Evaluation for algae and microcystins in several reservoirs in Haihe River Basin

J Zhan\textsuperscript{1,2}, X Z Meng\textsuperscript{1}, S L Zhang\textsuperscript{1}, Y Z Wang\textsuperscript{1}, W Xu\textsuperscript{1}, M X Xu\textsuperscript{1} and X S Zhou\textsuperscript{1}

\textsuperscript{1}Center of Monitoring and Scientific Research of Ecology and Environment, Administration of Ecology and Environment of Haihe River Basin and Beihai Area, Ministry of Ecology and Environment of People’s Republic of China, Tianjin, 300170, China

E-mail: zhangjun506@163.com

Abstract. In this study, algae and microcystins (MC-LR and MC-RR) were studied in 6 reservoirs in the northeastern part of Haihe River Basin. The results indicate that the eutrophication degree of most reservoirs has been obviously improved, but Yuqiao reservoir and Yanghe reservoir are still in the state of eutrophication. The MC-LR and MC-RR were also detected in Yuqiao reservoir and Yanghe reservoir. In addition, cyanobacteria were dominant spices in the eutrophic reservoirs, and the concentration of microcystins was positively correlated with algae density. Although the concentration of MC-LR is lower than the concentration limits of sanitary standard for drinking water. It is suggested that it should be monitored during the outbreak of cyanobacteria, especially in the later stage of outbreak considering a risk for the health of the water supply population.

1. Introduction
The occurrence of cyanobacteria associated with their toxins in surface water has become a worldwide problem [1-3]. According to the data released by the United Nations Environment Programme, 70% of freshwater bodies have a problem of eutrophication in the world [4]. An ecological survey of 531 representative lakes and reservoirs in China indicated that 95% of lakes and reservoirs were under the impacts of eutrophication at different levels [5]. As we all know, the carrying capacity of water resources in Haihe River Basin is the most serious in the seven river basins in China, facing the situation of "all rivers are dry and polluted" [6]. The concentration of nitrogen and phosphorus nutrients is relatively high in rivers and lakes in Haihe river basin, in which cyanobacteria bloom easily occurs especially in summer when temperature rises. Some reservoirs are increasingly experiencing algae blooms in Haihe river basin such as Yanghe Reservoir, Yuqiao Reservoir [7-9]. As the growth of abundant algae, dissolved oxygen in water decreases rapidly, resulting in the death of aquatic organisms such as fish, shrimp and snails and block of water supply pipelines [10]. Another hazard of algae blooms is metabolites after algae death, which can easily form odorous substances and toxins affecting water supply safety [11,12]. It is thought that the microcystins formed by the outbreak and metabolism of cyanobacteria are bound to be more harmful to human health [13]. Therefore, the problem of algae and toxic metabolites in surface water sources has become one of the major concerns of drinking water safety.

2. Materials and methods
2.1. Study area
The study area is located in the northeastern part of Haihe River Basin, which mainly includes the lower reaches of Luanhe River Basin, the coastal areas of Eastern Hebei Province and the route from Luanhe River to Tianjin. The water quality is relatively good in the 6 reservoirs in the study area, which supply drinking water for Tianjin, Tangshan and Qinhuangdao. However, eutrophication has also occurred in summer in these reservoirs. Considering the challenge to drinking water safety, algae and microcystins has been studied in the six reservoirs of the study area, which is shown in figure 1.

![Figure 1](image_url)

Figure 1. The location of 6 reservoirs in Haihe River Basin.

2.2. Sample collection and preparation
The samples of phytoplankton and microcystins were collected from six reservoirs in the summer of 2017, including Panjiakou Reservoir, Daheiting Reservoir, Yuqiao Reservoir, Taolinkou Reservoir, Yanghe Reservoir and Shihe Reservoir. Based on the regular monitoring task of water source, the sampling points are located in the open water area near the intake of the six reservoirs, which directly reflects the water quality supplying to surrounding cities. Qualitative samples of phytoplankton were dragged for 3 minutes at 0.15m under water by a plankton net No. 25 (0.074mm). The samples were fixed with 3mL formalin and stored in 100mL sample bottle for qualitative analysis. The samples of phytoplankton were taken from 1L water sample bottle fixed with 15mL Lugol's solution for quantitative analysis in the laboratory. 1L water samples were collected from surface water of reservoirs using brown glass bottle for preservation and brought back to the laboratory for preparation and analysis.

2.3. Identification of phytoplankton
Phytoplankton microscopy was performed under Zeiss Scope A1 microscope. Qualitative samples were classified mainly according to morphological classification methods. Species were identified
with reference to Freshwater Algae of North America: Ecology and Classification [14] and Freshwater Algae-Systems, Classification and Ecology of China [15]. Quantitative samples were taken back to the laboratory for 24 hours, then concentrated to 30 ml, and observed with plankton counting boxes. The algae cell density was calculated according to the concentration multiple. Based on the quality control method from other researchers [16], another professional person checked the same samples again after the samples were identified for the accuracy of classification and identification. The proportion of checking samples is about 10%.

2.4. Determination of microcystins
All water samples were filtered through 0.45μm glass fiber filters, and then were acidified to pH=4 by adding formic acid. Oasis HLB cartridges were preconditioned with 5.0ml of methanol and 5ml pure water. Acidified water samples of 500 ml were then passed through Oasis HLB columns at a flow rate of approximately 5mL/min. After drying, microcysins were eluted from each cartridge using 2mL of methanol for three times. Extracts were collected in a glass vial, reduced to a minimum volume, and then dissolved in 20% methanol to a final volume of 1.0mL. The concentrated samples were detected by high performance liquid chromatography-tandem mass spectrometry. The mobile phases consisted of deionized water containing 0.1% formic acid (A) and methanol (B). The gradient was set up as follows: 0-8min, 20%-80% B; 8-10min, 80% B; 10-12min, 80%-20% B; 12-25min, 20% B. The correlation coefficients of MC-LR and MC-RR standard curves are 0.9995 and 0.9991, respectively. The detection limits of MC-LR and MC-RR were 0.06 and 0.1 ug/L detected by LC-MS directly. The theoretical detection limits of method were 0.06 and 0.1ng/L calculated by 100% recovery. The recovery rates of MC-LR and MC-RR were actually 88.5% and 72.6%.

3. Results and discussion

3.1. Algae and eutrophication

| Evaluation Index | Eutrophication grade | extremely poor | poor | poor middle | middle | middle nutrition | nutrition | extremely nutrition |
|------------------|-----------------------|----------------|------|-------------|--------|-------------------|-----------|---------------------|
| algae density (10^6 ind./L) | ≤0.5 | ≤1.0 | 1-9 | 10-40 | 41-80 | 81-99 | ≥100 |

| content | Panjiakou | Daheiting | Yuqiao | Yanghe | Taolinkou | Shihe |
|---------|-----------|-----------|--------|--------|-----------|-------|
| algae density (10^6 ind./L) | 7.82 | 6.44 | 122.20 | 132.18 | 0.68 | 17.53 |
| cyanobacteria density (10^6 ind./L) | 6.30 | 6.00 | 120.78 | 123.32 | 0.12 | 17.10 |
| proportion of cyanobacteria (%) | 80.1 | 93.2 | 98.8 | 93.3 | 17.7 | 97.6 |
| Eutrophication types | Poor | Poor | extremely | extremely | poor | middle |
| middle | nutrition | nutrition |

The results indicate that the algae density of Yanghe and Yuqiao reservoirs is relatively high in the summer in 2017, while that of Taolinkou, Panjiakou and Daheiting reservoirs is relatively low. In addition, the dominant species are cyanobacteria when the algae density is high in these reservoirs. The proportion of cyanobacteria in Yuqiao reservoir is up to 98.8%. On the contrary, the proportion of cyanobacteria will decrease. The proportion of cyanobacteria in Taolinkou reservoir is only 17.7%. Therefore, cyanobacteria would be the dominant species when algae-blooming happen in the reservoir.
Based on the evaluation criterion (table 1) of algae density in lake water body [17], the eutrophication status of each reservoir was calculated from the results of algae density identification in six reservoirs. The results indicate that Yuqiao reservoir and Yanghe reservoir are at the status of extremely nutrition, Shihe reservoir is at the status of middle nutrition, Panjiakou reservoir and Daheiting reservoir are at the status of Poor middle, Taolinkou reservoir is at the status of poor, which is showed in the table 2. It is reported that water eutrophication have occurred several times in these reservoirs of the study area [7-9]. With the requirement of ecological civilization construction, the water quality has been significantly improved, especially for Panjiakou and Daheiting Reservoirs. The result showed that the eutrophication degree of Panjiakou and Daheiting Reservoirs has been significantly improved after all fish cages were cleaned at the request of the State Council and the Hebei Provincial Government in November, 2016. However, the eutrophication degree of Yuqiao Reservoir and Yanghe Reservoir is still critical at present.

3.2. Determination of microcystins

The results indicated that MC-RR and MC-LR were detected in micro-water samples of Yuqiao and Yanghe reservoirs (table 3), but the content of MC-LR was lower than the concentration limits of Sanitary Standards for Drinking Water Quality (GB 5749-2006) [18], which could not be higher than 1.0 μg/L. Both MC-LR and MC-RR were not detected in Panjiakou, Daheiting, Taolinkou and Shihe reservoirs. Generally speaking, the potential risk of microcystins in the water source of northeastern Haihe River Basin is relatively low, but it should pay more attention to microcystins in the water sources, especially in Yuqiao Reservoir and Yanghe Reservoir.

| Type            | Panjiakou | Daheiting | Yuqiao | Yanghe | Taolinkou | Shihe |
|-----------------|-----------|-----------|--------|--------|-----------|-------|
| MC-LR (ng/L)    | <0.06     | <0.06     | 57.87  | 35.40  | <0.06     | <0.06 |
| MC-RR (ng/L)    | <0.1      | <0.1      | 41.32  | 28.88  | <0.1      | <0.1  |

Figure 2. Relationship between cyanobacteria density and microcystins in 6 reservoirs.

3.3. The relationship between eutrophication and microcystins

Microcystins are toxic by-products of some natural cyanobacteria in eutrophic freshwater. It is found that more than 40 cyanobacteria could produce microcystins, which is one of the most harmful pollutants in cyanobacterial blooms. The results of this study indicated that the eutrophication degree of water body was positively correlated with the concentration of microcystins, which is shown in figure 2. Cyanobacteria are dominant spices in severe eutrophication water body, which are the main sources of microcystins. Therefore, there are increasing risks after the outbreak of cyanobacteria. The results showed that MC-LR and MC-RR were detected in Yuqiao and Yanghe reservoirs, which were
in extremely eutrophic condition, while microcystins were not detected in other reservoirs. It is reported that microcystins are a kind of intracellular compounds which are stable in nature and mainly come from the death and metabolism of cyanobacteria such as Microcystis aeruginosa [18]. In addition, microcystins could be produced by Microsystis spp., Anabaena spp., and Oscillatoriaspp. [19]. In this study, Microcystis and Anabaena should be the main sources of microcystins, which were found in Yuqiao Reservoir and Yanghe Reservoir.

4. Conclusions
The eutrophication degree of the middle water body in six reservoir-type water sources in the northeast of Haihe River Basin has been significantly improved. The results indicate that Yuqiao reservoir and Yanghe reservoir are at the status of extremely nutrition, Shihe reservoir is at the status of middle nutrition, Panjiakou reservoir and Daheiting reservoir are at the status of Poor middle, Taolinkou reservoir is at the status of poor. In addition, it is found that cyanobacteria are dominant spices in eutrophicwater body.

Both MC-LR and MC-RR were detected by solid phase extraction-liquid chromatography-tandem mass spectrometry in Yuqiao and Yanghe reservoir. However, the concentration of MC-LR was lower than the concentration limit of Sanitary Standards for Drinking Water. In addition, MC-RR and MC-LR were not detected in Panjiakou, Daheiting, Taolinkou and Shihe Reservoirs.

The results indicate that the concentration of microcystins was positively correlated with algae density. Although the concentration of MC-LR is lower than limit that Sanitary Standards for Drinking Water Quality. It is suggested that it should be monitored during the outbreak of cyanobacteria, especially in the later stage of outbreak considering a risk for the health of the water supply population.

Acknowledgments
This work was supported by the CRSRI Open Research Program (No. CKWV2018495/KY) and Program of water quality regular monitoring in Haihe River Basin.

References
[1] Pham T and Utsumi M 2018 An overview of the accumulation of microsystins in aquatic ecosystems J. Environ. Manage. 213 520-9
[2] Gurbuz F, Uzunmehmetoglu O Y, Diler O et al 2016 Occurrence of microsystins in water, bloom, sediment and fish from a pubic water supply Sci. Total Environ. 562 860-8
[3] Su X, Steinman A D, Xue Q, Zhao Yet al 2018 Evaluating the contamination of microcystins in lake Taihu, China: The application of equivalent total MC-LR concentration Ecol. Indic. 89 445-54
[4] Zhuang Y T 2008 Monitoring and evaluation of algal toxin pollution in water environment Environ. Monitor. China 24 84-7
[5] Li Z C, Deng Y X and Zheng B H 2012 Investigation and analysis of nutritional status of lakes and reservoirs in China Environ. Sci. Technol. 35 209-13
[6] Zhao J S, Wang Z J and Qin T 2008 Analysis of evolution of water resources carrying capacity in Haihe River Basin J. Hydraul. Eng. 39 647-51
[7] Wang M, Liu X, Chen Q W et al 2017 Spatial and temporal distribution characteristics of microcystins and toxigenic strains in the Yanghe River Reservoir Environ. Sci. 37 1307-15
[8] Xie R Q, Xu Y and Wang Y Q 2014 Characteristics of eutrophication time and analysis of pollution sources in Yuqiao Reservoir J. Water Resour. Water Eng. 25 132-6
[9] Wang Y, Xing H Y, Zhao E L et al 2016 Present situation and control measures of water pollution in Panjiakou and Daheiting Reservoir J. Haihe Water Conservancy 3 17-9
[10] Jia R B 2006 Study on Algae Pollution Control of Urban Water Supply (Jinan, China: Shandong University Press)
[11] Park J A, Jung S M and Yi I G 2017 Adsorption of microcystin-LR on mesoporous carbons and
its potential use in drinking water source. Chemosphere 177 15-23
[12] Li L, Wan N, Gan N Q et al 2007 Annual dynamics and origins of the odorous compounds in the pilot experimental area of Lake Dianchi, China Water Sci. Technol. 55 43-50
[13] Hernandez J M, Rodas V L and Costas E 2009 Microcystins from tap water could be a risk factor for liver and colorectal cancer: A risk intensified by global change Med. Hypotheses 72 539-40
[14] John D and Robert G 2003 Freshwater Algae of North America: Ecology and Classification (Amsterdam; Boston: Academic Press)
[15] Hu H J and Wei Y X 2006 Freshwater Algae in China: Systematics, Classification and Ecology (Beijing, China: Science Press)
[16] Jin X W, Wang Y Y, Wang B X et al 2017 Methods development for monitoring and assessment of ecological integrity of surface waters in China Environ. Monit China 33 88-94
[17] Liu S Y, Ma F and Zhang J Q 2007 Study on algal succession and diversity index in the process of eutrophication of landscape water body J. Environ. Sci. China 02 337-41
[18] Laflative J and Ten-gage L 2007 Algal and cyanobacterial secondary metabolites in freshwaters: A comparison of allelopathic compounds and toxins Freshwater Biol. 52 199-214
[19] Pearson L, Mihali T, Moffitt M et al 2010 On the chemistry, toxicology and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin Mar. Drugs 81 650-80