46	1.10	10.00	10.10
08-16	30	0.70	0.89	12.50	12.74
08-23	33	0.31	3.00	22.22	22.39
08-30	27	0.52	1.83	9.75	9.95
09-07	22	0.69	1.40	7.75	7.96
09-13	24	0.78	1.20	6.25	6.56
09-19	27	1.48	2.20	5.68	5.74
09-27	25	2.34	10.20	6.78	6.82
10-10	26	0.66	1.40	2.77	3.03
10-17	17	0.57	0.80	3.63	3.73
10-24	19	0.57	1.20	2.55	2.70
10-31	16	0.62	0.90	4.06	4.31
11-14	12	0.62	1.70	3.97	4.13

As also shown in Table 2, ammonium concentration in the pond was high. Throughout the blooms, it fluctuated from 0.25 to 22.22 $mg \cdot L^{-1}$ with an average up to 5.96 $mg \cdot L^{-1}$. As already noted above, the ratio of N:P was much less than 16, thus blooming was more likely limited by nitrogen content. Moreover, as algae took nitrogen in the form of ammonium rather than in other forms[6,10,11], ammonium might play a leading role in the blooming of *Microcystis*, and this is in agreement with the SPSS correlation analysis, which presents positive correlation between Chla content and ammonium level with a significance level of 82%.

The development of an algal bloom consists of three stages: initiation, development, and decay. Initiation may be the most important one to the ecological study of blooming[12]. As mentioned above, blooming of *Microcystis* mainly occurred from May to October with three growth peaks on May 13, July 8, and August 16, respectively (Fig. 1). Therefore, we analyzed the effect of ammonium concentration on initiation of the blooms (Table 3).

Table 3. Initial content of ammonium and change of biomass during the three stages of *M. aeruginosa* blooms

Stage	Bloom rise period (date)	Maintenance duration (day)	Chla ($mg \cdot L^{-1}$)[a)]	Average rate[b)]	Ammonium ($mg \cdot L^{-1}$)[c)]
1	05.23~06.13	21	0.66	0.03	10.80
2	06.27~07.08	12	1.32	0.11	9.51
3	08.09~08.16	8	0.98	0.11	10.00

a) Biomass difference between peak and initiation; b) ratio of the difference of biomass to maintenance days; c) concentration at initiation.

As clearly shown in Table 3, the concentration of ammonium at the three initiation stages of the blooms was as high as 10 $mg \cdot L^{-1}$. Analyzing the results of some long-term investigations by other researchers, Braj et al.[13] noted that the contents of dissolved ammonium in water bodies at the initiation of cyanobacterial blooms were significantly higher than those in waters without blooms. Christina et al.[14] discovered that high content of nitrogen was responsible for initiating *Microcystis* blooms in the Paranoa Reservoir of Brazil. Accordingly, high content of ammonium was likely the initiator of *Microcystis* blooms in the pond surveyed, with the critical concentration around 9.5 $mg \cdot L^{-1}$.

It should be noted that ammonium level was observed to be low within a few days of the period, though it was considerably high most of the time (Table 4).

Table 4. Change in biomass at low levels of ammonium

Stage	Date	Ammonium($mg \cdot L^{-1}$)	Chla($mg \cdot L^{-1}$)[a)]
1	05.17	0.65	0.01
2	06.07	0.25	0.49
3	06.13	0.50	-0.64
4	08.02	0.89	-0.10

a) Biomass difference within a week of each blooming.

As shown in Table 4 and Fig. 1, low levels of ammonium did not lead to a significant change in biomass in the following 7 days. That is, there was no clear sign for rapid growth or sudden death of *M. aeruginosa*. On the contrary, when ammonium concentration decreased to 0.5 $mg \cdot L^{-1}$ on 13 June (stage 3), the bloom decayed drastically. Since the

essential concentration of ammonium for the growth of algae is 1.0 mg·L^{-1}[15], we think that ammonium at the level of ca. 0.89 mg·L^{-1} could limit the growth of *M. aeruginosa*. If ammonium level was lower, the cyanobacteria could hardly grow. Furthermore, ammonium below 0.5 mg·L^{-1} caused the bloom to decay rapidly. As an exception, the biomass of *Microcystis* blooms increased substantially within a week in stage 2 even though the corresponding ammonium content was only 0.25 mg·L^{-1}. This might be associated with an input of nutrients during the period.

The details of the decay of the blooms are shown in Table 5.

Table 5. Biomass pattern and decay rate

Stage	Decay period	Maintenance duration/(day)	Chla/(mg·L^{-1})[a)]	Average rate of decay
1	06.13~06.21	9	-0.64	-0.07
2	07.08~07.11	4	-1.32	-0.33
3	08.16~08.23	8	-0.96	-0.12
4	08.23~09.13	21	-0.23	-0.01

a) Difference of biomass during the decay period.

The decline of the bloom in stage 1 resulted from low concentrations of ammonium, as discussed above, but the rapid decay of blooms in stage 2 and stage 3 was merely a natural process. Since the content of both N and P at the beginning of stage 2 and stage 3 was high (TN were 8.39 mg·L^{-1} and 12.74 mg·L^{-1} respectively, TP were 1.45 mg·L^{-1} and 0.89 mg·L^{-1} respectively), the decay of the blooms could not be attributed to nutrient deficiency. Rather, it can be considered as a natural disappearance following the growth peak. The slow decline of the biomass in stage 4 might be related to a high level of ammonium (22.22 mg·L^{-1}) on 23rd August and a decrease in temperature from 30th August to 7th September. As mentioned above, low temperature is unfavorable for the growth of *Microcystis*. Moreover, excessive ammonium is harmful to algae[15]. It is not clear whether ammonium in very high concentrations could inhibit growth of *Microcystis*. If so, what is the inhibiting concentration then?

To summarize, ammonium is closely related to the growth of *Microcystis*. Specifically speaking, ammonium at high level initiates *Microcystis* blooms, while that at low level inhibits it. Moreover, if ammonium is below a certain level, the blooms might fade away in a short period.

2.1.3 Relationship between blooms and other factors The SPSS correlation analysis showed no significant relationship between the biomass of the blooms and other physicochemical factors monitored, such as nitrate, nitrite, pH, and electric conductance.

2.2 Relationship between the toxicity and the growth

Examination of the mice treated with i.p. injection revealed that the toxicological symptons were typical of hepatotoxins or microcystins occurring in the cells of *Microcystis* blooms. With $1000/LD_{50}$ substituting for LD_{50}, the dynamics of toxicity is presented in Fig.2.

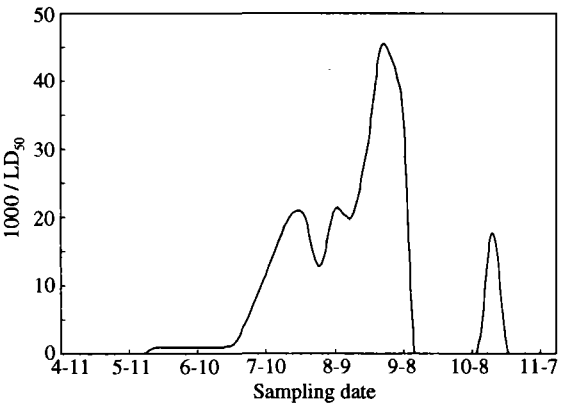

Fig.2. Changes in toxicity of the bloom. $1000/LD_{50}$ is defined by the weight of mice (per gram) killed by 1 mg of dry alga.

As clearly shown in Fig. 2, the toxicity represented a trend of gradual increase throughout the blooms from mid-May to end-September. The development of toxicity can be divided into 4 stages (Table 6).

Table 6. Change in toxicity

Stage	Initiation date	End date	Toxicity (LD_{50}) (mg·kg^{-1})
1	05.23	06.21	>1000
2	06.27[a)]		485
3	07.08	08.15	47~99
4	08.23	09.07	22~33

a) Measured between initiatoin and end date.

The statistical analysis with SPSS showed that the toxicity was positively correlated with the time course of blooming and the significance level was 100%. It means that toxin was not produced at the initiation period of the blooms, but gradually accumulated with the development of the bloom and finally reached its maximum when the blooms were about to disappear. Lee[9], dealing with microcystin yield of

M. aeruginosa relative to N: P ratio and growth stage, discovered a highly significant relationship between microcystin content and Chla concentration and accordingly, the microcystin content of *M. aeruginosa* can be easily estimated and monitored by measuring the *in vivo* fluorescence changes in the culture. Under all kinds of medium conditions, Rao[16] observed that the toxicity of cells increased with the growth process of *M. aeruginosa*. Other investigations on the pattern of microcystin production during an entire life cycle came to a common conclusion that the toxicity of *M. aeruginosa* increased with time, reached its peak at stationary stage and decreased quickly thereafter[17,18]. Similarly, the toxin-producing pattern we noted happened in a relatively long ecological period during which the blooms had experienced a number of growth cycles of the cyanobacteria.

Again, by comparing the curves of growth (Fig. 1) and toxicity (Fig. 2), it was noted that the toxic peaks lagged behind the corresponding growth peaks. Moreover, the higher the growth peaks, the longer the toxicity peaks lagged behind.

As seen in Table 7, following the prominent growth peaks 1 and 3, the relevant toxicity peaks appeared about half a month later, while in the case of smaller growth peak 2, the corresponding toxicity peak appeared a week later. This lagging phenomenon is most likely due to a need of time for accumulation of toxins in cells.

Table 7. Comparison of growth peaks and toxicity peaks

Growth peak	Date	Chla/ mg·L^{-1}	Toxicity peak	Date	Lagging time /day
1	07.08	1.44	A	07.26	18
2	08.02	0.42	B	08.09	7
3	08.16	1.30	C	08.30	14

From the above analysis, it is suggested that the periodical change in toxicity and the lag of toxin production may help predict the trend of toxicity change. More important, a marked increase in toxin accumulation in *Microcystis* cells after the collapse of bloom might hint some special ecological functions of microcystin. A number of reports had pointed out that conditions most favorable to the growth of *M. aeruginosa* may be unfavorable to the production of microcystin, and toxin yields might increase on the condition that certain nutrients were deficient[16~18]. Lukac et al.[19] took it into consideration that microcystins with complex structures were unlikely waste products or that their presence is fortuitous or accidental, since the energy cost involved in their production is likely to be high. From this point of view, they may meet particular needs or requirements of the organisms that produce them. When the growth of *M. aeruginosa* reached the maximum level, some adverse conditions like nutrient deficiency and feeding pressure emerged, thus toxins in the cells of the cyanobacterium accumulated rapidly and were released to water after cell breakdown, result in poisoning and death of other organisms[20,21] or changing of the competition patterns of zooplanktons in the water ecosystem[22]. Besides, because of the nutrients returning to water after cyanobacteria died, the environments became favorable to cyanobacterial growth and even formation of another bloom.

From the results above, it can be concluded that there is no relationship between phosphorus and *Microcystis* blooming. In contrast, ammonium plays a leading role in the occurrence and disappearance of the blooms, that is, the blooms are initiated by adequate ammonium, and can be inhibited by excessive and inadequate one, or even terminated when ammonium content is sufficiently low. In addition, the consequent toxicity presents a remarkable rising tendency and its maximum lags behind the growth peaks all along. This phenomenon may somewhat reflect the relationship between toxin production and cyanobacterial growth. The ecological significance of toxin production is at least related to suppression of the growth of other organisms and resumption of growth of toxin producers in adverse environments. The rapid growth mechanism of *Microcystis*, the physiological and ecological functions of toxin production, however, remain to be investigated by defining important factors under laboratory and model conditions.

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