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Effects of Rainfall in Kunming on the Growth and Alkaline Phosphatase Activity of the Cyanobacterium *Microcystis aeruginosa*

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Abstract

The effects of rainwater on freshwater ecosystems have received increasing attention worldwide. Alkaline phosphatase activity (APA) affects the biochemical cycles of phosphorus in the water, thereby affecting the proliferation and outbreak of cyanobacteria blooms. However, it is still unclear whether the complex composition of rainwater has a significant effect on the alkaline phosphatase activity. In this study, the effects of rainfall on *Microcystis aeruginosa* in Kunming were evaluated based upon changes in alkaline phosphatase activity, algal growth, and chlorophyll *a*. The results showed that the addition of rainwater brought about different changes in alkaline phosphatase activity, algal growth, and chlorophyll *a*. The general trend showed that a certain amount of rainwater (equivalent to light, moderate rain, and heavy levels) had a positive effect on the alkaline phosphatase activity, algal growth, and chlorophyll *a* in the cultivation system. Therefore, the following preliminary inference can be drawn: in the Kunming area, a certain amount of rainwater may promote the growth of blue-green algae, exacerbating an outbreak of cyanobacteria blooms.

Keywords: *Microcystis aeruginosa*; Rainwater; Alkaline phosphatase activity; Chlorophyll *a*

Introduction

Lake pollution and eutrophication is a severe environmental problem in China [1,2], well in line with global trend. In particular, eutrophication can lead to an outbreak of cyanobacteria blooms [3], where cyanobacteria *Microcystis aeruginosa* is one of the dominant species. Toxic *M. aeruginosa* is one of the most serious symptoms, which could cause severe health issues and increase financial pressure [4,5]. The excessive growth of toxic *M. aeruginosa* greatly deteriorate the water quality, damaging lakes' natural functions, and even threatening the drinking water resources [6-10]. The scientific and technical problems related to the reduction of nitrogen, phosphorus, and other nutrients and controlling algal blooms continue to remain as unsolved areas in terms of ecological restoration and pollution control in lakes.

Phosphorus is considered the main limiting nutrient in freshwater ecosystems. The study of phosphate regeneration is essential for the understanding of phosphorus cycling in aquatic ecosystems. Phytoplankton must constantly absorb inorganic phosphorus, which gradually reduces the content of inorganic phosphorus in the water [11,12]. The concentration of inorganic phosphorus in a lake is often less than 5% of the total phosphorus concentration. Therefore, the rate of organic phosphate turning into inorganic phosphate significantly affects the eutrophication and the formation of cyanobacterial blooms in lakes [13]. The ability of algae to acquire phosphorus from dissolved organic phosphorus compounds, depends on the activity of phosphatases, catalyzing the hydrolysis of phosphate monoesters and liberating inorganic phosphate and organic matter. Alkaline phosphatase in lakes comes mainly from phytoplankton [14], bacteria [15], and zooplankton; this process plays a catalytic role in the process of conversion of organic phosphorus into inorganic phosphorus. The enhancement in the alkaline phosphatase activity can accelerate the conversion of organic phosphorus to inorganic phosphorus. Its purpose is to provide nutrients that are more absorbable for cyanobacterial blooms, and it plays a key role in the formation of cyanobacterial blooms and the biogeochemical cycles of phosphorus in water [16-18]. It has often been used as an indicator of the nutritional status of the phytoplankton communities in terms of phosphorus concentration [14,19,20].

Rainfall could bring atmospheric pollutants into the lakes. So rain and its runoff has an important impact on the water quality of lakes, and even the surface of the sea [21-23]. Alkaline phosphatase activity affects the biochemical cycles of phosphorus in the lake, thereby affecting the proliferation and outbreak of cyanobacterial blooms. While previous studies have suggested that the changed in rainfall may severely promote the occurrence of cyanobacteria bloom [24], it is still unclear whether the complex composition of rainwater has a significant effect on the alkaline phosphatase activity. The effects of rainfall on *Microcystis aeruginosa* in Kuming have been examined in this study. The effects have been evaluated based on changes in alkaline phosphatase activity, algal growth, and chlorophyll a. The main purposes of our study are to study the effect of rainwater on the lake eutrophication ecosystem in the Dianchi Lake, and to provide a scientific foundation for research in this regard.

Materials and Methods

Sample collection

The rainwater samples were collected from July to August, which was considered the rainy season in Kunming. Rainwater was collected using a polypropylene box. First, the box was soaked in a HCl (1:5) solution for a few days, followed by rinsing with deionized water. The box was placed on a platform 3 m above the ground, and it was opened immediately before precipitation occurred. Immediately after collection, the rainwater sample was transferred to a polyethylene bottle and stored at 10°C until analysis. Before the experiment, the rainwater samples were filtered through a 0.45 μm Millipore filter. The environmental

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conditions (wind speed, precipitation, atmospheric pressure, average wind velocity, etc.) were recorded in Table 1.

Algae cultivation

Microcystis aeruginosa (*M. aeruginosa*) (FACHB-927) was provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Cells were cultivated in a BG11 medium [25,26] at 25 ± 1°C in autoclaved conical flasks with a 12 h light-dark cycle and irradiance of 58 μmol photons/(m^2·s), and they were shaken four times every day. In this study, the cells in the exponential growth phase were used, which were ascertained by measuring the cell density every day.

Measurements of alkaline phosphatase activity

Alkaline phosphatase activity was assayed by p-nitrophenyl phosphate (pNPP), which was hydrolyzed by the enzyme to yield yellow p-nitrophenol (PNP). The rate of PNP production was measured using the colorimetric method, and the rate was used as an indicator of alkaline phosphatase activity. With this system, enzyme activity is indicated by an increase in light absorbance. Previous studies and our pre-experiments found that the pH, reaction temperature, reaction time, and the volume of reactants could affect the enzymatic reaction [14,27,28]. The modified procedure followed that described by Berman [14]. The samples were placed into a cuvette containing a solution of Tris-HCl buffer (pH 8.4) and 2 mL of p-nitrophenyl phosphate (pNPP). After incubation (6 h at 30°C), the absorbance was then measured at 410 nm to determine the production of PNP. Controls containing no substrate and no cells were included to correct the absorbance changes due to cell density and spontaneous hydrolysis of the p-NPP. APA was assayed at eight different concentrations of pNPP from 0.3 to 3 mmol/L and the initial velocity was determined for each concentration. The Lineweaver-Burke transformation of the Michaelis-Menten equation was used to calculate the Michaelis constant (K_m) and maximum velocity of the enzyme (V_{max}). K_m and V_{max} were computed by linear regression analysis of the values obtained in the assay. All samples were run in triplicate.

Measurements of cell density and Chl *a*

The cell density was determined by a microscope (Shanghai Dilun Optical Instrument Co., Ltd., XSP-8CA) using the hemacytometer counting method. Cell density was determined by measuring its absorbance at 680 nm (OD_{680}) with an ultraviolet-visible (uv-vis) spectrophotometer. The regression equation between OD_{680} (Y) and the number of cells (X, × 10^6 cell mL^{-1}) was established as Y=0.055X-0.005 (R^2=0.99).

Chl *a* content was measured after extraction with 90% acetone. Briefly, 5-mL samples were filtered through a 0.45-μm Millipore filter. Chl *a* was extracted with 3 mL of 90% acetone at 4°C for 24 h. The extract was then centrifuged and the Chl *a* content of the supernatant was measured with a spectrophotometer (752N; Shanghai Lengguang Industrial Co., Ltd., Shanghai, China) at wavelengths of 630, 645, 663, and 750 nm. Chl *a* content was calculated as follows:

Parameters	Data
Air temperature (°C)	18.34
Atmospheric pressure (mm Hg)	605.06
Average wind velocity (ms^{-1})	2.29
Precipitation/RRR (mm)	23
pH	7.6

Table 1: Sampling of environmental conditions and rainwater of the main physical and chemical indexes.

$$Chl\,a\left(\mu g\cdot L^{-1}\right)=\frac{\left(11.64\times(A\,663-A\,750)-2.16\times(A\,645-A\,750)+0.10\times(A\,630-A\,750)\right)\times V1}{V} \quad (1)$$

A: absorbance, V1: volume of 90% acetone (mL), V: volume of water sample (L) (State Environmental Protection Administration (SEPA) Water and wastewater monitoring analysis method. 4th edn. Beijing: China Environmental Science Press (in Chinese)).

Experimental design

All experiments were conducted in triplicates. The standard BG11 medium was prepared and sterilized at high temperature (120°C for 30 min. It was then cooled, and 150 mL was placed into each sterilized tissue culture flask. Simulating of the effect of different rainfall amounts (light rain, moderate rain, and heavy rain) falling into the lake, the rainwater (0.45 μm Millipore filtered) measuring 6, 12, and 24 mL were added to the tissue culture flasks. This was equivalent to rain addition for a total volume of 2%, 4%, and 8%, respectively, after inoculation. We then added the *M. aeruginosa*, in the exponential growth phase, to the tissue culture flasks, and this made the total volume of the solution to 300 mL. In addition, 150 mL of deionized water were also added to the flasks, which had the same volume of culture solution as the control group. The cell density, Chl *a*, and alkaline phosphatase activity were carried out at 12, 24, 48, 72, 96, and 120 h after the onset of different treatments. In addition, the rainwater samples were measured and observed for the occurrence of alkaline phosphatase activity.

Statistical analysis

All experiments were performed in 3 replicates. Means and standard deviation (S.D.) were calculated and presented. All statistical analyses were conducted using SPSS 19.0. All figures were plotted using Origin 8.0.

Results

Alkaline phosphatase activity of the rainwater

According to the method described in Section 2.3, the APA of the rainwater was 4.12 nmol/ (L·min). This shows that the rainwater samples have some alkaline phosphatase activity. However, compared with the activity value and the determination of lake eutrophication [29,30], the APA of rainwater was relatively small.

Effects of rainwater on growth of *M. aeruginosa*

The growth of *M. aeruginosa* was promoted to different extents under different rainwater concentrations. As seen in Figure 1a, all experimental groups and control groups appeared increasing tendency, but the cell number of control were significantly less than the experiment group. The changes of cell number between every experiment group and control group were as following: 2% rainwater treatment significantly increased the cells from 1.346 to 3.770 × 10^6 cells·mL^{-1}, 4% and 8% rainwater treatment increased from 1.291 to 3.667 × 10^6 cells·mL^{-1} and 1.327 to 4.091 × 10^6 cells·mL^{-1}, respectively. These three treatment increased by 180.17%, 184.04%, 208.29%, respectively. 2% rainwater control significantly increased the cells from 1.036 to 3.509 × 10^6 cells·mL^{-1}, 4% and 8% rainwater control increased from 1.236 to 2.752 × 10^6 cells·mL^{-1} and 1 to 3.218 × 10^6 cells·mL^{-1}, respectively. These three control increased by 238.71%, 122.65%, 221.8%, respectively (Figure 1).

Effects of rainwater on Chl *a* content of *M. aeruginosa*

The trends in Chl a content under different doses of rainwater showed patterns similar to that of cell density (Figure 1b). 2% rainwater treatment significantly increased the cells from 0.274 to 1.034 μg·L^{-1}, 4%

and 8% rainwater treatment increased from 0.273 to 0.92 μg·L^{-1} and 0.273 to 0.807 μg·L^{-1}, respectively. These three treatment increased by 277.37%, 237%, 195.6%, respectively. 2% rainwater control significantly increased the cells from 0.182 to 0.456 μg·L^{-1}, 4% and 8% rainwater control increased from 0.182 to 0.667 μg·L^{-1} and 0.183 to 0.876 μg·L^{-1}, respectively. These three control increased by 150.55%, 266.48%, 378.69%, respectively.

Effects of rainwater on APA of *M. aeruginosa*

The kinetics of alkaline phosphatase was assayed for the rainwater concentration at different substrate concentrations (*p*-NPP, from 0.3-3.0 mM) and the initial velocity was determined for each concentration. The total APA activity varied strongly over time, K_m had a downward trend over the time in the treatment group, but the control had the similar trend before 96 h, and then was rising (Figure 2). K_m of 2% rainwater treatment significantly decreased from 0.246 to 0.095 μmol·L^{-1}, 4% and 8% rainwater treatment decreased from 0.181 to 0.083 μmol·L^{-1} and 0.246 to 0.095 μmol·L^{-1}, respectively. These three treatment decreased by 56.68%, 54.06%, 61.19%, respectively. For the control groups, the minimum value of K_m appeared at 96 h: 0.1090, 0.1063, 0.1444 μmol·L^{-1}.

In contrast to K_m, V_{max} had the trend of increase over the time. V_{max} of 2% rainwater treatment significantly increased from 10.020 to 20.121 μmol·min^{-1}·L^{-1}, 4% and 8% rainwater treatment increased from 9.950 to 20.790 μmol·min^{-1}·L^{-1} and 10.504 to 19.881 μmol·min^{-1}·L^{-1}, respectively. These three treatment increased by 100.81%, 108.94%, 89.26%, respectively. 2% rainwater control significantly increased from 11.390 to 22.472 μmol·min^{-1}·L^{-1}, 4% and 8% rainwater control increased from 8.518 to 20.080 μmol·min^{-1}·L^{-1} and 8.496 to 23.810 μmol·min^{-1}·L^{-1}, respectively. These three control increased by 97.30%, 135.74%, 180.24%, respectively (Figure 2).

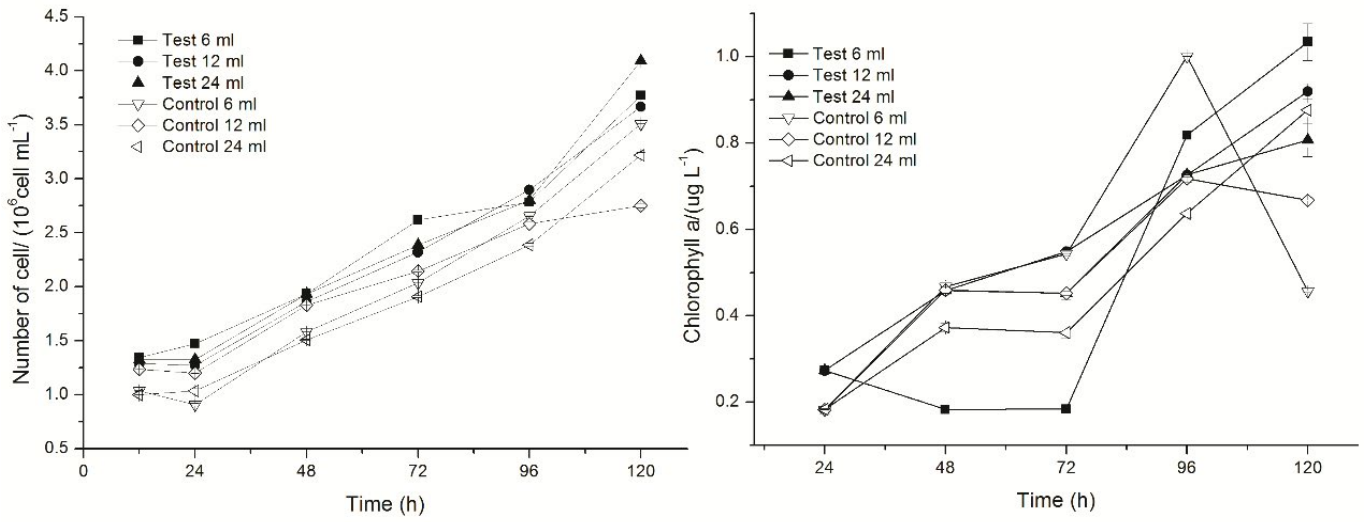

Figure 1: Effect of rainwater on cell density and Chl *a* of *M. aeruginosa*.

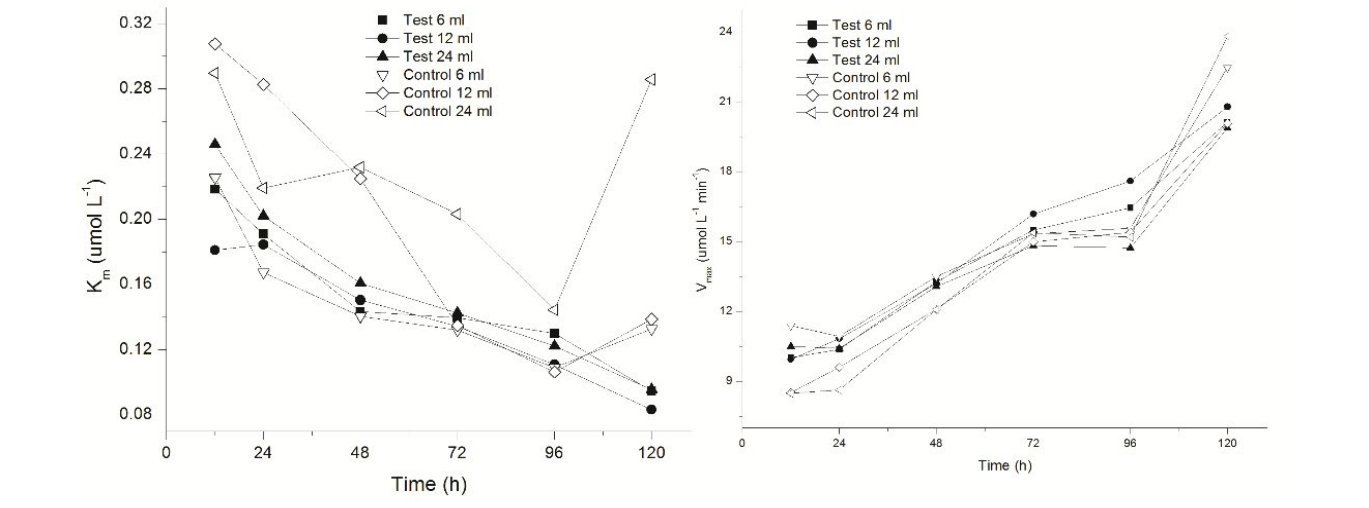

Figure 2: Effect of rainwater on APA of *M. aeruginosa*.

Discussion and Conclusion

The control of cyanobacterial blooms has become an important target worldwide and rainfall might lead to large changes in the physicochemical structure of water bodies [31]. Our results demonstrated that rainfall could effectively increase the growth of *M. aeruginosa*. The action of rainwater on cell number of *M. aeruginosa* increased dramatically over time (Figure 1). The results showed that the cell number of *M. aeruginosa* in treatment group was higher compared with that of the control group. This suggests that appreciated rainwater might improve the growth of *M. aeruginosa*.

Alkaline phosphatase is located on the cell membrane and can hydrolyze organic phosphorus-containing compounds when inorganic phosphate is deficient [32]. In the present study, the patterns of APA implied that the rainwater would be an important factor affecting the kinetics of alkaline phosphatase. The effects of rainwater on the APA of the system that cultivated *M. aeruginosa* showed a positive impact as a whole. However, the positive impact showed a decreasing trend when rainwater content increased. The alkaline phosphatase activity followed the regulatory mechanism of induction-inhibition. As the activity increased, it was shown that algae growth in the cultivation system leads to an increased demand for inorganic phosphorus, and vice versa. A between-basin comparison of kinetics of APA was made. The variability in V_{max} was large, the treatment was higher than the control. This mean that rainwater could improve the activity of alkaline phosphatase in the water body. There is no clear trend of changes in K_m values, but the highest values appeared at 12 h of the treatment, and 120 h of the control.

This study showed that different amounts of rainwater added to a cultivation system result in similar influences from alkaline phosphatase activity, algal growth, and chlorophyll *a*. The general trend was that a amount of added rainwater (equivalent to a light, moderate, and heavy rain) may have a positive effect on alkaline phosphatase activity, algal growth, and chlorophyll fluorescence parameters in the cultivation system. Thus, in the Kunming area, a certain amount of rainfall may promote the growth of blue-green algae in a lake with local eutrophication, exacerbating the risks and hazards of an outbreak of cyanobacterial blooms. In addition, alkaline phosphatase activity was detected under the influence of rainfall, but its values were lower than that observed in the eutrophic lakes.

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