# INTERANNUAL MONITORING OF WATER ENVIRONMENT AND SPATIOTEMPORAL DYNAMICS OF PHYTOPLANKTON COMMUNITY AND BLOOMING RISK IN A TROPICAL WATER SOURCE RESERVOIR, CHINA

 $\begin{array}{l} \text{Song, Y. } L.^{1,2*}-\text{Wang, L.}^3-\text{Chang, A. } M.^3-\text{Fei, S. } D.^3-\text{Fang, J. } F.^3-\text{Liang, } D.^3-\text{Qin, X. } M.^3-\text{Ke, X. } S.^{1,4}-\text{Li, Y. } X.^1-\text{Yan, C. } J.^5 \end{array}$ 

<sup>1</sup>VAST Institute of Water Ecology and Environment, Shenzhen 518101, China

<sup>2</sup>School of Civil and Environment Engineering, Harbin Institute of Technology (Shenzhen), Shenzhen 518055, China

<sup>3</sup>Shenzhen Water Quality Testing Center, Shenzhen 518055, China

<sup>4</sup>PowerChina Eco-environmental Group Co., Ltd., Shenzhen 518133, China

<sup>5</sup>Belt and Road Environmental Technology Exchange and Transfer Center, Shenzhen 518100, China

\*Corresponding author e-mail: Songyunlongwater@163.com

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Abstract. Shiyan Reservoir is situated in a densely populated urban area in Shenzhen, China, where its water quality and ecological health are crucial for the safety and security of the city's drinking water supply. To assess these factors, 13 water quality and ecological indicators were monitored over a 14month period. Chlorophyll-a concentrations in Shiyan Reservoir ranged from 21.25 to 88.77 µg/L, with pronounced temporal heterogeneity and substantial variation. The highest chlorophyll-a levels, averaging around 85 µg/L, were recorded from April to July, indicating a high risk of algal blooms during this period. The reservoir's annual average water temperature (WT) was 25.31°C, with temperatures rising to approximately 30°C from May to September, further increasing bloom risk. Chemical oxygen demand (COD) ranged from 1.71 to 3.08 mg/L, and total organic carbon (TOC) varied from 2.22 to 5.13 mg/L, indicating minimal organic pollution that meets Class I surface water standards. However, total nitrogen (TN) pollution remains severe, with concentrations between 1.66 and 2.49 mg/L, aligning with Class V surface water standards. Although recent reductions in TN levels have been observed, it continues to be the primary pollutant in the reservoir. Total phosphorus (TP) concentrations, ranging from 0.01 to 0.043 mg/L, are sufficiently low to meet Class I-II surface water standards. The reservoir's monthly average integrated trophic state index fluctuated between 43.13 and 53.81, with an annual average of 50.49, indicating a state of light eutrophication from April to May and moderate eutrophication in other months. Correlation analyses revealed WT as the primary factor influencing phytoplankton dynamics during the flood season, with a correlation coefficient of 0.69. In the flood season, WT, TOC, nitrate nitrogen, and transparency were the main factors affecting phytoplankton growth, while in the dry season, TOC, dissolved oxygen (DO), and WT were the primary drivers. Two-way ANOVA results indicated that temporal factors and sampling location, along with their interactions, explained the variations in environmental factors, with temporal heterogeneity significantly exceeding spatial heterogeneity. Cyanobacteria dominated from May to October, while diatoms were prevalent from November to February. Correlation and canonical correspondence analyses (CCA) highlighted significant differences in the environmental drivers of cyanobacteria, diatoms, and green algae. WT was the primary influencing factor for all three algae types, while organic matter and nutrients from runoff during the pre-flood season acted as critical triggers for cyanobacterial blooms. Additionally, a negative correlation was found between the nitrogen-to-phosphorus ratio and the abundance of cyanobacteria, green algae, and diatoms, suggesting that phosphorus may be a limiting factor for algal growth in Shiyan Reservoir.

**Keywords:** Shiyan Reservoir, integrated nutrient status, harmful algae composition, two-factor ANOVA, water environment health assessment, canonical correspondence analysis

#### Introduction

Reservoirs are artificial lakes formed by dams, which play an important social and economic value in water security, flood control and drought relief (Simões et al., 2022; Bai et al., 2023; Mineeva, 2023). In recent decades, due to human activities and the impact of climate change, the number and area of natural lakes in China have decreased while the number of reservoirs has increased rapidly, and the water storage capacity of reservoirs has reached three times that of lakes. With the increasing water consumption in cities, the dependence of cities on reservoirs for water supply has increased, and the function of urban reservoirs has changed from agricultural irrigation to water resources protection and water ecological health (Cetin, 2023; Cabecinha et al., 2024; Elser et al., 2009). The processes of water storage and water supply in reservoirs have typical anti-seasonal regulation characteristics, resulting in the physical, chemical and biological processes in reservoir ecosystems being very different compared to natural lakes (Barathan et al., 2023; Panikkar et al., 2022). Currently, the main threats to the safety of reservoirs in China's water sources include eutrophication, excessive odor substances, algal blooms, algal toxins, and bottom hypoxia (Mpakairi et al., 2024; Dervisoglu et al., 2024). Due to the unique hydrological characteristics of reservoirs, the characteristics and mechanisms of algal blooms are often different from those of lakes in general (Fan et al., 2021; Yuan et al., 2024; Liu et al., 2016).

Shiyan Reservoir is located in the high-density built-up area of Bao'an District. It is one of the backbones of Shenzhen's urban water supply network, and ranks 2nd among all the reservoirs in the city in terms of water supply capacity, and guarantees the production and living water supply for more than 6 million people in the western part of Bao'an. Due to the dense population in the basin, although closed management is implemented, domestic, agricultural and industrial wastewater still flows into the Shiyan Reservoir, making it the most polluted of the 12 major water supply reservoirs in Shenzhen, and the risk of seasonal algal outbreaks is increasing. 2020 onwards, the Shenzhen Municipality has implemented the Tiegang-Shiyan Reservoir Water Quality Assurance Project, which is based on a program of projects that include physical isolation, sewage diversion, ecological restoration, and storage and replenishment. Through the construction of clear water interceptor ditches and the construction of estuarine ecological reservoirs, to achieve physical isolation of drinking water reservoirs from incoming rivers and estuarine ecological reservoirs, and then realize the clean and polluted diversion of clean water in ecological zones and rainwater or river water in built-up areas that do not meet the standards.

Currently, there are fewer studies on Shiyan Reservoir and most of them focus on water quality, and there is a relative lack of studies reflecting the inter-annual algal succession characteristics of Shiyan Reservoir. The research on algal blooms by scholars in China and abroad mostly focuses on various natural lakes in spring and summer (Santos et al., 2022; Christensen et al., 2019; Loaiza-González et al., 2021; Tsunashima et al., 2021), and pays little attention to drinking water source reservoirs. This study investigated 13 water quality and water ecology indicators of Shiyan Reservoir through 14 months of continuous monitoring, comprehensively analyzed the

water quality condition and water ecology health level of Shiyan Reservoir, studied the inter-annual change pattern of phytoplankton biomass in Shiyan Reservoir, researched the characteristics of the phytoplankton outbreaks and spatial-temporal succession of dominant species, and focused on the analysis of the characteristics of the algae that triggered the exceedance of algal toxins and odorous substances in the water body. The study provides scientific basis for safeguarding the water ecological health and drinking water safety of Shiyan Reservoir, and is of reference significance for the protection and water quality improvement of small and medium-sized reservoirs of the same type in Shenzhen and even the Pearl River Delta region.

#### Sampling and analysis

#### Introduction of experimental reservoir

Shiyan Reservoir is located in the upper stream of Maozhou River in Shiyan Street, Bao'an District, Shenzhen, China. Above the dam site of Shiyan Reservoir, the length of the main stream is 12.2 km, the rain catchment area is 44 km<sup>2</sup>, the total capacity is  $3.2 \times 10^7$  m<sup>3</sup>, it is a medium-sized water conservancy hub project which mainly supplies water and also has the comprehensive benefit of flood control. Reservoir was basically built in 1960 and began to store water, the original function of farmland irrigation is mainly, after 1990, gradually converted to urban water supply, agricultural water use is reduced year by year, to November 1994, all the switch to industrial and residential water use. With the completion of the construction of water intake projects in the eastern part of Shenzhen City, the water is lifted to the West Lek Reservoir and released to the Tiegang Reservoir, and the Tangtou Pumping Station was constructed in 2001, which is a water lifting project from the Tiegang Reservoir to the Shiyan Reservoir. Therefore, Shivan Reservoir has become one of the backbone projects of Shenzhen city water supply network, and is also the most important storage reservoir in the western water supply system of Bao'an District, which is responsible for the water supply of 6 areas in the western part of Bao'an District, such as Shiyan, Gongming, Guangming, Shajing, Fuyong, Songgang, etc. The topography of the reservoir basin is generally in the south-east.

The topography of the reservoir basin is generally high in the southeast and low in the northwest. In the southeast of the basin is the second highest peak in the west of Shenzhen, with an altitude of 587 m. If we take the Shiyan River as the boundary, there is a high north and a low south to the north of Shiyan River, and a high south and a low north to the south of Shiyan River. The geological type of the Shiyan Reservoir basin is dominated by fine- to medium-grained black mica granite intruded during the Yanshan period. The Quaternary sediments in the largest area of the reservoir basin are residual thick red loam-type weathered crusts, the sediments near the Shiyan Lake Hot Spring Resort are residual slope deposits of granite eggs, and the alluvial sandy clay near the Shiyan Street on both sides of the Shiyan River. The average rainfall of Shiyan Reservoir for many years is 1568.5 mm, and the average runoff depth of the area is 1100 mm for many years, with large intra- and inter-annual variations in runoff. Groundwater is shallow and abundant, mostly pore water, with medium water-richness, and Yanshan period granite, underground runoff modulus is generally 6~10 L/s<sup>-1.</sup>km<sup>2</sup>.

Shiyan Reservoir has 6 main tributaries, among which 4 tributaries, namely, Wangjiazhuang, Shiyan River, Shamkengli and Baikengwu enter into the reservoir on the east bank, and 2 tributaries, namely, Mabushui and Yunnukeng, enter into the

reservoir on the west bank, and the watersheds of these 6 tributaries are all in the Shiyan Street. Shiyan River is the main water source of Shiyan Reservoir, Shiyan River originates from Shilongzai in the east of Shiyan Street Office, and injects into Shiyan Reservoir in the west from east to west, with a total length of 5 km, and the water area of Shiyan River is 0.14 km<sup>2</sup>. The characteristics of the above tributaries in Shiyan are shown in *Table 1*. Tiegang Reservoir is adjacent to the south-western boundary of Shiyan Street, and according to the needs of urban water supply, Tiegang Reservoir and Shiyan Reservoir have been connected through the water transmission channel to form a chain of reservoirs.

No.	Name of tributary	Location of inflow	Place of origin	River length/km	Rain catchment area/km <sup>2</sup>	
1	Baikengwu Trailback	New Village Baikengwu	Aikengwu	0.5	3.36	
2	Shamkengli	Shiyan River mouth	Shamkengli	2.6	2.06	
3	Shiyan River	Shiyan River mouth	Shamkengli	7.1	22.34	
4	Wangjiazhuang Stream	Xinyi Village	YangtaiMountain	1.2	7.55	
5	Mabu Water	West Bank Xilian	Mabu	0.8	3.97	
6	Yunnukeng Abaun Water	Abaun East	Yunnukeng	0.6	4.72	

Table 1. Status of all branches of Shiyan reservoir

# Sampling points layout

In Shiyan Reservoir, 8 sampling points were set up from south to north, among which sampling point 1 is near the intake of Tiegang Reservoir, sampling point 2 has industrial and agricultural wastewater entering nearby, sampling point 3 is near the river, sampling points 4, 5 and 6 are in the center of the reservoir and sampling points 7 and 8 are near the intake of the water plant. The distribution of sampling points is shown in *Figure 1*. The precise latitude and longitude of sampling points 1 to 8 are as follows: N22°42'17.97" E113°54′12.97″, N22°41'12.32" E113°54'17.47", N22°41'39.28" E113°54'09.07", N22°42'12.73" N22°41′29.25″ E113°54′13.72″, E113°54'11.87", N22°41'52.15" E113°54'06.62", N22°42'26.30" E113°54'03.85", N22°42'18.36" E113°54'29.92".

## Monitoring and analyzing methods

The monitoring period spanned from April 2021 to June 2022, totaling 14 months, with sampling conducted on a monthly basis. Based on seasonal weather patterns, the sampling period was divided into five distinct phases: the Pre-Rainy Period (PRP) from April to June in both 2021 and 2022, the Latter Rainy Period (LRP) from July to September 2021, the High Temperature and Rain-Free Period (HTRFP) from October to November 2021, the Winter Drought Period (WDP) from December 2021 to February 2022, and the Temperature Jump Period (TJP) in March 2022.

The samples were collected using a ZPY-1 water collector and stored separately. The water samples were transferred to the laboratory within 2 h after collected and they were preserved at 4°C. The chemiluminescence detection of the permanganate index (CODMn) and determination of  $\rho$ (Chla), total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), ammonia nitrogen (NH<sub>4</sub><sup>+</sup>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N), iron (Fe) and silicate were carried out within two days. Chl-a was measured by using a

modulated fluorometer (WALZ Phyto-PAM, Germany) that was periodically calibrated by acetone extraction spectrophotometry. The depth of the water, WT, pH, DO and turbidity were measured in-site using a multi-parameter water quality analyzer (YSI 6600V2, USA). The transparency (SD) was measured in-site using a secchi disk. COD was measured by the acidic potassium permanganate method; TP was determined by ammonium molybdate spectrophotometry (Shimadzu UV-2700 Ultraviolet-Visible Spectrophotometer, Japan); silicate was determined by silicon molybdenum blue spectrophotometry (Shimadzu UV-2700 Ultraviolet-Visible Spectrophotometer, Japan); TN, NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N were analyzed by a flow analyzer (AMS-Alliance-Futura, French); and TOC was determined using a TOC analyzer (GE-Siever 5310C) (APHA, 2012; He et al., 2024; Yuan et al., 2017). Heat map analysis of water quality factors was produced using origin2021. IBM SPSS Statistics 20 was used for ANOVA analysis and CCA analysis. ARCGIS 10.8 was used to map the spatial and temporal distribution of phytoplankton biomass in Shiyan Reservoir.



Figure 1. Sampling sites layout map for Shiyan Reservoirs

Qualitative and quantitative phytoplankton samples were collected separately. Qualitative samples were taken by using No. 25 plankton net in the corresponding water layer using the outer 8 characters, and fixed by adding 4% formalin Rugo reagent on the spot. The samples were brought back to the laboratory and then observed and identified with a 400x microscope (OLYMPUS BX51) with reference to the Chinese Journal of Freshwater Algae. Quantitative samples were collected by stainless steel water sampler in the corresponding water layer with 2L water samples, fixed by adding Rugo reagent. And The samples were settled with the laboratory for 48 h, and finally concentrated to 30 mL, using Hangzhou Xunji Algae Intelligent Identification Counter (Shineso A Algacount® M300), under the microscope (OLYMPUS BX51) using phytoplankton counting frame rows of lattice method counting. Specific counting methods were referred to the 21st edition of the U.S. Standard Methods for the Examination of Water and Wastewater (APHA, 2012).

#### **Results and discussion**

#### Single factor water quality analysis

The inter-annual variation of water quality factors is shown in *Figure 2*. The annual average WT of Shiyan Reservoir is 25.31°C, the lowest WT is 14.50°C, which occurs in January, and the highest WT is 31.98°C, which occurs in August. Shiyan Reservoir is located in the southern tropical zone, where high temperature and strong sunshine are conducive to the growth of phytoplankton all year round, especially from May to September when the WT is about 30°C for a long period of time, and the risk of algal bloom is high. The dissolved oxygen in the surface layer of the reservoir is more than 7.33 mg/L throughout the year, which meets the environmental quality class I standard for surface water. The dissolved oxygen flood season (Apr.~Sep.) is low, especially the pre-flood season which is the lowest period of dissolved oxygen in the whole year. In the dry season (Oct.~Mar.), the dissolved oxygen rises and reaches a maximum of 11.12 mg/L in January. The turbidity of the reservoir ranges from 3.85 to 11.66 NTU, which rises rapidly in the pre-flood season (Apr.~Jun.) and decreases in the post-flood season. The correlation between turbidity and algal biomass was low, and the main factor affecting turbidity was the surface source pollution brought by rainfall runoff. Transparency ranged from 0.73 to 1.16 m. Transparency was higher than 1 m from November to March, and around 0.8 m during the flood season. Compared with 2010~2015, the transparency and water quality of Shiyan Reservoir increased significantly (Peng et al., 2012; Fu et al., 2014; Zhang et al., 2016).

The COD of the reservoir ranged from 1.71 to 3.08 mg/L, and the organic pollution was very low, meeting the environmental quality of surface water I standard. The mean value of COD was 2.53 mg/L in the flood season and 2.12 mg/L in the dry season, and the temporal heterogeneity was not significant. The TOC of the reservoir ranged from 2.22 to 5.13 mg/L, and the mean value of COD was significantly higher in the flood season than in the dry season. Shiyan Reservoir TN was seriously polluted, with TN ranging from 1.66 to 2.49 mg/L during the investigation period, which was class V water quality. Temporal heterogeneity was obvious, TN concentration before flood season > after flood season > dry season. Previous relevant studies have shown that in 2009, Shiyan Reservoir TN highest 13.80 mg/L (Wen et al., 2009). In recent years, Shenzhen has carried out a systematic comprehensive treatment of the water environment project, TN decreased significantly, but still Shiyan Reservoir is the primary pollutant. Nitrate nitrogen in Shiyan Reservoir is 1.18~1.85 mg/L, TN pollutant mainly exists in the form of nitrate. Ammonia nitrogen in Shiyan Reservoir was 0.17~0.45 mg/L, which was class II water quality, thanks to the high DO concentration in the reservoir. The TP of Shiyan Reservoir was 0.01~0.043 mg/L, and the total phosphorus concentration was low, which met the environmental quality standard of surface water I ~ II. The temporal heterogeneity of TP was low, and the significant increase of TP in March was closely related to the upward movement of the bottom layer of the reservoir caused by the temperature

jump of the water body, and pollutants at the bottom of the water body were taken into the middle and upper layers of the water body.

Chlorophyll a is an important pigment for photosynthesis in phytoplankton, and can accurately reflect phytoplankton biomass and primary productivity (Pilla and Griffiths, 2024; Papenfus et al., 2020). During the observation period, the chlorophyll a concentration in Shiyan Reservoir ranged from 21.25 to 88.77  $\mu$ g/L. The highest value of 88.77  $\mu$ g/L was reached in May, and the lowest value of 21.25  $\mu$ g/L was reached in February. Compared with domestic and international drinking water sources, Shiyan Reservoir's chlorophyll a is at a low level and meets the requirements for drinking water sources (Li et al., 2024; Brinovcar et al., 2022; Guimarães et al., 2023).

The temporal heterogeneity of the chlorophyll a concentration was obvious, and the changes were drastic. April was the outbreak of the phytoplankton, and the concentration of chlorophyll a could be increased from 38 µg/L to 80 µg/L drastically. From April to July, the highest chlorophyll a concentration was maintained at about 85 µg/L. From August to February, the chlorophyll a concentration was in a period of decline, and from March onwards, with the increase of WT, the phytoplankton recovered, and the chlorophyll a concentration increased to 40 µg/L. From April to May, with the increase of air temperature to 28-30°C, a large number of surface pollutants entered into the water body in the pre-flood season. Suitable temperature and sufficient nutrients created favorable conditions for algal outbreaks. Higher chlorophyll a concentration in summer brings the risk of cyanobacterial blooms, and the accompanying production of algal toxins seriously endangers human health (Yan et al., 2022; Wijerathna et al., 2024; Vianney et al., 2024). Reservoir management needs to strengthen the monitoring of water quality and phytoplankton, and take precautionary measures against cyanobacterial blooms in order to ensure the safety of drinking water.



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Figure 2. Inter-annual variation patterns of water environment factors in Shiyan Reservoir

#### Analysis of the integrated nutrient status of Shiyan Reservoir

The integrated trophic state evaluation method was used to synthesize and evaluate the quality of the water environment from five indicators, namely chlorophyll a, total phosphorus, total nitrogen, transparency and permanganate index (Fig. 3). The main water quality factor affecting the trophic state of Shivan Reservoir is TN. The monthly average integrated trophic state of Shiyan Reservoir is located between 43.13 and 53.81, with an annual average value of 50.49. The monthly average integrated trophic state of April and November 2021, and April and May 2022 was less than 50, which was in the light eutrophic state, and the other months were in the moderate trophic state. Comparison with similar reservoirs in China and abroad shows that the combined trophic state of Shiyan Reservoir is on the high side, mainly due to the high concentration of TN (Tekanova et al., 2023; Semensatto et al., 2023; Jachniak and Jaguś, 2023). Shiyan Reservoir is in mildly eutrophic state all year round, except for No. 7 and No. 8, where the water quality is slightly better and the trophic state is lower. Most of the points in Shiyan Reservoir were higher than 50 and in moderate trophic state all year round. Seasonal changes in the nutrient status of the eight points in Shiyan Reservoir were not obvious, and there was some spatial heterogeneity. the highest integrated nutrient status was found in sampling points No. 3 and 4, and the integrated nutrient status of sampling points No. 7 and 8 was slightly lower than that of the other points. Vertically, there was little difference between the nutrient status of the surface layer and the light-permeable layer. The nutrient status of the surface layer and translucent layer was 12.13% higher than that of the bottom layer. Water quality data from Shenzhen Water Quality Inspection Centre show that the comprehensive trophic state of Shiyan Reservoir was always higher than 50 and reached a maximum of 63 from 2010 to 2015. In recent years, the trophic state index of Shiyan Reservoir has decreased significantly, and the quality of the water environment and the health of the water ecology have continued to improve. Shenzhen has implemented the Tiegang-Shiyan Reservoir Water Quality Protection Project, which has achieved remarkable results. The project has reduced the pollution load of Shiyan Reservoir and safeguarded the safety of drinking water by means of engineering programs such as physical isolation, sewage diversion, ecological restoration and water transfer and replenishment.



*Figure 3.* Interannual variation patterns of integrated trophic state in Shiyan Reservoir. The 8 bars represent the composite trophic state indices of sampling sites 1 to 8 in different months

#### Single factor water quality analysis

Chlorophyll a is an important pigment for photosynthesis in phytoplankton and reflects the primary productivity of the water column. Estimation of phytoplankton biomass in water bodies by detecting  $\rho$ (Chla) has become a common technical tool used internationally (Guimarães et al., 2023). Correlation analysis is an important tool to analyze the correlation between phytoplankton biomass and environmental factors (Melese and Debella, 2024; Cao et al., 2023). *Figure 4* reflects the correlation between phytoplankton varied greatly in different periods, and the main correlation factors of phytoplankton varied greatly in different periods. The main correlation factors of phytoplankton in flood season are WT, TOC, nitrate nitrogen, SD, TN, COD, pH, etc., among which SD, nitrate nitrogen and TN are negatively correlated. The main correlation factors in the dry period of phytoplankton were TOC, DO, WT, nitrate nitrogen, silicate, and p, which were all positively correlated. Throughout the year, phytoplankton highly positively correlated with WT and TOC, moderately positively correlated with COD, pH and turbidity, and moderately negatively correlated with transparency (SD).

There were also some correlations between water quality factors. During the flood season, WT was highly correlated with TOC, with a correlation coefficient of 0.51. WT was highly negatively correlated with TN, with a correlation coefficient of -0.70, which was mainly due to the high temperature and rainy weather during the flood season that brought a large amount of surface runoff into the reservoir, and the TN content of surface runoff was significantly lower than that of TN in the reservoir. During the flood season, WT was highly negatively correlated with SD, with a correlation coefficient of -0.64, which was mainly due to the dual effects of surface runoff and algal value-added brought about by the high temperature and rainy weather during the flood season, resulting in a decrease in the transparency of the water body. During the dry period, WT was highly positively correlated with TOC and silicate, with correlation coefficients of 0.83 and 0.67, respectively. Throughout the year, WT was highly positively correlated with TOC, with a correlation coefficient of 0.80. SD was negatively correlated with turbidity poisoning, with a correlation coefficient of -0.43.

WT was the primary influencing factor of phytoplankton in flood season, the correlation coefficient was as high as 0.69. WT was the primary factor influencing the growth of algae in tropical lakes and reservoirs, which was consistent with the conclusion of most scholars' findings (Krasheninnikova et al., 2024; Gao et al., 2024). Shenzhen is located in the tropical region, and the WT fluctuated in the range of 14.08~32.33°C during the monitoring period. During the flood season, the WT reached more than 29°C from May to September, which overlapped with the optimum growth temperature of cyanobacteria. As the temperature increased, the cyanobacteria gained an advantage in interspecies competition and inhibited the growth of diatoms. During the dry period, the WT ranged from 14.08 to 23.57°C, which was suitable for the growth of diatoms, and diatoms replaced cyanobacteria as the dominant algae. TOC and COD represented the organic matter content of the water body, and there was a high correlation between the two and the algae. This is due to the fact that the vast majority of organic matter in the water body will be detected in the form of TOC, and algae itself is organic matter, an important component of TOC. Transparency, turbidity and algae, especially cyanobacteria, were significantly negatively correlated during the flood season, which indicates that the increase in algae during the flood

season increases the turbidity of the water body, reduces the transmittance of solar radiation in the water body, and restricts algal photosynthesis. Nitrate nitrogen, TN and phytoplankton were negatively correlated, and the average concentration of TN in Shiyan Reservoir was 2.03 mg-L-1, which exceeded the standard for surface water class V. Total nitrogen exceeded the standard significantly. The increase of TN in flood season instead caused the decrease of phytoplankton biomass, which indicated that the high concentration of nitrogen in Shiyan Reservoir could meet the demand of algal growth, while the low concentration of TP was a limiting factor for algal growth.



(b) dry season (October to March)



(c) The entire monitoring period runs from April

Figure 4. Heat map for water quality correlation analysis of Shiyan Reservoir

# Two-factor analysis of variance (ANOVA) for water environment factors in Shiyan Reservoir

Two-factor ANOVA (*Table 2*) showed that the variance of each environmental factor was better explained by periodical and sampling point and their interaction effects ( $R^2$ of 23.7% to 78.1%). Periodical was the most important factor affecting environmental factors, with significant effects on WT, SD, COD, TOC, Silicate, NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N (P < 0.01), and some correlation with TN (P = 0.087), and less effect on pH, DO and TP. Sampling points had a more significant effect on SD (P = 0.068) and little effect on other environmental factors (P > 0.3). The interaction of time periodical and sampling point had a weak effect on Fe and SD, and little effect on other environmental factors (P > 0.8). Taken together, the main influence of environmental factors in Shiyan Reservoir was periodical, and the temporal heterogeneity of environmental factors was significantly higher than the spatial heterogeneity.

Table 2. Two-way ANOVA of periodical and sampling sites to environmental factors

Water quality indicators	pН	DO	WT	SD	COD	TOC	Silicate	$\mathbf{NH_4^+}$ -N	NO <sub>3</sub> -N	TN	ТР
Periodicals Sig.	0.294	0.196	0.000	0.000	0.005	0.007	0.000	0.001	0.000	0.087	0.152
Sampling point Sig.	0.991	0.993	1.000	0.068	0.559	0.467	0.994	0.690	1.000	0.755	0.801
Interactive effect Sig.	1.000	0.912	1.000	0.798	0.899	0.876	1.000	1.000	1.000	0.961	0.987
Judgement coefficients R <sup>2</sup>	0.299	0.270	0.781	0.452	0.465	0.460	0.465	0.314	0.314	0.237	0.241

## Analysis of typical hazardous algae in Shiyan Reservoir

A total of 21 genera of cyanobacteria, 24 genera of diatoms, 39 genera of chlorophyta, 3 genera of euglenophyta, 2 genera of pyrrophyta, 1 genus of chrysophyta

and 2 genera of cryptobranchs were detected in Shiyan Reservoir. The main dominant phytoplankton are shown in *Figure 5*.

In Shiyan Reservoir, cyanobacteria dominated from May to October, and diatoms dominated from November to February. Other algae such as Chlorophyta, Pyrrophyta and Euglenophyta had lower dominance throughout the year. March~April was the recovery period of cyanobacteria, and with the increase of temperature, the cyanobacteria proliferated, and the dominance of diatoms was gradually reduced. At the end of April, cyanobacteria completely replaced diatoms, and became the dominant algae in Shiyan Reservoir.

There were 11 species of diatoms are dominant in Shivan Reservoir, including Melosira. Skeletonema. Skeletonema. Coscinodiscus. Nitzschi. Cyclotella, Rhizosolenia, Synedra, Navicula, Amphipleura and Diploneis. Rhizosolenia tends to proliferate during the winter months, causing clogging of waterworks filter tanks and reducing the treatment efficiency of the tanks. Chlorophyta is the most diverse alga in Shiyan Reservoir. Thirteen generas of diatoms are dominant in Shiyan Reservoir, including Pediastrum, Sphaerocystis, Scenedesmus Asterionella, Micractinium, Staurastrum. Closterium, Attheya, Actinastrum, Crucigenia, Tetraedron and Selenastrum.

Cyanobacteria are the most harmful algae in drinking water sources (Christensen et al., 2019; Chakraborty et al., 2019). The proliferation of cyanobacteria will not only cause the DO of the water body to decrease, destroying the ecological balance of water. Cyanobacterial blooms also cause a surge of algal toxins and odours in the water column, seriously endangering the safety of drinking water (Kuhlisch et al., 2024; Yan et al., 2022; Hou et al., 2022). Table 3 summarizes common harmful cyanobacteria that release phycotoxins and odorous substances and lists their frequency of occurrence in Shiyan Reservoir. There were 9 generas of cyanobacteria dominant in Shiyan Reservoir, including Raphidiopsis, were Oscillatoria, Phormidium, Planktothrix, Anabaena, Merismopedia, Chroococcus, Microcystis and Snowella. During the monitoring period, small localized cyanobacterial blooms occurred in the vicinity of sampling sites No. 1, No. 2 and No. 3 of the reservoir, and the algae causing the blooms were mainly Planktothrix, Microcystis, Lyngbya and Aphanizomenon.

Cyanobacteria are phycotoxin-producing algae see Table 3 for details) and are prone to forming blooms. A total of 19 genera of cyanobacteria producing phycotoxins and odorous substances were detected in the Shiyan Reservoir (Fig. 5), including the Hirakatsura, Aphanizomenon, Microcystis, Planktothrix, Pseudofusarium, Fischeria, and Acanthopanax. Cyanobacteria were mainly Pseudoichthys olivaceous, Floating Filamentous Algae, Hiraclean Algae, Chromococcus spp, Acanthamoeba, Fasciculata spp and Chlorophyceae spp. Among them, Acanthamoeba spp, Hiraclean Algae, Chromococcus spp., and Pseudoichthys olivaceous were detected in almost all the months of use, and the common algal species that caused lake blooms such as Microcystis spp. and Fishybacteria spp. were detected in more than one period of time, which indicated that the risk of bloom was relatively high. The highest number of cyanobacteria in Shiyan Reservoir was in the genus Hirundinia, and the highest biomass was in the genera Pseudopuccinia. The diatoms were mainly Chlamydomonas, Acropora, Cyclophyllum, Tetrahymena, Dictyostelium, Ophiophora, Rhodophyllum, Curculionema, Bilobium, Bony Stripes and Boat-shaped Algae. Green algae is the most diverse algae genus, mainly in the genus Gasterophyta, Dolichospermum, Dolichospermum, Drusilla, Discospermum, Chlorella, Crescentus, Chlorella, Ocellulospermum, Vesiculospermum, Chlamydomonas, Tetraspermum, Crassostrea gigantea, Tetraspermum, Dolichospermum, and so on. The methanotrophs are *Polychaeta* and *Nudibranchia*, the nudibranchs are *Nudibranchia*, *Nudibranchia*, *Nudibranchia*, and Nudibranchia, the cryptophytes are Cryptophyta and Cryptophyta, and the golden algae are *Ichthyophthora*.

Algal toxin releases are a major cause of concern for cyanobacterial blooms. Most of the common algae in the cyanobacteria phylum that form blooms are capable of producing algal toxins, which poses a greater challenge to the safety of water quality in source reservoirs. The most common algal toxin is microcystin (including several isomers, abbreviated as MC), which is a class of polypeptide toxins, and the upper limit of the concentration of MCR (the most toxic of the several isomers of MC) in drinking water is set at 1 µgL by CB 5749-2022. According to the analysis, there were 37 reservoirs in China where MCs were reported to have been investigated in the literature, of which 22 had MCs detected, and the reservoirs where the total amount of MCs (including intracellular and extracellular MCs) exceeded 1 µgL were Guanting Reservoir in Beijing, Yanghe Reservoir in Qinhuangdao, and Shanzai Reservoir in Fuzhou, while the reservoirs where the concentration of extracellular MCs occasionally exceeded 1 µgL were Shanzai Reservoir, Guanting Reservoir, and Aha Reservoir in Guiyang City (Wei et al., 2022). In the source reservoirs where cyanobacterial blooms occurred, the period of algal cell death was the critical period for the greater harm of MCs due to the large release of intracellular MCs. In addition, with the frequent occurrence of extreme weather and sudden algal blooms in recent years, the problem of excessive levels of algal toxins in water source reservoirs may become more common.

2-MIB and Geosmin are the most frequently occurring off-flavors in Chinese water reservoirs. China's drinking water hygiene standard (CB 5749-2022) has a limit of 10 ngL for both 2-MIB and Geosmin, and Shanghai Qingcaosha Reservoir has experienced elevated concentrations of 2-MIB or exceedances of the raw water limit every summer since the second year of official water supply (2011) (Chen et al., 2018). From 9 July to the end of August 2007, high concentrations of Ceosmin occurred in Yanghe Reservoir for more than 50 days during the outbreak of water blooms of mixed populations of Cichlidia and Microcystis aeruginosa (Li et al., 2010). 2009-2012, in the autumn of each year, a large number of *Planktothrix* bloomed in the sub-surface layer of the Miyun Reservoir, leading to local 2-MIB concentrations of more than 100 ng/L in the water body (Su et al., 2015). During the survey in July-August 2020, it was found that the concentration of 2-MIB exceeded the standard in Tianmu Lake Shahe Reservoir, the water source of Liyang city, and the concentration of Geosmin exceeded the standard in Tangpu Reservoir, the water source of Shaoxing city (Wu et al., 2021). On 8 May 2017, 12 odorous substances were detected in the Shiyan Reservoir, the water source of Shenzhen, with the highest concentration of 2-MIB (Rong et al., 2018). The odor substances still pose a threat to the safety of water supply in Shiyan Reservoir. Although exceeding the concentration of odorous substances in the water body of the water source does not mean that the effluent of the water plant exceeds the standard (GB 5749-2022 requires that the effluent meets the standard), it poses a great risk of exceeding the standard for the effluent of the water plant, increases the cost of treating the raw water, and needs to be paid great attention to.

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Cyanobacteria-Merismopedia

Cyanobacteria-Chroococcus

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Chlorophyta-Staurastrum Chlorophyta-Closterium Figure 5. Dominant phytoplankton in Shiyan Reservoir

Table 3.	Potentially	hazardous	algae in	Shiyan	Reservoir	and the	toxins	and t	ypes th	ey
produce										

Cyanobacterial	Toxins											Found at	
genera	Dermatoxins			Hepatotoxins			Neurotoxins				Tastes a	Shiyan	
Colonial/filamentous	LYN	APL	LPS	CYL	МС	NOD	ANA	BMAA	NEO	SAX	GEOS	MIB	Reservoir
Anabaena			+	+	*		+	+	+	+	+		
Anabaenopsis			+		*		+	+	+	+	+	+	
Aphanizomenon			+	+			+			+	+		
Aphanocapsa			+		*								
Cylindrospermorpsis			+	+			+	+			+		
Phormidium			+				+	+			+	+	
Microcystis			+		*			+			+		
Nodularia			+			+		+					
Nostoc			+		*			+			+	+	
Oscillatoria	+	+	+		*		+	+		+	+	+	
Planktothrix	+	+	+		*		+	+		+	+	+	
Pseudanabaena			+		*							+	
Raphidiopsis			+	+			+				+		
Hepalosiphon					*								
Umezakia			+	+									
Snowella					*								
Cylindrospermum				+			+				+		
Lyngbya							+			+	+		
Radiocystis					*								
Woronichinia					*						+		
Unicellular													
Synechococcus			+		*			+			+	+	-
Synechocystis			+		*			+					

"+" indicates that this type of toxin can be produced; "\*" is a warning indication that this genus can produce microcystin GEOS and MIB are two metabolites produced by algal blooms, known as genotoxin and dimethyl GEOS and MIB are two metabolites produced by algal blooms, known as geosmin and dimethyl isotretinoin, respectively, and can be detected at concentrations of 10-29 ng

#### Identification of areas at risk of high phytoplankton outbreaks

Figure 6 shows the inter-annual pattern of change in the Chlorophyll a in Shiyan Reservoir. The  $\rho$ (Chla) of Shiyan Reservoir gradually decreased from south to north, and overall showed the characteristics of high in the middle-south reservoir area and low in the north reservoir area. During the flood season and high-temperature no-rain period,  $\rho$ (Chla) was high in most of the sampling sites, and there was a high risk of algal bloom.  $\rho(TChla)$  in the southern half of the reservoir, where sampling sites 1, 2 and 3 were located, was 60.35  $\mu$ g/L, which was the area with the highest  $\rho$ (Chla) in Shiyan Reservoir. Sampling point 1 is the location where the incoming water from Tiegang Reservoir enters Shiyan Reservoir, and the  $\rho$ (Chla) ranged from 14.06 to 123.29  $\mu$ g-L-1, with a mean value of 45.69 µg/L. The incoming water from Tiegang Reservoir contained high concentrations of algae and nutrients, which resulted in a large base of  $\rho$ (Chla) in this area and a high rate of algal growth. Sampling points 2 and 3 were more polluted by runoff, and the nutrient status of the water body was The southern half of the reservoir had a high risk of algal bloom during flood season and high temperature without rain. Sampling sites 4, 5 and 6 were located in the live water area of the reservoir core, where water exchange was frequent and the water flow rate reached 15-30 cm/s, which to some extent inhibited the rapid algal Sampling sites 7 and 8 were near the water intake of the water plant, far away from the runoff pollution, which was the best place for the water quality of Shiyan Reservoir, and the total algal  $\rho$ (Chla) was 8.96-89.69 μg/L and 5.11-77.89 μg/L, respectively, and there was a higher risk of algal bloom during the flood season. In general, it seems that Shiyan Reservoir is small in size and regular in shape, with sufficient water exchange within the reservoir and low spatial heterogeneity at each sampling point. Runoff pollution and the sink of industrial and agricultural pollution sources were the main reasons for the spatial heterogeneity of algal chlorophyll a in Shiyan Reservoir, so strengthening the protection of water sources and reducing the entry of exogenous pollutants from the source are the fundamental ways to reduce the risk of algal bloom.



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**Figure 6.** Interannual spatiotemporal succession patterns of phytoplankton biomass (represented by chlorophyll-a concentration) in Shiyan Reservoir: (a) phytoplankton biomass during the Pre-Rainy Period (PRP) in 2021; (b) phytoplankton biomass during the Latter Rainy Period (LRP) in 2021; (c) phytoplankton biomass during the High Temperature and Rain-Free Period (HTRFP) in 2021; (d) phytoplankton biomass during the Winter Drought Period (WDP) from 2021 to 2022; (e) phytoplankton biomass during the Temperature Jump Period (TJP) in 2022; (f) phytoplankton biomass during the Pre-Rainy Period (PRP) in 2022

# Canonical correspondence analysis of phytoplankton biomass and environmental factors

The water quality and meteorological factors in the flood and dry periods were analyzed in canonical correspondence with the algae  $\rho$ (Chla), and the effects of the combined effect of the two on the growth of the algae were investigated, and the results are shown in *Figure 7*. Cyanobacteria, green algae and diatoms were located in the first,

second and third quadrants during the flood season, and cyanobacteria, green algae and diatoms were located in the first, third and fourth quadrants during the dry season, indicating that there were large differences in the environmental factors affecting cyanobacteria, green algae and diatoms. The eigenvalues of F1 and F2 in the flood season were 0.015 and 0.012, with an explanation rate of 55.71% and 44.29%, respectively; and the eigenvalues of F1 and F2 in the dry season were 0.067 and 0.011, with an explanation rate of 86.87% and 13.13%, respectively.



Figure 7. Canonical correspondence analysis of environmental factors and phytoplankton of Shiyan Reservoir. Blue, green, purple and black dots represent cyanobacterial, green algal, diatom and total algal biomass, dark blue dots indicate sampling points, and arrow lines indicate environmental factors

The primary factor affecting the growth and distribution of algae during the flood season was runoff, especially cyanobacteria were highly positively correlated with runoff. Shiyan Reservoir is surrounded by dense population, and the tributaries into the reservoir include Shiyan River, Wangjiazhuang River, Mabu Water, Houqiao River, Shangya River, Yungnukeng Water, and Baikengwu Water, which bring domestic sewage and industrial and agricultural effluents in the watershed into the reservoir, which increase the nutrient levels. At the same time, the algae attached to the surface of the ground and plant surfaces are flushed by rainfall and enter the reservoir with surface runoff, and the algal biomass increases accordingly. Therefore, strengthening the interception and removal of sewage and endogenous pollution in the reservoir, and limiting the sources of pollution from agriculture and farming in the reservoir area are the primary ways to solve the eutrophication of the water body in Shiyan Reservoir. Air temperature, WT, air pressure, sunshine hours are the second type of influencing factors affecting the growth of algae, most of the studies. So we should strengthen the monitoring of algae in high temperature weather during the flood season to prevent the outbreak of local algal blooms. organic matter and nutrients such as TOC, COD, TN, ammonia nitrogen, and TP are the third type of influencing factors on algae. the correlation between DO, transparency, HRT, and evaporation and on algae is relatively small, and the silicate, rainfall and Fe had no significant correlation with algae.

The primary factor affecting algal growth and distribution during the dry period was silicate, and the effects of water and air temperatures ranked second. It was found that diatoms in Taihu Lake grew rapidly at temperatures of 16-26°C, and the biomass of diatoms began to decrease when the temperature exceeded 26°C (Zhu et al., 2020). The surface WT of Shiyan Reservoir was 13~24°C in winter, and the WT was more suitable for the growth of diatoms, which replaced cyanobacteria as the dominant algae. During the dry period, precipitation was scarce, and the silicate entering the reservoir with surface runoff was significantly reduced, which is the necessary nutrient salt required for the growth of diatoms, so the correlation between silicate and algae became more and more significant.

TOC and COD represent the organic matter content in the water, which has a high correlation with algae, and is the third type of. NO<sub>3</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N, TN and TP represent the nutrient salt level, which is a necessary factor for the growth of algae, and  $NO_3^{-}N$ , NH4<sup>+</sup>-N, TN are significantly correlated with algae, while TP has a lower correlation with algae. Fe has a significant correlation with algae, because Fe is an essential element for photosynthesis and metabolism of algae, which is the most important factor for the growth of algae. essential element for photosynthesis and metabolism, and has a significant effect on both algal biomass and growth rate. Meteorological factors such as barometric pressure, sunshine hours, rainfall, runoff, humidity, etc. were correlated with algae, but the significance was lower than that of the flood significant and there was no correlation between DO. season. HRT. evapotranspiration, and TN:TP and algae.

## Conclusions

Shiyan Reservoir is located in a typical high-density built-up area in Shenzhen, and its water environment quality and water ecological health are key to the safety and security of Shenzhen's drinking water. The 13-way water quality and water ecological indicators of Shiyan Reservoir were monitored for 14 months.

(1) The chlorophyll a concentration in Shiyan Reservoir ranged from 21.25 to 88.77  $\mu$ g/L. The highest value of 88.77  $\mu$ g/L was reached in May, and the lowest value of 21.25  $\mu$ g/L was reached in February. The temporal heterogeneity of the chlorophyll a concentration was obvious, and the changes were drastic. The highest concentration of

chlorophyll a was found in the period of April to July, which was maintained at about  $85 \mu g/L$ , and the risk of algal bloom was high.

(2) The annual average WT of Shiyan Reservoir is 25.31°C, and the WT from May to September is about 30°C for a long time, so the risk of algal bloom is high. The COD of the reservoir is 1.71~3.08 mg/L, and the TOC is 2.22~5.13 mg/L. The organic pollution of the reservoir is very low, and it reaches the environmental quality of surface water I standard. The dissolved oxygen in the surface layer of the reservoir is more than 7.33 mg/L throughout the year, reaching the environmental quality of surface water I standard. The transparency is 0.73~1.16 m. Relative to 2010~2015, the transparency of Shiyan Reservoir increased significantly. Shiyan Reservoir TN pollution is serious, during the investigation period, TN was 1.66~2.49 mg/L, the water quality of Class V. TN temporal heterogeneity is obvious, TN concentration in the pre-flood season > post-flood season > dry season. In recent years, Shenzhen has carried out a systematic comprehensive water environment management project, TN decreased significantly, but it is still the primary pollutant in Shiyan Reservoir. The TP in Shiyan Reservoir was 0.01~0.043 mg/L, and the total phosphorus concentration was low, which reached the environmental quality of surface water  $I \sim II$  standards. the temporal heterogeneity of TP was low, and the spatial heterogeneity along the water depth was high.

(3) The monthly average integrated trophic state of the reservoir is located between 43.13 and 53.81, with an annual average value of 50.49. The monthly average integrated trophic state of April and November 2021 and April and May 2022 is less than 50, which is in the state of light eutrophication, and the other months are in the state of moderate eutrophication.

(4) The correlation analysis showed that WT was the primary influencing factor of phytoplankton in flood season, and the correlation coefficient was as high as 0.69. The main correlation factors of phytoplankton in flood season were WT, TOC, nitrate nitrogen, and SD, and the main correlation factors of phytoplankton in dry season were TOC, DO, and WT. Throughout the whole year, phytoplankton highly positively correlated with WT, TOC, moderately positively correlated with COD, pH, and turbidity, and moderately negatively correlated with transparency. Transparency were moderately negatively correlated.

(5) Two-factor ANOVA showed that the variance of each environmental factor was better explained by the period and sampling site and the interaction effect between the two (R2 ranging from 23.7% to 78.1%). The main influence of environmental factors in Shiyan Reservoir was period, and the temporal heterogeneity of environmental factors was significantly higher than the spatial heterogeneity.

(6) A total of 21 genera of cyanobacteria, 24 genera of diatoms, 39 genera of chlorophyta, 3 genera of euglenophyta, 2 genera of pyrrophyta, 1 genus of chrysophyta and 2 genera of cryptobranchs were detected in Shiyan Reservoir. In Shiyan Reservoir, cyanobacteria dominated from May to October, and diatoms dominated from November to February. There were 9 genera of cyanobacteria were dominant in Shiyan Reservoir, including Raphidiopsis, Oscillatoria, Phormidium, Planktothrix, Anabaena, Merismopedia, Chroococcus, Microcystis and Snowella.

(7) The change pattern of  $\rho$ (Chla) in Shiyan Reservoir in time is pre-flood period (April to June in 2021 and 2022) > post-flood period (July to September 2021) > high-temperature rainless period (October to November 2021) > temperature jumping period (March 2021) > autumn and winter dry period (December 2021 to February 2022), and

in space, it decreases gradually from south to north. There is a risk of cyanobacterial bloom in the whole reservoir area during the flood season and in the south-central reservoir area during the high-temperature rainless period, and the diatom outbreaks in winter and spring do not reach the level of bloom, but they are easy to block the filter ponds of the water supply plant, which is detrimental to the safety of water supply.

(8) Correlation and CCA analyses showed that the key environmental impact factors of cyanobacteria, diatoms and green algae differed greatly, with WT being the primary impact factor for the three algae, and organic matter and nutrients brought by runoff during the pre-flood season being an important trigger for cyanobacterial outbreaks. Nitrogen-phosphorus ratio was negatively correlated with cyanobacteria, green algae and diatoms. Phosphorus may be the limiting factor for algal growth in Shiyan Reservoir. The main correlation factors of cyanobacteria also included TOC, TN, pH, transparency, turbidity and nitrate nitrogen, etc. The main correlation factors of diatoms also included silicate, TOC, nitrate nitrogen and COD, etc. The main correlation factors of green algae also included TOC, COD, pH, transparency and nitrate nitrogen, etc. The main correlation factors of cyanobacteria also included nitrate nitrogen, etc. The main correlation factors of cyanobacteria also included nitrate nitrogen, etc. The main correlation factors of green algae also included TOC, COD, pH, transparency and nitrate nitrogen, etc. The main correlation factors of cyanobacteria also included TOC, COD, pH, transparency and nitrate nitrogen, etc. The main correlation factors of cyanobacteria also included TOC, COD, pH, transparency and nitrate nitrogen.

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