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The Correlation between the Variation of Dissolved Inorganic Carbon Content and Cyanobacterial Blooms in Fubao Bay of Dianchi Lake

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Abstract To explore variation of dissolved inorganic carbon content (DIC) caused by cyanobacterial blooms and provide a basis for formulating effective preventive and control measures of cyanobacterial blooms, the concentration of inorganic carbon and the concentration of planktonic algae were studied by sampling method, and the distribution and variation of the DIC and physicochemical factors in the ecological restoration area of Fubao Bay of Dianchi Lake were analyzed. Results indicated that the distribution of chlorophyll-a was significantly positive correlated with CO_3^{2-} ($P < 0.01$) and pH values ($P < 0.05$); and the distribution of chlorophyll-a was significantly negative correlated with CO_2 ($P < 0.01$), DIC and HCO_3^- ($P < 0.05$). In conclusion, the outbreak and extinction of cyanobacterial blooms is one of the important reasons for the variation of inorganic carbon form and concentration.

Key words Dianchi Lake, Dissolved inorganic carbon (DIC), Cyanobacterial blooms

1 Introduction

Dianchi Lake is the largest lake in Yunnan-Guizhou Plateau and it is located in southwest of Kunming City. Fubao Bay, situated in northeast shore of Dianchi Lake, is an alluvial fan formed by Daqing River and Haihe River. The shore line is about 3.16 km long and the area is about 1 km^2, as shown in Fig. 1. At present, the eutrophication of Fubao Bay is increasingly deteriorating. Its original lake beach wetland was replaced by man-made concrete "breakwaters", storms in winter and spring strongly erode the lake embankment, sediments become hard and barren, aquatic vegetation disappears, cyanobacterial blooms occur frequently, and lake ecosystems are severely damaged[1]. Dissolved inorganic carbon exists in three different forms: free CO_2 (form of molecules dissolved in water are CO_2 and H_2CO_3), ion form HCO_3^- and CO_3^{2-}. In eutrophic water body of Dianchi Lake, outbreak of cyanobacterial blooms reduces the concentration of CO_2 and HCO_3^- in water body; the diffusion rate of CO_2 in air is difficult to meet the use rate of carbon by cyanobacterial blooms, resulting in extremely low inorganic carbon level[2,3].

Using water body of ecological restoration area in Fubao Bay of Dianchi Lake as research object, we studied temporal and spatial dynamic variation of dissolved inorganic carbon (DIC) in eutrophic water body, and explored rules of variation in DIC in natural water body in the process from outbreak to extinction of cyanobacterial blooms, to provide a basis for effective prevention and control of cyanobacterial blooms.

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2 Materials and methods

2.1 Distribution of sampling points and sampling method

The east shore of Fubao Bay belongs to a whole area. Considering deep water of vertical embankment, poor water quality, and low transparency, strong erosion of winds and waves, and impossible to restore aquatic vegetation in a short term, it has implemented sediment dredging and filling demonstration project. According to dredger filling, we divided this area into two dredger filling sections (dredger filling section I and dredger filling section II), non-dredger filling section, and an undeveloped area (good hope cape).

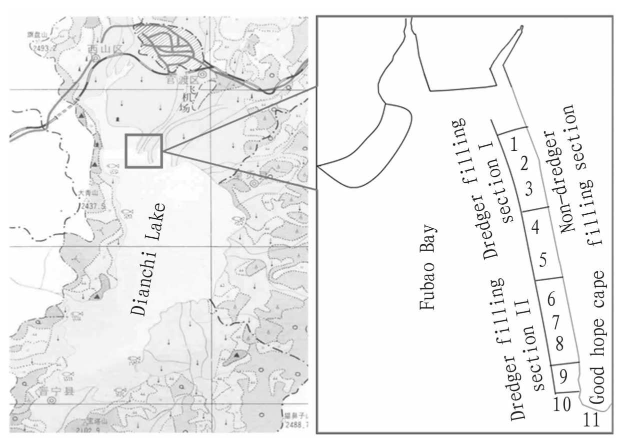


Fig. 1 Map for distribution of sampling points in Fubao Bay of Dianchi Lake

In ecological restoration area of Fubao Bay, we selected 11 sampling points: 3 areas of dredger filling section I (dredger filling section 1, 2, and 3), 2 areas of non-dredger filling section II (non-dredger filling section 4 and 5), 3 areas of dredger filling section II (dredger filling section 4 and 5), and the 3 areas of good hope cape (good hope cape 9, 10, and 11); statistical results were average value of sampling points in each area. In March to December, 2007, we collected surface water of each sampling point in ecological restoration area of Fubao Bay of Dianchi Lake by the organic glass sampler. At the same time, we measured the water temperature and pH value. Sampling frequency was 2 times a month (one time for some month due to force majeure). Sampling time was controlled in the morning 9: 00 to 11: 00.

2.2 Measurement and calculation method of dissolved inorganic carbon We measured the alkalinity of water in accordance with the national standard[4], and calculated the inorganic carbon concentration by the following method[5].

$$\alpha_0 = \left(1 + \frac{K_1}{[H^+]} + \frac{K_1 K_2}{[H^+]^2}\right)^{-1}$$

$$\alpha_2 = \left(1 + \frac{[H^+]^2}{K_1 K_2} + \frac{[H^+]}{K_2}\right)^{-1}$$

$$\alpha_1 = \left(1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}\right)^{-1}$$

$$C_T = \{[\text{total alkalinity}] + [H^+] - [OH-]\} / (\alpha_1 + 2\alpha_2)$$

$$[CO_2] = C_T \alpha_0$$

$$[HCO_3^-] = C_T \alpha_1$$

$$[CO_3^{2-}] = C_T \alpha_2$$

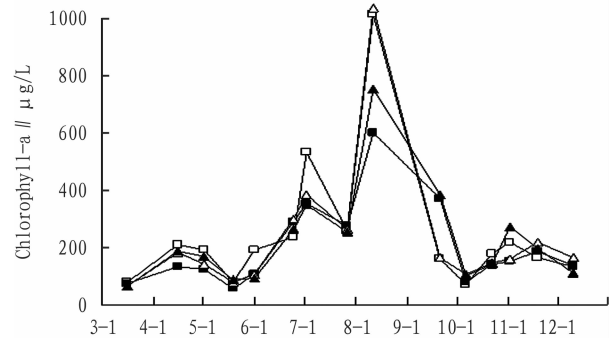
where K_1 and K_2 are first and second order dissociation constants of H_2CO_3, α is carbonic acid equilibrium coefficient, C_T and is concentration of inorganic carbon.

2.3 Measurement of chlorophyll-a concentration Within 12 hours after sampling, we measured chlorophyll-a concentration by 90% acetone extraction method[6], and measured the absorbance using UV2401 ultraviolet-visible Spectrophotometer (Japanese GL Sciences).

2.4 Statistical analysis We used Spearman correlation analysis tool and linear regression analysis tool of SPSS 12.0.

3 Results and analysis

3.1 Distribution and variation of chlorophyll-a in water body of Fubao Bay In March to December, 2007, the average chlorophyll-a content in water body of dredger filling section I and dredger filling section II (249.6 μg/L and 236.4 μg/L respectively) was higher than in non-dredger filling section and good hope cape. From Fig. 2, it can be seen that the variation trend of chlorophyll-a in 4 sampling areas was basically the same. The chlorophyll-a in water body rose from March, reached a peak in the middle of April, and started to decline to the lowest value in the middle of May. In this period, chlorophyll-a in water body of Fubao Bay was consistent with outbreak and extinction of cyanobacterial blooms. From the end of May to the beginning of June, it was the recovery period of cyanobacterial blooms, till July to September, it was large outbreak of cyanobacterial blooms, and the chlorophyll-a in water body reached the maximum value in middle of August, and later, it declined with the lapse of time; from October to December, it was extinction period of cyanobacterial blooms, and planktonic algae with *aphanizomenon flo-saquae* as major part appeared again[7].



Note: –□– Dredger filling section I; –■– Non-dredger filling section; –△– Dredger filling section II; –▲– Good hope cape

Fig. 2 Variation of chlorophyll-a in water body of Fubao Bay

3.2 Distribution and variation of inorganic carbon, temperature and pH value in water body of Fubao Bay From Fig. 3, we can see that 3 forms of inorganic carbon content, total inorganic carbon content and pH value were basically the same in dredger filling section II, non-dredger filling section, and good hope cape, and the variation with time was basically consistent; in March to December, 2007, total dissolved inorganic carbon, HCO_3^- and CO_2 in water body of three sections reached the maximum value in March; later, they gradually dropped with growth of *aphanizomenon flo-saquae*, and dropped to the minimum value in the middle of April with outbreak of *aphanizomenon flo-saquae*. Later, they gradually rose. However, with recovery and outbreak of microcysisaeruginosa in the end of May and the beginning of June, they dropped; till the beginning of November, they rose again; CO_3^{2-} content and pH value showed opposite variation of total dissolved inorganic carbon, HCO_3^- and CO_2.

By contrast, the situation in dredger filling section I was significantly different from other three sections possibly because it remains close to estuary of Haihe River and the physical and chemical indicators of water body suffered the most serious pollution. In March to December, 2007, total dissolved inorganic carbon, average content of HCO_3^- and CO_2 in water body of dredger filling section I reached the highest value (1.682 mmol/L, 1.557 mmol/L, and 0.012 mmol/L respectively), the average content of CO_3^{2-} and pH value was the lowest (0.112 mmol/L for the average content of CO_3^{2-} and 9.09 for average pH value).

The variation of water temperature with time in four sections was basically consistent. In March to December, 2007, the average temperature was higher than 19.7 ℃.

3.3 Correlation between planktonic algae and environmental factors like inorganic carbon in water body of Fubao Bay Results of correlation analysis indicated that chlorophyll-a concentration was significantly positive correlated with CO_3^{2-} concentration in non-dredger filling section, dredger filling section II, and good hope cape ($P < 0.01$), it was significantly negative correlated with CO_2 concentration and total inorganic carbon concentration and HCO_3^- concentration ($P < 0.05$). The chlorophyll-a concentration was significantly correlated with pH value of dredger filling section II and good hope cape ($P < 0.05$). The chlorophyll-a concentration was significantly correlated with pH value of non-dredger filling section ($P < 0.01$). In dredger filling section I, the chlorophyll-a concentration was significantly positive correlated with CO_3^{2-} concentration ($P < 0.05$), but not correlated with other factors. Only in good hope cape, the chlorophyll-a concentration was significantly positive correlated with water temperature ($P < 0.05$).

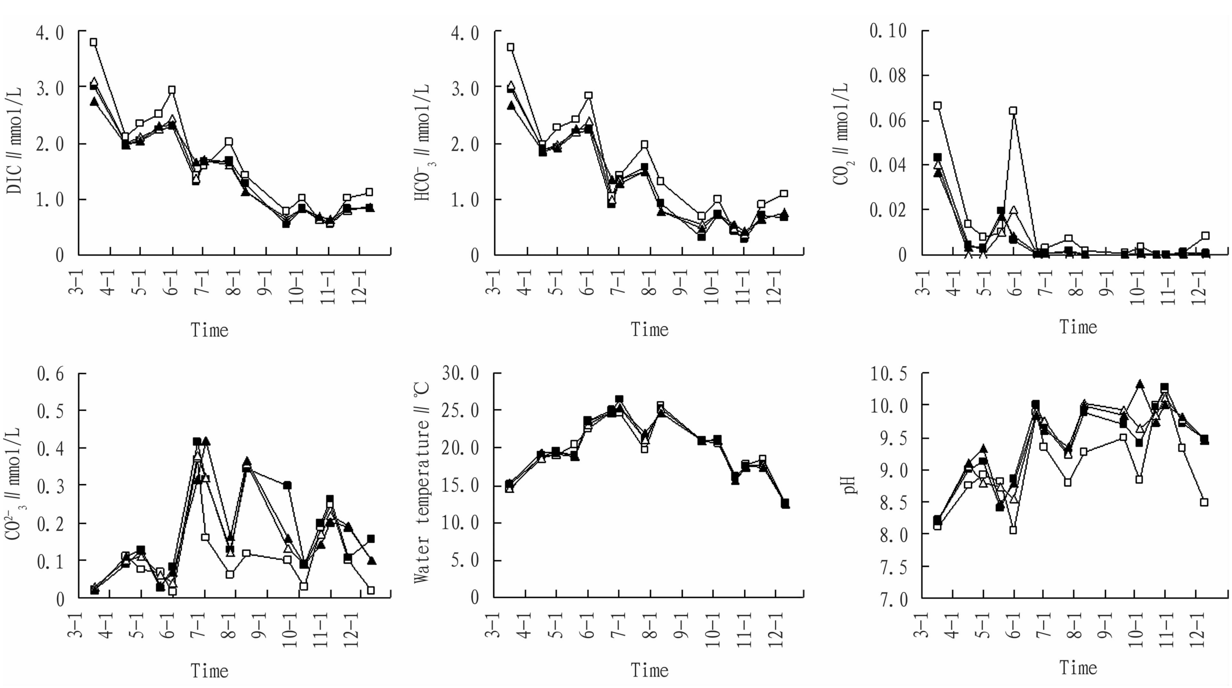
4 Discussions

4.1 Reasons for dynamic variation of planktonic algae and inorganic carbon The high average content of chlorophyll-a in dredger filling section I and dredger filling section II is possibly due to slow exchange of water in the lake because of closure of enclosure. Besides, the stability of the sediment is poor in the short time, vulnerable to violent erosion of winds and waves, leading to release and re-suspension of nutrient salts in sediment, and also leading to massive growth of planktonic algae. The Haihe River is one of the major rivers receiving pollutants in Fubao Bay, and one of the main channels for receiving pollutants of surrounding areas. The water quality is class V. Every year, large volume of pollutants such as nitrogen and phosphorus are discharged to Fubao Bay. The north side of dredger filling section I faces the estuary of Haihe River, leading to high content of nitrogen and phosphorus in water body of this area and providing nutrient foundation for massive growth of phytoplankton.

In addition, from May to October, it is the rainy season of Yunnan-Guizhou Plateau. Large amount of precipitation flows to Fubao Bay from Haihe River, which exerts a huge impact on water body of Fubao Bay, especially the dredger filling section I facing the estuary of Haihe River, leading to decline of pH value, consequently influencing inorganic carbon concentration. This is the main reason for high average content of total dissolved inorganic carbon, HCO_3^- and CO_2, average content of CO_3^{2-} and the lowest pH value in dredger filling section I.

The pH value of lake water is the result of combined action of natural and geographical factors of the lake and the river basin. In normal natural lake water, the hydrogen ion concentration depends largely on the relation between CO_3^{2-}, HCO_3^- and CO_2 of lake water. CO_2 is raw material for photosynthesis of planktonic algae. The planktonic algae have high reproduction speed. Especially, the blue-green algae consume CO_2 very rapidly. As a result, it fails to balance with CO_2 in air, leading to pH value rising to 9 or higher[8], which is consistent with the monitoring data of our experiment. In March to December, 2007, pH value of water body in Fubao Bay remained 8.1 – 9.3, and the highest value was 10.3.

An essential factor influencing the inorganic carbon concentration in water is the variation of pH value, which is related to the karstification of carbonate rock widely distributed in Dianchi Lake basin under suitable hydro-geological and tectonic conditions; decomposition of carbonate rock in water leads to high content of HCO_3^- and $OH-$ in water body, and consequently leads to slight alkaline water of Dianchi Lake[9]. Researches indicate that high pH value (above 8) is more favorable for growth of prokaryote algae[10], alkaline water of Dianchi Lake is very conducive to growth of prokaryote algae; once other conditions for growth of prokaryote algae are satisfied, there will be outbreak of water bloom. Therefore, inorganic carbon source and pH value of water body are essential factors influencing growth and reproduction of planktonic algae and biomass accumulation[11, 12].



Note: –□– Dredger filling section I; –■– Non – dredger filling section; –△– Dredger filling section II; –▲– Good hope cape

Fig. 3 Variation of inorganic carbon, water temperature and pH value of water body in Fubao Bay of Dianchi Lake

Table 1 Correlation between chlorophyll-a concentration and inorganic carbon

Chlorophyll – a	Dredger filling section I			Non – dredger filling section			Dredger filling section II			Good hope cape		
	Spearman Correlation coefficient	P	N	Spearman Correlation coefficient	P	N	Spearman Correlation coefficient	P	N	Spearman Correlation coefficient	P	N
DIC	–0.029	0.919	15	–0.556*	0.031	15	–0.632*	0.021	15	–0.532*	0.041	15
HCO_3^-	–0.057	0.840	15	–0.525*	0.044	15	–0.632*	0.021	15	–0.550*	0.034	15
CO_2	–0.307	0.266	15	–0.709**	0.003	15	–0.694**	0.004	15	–0.718**	0.003	15
CO_3^{2-}	0.584*	0.022	15	0.893**	0.000	15	0.817**	0.000	15	0.918**	0.000	15
pH	0.422	0.117	15	0.728**	0.002	15	0.637*	0.011	15	0.614*	0.015	15
Water temperature	0.477	0.072	15	0.441	0.099	15	0.492	0.063	15	0.556*	0.031	15

Note: * significantly correlated ($P < 0.05$); ** extremely significantly correlated ($P < 0.01$).

4.2 The correlation between planktonic algae and inorganic carbon of water body Researches indicated that when large scale blue green algae blooms broke out in Tangxi Reservoir in July, the abundance of blue green algae was significantly positive correlated with pH value[13], which is consistent with our experiment results. The pH value of lake water rose with growth of algae, mainly because photosynthesis of algae especially the blue green algae consume CO_2, most blue green algae can use HCO_3^- as inorganic carbon source, so that the concentration of dissolved inorganic carbon is reduced, H^+ concentration is reduced, and finally leading to rise of pH value[14]. The mutual conversion of 3 types of inorganic carbon sources follows the chemical equilibrium equation:

$$CO_2 + H2O = H^+ + HCO_3^- ; HCO_3^- = H^+ + CO_3^{2-}$$

According to the principle of chemical equilibrium shift, photosynthesis of algae absorbs and uses dissolved CO_2, making chemical equilibrium of 3 carbon sources shift. As a result, H^+ concentration drops, while CO_3^{2-} concentration rises, finally leading to rise of pH value. Outbreak of blue green algae blooms and drop of inorganic carbon source CO_2 and HCO_3^- concentration just manifest the negative correlation between chlorophyll-a concentration and inorganic carbon source CO_2 and HCO_3^- concentration.

In eutrophic water, the pH value of water is mainly controlled by biological processes. Wang Zhihong *et al.* [15] studied the correlation between reservoir eutrophication and pH value and concluded that in the eutrophication process, especially at the stage of the outbreak of bloom, pH value of upper layer of water will rise rapidly, and the daily fluctuation of pH value is large. Therefore, life activities of planktonic algae promote rise of pH value to a certain extent.

Besides, the water temperature is closely connected with growth of planktonic algae, abundance of bloom of blue green algae and chlorophyll-a concentration are significantly positive correlated with water temperature, and higher water temperature is favorable for blue green algae becoming dominant population and outbreak of blooms of blue green algae[16–21]. This is basically the same as this study, indicating that water temperature has obvious inhibitory function for growth of blue green algae.

5 Conclusions

(i) In water body with relatively abundant nutrient salts, due to promotion of rise of water temperature, violent changes in CO_2, HCO_3^-, and CO_3^{2-} concentration are directly correlated with rapid growth of planktonic algae. For the eutrophic water body of Dianchi Lake, outbreak of cyanobacterial blooms reduces the concentration of CO_2 and HCO_3^- in water body; the diffusion rate of CO_2 in air is difficult to meet the use rate of carbon by cyanobacterial blooms, leading to extremely low inorganic carbon level.

(ii) When the water body is under the restriction of inorganic carbon, blue green algae will rapidly grow, and then form the dominant population, finally evolve into blooms of blue green algae. Under dual action of restriction of inorganic carbon and shading of blooms of blue green algae, it leads to extinction of submerged plants and deteriorates the eutrophication process.

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cluded chicken, pigeon and quail. Using the chicken primer, only chicken DNA template could be amplified into 444 bp target band; using the pigeon primer, only pigeon DNA template could be amplified into 600 bp target band; using the quail primer, only quail DNA template could be amplified into 767 bp target band.

4 Discussions

In this study, the specific primers of different species were designed according to the site difference in 16s RNA sequence of chicken, pigeon and quail mitochondrial genome, for PCR amplification and detection. After repeated tests, a quick and accurate chicken, pigeon and quail PCR identification method was established, with less sample consumption, simple reaction system and low cost. In the next step, we will carry out the 16s RNA sequence RTFQ PCR technology, further develop different methods for analysis of animal-derived ingredients in foods, explore new ways for meat quality and safety control, provide technical support to protect the interests of consumers, and provide technical supervision to ensure healthy development of the meat industry.

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