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*. 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*. 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*. 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*. 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*.

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 change 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 Kunming 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...
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/m2-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 (Km) and maximum velocity of the enzyme (Vmax). Km and Vmax 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 (OD680) with an ultraviolet-visible (uv-vis) spectrophotometer. The regression equation between OD680 (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:

\[
\text{Chl } a [\mu g \cdot L^{-1}] = \frac{1.16 \times (A_{663} - A_{750}) - 2.16 \times (A_{645} - A_{750}) + 0.10 \times (A_{630} - A_{750}) - V1}{V} \cdot 10^{-4}
\]

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 rainfall 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 experimental groups and control groups. 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 experimental groups and control groups. 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 experimental groups and control groups.

<table>
<thead>
<tr>
<th>Parameters</th>
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<tr>
<td>Air temperature (°C)</td>
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<tr>
<td>Atmospheric pressure (mm Hg)</td>
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<tr>
<td>Average wind velocity (ms^-1)</td>
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<td>Precipitation/RRR (mm)</td>
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<tr>
<td>pH</td>
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Table 1: Sampling of environmental conditions and rainwater of the main physical and chemical indexes.

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%
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, with *K_m* having a downward trend over the treatment group, but the control had a 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, while the 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 treatments 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, while the 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 treatments 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, while the 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).

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**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 physiocochemical 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 algal 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 Km control. This mean that rainwater could improve the activity of alkaline phosphatase in the water body.

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 added rainwater (equivalent to a medium to heavy 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.

References