Influencing factors and health risk assessment of microcystins in the Yongjiang river (China) by ~~the~~ Monte Carlo simulation ~~method~~

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ABSTRACT

The Yongjiang river is a large, shallow, hyper-trophic, freshwater river in Guangxi, China. To investigate the ~~exposure to~~presence of microcystin-RR, microcystin-LR, and microcystin-YR (~~-~~MC-RR, MC-LR, and MC-YR) ~~of~~ in the Yongjiang river and describe their correlation with environmental factors, as well as, assess ~~the~~ health risk using Monte Carlo simulation ~~method~~ , 90 water samples ~~with environmental data~~ were collected ~~from~~ at three sample points from March–December 2017 ~~in river Yongjiang~~. ~~The r~~Results showed that during the monitoring period, ~~the~~ total concentrations of MC-RR (TMC-RR), MC-YR (TMC-YR), and MC-LR (TMC-LR) ~~were~~ varied from 0.0224 to 0.3783 μg/L, 0.0329 to 0.1433 μg/L, and 0.0341 to 0.2663 μg/L, respectively. ~~The t~~Total phosphorus (TP) content ~~may~~ appeared to be related to TMC-LR and the total content of microcystins (TMCs)~~-~~, while pH and total nitrogen/total phosphorus (TN/TP) ratio ~~may~~ appeared to be related to TMC-RR and TMC-YR, respectively. ~~By u~~Using the professional health risk assessment software @Risk7.5, the risks of dietary intake of microcystins (MCs), ~~include~~ including ~~of~~ the carcinogenic risk and non-carcinogenic risk, were evaluated. It was found that the carcinogenic risk of MC-RR from drinking water ~~source~~ was higher than ~~those of~~ MC-LR and MC-YR, and the ~~pollution~~ presence of MCs would lead to high potential health risks, especially in children. The carcinogenic risk of MC-RR to children was ~~higher than~~≥ 1×10^{-4}, the maximum allowance level~~s~~ recommended by the US Environmental Protection Agency (USEPA); ~~A~~as ~~to~~ for adults, it was ~~higher than~~≥ 5×10^{-5}, the maximum allowance level~~s~~ recommended by the International Commission on Radiological Protection (ICRP). The non-carcinogenic hazard index of MC-RR, MC-YR, and MC-LR increased successively, ~~indicated~~ indicating that MC-LR was more hazardous to human health

than MC-YR and MC- RR, but its was found that the hazard index is was < less than 1; . Those This suggested suggests that MCs pose less risk to health. ThereforeHowever, it is necessary to strengthen the protection and monitoring of drinking water sources for effective control of water pollution and safeguarding of human health.

1. INTRODUCTION

Eutrophication of freshwater bodies can result in algal blooms, especially those caused by cyanobacteria. Cyanobacteria can secrete algal toxin, most of which are potentially harmful to plants, animals and humans (Holland & Kinnear, 2013; Cao et al., 2017). The majority of the approximately 90 known microcystins(MCs) have been isolated from species and strains of *Microcystis* (Pham &Utsumi, 2018). Of these, the most widely distributed are microcystin-LR (MC-LR), microcystin-RR (MC-RR), and microcystin-YR (MC-YR) (Zegura, 2016). These toxins are synthesized in the cells and released after cell rupture, resulting in the appearance of microcystins in the water source.

Cyanobacteria blooms appear in eutrophicated waters worldwide, and MCs can be bioaccumulated by aquatic animals and can reach humans, severely endangering human health and leading to illness or death. In 1975, the drinking water source in the small town of Pennsylvania was contaminated with Microcystis, which resulted in acute gastroenteritis in more than half of the local population (Keleti et al., 1979; Keleti & Sykora, 1982; Lippy & Erb, 1976). In 1996, a hemodialysis center in Brazil was contaminated with MCs , causing 116 of 130 patients to have abnormal symptoms such as blurred vision, nausea, and >50 individuals succumbed to mortality (Pouria et al., 1998). Studies on drinking water showed that in Haimen and Fu Sui, the mortality rate of hepatocellular carcinoma (HCC) in drinking ditch pond water with MCs was about 100/100 000, which was significantly higher than that of shallow wells or deep wells (20/100 000) (Ueno et al., 1996). In 2010, the International Agency for Research on Cancer (IARC) listed MCs as a “possible human carcinogen” (Group 2B) based on its potential carcinogenicity (IARC, 2010).

Although MCs have been confirmed to cause acute and chronic damage to the human body, reports on MC-LR, MC-RR, and MC-YR and their risk assessment in Yongjiang river of China are yet lacking. The environmental conditions of water source are crucial in concentration levels of toxins. However, the factors affecting the concentration levels of MCs (nutrient levels and climatic conditions) in Yongjiang have not yet been elucidated. Therefore, the present study investigated the concentration and distribution of MCs in Yongjiang river as influenced by seasonal changes in water quality and the related parameters.

Owing to the requirement of prolonged duration and inoperability of toxicological health risk assessments, the present study could not be conducted in a population. Thus, the Monte Carlo simulation mathematical and logical model was used. In recent years, this model has been used to understand the behavior of water systems that would aid in decision-making based on the advantage to model any appropriate assumptions of the problem or the system (Clausen et al., 2017). Moreover, the Monte Carlo simulation of uncertainties was applied in the risk assessment model by collecting limited samples to predict the overall situation. In this regard, the risk uncertainty was expressed intuitively, which was in agreement with the nature of the uncertainty and predictability of the risk; The risk managers and policymakers can provide a favorable intuitive and scientific basis (Paladino, Moranda & Seyedsalehi, 2017; Sasi, Yozukmaz & Yabanli, 2017). The US Environmental Protection Agency (USEPA) has set Monte Carlo simulation as a basic method in the risk analysis policy (Moolenaar, 1996). Currently, American Palisade developed @Risk Monte Carlo software that is loaded into Excel simulation technology for risk. The professional software is mainly based on the analysis of stochastic simulation method of Monte Carlo that provides various potential results using a variety of probabilistic simulations, the probability of occurrence of events that constitute a risk, forecast the risk of uncertainty quantitatively, and summarize the characterization results. Li et al. (2017) used the Monte Carlo model to assess the quantitative risk of aluminum in Youtiao, which did not exceed the provisional tolerable weekly intake (PTWI) set by the Joint Expert Committee on Food Additives (JECFA) for the public. Jia et al. (2018) used the Monte Carlo simulation to evaluate the trace elements in four freshwater fish from a mine-impacted river, and found that consumption did not exert any appreciable adverse impact on human health due to the exposure to trace elements in fish muscle.

The present study investigated the current status of drinking water sources in the Yongjiang river in China regarding the contamination of MCs. By using the professional health risk assessment software @Risk7.5, the risks of dietary intake of microcystins (MCs) include of the carcinogenic risk and non-carcinogenic risk were evaluated. These findings provide a basis to undergo an effective control of water pollution and quality in order to protect the human health of the area.

2. MATERIALS AND METHODS

2.1. Sampling location

Yongjiang river is a major water resource with an average annual flow of 1292 m^3/s in the Nanning City area (Figure 1) . It is the main urban water source in Nanning city, China, and the tributary channel is also a vital transportation route. The surface area of the river is 2676 ha with a maximum depth of 23 m and is used for entertainment purposes as well as a source of water for domestic use, agriculture, fishery, and industry.

2.2. Sampling

Water samples were collected from the Yongning river in Nanning City from March to December in 2017. For this, the river section was set into three sampling points: Qingxiu District, Jiangnan District, and Yongning District (Figure 1). The water samples were collected at a depth of 0.5 m and 3 times/month from each sampling point, a total of 90 samples were collected in 10 months, and were taken during the most active daylight period (11:00–14:00). Sample collection, containers, stabilization, and transportation to the laboratory were in accordance with the methods described in Wunderlin et al. (2001). Water samples were passed through the 500 mesh stainless steel screensto remove large particles of impurities and were stored at 4°C and protected from light, and process samples within 24 hours. A volume of 2000 mL water sample was collected, and 500 mL water sample is passed through the 0.45 μm filter (Jinteng, China) under reduced pressure filtration. The filter containing the algae was subjected to an extraction process in order to recover the intracellular MCs, followed by two extractions with 5 mL ultra-pure water after five times freezing-thawing at -80°C/37°C. After filtering through the 0.45 μm filter for removing the algal cells, the filtrate and the extract from the filter were passed through solid phase extraction (SPE) (500 mg/6 mL) (SUPELCO, USA). The SPE was rinsed with 20 mL of 20% methanol and 10 mL deionized distilled water. The toxin was eluted from the stationary phase with 80% methanol (containing 0.05% TFA), and each sample was dried in a water bath under control temperature (60 °C).

2.3. Water quality analysis

Water parameters χ^1=Water Temperature, χ^2=pH, χ^6=Dissolved Oxygen (DO) were measured in situ and χ^3=Total phosphorus (TP), χ^4=PO_4^{3-}-P, χ^5=Total Nitrogen (TN) were measured in the laboratory. Each experiment was performed in triplicate, and the average values are reported. All water samples were analyzed using standard methods (GB, 2002). Instruments used were YSI Model 58 thermometer, Knick Portamess 911 for pH measurement. DO using iodometric method. TN and TP were analyzed using Kjeldahl method and persulfate digestion. PO_4^{3-}-P was determined according to stannous chloride method.

2.4. Determination of MCs

The air-dried samples were suspended into 1 mL deionized distilled water for high performance liquid chromatography (HPLC) (Shimadzu LC-20A, Japan) analysis. The solubilized toxin samples were analyzed using HPLC with UV detector at 238 nm and symmetrical C18 column (3.9×150 mm) (Waters, USA). The mobile phase constituted of 33% acetonitrile and 67% deionized distilled water in 0.1% phosphate buffer (pH=3.0). The flow rate was set at 1 mL/min. The injection volume is 20 µL and the column temperature is 45°C. MC-LR and MC-RR (Solarbio, approximate purity, 95%) and MC-YR (Alexis, approximate purity 98%) were used as standards. Furthermore, the concentrations of MCs were determined by calibrating such area under the peak with the corresponding standard curves. MC-LR, MC-RR, and MC-YR showed good linearity in the range of 0.025~2 µg/L（r^2 =0.9987, 0.9992, 0.9997）. Under the condition that the signal to noise ratio (S/N) is 3, the detection limits of MC-RR and MC-LR were 0.0125 μg/L, and the detection limit of MC-YR was 0.014 μg/L. The recoveries ranged from 91% to 110%, the relative standard deviation (RSD) was 3.0%~5.6%. A series of toxin peaks was identified using retention time and comparison with spikes and known standard in the blank samples. Furthermore, the concentrations of microcystins were determined by calibrating such area under the peak with corresponding standard curves. The order of the peaks and time of each standard substance were as follows: MC-RR (7.599 min), MC-YR (14.225 min), and MC-LR (17.601 min). The test sample was analyzed in 18 min (Figure 2).

2.5. Method of risk assessment

2.5.1. Construction of exposure assessment model

The three main route of exposure to pollutants were consumption, inhalation, and skin absorption; the proportion of each was different due to the variations in the pollutants. The present study assessed the risk exposure caused by drinking water. The daily exposure to MC-RR, MC-YR, and MC-LR by direct drinking water was assessed using the Monte Carlo simulation by @Risk7.5 software operating platform; the Bootstrap sampling method was used for the quantification of uncertainty. Each Bootstrap sample was simulated with 10,000 Monte Carlo simulations to obtain different percentile values (P5–P95) for determining its uncertainty. The probabilistic assessment method was used to construct the exposure evaluation model (Zobitz et al., 2011).

The mechanism underlying the different exposures and various routes with different exposure dose formula were employed for the exposure assessment model of the health risk of chemical pollutants from the US environmental protection agency. Chronic daily intake (CDI) evaluated the safety of MCs in drinking water and the health risks of diverse routes in different populations. The CDI (μg/kg/day) formula is as follows.

Daily exposure of drinking water (Duy et al., 2000; Funari & Testai, 2008):

$$\text{CDI} = \frac{\text{Cw} \times \text{IR} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT}}$$

Here, Cw is the concentration of pollutants in the water, μg/L; IR is the volume of drinking water (L/day); EF is the frequency of exposure, drinking water for daily necessities (365 days/years) ; ED is exposure duration (years); BW is the average body weight, kg; AT is the average time, equal to ED multiplied by 365 days/years. According to the WHO, the standard weight of adults is 70 kg, and the daily drinking volume is 2 L/day; While the weight of children is 16 kg and the daily drinking volume is 1 L/day (WHO, 2017).

2.5.2. Construction of risk description model

The characteristics of the pollutants in a water environment are generally divided into genetic, toxic substances (for instance, chemical carcinogens) and somatic toxic substances (for instance, non-carcinogens) as recommended by the USEPA water environmental health risk assessment model. Calculating the carcinogenic and non-carcinogenic risks of MCs in source waters from exposure pathways.

(1) Health hazard risk model of chemical carcinogens

The formula of health hazard risk caused by chemical carcinogens recommended by USEPA (Duy et al., 2000):

$$R_i^c = \frac{1-\exp(-D_i q_i)}{70}$$

In the formula, R_i^c is the average personal carcinogenic annual risk of chemical carcinogen i through drinking water, $(\text{years})^{-1}$; 70 indicates the average life expectancy of Chinese population, years; D_i is the daily average exposure to chemical carcinogen i through drinking water, i.e., CDI, μg/kg/day; q_i is the carcinogenic strength coefficient of the chemical carcinogen i through the drinking water, and currently, there is no recognized carcinogenic intensity coefficient of MCs. Based on the formula of carcinogenic strength coefficient of carcinogens (Hitzfeld, Hoger & Dietrich, 2000), this study deduced the formula as follows:

$$CPI = \frac{(OR-1) \times LR}{D}$$

Where, carcinogenic potency index (CPI) is the coefficient of carcinogenic strength estimated from the population data: q_i, kg/day/μg/L. Odds ratio (OR) refers to the ratio of the number of exposed and non-exposed people in the case group divided by the ratio of the number of exposed and non-exposed in the control group. According to the population of 80,000 inhabitants, the study showed that the person who drank the river water presented a liver cancer OR of 1.246 (Falconer & Buckley, 1989; Yeh et al., 1989; Yu, Chen & Li, 1995). Lifetime risk (LR) indicated the risk of cancer among individual in the whole local population, and the parameter was not dimensionless; According to the risk of cancer during the individual's lifespan exposed to MC-LR in the population of China: 6.2×10^{-3} (Yeh et al., 1989; Fan et al.,2009). D indicated the calculation of the average daily life exposure dose, μg/kg/day. According to the results of the study by Wolf et al., the lifetime carcinogenic strength of MC-RR was set to 1/10th of that of MC-LR, while the strength of carcinogenicity of MC-YR and MC -LR was equivalent (Wolf & Frank, 2002).

(2) Non-carcinogenic health risk assessment model

The health risk assessment model recommended by USEPA was used to evaluate the non-carcinogenic health risk of MCs in the Yongjiang river source water. The non-carcinogenic risk was described using a hazard index (HI) by the following formula:

$$HI = \frac{CDI}{RfD}$$

Here, reference dose (*RfD*) is the reference dose for MCs: the internationally accepted tolerable daily intake (*TDI*) instead of MC-LR *RfD* was 0.04 μg/kg/d. According to the equivalent toxicity relationship among MC-RR, MC-YR and MC-LR, the *RfD* values of MC-RR and MC-YR were 0.4 and 0.04 μg/kg/d, respectively (Wolf & Frank, 2002; Lee et al., 2017).

The HI to 1 is usually calculated for the benchmark: HI>1 indicates harm to the human body; The value higher than the reference dose for exposure may be dangerous; HI≤1 indicates that the extent of damage is smaller and the exposure level is lower than the reference dose, which is unlikely to be detrimental (Younes, 1999).

2.6. Statistical analysis

IBM SPSS Statistics 22.0 software was used to perform all descriptive statistical analysis including minimum value, maximum value, mean value, standard deviation, Pearson's correlation analysis and stepwise multiple linear regression. Moreover, the risk assessment of MC-RR, MC-YR, and MC-LR exposure in water source was carried out using @Risk7.5 probabilistic evaluation software based on the Monte Carlo simulation technique.

3. RESULTS

3.1. Concentration level, distribution characteristics, and environmental impact factors of MCs in source water

3.1.1. Concentration distribution characteristics of MCs in source water

The concentration of MC-LR, MC-RR, and MC-YR in water samples were detected. The total concentrations of MCs (TMCs) in water is the sum of the concentrations of extracellular MCs (EMCs) and intracellular MCs (IMCs) dissolved in the water, and the results summarized in Table 1.

3.1.2. Seasonal distribution characteristics of MCs in water samples

The seasonal distribution of MCs in Yongjiang river source is shown in Figure 3, which demonstrated that the concentration of TMC-YR (refers to the sum of intracellular and extracellular and the same below) was significantly lower than the other two MCs. The concentrations of TMC-RR and TMC-YR at the same time reached maximum levels in October.

3.1.3. Pearson's correlation analysis of environmental factors and MCs' concentration

Pearson's correlation analysis of environmental factors and MCs' concentration was analyzed and the results shown in Table 2. According to the correlation analysis, the concentration of

TMC-RR was positively correlated with water temperature and TN ($p<0.01$), with a significant negative correlation with pH ($p<0.01$). The concentration of TMC-YR was negative correlated with TN:TP ratio ($p<0.05$). The concentration of TMC-LR was positively correlated with water temperature, TP, and PO_4^{3-}-P ($p<0.01$), with a significant negative correlation with DO and TN:TP ratio ($p<0.01$). The concentration of TMCs was positively correlated with water temperature and TP ($p<0.01$), with a significant negative correlation with pH, DO, and TN:TP ratio ($p<0.01$).

3.1.4. Stepwise multiple linear regression analysis of MCs' concentration and environmental factors

A stepwise multiple linear regression analysis of MCs' concentration and environmental factors is shown in Table 3. The results indicated that TP (χ^3) is the dominant factor affecting the contents of TMC-LR and TMCs. pH (χ^2) and TN:TP ratio (χ^7) are the primary factors affecting the content of TMC-RR and TMC-YR, respectively. These findings were in agreement with the results of the correlation analysis.

3.2. Assessment of MCs' exposure in source waters

3.2.1. Distribution fitting of the concentration of MCs in source water

The present study used @Risk7.5 software to fit the processed samples. The sample data of the concentration of MCs in source water was characterized as continuous data. The fitting results were Gamma, Invgauss, Lognorm, Expon, and Loglogistic, followed by the optimal fitting distribution model. Three main methods were used to test the goodness of fit: Chi-Sq (Chi-squared) test, K-S (Kolmogorov–Smirnov) test, and A-D (Anderson–Darling) test (Lipton et al., 1995; Cummins et al., 2009). Taken together, the sample fitting results (Table 4) were used to determine the fitting distribution types of the optimal probability of the pollution data: MC-RR had Gamma and Invgauss distribution, MC-YR had Lognorm, Expon, and Loglogistic distribution, and MC-LR had Gamma and Expon distribution. Furthermore, the results of the distribution parameters after fitting and comparison with the sample data parameters are summarized in Table 5. The probability distribution of the mass concentration of microcystins MC-RR, MC-YR and MC-LR in source water is shown in Figures 4, 5, and 6. The fitting results can be visually observed from the coincidence of the blue rectangular shape and the area under the red curve.

3.2.2. Daily exposure calculation

The @Risk7.5 software was utilized for the random extraction of the MCs concentration profiles from the water to calculate the daily exposure of direct drinking water by different populations to MC-RR, MC-YR, and MC-LR. Each simulation cycle was performed for 10,000 cycles, and the simulation results are shown in Table 6. In adults and children, a significant difference was observed in daily exposure. P50, P85, P90, and P95 (Table 6) represented the high exposure sites of each population. The MCs exposed to drinking water showed that the children's daily intake was 2-fold higher than that of the adults, suggesting that children are more susceptible to the pollution of MCs than adults.

3.3. Risk characterization of MCs in source water

3.3.1. Carcinogenic risk of MCs in source water

Based on the exposure parameters and carcinogenic risk formula, @Risk7.5 risk analysis software was used to extract the numerical the value of MCs concentration in water randomly and calculate the carcinogenic risk of MC-RR, MC-YR, and MC-LR intake by different groups of individuals through direct drinking water. Each simulation cycle of 10,000 displayed the statistical simulation results summarized in Table 7. The carcinogenic annual risk of MC-YR was less than that of MC-LR and MC-RR, and MC-RR was the primary hazard in the source water. The maximum acceptable level and the negligible level of carcinogenic risk for the population recommended by some institutions is listed in Table 8. The annual risk of carcinogenesis of MCs in a water source is 10^{-6}–10^{-4}. The carcinogenic risk of MC-YR and MC-LR in adults and children is lower than the maximum acceptable risk level designated by USEPA (1×10^{-4}) and ICRP (5×10^{-5}), and the risk of carcinogenic in children is higher than that in adults. The health risks caused by MC-RR from drinking water source for children was significantly higher than the maximum allowance levels recommended by USEPA (1×10^{-4}). Also, the health risks caused by the MC-RR from drinking water source for adults were significantly higher than the maximum allowance levels recommended by ICRP (5×10^{-5}). These statistical details indicated that MC-RR in water bodies exhibited a significant carcinogenic risk to the health of adults and children.

3.3.2. Non-carcinogenic risk of MCs in source water

The exposure parameters and non-carcinogenic hazards index formula was used to calculate the value of different populations through direct drinking water intake of MC-RR, MC-YR, and MC-LR (Table 9), which demonstrated that the average non-carcinogenic hazards index of MCs in different populations through drinking water intake and the non-carcinogenic hazards index of P90 and P95 at high levels of exposure was <1. This suggested that MCs, which are ingested through drinking water, pose less risk to health. The non-carcinogenic hazard index of MC-RR, MC-YR and MC-LR increased successively, indicated that MC-LR was more hazardous to human health than MC-YR and MC- RR. Although the MC-RR, MC-YR, and MC-LR display a non-carcinogenic index in children than adults, and thus, MCs are detrimental to children.

4. DISCUSSION

The World Health Organization (WHO) established a guide value of 1 μg/L for MC-LR concentration in drinking water (WHO, 2017). A comprehensive Australian report suggest the concentration of total MCs in drinking water should not exceed 1.3 μg/L expressed as MC-LR toxicity equivalents. Furtherly, a cell density of approximately 6,500 cells/mL (biovolume of 0.6 mm^3/L) would be equivalent to the guideline of 1.3 μg/L MC-LR toxicity equivalents if the toxin were fully released into the water (NHMRC & NRMMC, 2011). In a 1996 Canadian publication, the recommended maximum acceptable level (MAL) in drinking water is 0.5 μg/L of MC-LR, or in the absence of potency equivalency values for other microcystins, 1 μg/L of total MCs (Watanabe et al., 1996).

The monitoring of the water quality in Yongjiang river demonstrated that although no major algal bloom occurred, MC-RR, MC-YR, and MC-LR were present in the water column during the monitoring period. The TMCs concentrations varied from 0.0313 to 0.4585 μg/L during the monitoring period. An earlier study of microcystins in Guangxi showed that the average concentration of microcystins in source water and treated water supplies were 0.277 μg/L and 0.221 μg/L (Lv et al., 2005). Another survey showed that the concentration of microcystins in the source water of high-incidence areas of liver cancer in Guangxi was (15.64±2.08) ng/L, and the concentration in treated water supplies was (14.42±2.28) ng/L (Li et al., 2016). These results suggest that microcystins are detected in parts of Guangxi, but no one study considered the influence factors and health risk assessment of microcystins. Data collected indicated when using TMCs content as an indicator that a peak level in October followed by a sharp drop in concentration (Figure 3). The significant decrease in TMCs content may be due to lower temperature from November to December and a slow growth of *Microcystis*. These phenomena were similar to those described from previously in Tai, Yang-cheng, and Xuanwu lakes in China (Xu et al., 2010; Li, Gu & He, 2014). TMC-RR concentration reached maximum levels in October, and then decreased to an average concentration level in November. As compared to the concentration of the above two toxins, TMC-YR concentration was lowest of the three toxins studied; these results were identical with findings by other researchers, which suggested that the MCs are primarily dominated by TMC-RR and TMC-LR (Yang et al., 2006; Bi et al., 2017). It was clearly shown that TMC-LR gradually increased in concentration from September to November. Such variation may be due to the influence of differences in nutrients and climates which are in favor of TMC-RR, TMC-LR and to a lesser extent TMC-YR.

Previous studies demonstrated that the algal toxins are produced by algae and consequently the concentration of toxins in water depends mainly on algal abundance (or biomass) such as chlorophyll-a concentration or algal cell counts , in turn, which is regulated by environmental factors. The relationship of physical and chemical water parameters to concentration levels of toxins are shown in Table 2. In this study, it was evident that temperature was positive and significantly correlated with concentration levels of TMCs, TMC-LR, TMC-

RR and weakly associated with concentration levels of TMC-YR. The highest TMCs and TMC-RR, TMC-LR concentration was observed in October and November with surface water temperature were around 25.6℃and 26.2℃, respectively. When water temperature increased, further higher concentration of TMCs and TMC-RR, TMC-LR concentration were detected. These findings are in agreement with previous reports which showed TMCs and TMC-RR, TMC-LR concentration were temperature dependent, and TMC-RR which are generally detected at lower temperatures as compared to TMC-LR which favor at higher temperatures (Wang et al., 2010; Mantzouki et al., 2018). Intriguingly, water pH was also shown to be related to the concentration levels of toxins in Nanning Yongjiang river. The maximum toxin concentration was detected at a pH below or above the medium level. As a result, TMC-RR and TMCs were negatively correlated with pH (Table 2), which was similar to the results from other studies. Notably, the phytoplankton is known to affect pH and then further affect the concentration levels of toxins. Therefore, the pH value cannot be used as an appropriate parameter to determine the concentration levels of toxins. A majority of the blue-green algae can grow adequately in the water at pH 6.5–7.9 (Wang et al., 2002). The pH of Yongjiang river was within this range. Dissolved oxygen (DO) concentration ranged from 2.0 to 12.5 mg/L during the study period. The reported environmental standard for river water is 5 mg/L (GB, 2002). DO of Yongjiang river was partially lower than the reported standard during the monitoring period; these results show that the water is contaminated by organic matter, the oxygen consumption is severe, dissolved oxygen can not be replenished in time, and the anaerobic bacteria in the water will multiply quickly (Wang et al., 2002). DO showed a negative correlation of TMC-LR with TMCs in Yongjiang river. However, some researchers indicated that increases in oxygen saturation were correlated with algal biomass (Bi et al., 2017). Unfortunately, the algal abundance (or biomass) such as chlorophyll-a concentration or algal cell counts were not measured, thus, DO has no direct effect on concentration levels of toxins. Correlation analysis results indicated that increasing the TP concentration could increase the concentration levels of toxins, especially that of TMC-LR. The current observations were in agreement with those from a study conducted in the large eutrophic Lake Erie in the USA (Harke et al., 2016) that demonstrated positive correlations between TP and the abundance of toxic Microcystis and MCs. Consistent with the trend, Vézie et al. (2002) also found that higher P concentrations were beneficial to the growth of toxic Microcystis. Although TP was a dominant explanatory variable, the effect of TN on the concentration levels of toxins could not be ignored. The concentration of TMC-YR was negatively correlated with the TN/TP ratio. Previous studies also demonstrated that decreasing the TN/TP ratio concentration could promote the growth and toxin concentration of Microcystis (Yu et al., 2014; Lei et al., 2015). According to stepwise multiple linear regression (Table 3), TP was found to be the dominant factor affecting the contents of TMC-LR and TMCs, and pH and TN/TP ratio as the main factors affecting the content of TMC-RR and TMC-YR. These findings were in agreement with the results of correlation analysis.

The Monte Carlo simulation model determined the risk levels and putative human exposure scenarios associated with the blooms in Yongjiang river used for drinking. The whole process of security risk assessment was always accompanied by the uncertainty. The entire process of risk assessment was conducted two in steps: exposure assessment and hazard characterization. Although the extrapolation of the experimental results does not lead to certainty, it could be carried out from experimental animals to the general population and from the general population to specific populations (sensitive populations). The variations in human individuals involved parameters such as genetics, age, sex, environment (nutritional status) and other factors. On the other hand, missing data or limitations led to uncertainties, including NOVEL, time differences, and lack of exposure data. Recent studies have gradually established superior methods, such as benchmark dose (BMD) and chemical-specific adjustment factor (CSAF), to address and reduce the uncertainty in the risk assessment (Ibelings et al., 2015). The USEPA and Health Canada have gradually started utilizing the BMD and CSAF methods to develop the health guidance values (Zeller, Duran-Pacheco & Guerard, 2017).

Several countries that regulate cyanotoxins in drinking water use a parametric value based on the WHO Guidelines for 1 μg/L MC-LR (WHO, 2017). With respect to the drinking source waters, most countries use guidance values based on cyanobacterial biomass (cell density, chlorophyll-a, biovolume) indirectly reflecting the potential hazardous MCs concentrations (Valerio et al., 2009; Menezes, Churro & Dias, 2017). Our results indicated that the risk of carcinogenicity of MC-RR to children health under high exposure was greater than the maximum acceptable risk level recommended by USEPA (1×10^{-4}); the annual risk of carcinogenic exposure in adults with MC-RR was greater than the maximum acceptable risk level recommended by the ICRP (5×10^{-5}). The TMC-RR concentrations varied from 0.0224 to 0.3783 μg/L during the monitoring period. Therefore, the guideline value of TMC-RR in the Yongjiang river should be less than 0.3783 μg/L, so as not to pose a health risk to humans. Furthermore, we must take into account that the use of Yongjiang river is increasing, not only for the production of drinking water, but also for ludic activities, such as water sports, fishing, sailing and swimming. Thus, the relevant departments must attach great importance to the potential risks associated with Yongjiang river in order to protect the health of their users.

5. CONCLUSION

This study analyzed the influencing factors and the health risk assessment of MCs by Monte Carlo simulation method in Yongjiang river, China. The results showed that TP content may be related to TMC-LR and TMCs concentration, while pH and TN/TP ratio may be related to TMC-RR and TMC-YR concentration, respectively. The health risk assessment results showed that the risk of MC-RR for human health hazards is higher than that of MC-LR and MC-YR, and children are more vulnerable to MCs contamination than the adults. The risk of carcinogenicity of MC-RR to children health under high exposure was greater than the maximum acceptable risk level recommended by USEPA. The annual risk of carcinogenic exposure in adults with MC-RR was greater than the maximum acceptable risk level recommended by the ICRP. The non-carcinogenic hazard index for MCs was <1. Therefore, MCs in the water bodies should be monitored in regards to the carcinogenic risk to human health.

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