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

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The Yongjiang river is a large, shallow, hyper-trophic, freshwater river in Guangxi, China. To investigate the presence of microcystin-RR, microcystin-LR, and microcystin-YR (MC-RR, MC-LR and MC-YR) in the Yongjiang river and describe their correlation with environmental factors, as well as, assess health risk using Monte Carlo simulation, 90 water samples were collected at three sample points from March–December 2017. Results showed that during the monitoring period, total concentrations of MC-RR (TMC-RR), MC-YR (TMC-YR), and MC-LR (TMC-LR) 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. Total phosphorus (TP) content appeared to be related to TMC-LR and the total content of microcystins (TMCs), while pH and total nitrogen/total phosphorus (TN/TP) ratio appeared to be related to TMC-RR and TMC-YR, respectively. Using the professional health risk assessment software @Risk7.5, the risks of dietary intake of microcystins (MCs), including the carcinogenic risk and non-carcinogenic risk, were evaluated. It was found that the carcinogenic risk of MC-RR from drinking water was higher than MC-LR and MC-YR, and the presence of MCs would lead to high potential health risks, especially in children. The carcinogenic risk of MC-RR to children was > 1×10^{-4}, the maximum allowance level recommended by the US Environmental Protection Agency (USEPA); as for adults, it was $> 5 \times 10^{-5}$, the maximum allowance level recommended by the International Commission on Radiological Protection (ICRP). The non-carcinogenic hazard index of MC-RR, MC-YR, and MC-LR increased successively, indicating that MC-LR was more hazardous to human health than MC-YR and MC- RR, but its hazard index was <1. This suggests that MCs pose less risk to health. However, it is necessary to strengthen the protection and monitoring of drinking water source for effective control of water pollution and safeguarding of human health.

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ABSTRACT

The Yongjiang river is a large, shallow, hyper-trophic, freshwater river in Guangxi, China. To

investigate the presence of microcystin-RR, microcystin-LR, and microcystin-YR (MC-RR,

MC-LR and MC-YR) in the Yongjiang river and describe their correlation with environmental

factors, as well as, assess health risk using Monte Carlo simulation, 90 water samples were

collected at three sample points from March–December 2017. Results showed that during the

monitoring period, total concentrations of MC-RR (TMC-RR), MC-YR (TMC-YR), and MC-LR

(TMC-LR) varied from 0.0224 to 0.3783 µg/L, 0.0329 to 0.1433 µg/L, and 0.0341 to 0.2663

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total content of microcystins (TMCs), while pH and total nitrogen/total phosphorus (TN/TP)

ratio appeared to be related to TMC-RR and TMC-YR, respectively. Using the professional

health risk assessment software @Risk7.5, the risks of dietary intake of microcystins (MCs),

including the carcinogenic risk and non-carcinogenic risk, were evaluated. It was found that the

carcinogenic risk of MC-RR from drinking water   was higher than MC-LR and MC-YR, and

the presence of MCs would lead to high potential health risks, especially in children. The

carcinogenic risk of MC-RR to children was >1×10^{-4}, the maximum

allowance level recommended by the US Environmental Protection Agency (USEPA); as for

adults, it was >5×10^{-5}, the maximum allowance level recommended by the International

39   Commission on Radiological Protection (ICRP). The non-carcinogenic hazard index of MC-RR,

40   MC-YR, and MC-LR increased successively, indicating that MC-LR was more hazardous to

41   human health than MC-YR and MC- RR, but its hazard index was <1. This suggests that MCs

42   pose less risk to health. However, it is necessary to strengthen the protection and monitoring of

43   drinking water source for effective control of water pollution and safeguarding of human health.

44   **1. INTRODUCTION**

45   Eutrophication of freshwater bodies can result in algal blooms, especially those caused by

46   cyanobacteria. The algal toxins secreted from cyanobacteria are possibly harmful to plants,

47   animals, and humans (Holland & Kinnear, 2013; Cao et al., 2017). So far, most of as-known 90

48   microcystins(MCs) have been isolated from species and strains of *Microcystis* (Pham &Utsumi,

49   2018). Among them, the most widely distributed are microcystin-LR (MC-LR), microcystin-

50   RR(MC-RR), and microcystin-YR (MC-YR) (Zegura, 2016). These toxins are synthesized in the

51   cells and released after cell rupture, finally appeared as MCs in the water source.

52   Cyanobacteria blooms exist in eutrophicated waters worldwide, so that MCs can be

53   bioaccumulated by aquatic animals and reach human bodies. These would severely harm human

54   health and cause illness or deaths. In 1975, the drinking water source in the small town of

55   Pennsylvania was contaminated by *Microcystis*, which resulted in acute gastroenteritis for over

56   half of the local population (Keleti et al., 1979; Keleti & Sykora, 1982; Lippy & Erb, 1976). In

57   1996, due to contamination by MCs occurring in a hemodialysis center in Brazil, 116 of 130

58   patients developed symptoms of blurred vision and nausea, and >50 individuals succumbed to to

59   mortality (Pouria et al., 1998). Studies on drinking water showed that with drinking ditch pond

60   water containing MCs, the mortality rate of local people in Haimen and Fu Sui caused by

61   hepatocellular carcinoma (HCC) reached about 100/100,000, which was significantly higher than

62   that of shallow wells or deep wells (20/100,000) (Ueno et al., 1996). In 2010, the International

63   Agency for Research on Cancer (IARC) listed MCs as a "possible human carcinogen" (Group

64   2B) based on its potential carcinogenicity (IARC, 2010).

65   Although it has been confirmed that MCs can cause acute and chronic damage to human

66   bodies, the risk assessment of MC-LR, MC-RR, and MC-YR in the Yongjiang river of China

67   remains lack of reports. The environmental conditions of water source are crucial in the

68   concentration levels of toxins. However, the factors affecting the concentration levels of MCs

69   (nutrient levels and climatic conditions) in Yongjiang have not yet been elucidated. Therefore, it

70   is urgent to investigate the concentration and distribution of MCs in Yongjiang river as

71   influenced by seasonal changes in water quality and the related parameters.

72   Owing to the limitations of conducting toxicological health risk assessments in a population,

73   the Monte Carlo simulation (mathematical and logical model) has been widely used in recent

74   years. It was used to understand the behavior of water systems by assuming different problems

75   and systems, showing an advantage over experiments(Clausen et al., 2017). Moreover, the

76   Monte Carlo simulation of uncertainties was applied in the risk assessment model by collecting

77   limited samples to predict the overall situation. As a result, the risk uncertainty was expressed

78   intuitively, in agreement with the order of the nature, which favors for a decision-making for risk

79 managers and policymakers(Paladino, Moranda & Seyedsalehi, 2017; Sasi, Yozukmaz &

80 Yabanli, 2017). The US Environmental Protection Agency (USEPA) has set Monte Carlo

81 simulation as a basic method in the risk analysis policy(Moolenaar, 1996). Currently, American

82 Palisade has developed the @Risk Monte Carlo software loading into Excel simulation

83 technology for risk assessments. It is mainly based on the analysis of stochastic simulation

84 method of Monte Carlo, which provides various predicted results by using a variety of

85 probabilistic simulations, including the occurrence probability of events with a risk, forecasting

86 the risk of uncertainty quantitatively, and summarizing the characterization results. For instance,

87 Li et al. (2017) used the Monte Carlo model to assess the quantitative risk of aluminum in

88 Youtiao, which did not exceed the provisional tolerable weekly intake (PTWI) set by the Joint

89 Expert Committee on Food Additives (JECFA) for the public. Jia et al. (2018) used the Monte

90 Carlo simulation to evaluate the trace elements in the four freshwater fishes from a mine-

91 impacted river, and found that the consumption did not exert any appreciable adverse impact on

92 human health due to the exposure to trace elements in fish muscle.

93 This study investigated the current status of drinking water sources in the Yongjiang river in

94 China with respect to the contamination of MCs. The professional health risk assessment

95 software @Risk7.5 was used to evaluate the risks of dietary intake of MCs, including the

96 carcinogenic and non-carcinogenic risks. These findings provide a basis to develop an effective

97 control of water pollution and quality in order to protect the human health in the specific area.

98 **2. MATERIALS AND METHODS**

99   **2.1. Sampling location**

100   The Yongjiang river is a major water resource with an average annual flow of 1292 m^3/s in the

101   Nanning City area (Figure 1). The surface area of the river is 2676 ha with a maximum depth of

102   23 m. It is the main urban water source in Nanning city, China, and the tributary channel is also a

103   vital transportation route. Except for that, it is used for recreation as well as the source of water

104   for domestic use, agriculture, fishery, and industry.

105   **2.2. Sampling**

106   Water samples were collected from the Yongjiang river in Nanning City from March to

107   December in 2017. For this, the river section was set into three sampling points: Qingxiu District,

108   Jiangnan District, and Yongning District (Figure 1). The water samples were collected at a depth

109   of 0.5 m and 3 times/month from each sampling point. Thus, a total of 90 samples were collected

110   during the most active daylight period (11:00–14:00) in 10 months. Sample collecting, containers,

111   stabilization, and transportation to the laboratory were in accordance with the methods described

112   in Wunderlin et al. (2001). Water samples were filtered through the 500 mesh stainless steel

113   screensto remove large particles and were stored at 4°C with the protection from light, finally

114   processed within 24 hours. A volume of 2000 mL water sample was collected, and 500 mL water

115   sample is passed through the 0.45 μm filter (Jinteng, China) under reduced pressure filtration.

116   The filter containing the algae was subjected to an extraction process in order to recover the

117   intracellular MCs, followed by two extractions with 5 mL ultra-pure water after five times

118   freezing-thawing at -80°C/37°C. After filtering through the 0.45 μm filter for removing the algal

119 cells, the filtrates and the extracts from the filter were passed through solid phase extraction (SPE)

120 (500 mg/6 mL) (SUPELCO, USA). The SPE was rinsed with 20 mL of 20% methanol and 10

121 mL deionized distilled water. The toxin was eluted from the stationary phase with 80% methanol

122 (containing 0.05% TFA), and each sample was dried in a water bath under control temperature

123 (60 °C).

124 **2.3. Water quality analysis**

125 Water parameters χ^1=Water Temperature, χ^2=pH, χ^6=Dissolved Oxygen (DO) were measured

126 in situ and χ^3=Total phosphorus (TP), χ^4=PO_4^{3-}-P and χ^5=Total Nitrogen (TN) were measured in

127 the laboratory. Each experiment was performed in triplicate, and the average values were

128 reported. All water samples were analyzed using standard methods (GB, 2002). The instruments

129 used were YSI Model 58 thermometer, Knick Portamess 911 for pH measurement. DO using

130 iodometric method. TN and TP were analyzed using Kjeldahl method and persulfate digestion.

131 PO_4^{3-}-P was determined according to stannous chloride method.

132   **2.4. Determination of MCs**

133 The air-dried samples were suspended into 1 mL deionized distilled water for high performance

134 liquid chromatography (HPLC) (Shimadzu LC-20A, Japan) analysis. The solubilized toxin

135 samples were analyzed using HPLC with UV detector at 238 nm and symmetrical C18 column

136 (3.9×150 mm) (Waters, USA). The mobile phase consists of 33% acetonitrile and 67% deionized

137 distilled water in 0.1% phosphate buffer (pH=3.0). The flow rate was set at 1 mL/min. The

138 injection volume was 20 μL and the column temperature was 45°C. MC-LR and MC-RR

139 (Solarbio, approximate purity, 95%) and MC-YR (Alexis, approximate purity 98%) were used as

140 standards. Furthermore, the concentrations of MCs were determined by calibrating such area

141 under the peak with the corresponding standard curves. MC-LR, MC-RR, and MC-YR showed a

142 good linearity in the range of 0.025–2 μg/L(r^2 =0.9987, 0.9992, 0.9997). Under the condition that

143 the signal to noise ratio (S/N) is 3, the detection limits of MC-RR and MC-LR is 0.0125 μg/L,

144 and the detection limit of MC-YR is 0.014 μg/L. The recoveries ranged from 91% to 110%, the

145 relative standard deviation (RSD) was 3.0% to 5.6%. A series of toxin peaks were identified

146 using retention time and compared with spikes and known standards in the blank samples.

147 Furthermore, the concentrations of MCs were determined by calibrating such area under the peak

148 with corresponding standard curves. The order of the peaks and time of each standard substance

149 were as follows: MC-RR (7.599 min), MC-YR (14.225 min), and MC-LR (17.601 min). The test

150 sample was analyzed in 18 min (Figure 2).

151 **2.5. Method of risk assessment**

152 **2.5.1. Construction of exposure assessment model**

153 The three main routes of exposure to pollutants were consumption, inhalation, and skin

154 absorption. The proportion of each varied in different pollutants. This study assessed the

155 exposure risk caused by the drinking water. The daily exposure to MC-RR, MC-YR, and MC-LR

156 in the drinking water was assessed using Monte Carlo simulation by @Risk7.5 software

157 operating platform, while the Bootstrap sampling method was used for quantifying the

158 uncertainty. Each Bootstrap sample was simulated with 10,000 Monte Carlo simulations to

159   determine its uncertainty by obtaining different percentile values (P5–P95). The probabilistic

160   assessment method was used to construct the exposure evaluation model (Zobitz et al., 2011).

161   The mechanism underlying the different exposures and various routes with different exposure

162   dose formula were employed for the exposure assessment model of the health risk of chemical

163   pollutants from the USEPA. Chronic daily intake (CDI) evaluated the safety of MCs in drinking

164   water and the health risks of diverse routes in different populations. The CDI (µg/kg/day)

165   formula for daily exposure of drinking water (Duy et al., 2000; Funari & Testai, 2008) is as

166   follows:

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

167   Here, Cw is the concentration of pollutants in the water, µg/L; IR is the volume of drinking water

168   (L/day); EF is the frequency of exposure, drinking water for daily necessities (365 days/year);

169   ED is exposure duration (years); BW is the average body weight, kg; AT is the average time

170   equal to ED multiplied by 365 days/year. According to the World Health Organization (WHO),

171   the standard weight of adults is 70 kg, and the daily drinking volume is 2 L/day ; while the

172   weight of children is 16 kg and the daily drinking volume is 1 L/day (WHO, 2017).

173   **2.5.2. Construction of risk description model**

174   The characteristics of the pollutants in a water environment are generally divided into genetic,

175   toxic substances (for instance, chemical carcinogens), and somatic toxic substances (for instance,

176   non-carcinogens) as recommended by the USEPA water environmental health risk assessment

177   model. The carcinogenic and non-carcinogenic risks of MCs in source waters are evaluated from

178   exposure pathways.

179   (1) Health hazard risk model of chemical carcinogens

180   The formula of health hazard risk caused by chemical carcinogens recommended by USEPA

181   (Duy et al., 2000):

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

182

183   In the formula, $R_i{}^c$ is the average personal carcinogenic annual risk of chemical carcinogen i

184   through drinking water, $(years)^{-1}$; 70 indicates the average life expectancy of Chinese population,

185   years; D_i is the daily average exposure to chemical carcinogen i through drinking water, i.e., CDI,

186   µg/kg/day; q_i is the carcinogenic strength coefficient of the chemical carcinogen i through the

187   drinking water, and currently, there is no recognized carcinogenic intensity coefficient of MCs.

188   Based on the formula of carcinogenic strength coefficient of carcinogens (Hitzfeld, Hoger &

189   Dietrich, 2000), this study deduced the formula as follows:

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

190   Where carcinogenic potency index (CPI) is the coefficient of carcinogenic strength estimated

191   from the population data: q_i, kg/day/µg/L. Odds ratio (OR) refers to the ratio of the number of

192   exposed and non-exposed people in the case group divided in the control group. According to the

193   population of 80,000 inhabitants, the study showed that the person who drank the river water

194   presented a liver cancer OR of 1.246 (Falconer & Buckley, 1989; Yeh et al., 1989; Yu, Chen &

195   Li, 1995). Lifetime risk (LR) indicated the risk of cancer among individuals in the whole local

196   population; according to the risk of cancer during the individual's lifespan exposed to MC-LR in

197   the population of China, which was 6.2×10^{-3} (Yeh et al., 1989; Fan et al.,2009). D indicated the

198   calculation of the average daily life exposure dose, µg/kg/day. According to the study by Wolf et

199   al., the lifetime carcinogenic strength of MC-RR is $1/10^{th}$ of that of MC-LR, while the strength of

200   carcinogenicity of MC-YR and MC-LR was equivalent (Wolf & Frank, 2002).

201   (2) Non-carcinogenic health risk assessment model

202   The health risk assessment model recommended by USEPA was used to evaluate the non-

203   carcinogenic health risk of MCs in the Yongjiang river source water. The non-carcinogenic risk

204   was described using a hazard index (HI) by the following formula:

205

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

206

207   Here, reference dose (RfD) is the reference dose for MCs: the internationally accepted

208   tolerable daily intake (TDI) instead of MC-LR RfD was 0.04 µg/kg/d. According to the

209   equivalent toxicity relationship among MC-RR, MC-YR, and MC-LR, the RfD values of MC-RR

210   and MC-YR were 0.4 and 0.04 µg/kg/d, respectively (Wolf & Frank, 2002; Lee et al., 2017).

211   The HI is usually used as a benchmark of danger: HI>1 indicates that the exposure higher than

212 the reference dose is harmful to the human body; HI≤1 indicates that the exposure level is lower

213 than the reference dose, which is unlikely to be detrimental (Younes, 1999).

214 **2.6. Statistical analysis**

215 The IBM SPSS Statistics 22.0 software was used to perform all descriptive statistical analysis,

216 including minimum value, maximum value, mean value, standard deviation, Pearson's

217 correlation analysis, and stepwise multiple linear regression. Moreover, the risk assessment of

218 MC-RR, MC-YR, and MC-LR exposure in water source was carried out using @Risk7.5

219 probabilistic evaluation software based on the Monte Carlo simulation technique.

220 **3. RESULTS**

221 **3.1. Concentration level, distribution characteristics, and environmental impact factors of**

222 **MCs in source water**

223 **3.1.1. Concentration distribution characteristics of MCs in source water**

224 The concentrations of MC-LR, MC-RR, and MC-YR in water samples were detected. The total

225 concentrations of MCs (TMCs) in water are the sum of the concentrations of extracellular MCs

226 (EMCs) and intracellular MCs (IMCs) dissolved in the water. The results were summarized in

227 Table 1.

228 **3.1.2. Seasonal distribution characteristics of MCs in water samples**

229 The seasonal distribution of MCs in the Yongjiang river is shown in Figure 3. The concentration

230 of TMC-YR (referring to the sum of intracellular and extracellular, the same to the other twos)

231 was significantly lower than the other two MCs. The concentrations of TMC-RR and TMC-YR

232 at the same time reached the maximum levels in October.

233 **3.1.3. Pearson's correlation analysis of environmental factors and MCs' concentration**

234 Pearson's correlation analysis of environmental factors and MCs' concentration were analyzed

235 and the results were shown in Table 2. According to the correlation analysis, the concentration of

236 TMC-RR was positively correlated with water temperature and TN ($p<0.01$) with a significant

237 negative correlation with pH ($p<0.01$). The concentration of TMC-YR was negatively correlated

238 with TN:TP ratio ($p<0.05$). The concentration of TMC-LR was positively correlated with water

239 temperature, TP, and PO_4^{3-}-P ($p<0.01$) with a significant negative correlation with DO and

240 TN:TP ratio ($p<0.01$). The concentration of TMCs was positively correlated with water

241 temperature and TP ($p<0.01$) with a significant negative correlation with pH, DO, and TN:TP

242 ratio ($p<0.01$).

243 **3.1.4. Stepwise multiple linear regression analysis of MCs' concentration and**

244 **environmental factors**

245 A stepwise multiple linear regression analysis of MCs' concentration and environmental factors

246 is shown in Table 3. The results indicated that TP (χ^3) is the dominant factor affecting the

247 contents of TMC-LR and TMCs. pH (χ^2) and TN:TP ratio (χ^7) are the primary factors affecting

248 the content of TMC-RR and TMC-YR, respectively. These findings were in agreement with the

249 results of correlation analysis.

250 **3.2. Assessment of MCs' exposure in source waters**

251 **3.2.1. Distribution fitting of the concentration of MCs in source water**

252 The @Risk7.5 software to is used to fit the processed samples. The concentration of MCs in

253 source water were characterized as continuous data. The fitting results were followed by the

254 optimal fitting distribution models: Gamma, Invgauss, Lognorm, Expon, and Loglogistic. Three

255 main methods were used to test the goodness of the fittings: Chi-Sq (Chi-squared) test, K-S

256 (Kolmogorov–Smirnov) test, and A-D (Anderson–Darling) test (Lipton et al., 1995; Cummins et

257 al., 2009). Above all, the sample fitting results (Table 4) were used to determine the fitting

258 distribution types of the optimal probability of the pollution data: MC-RR had Gamma and

259 Invgauss distribution, MC-YR had Lognorm, Expon, and Loglogistic distribution, and MC-LR

260 had Gamma and Expon distribution. Furthermore, the results of the distribution parameters after

261 fitting and the comparison with the sample data parameters are summarized in Table 5. The

262 probability distribution of the mass concentration of MCs, MC-RR, MC-YR, and MC-LR in

263 source water is shown in Figures 4, 5, and 6. The fitting results can be visually observed from the

264 coincidence of the blue rectangular shape and the area under the red curve.

265 **3.2.2. Daily exposure calculation**

266 The @Risk7.5 software was utilized for the random extraction of the MCs concentration profiles

267 from the water to calculate the daily exposure of direct drinking water by different populations to

268 MC-RR, MC-YR, and MC-LR. Each simulation cycle was performed for 10,000 cycles, and the

269 simulation results are shown in Table 6. A significant difference between adults and children was

270   observed in the daily exposure. P50, P85, P90, and P95 (Table 6) represent the high exposure

271   sites of each population. The MCs exposed to drinking water showed that the children's daily

272   intake was 2-fold higher than that of the adults, suggesting that children are more susceptible to

273   the pollution of MCs than adults.

274   **3.3. Risk characterization of MCs in source water**

275   **3.3.1. Carcinogenic risk of MCs in source water**

276   Based on the exposure parameters and carcinogenic risk formula, @Risk7.5 risk analysis

277   software was used to extract the numerical the value of MCs concentration in water randomly

278   and calculate the carcinogenic risk of MC-RR, MC-YR, and MC-LR intake by different groups

279   of individuals through direct drinking water. Each simulation cycle of 10,000 displayed the

280   statistical simulation results summarized in Table 7. The carcinogenic annual risk of MC-YR

281   was less than that of MC-LR and MC-RR, and MC-RR was the primary hazard in the source

282   water. The maximum acceptable level and the negligible level of the carcinogenic risk for the

283   population recommended by some institutions are listed in Table 8; the annual risk in

284   carcinogenesis of MCs in a water source is 10^{-6} to 10^{-4}.(NHMRC & NRMMC, 2011). The

285   carcinogenic risk of MC-YR and MC-LR in adults and children was lower than the maximum

286   acceptable risk level designated by USEPA (1×10^{-4}) and ICRP (5×10^{-5}), and the risk of

287   carcinogenesis in children was higher than that in adults. The health risks caused by MC-RR

288   from drinking water source for children was significantly higher than the maximum allowance

289   level recommended by USEPA (1×10^{-4}). Similarly, the health risks caused by the MC-RR from

290   drinking water source for adults were significantly higher than the maximum allowance level

291   recommended by ICRP (5×10^{-5}). These statistical details indicated that MC-RR in water bodies

292   exhibited a significant carcinogenic risk to the health of adults and children.

293   **3.3.2. Non-carcinogenic risk of MCs in source water**

294   The exposure parameters and non-carcinogenic hazards index formula were used to calculate the

295   values of different populations through direct drinking water intakes of MC-RR, MC-YR, and

296   MC-LR (Table 9). These findings demonstrated that the average non-carcinogenic hazards index

297   of MCs in different populations through drinking water intake and the non-carcinogenic hazards

298   index of P90 and P95 at high level of exposure was <1. This suggested that MCs, which are

299   ingested through drinking water, pose less risk to health. The non-carcinogenic hazard index of

300   MC-RR, MC-YR, and MC-LR increased successively, indicating that MC-LR was more

301   hazardous to human health than MC-YR and MC-RR. Although the MC-RR, MC-YR, and MC-

302   LR display a non-carcinogenic index in children than adults, and thus, MCs are detrimental to

303   children.

304   **4. DISCUSSION**

305   The World Health Organization established a guide value of 1 µg/L for MC-LR concentration in

306   drinking water (WHO, 2017). A comprehensive Australian report shows that the concentration of

307   total MCs in drinking water should not exceed 1.3 µg/L, expressed as MC-LR toxicity

308   equivalents. Furthermore, a cell density of approximately 6,500 cells/mL (biovolume of 0.6

309   mm^3/L) would be equivalent to the guideline of 1.3 μg/L MC-LR toxicity if the toxin was fully

310   released into the water(NHMRC & NRMMC, 2011). In the study of a Canadian group, the

311   recommended maximum acceptable level (MAL) in drinking water is 0.5 μg/L of MC-LR, or in

312   the absence of potency equivalency values for other MCs, 1 μg/L of total MCs (Watanabe et al.,

313   1996).

314   By monitoring of the water quality in the Yongjiang river, we demonstrated that although no

315   major algal bloom occurred, MC-RR, MC-YR, and MC-LR were present in the water column

316   during the monitoring period. The TMCs concentrations varied from 0.0313 to 0.4585 μg/L. An

317   earlier study of MCs in Guangxi showed that the average concentration of MCs in source water

318   and treated water supplies were 0.277 μg/L and 0.221 μg/L, respectively(Lv et al., 2005).

319   Another survey showed that the concentration of MCs in the source water of high-incidence

320   areas of liver cancer in Guangxi was 15.64±2.08 ng/L, and the concentration in treated water

321   supplies was 14.42±2.28 ng/L(Li et al., 2016). These results suggest that MCs are detected in

322   parts of Guangxi, but without considering the influencing factors and health risk assessment of

323   MCs. The collected data indicated that the peak level in October followed by a sharp drop in

324   concentration when using TMCs content as an indicator (Figure 3). The significant decrease in

325   TMCs content may result from the decreasing of   temperature from November to December,

326   and result in a slow growth of *Microcystis*. These phenomena were similar to those described by

327   previous groups in Tai, Yang-cheng, and Xuanwu lakes in China(Xu et al., 2010; Li, Gu & He,

328   2014). TMC-RR concentration reached maximum level in October and then decreased to an

329　average concentration level in November. As compared to the concentration of the above two

330　toxins, TMC-YR concentration was the lowest of the three toxins studied; these results were

331　identical to findings by other researchers, which suggested that the MCs are primarily dominated

332　by TMC-RR and TMC-LR (Yang et al., 2006; Bi et al., 2017). It was clearly shown that the

333　concentration of TMC-LR gradually increased from September to November. Such variation

334　may be influenced by the differences in nutrients and climates, which are in favor of TMC-RR,

335　TMC-LR and to a lesser extent TMC-YR.

336　　　Previous studies demonstrated that the algal toxins are produced by algae and consequently

337　the concentration of toxins in water depends mainly on algal abundance (or biomass) such as

338　chlorophyll-a concentration or algal cell counts, which in turn, is regulated by the environmental

339　factors. The relationship of physical and chemical water parameters to the concentration levels of

340　toxins are shown in Table 2. In this study, it was evident that temperature was positive and

341　significantly correlated with concentration levels of TMCs, TMC-LR, TMC-RR and weakly

342　associated with concentration levels of TMC-YR. The highest concentrations of TMCs and

343　TMC-RR, TMC-LR were observed in October and November with surface water temperature

344　were around 25.6℃and 26.2℃, respectively. When water temperature increased, even higher

345　concentration of TMCs and TMC-RR, TMC-LR concentration were detected. These findings are

346　in agreement with previous reports which showed the concentrations of TMCs and TMC-RR,

347　TMC-LR were temperature dependent, and TMC-RR which are generally detected at lower

348　temperatures as compared to TMC-LR which favors at higher temperatures(Wang et al., 2010;

349 Mantzouki et al., 2018). Intriguingly, the pH value was also shown to be related to the

350 concentration level of toxins in the Yongjiang river. The maximum toxin concentration was

351 detected at a pH below or above the medium level. As a result, TMC-RR and TMCs were

352 negatively correlated with pH value   (Table 2), which was similar to the results of other studies.

353 Notably, the phytoplankton is known to affect the pH, and then, further affects the concentration

354 leves of toxins. Therefore, the pH value cannot be used as an appropriate parameter to determine

355 the concentration levels of toxins. A majority of the blue-green algae can grow adequately in the

356 water at pH of 6.5-7.9(Wang et al., 2002). The pH of Yongjiang river was within this range.

357 Dissolved oxygen concentration ranged from 2.0 to 12.5 mg/L during the study period. The

358 reported environmental standard for river water is 5 mg/L (GB, 2002). DO of Yongjiang river

359 was partially lower than the reported standard during the monitoring period; these results show

360 that the water is contaminated by organic matter, the oxygen consumption is severe, dissolved

361 oxygen can not be replenished in time, and the anaerobic bacteria in the water will multiply

362 quickly (Wang et al., 2002). DO showed a negative correlation of TMC-LR with TMCs in

363 Yongjiang river. However, some studies indicated that increases in oxygen saturation were

364 correlated with algal biomass (Bi et al., 2017). Nonetheless, the algal abundance (or biomass)

365 such as chlorophyll-a concentration or algal cell counts was not measured, and thus, DO has no

366 direct effect on the concentration levels of toxins. The correlation analysis results indicated that

367 increasing the TP concentration could increase the concentration levels of toxins, especially that

368 of TMC-LR. The current observations were in agreement with those from a study conducted in

369 the large eutrophic Lake Erie in the USA (Harke et al., 2016), which demonstrated positive

370   correlations between TP and the abundance of toxic Microcystis and MCs. Consistent with the

371   trend, Vézie et al. (2002) also found that higher P concentrations were beneficial to the growth of

372   toxic *Microcystis*. Although TP was a dominant explanatory variable, the effect of TN on the

373   concentration levels of toxins could not be ignored. The concentration of TMC-YR was

374   negatively correlated with the TN/TP ratio. Previous studies also demonstrated that decreasing

375   the TN/TP ratio concentration could promote the growth and toxin concentration of Microcystis

376   (Yu et al., 2014; Lei et al., 2015). According to stepwise multiple linear regression (Table 3), TP

377   was found to be the dominant factor affecting the contents of TMC-LR and TMCs, and pH and

378   TN/TP ratio as the main factors affecting the content of TMC-RR and TMC-YR. These findings

379   were in agreement with the results of correlation analysis.

380   The Monte Carlo simulation model determined the risk level and putative human exposure

381   scenarios associated with the blooms in the Yongjiang river used for drinking. The whole

382   process of security risk assessment was always accompanied by the uncertainty. The entire

383   process of risk assessment was conducted in two steps: exposure assessment and hazard

384   characterization. Although the extrapolation of the experimental results does not lead to certainty,

385   it could be carried out from experimental animals to the general population and from the general

386   population to specific populations (sensitive populations). The variations in human individuals

387   involved parameters such as genetics, age, sex, environment (nutritional status) and other factors.

388   On the other hand, missing data or limitations led to uncertainties, including NOVEL, time

389   differences, and lack of exposure data. Recent studies have gradually established superior

390   methods, such as benchmark dose (BMD) and chemical-specific adjustment factor (CSAF), to

391   address and reduce the uncertainty in the risk assessment (Ibelings et al., 2015). The USEPA and

392   Health Canada have gradually started utilizing the BMD and CSAF methods to develop the

393   health guidance values (Zeller, Duran-Pacheco & Guerard, 2017).

394   Several countries that regulate cyanotoxins in drinking water use a parametric value based on

395   the WHO Guidelines for 1 µg/L MC-LR (WHO, 2017). With respect to the drinking source

396   waters, most countries use guideline values based on cyanobacterial biomass (cell density,

397   chlorophyll-a, biovolume) indirectly reflecting the potential hazardous MCs concentrations

398   (Valerio et al., 2009; Menezes, Churro & Dias, 2017). Our results indicated that the risk of

399   carcinogenicity of MC-RR to children health under high exposure was greater than the maximum

400   acceptable risk level recommended by USEPA (1×10^{-4}); the annual risk of carcinogenic exposure

401   in adults with MC-RR was greater than the maximum acceptable risk level recommended by the

402   ICRP (5×10^{-5}). The TMC-RR concentrations varied from 0.0224 to 0.3783 µg/L during the

403   monitoring period. Therefore, the guideline value of TMC-RR in the Yongjiang river should be

404   <0.3783 µg/L, so as not to pose a health risk to humans. Furthermore, we must take into account

405   the increasing usage of the Yongjiang river, not only for the production of drinking water, but

406   also for ludic activities, such as water sports, fishing, sailing and swimming. Thus, the relevant

407   departments must attach great importance to the potential risks associated with the Yongjiang

408   river in order to protect the health of their users.

409   **5. CONCLUSION**

410 This study analyzed the influencing factors and the health risk assessment of MCs by Monte

411 Carlo simulation in the Yongjiang river, China. The results showed that TP content may be

412 related to TMC-LR and TMCs concentration, while pH and TN/TP ratio may be related to TMC-

413 RR and TMC-YR concentration, respectively. The health risk assessment results showed that the

414 risk of MC-RR for human health hazards is higher than that of MC-LR and MC-YR, and

415 children are more vulnerable to MCs contamination than the adults. The risk of carcinogenicity

416 of MC-RR to children health under high exposure was greater than the maximum acceptable risk

417 level recommended by USEPA. The annual risk of carcinogenic exposure in adults with MC-RR

418 was greater than the maximum acceptable risk level recommended by the ICRP. The non-

419 carcinogenic hazard index for MCs was <1. Therefore, MCs in the water bodies should be

420 monitored with regarding to the carcinogenic risk to human health.

421 **ACKNOWLEDGEMENTS**

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Table 1(on next page)

Results of analysis of MCs in water samples

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; standard deviation means SD.

Toxin types	Number of samples	EMCs	IMCs	TMCs (EMCs+IMCs) concentration (μg/L)	
		Detection Rate(%)	Detection Rate(%)	Range	Mean±SD
MC-RR	90	74.44	77.78	0.0224–0.3783	0.0727±0.0599
MC-YR	90	64.44	56.67	0.0329–0.1433	0.0424±0.0376
MC-LR	90	77.78	76.67	0.0341–0.2663	0.0763±0.0637

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Table 2(on next page)

Correlation coefficients between MCs and the influencing factors

*Correlation is significant at the 0.05 level (2-tailed).**Correlation is significant at the 0.01 level (2-tailed).

Environmental Factors	Correlation Coefficient			
	TMC-RR	TMC-YR	TMC-LR	TMCs
Water Temperature	0.436**	0.085	0.480**	0.614**
PH	−0.729**	−0.029	−0.164	−0.566**
DO	−0.063	−0.076	−0.768**	−0.570**
TN	0.286**	−0.179	−0.079	0.055
TP	0.043	0.073	0.851**	0.610**
PO_4^{3-}-P	−0.047	−0.071	0.399**	0.204
TN:TP ratio	0.040	−0.229*	−0.434**	−0.347**

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Table 3(on next page)

Results of stepwise multiple linear regression

The fitting result of TMC-YR after lg10 conversion; D-W statistic (Durbin-Watson statistic); Variance Inflation Factor(VIF).

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MCs	Fitting equation	Correlation coefficient R	Adjusted R^2	Tolerance	VIF	Model fitting		Partial regression coefficient test	D-W statistic
						F	P		
TMCs	$y=116.848+46.449\chi3$	0.581	0.337	1	1	44.792	<0.001	$\chi3$（t=6.693, P<0.001）	1.979
	$y=805.526+42.979\chi3-92.886\chi2$	0.716	0.513	0.989	1.001	45.87	<0.001	$\chi3$（t=7.148, P<0.001） $\chi2$（t=-5.608, P<0.001）	
	$y=538.069+35.557\chi3-75.968\chi2+6.452\chi1$	0.741	0.549	0.693	1.443	34.938	<0.001	$\chi3$（t=5.495, P<0.001） $\chi2$（t=-4.396, P<0.001） $\chi1$（t=2.622, P=0.01）	
TMC-RR	$y=799.874-99.263\chi2$	0.718	0.515	1	1	93.396	<0.001	$\chi2$（t=-9.664, P<0.001）	1.828
	$y=825.53-101.047\chi2-35.61\chi4$	0.738	0.544	0.994	1.006	51.98	<0.001	$\chi2$（t=-10.066, P<0.001） $\chi4$（t=-2.375, P=0.02）	
	$y=676.095-91.169\chi2-39.668\chi4+3.42\chi1$	0.759	0.576	0.846	1.181	38.908	<0.001	$\chi2$（t=-8.682, P<0.001） $\chi4$（t=-2.709, P<0.001） $\chi1$（t=2.522, P=0.014）	
TMC-YR	$y=1.54-0.013\chi7$	0.268	0.072	1	1	6.83	0.011	$\chi3$（t=-2.613, P=0.011）	2.299
TMC-LR	$y=11.216+41.829\chi3$	0.806	0.650	1	1	163.585	<0.001	$\chi3$（t=12.79, P<0.001）	1.461
	$y=63.349+32.481\chi3-6.653\chi6$	0.848	0.718	0.678	1.476	111.004	<0.001	$\chi3$（t=9.06, P<0.001） $\chi6$（t=-4.592 P<0.001）	

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Table 4(on next page)

Fitting distribution and related parameters of MCs in source water (μg/L)

MCs	Fitting of Distribution	Distributed parameters		Fit test sort			50% Confidence value	90% Confidence value	95% Confidence value
		Mean	Std Dev	K-S	A-D	Chi-Sq			
MC-RR	Gamma	0.073	0.061	1	1	1	0.056	0.154	0.194
	Invgauss	0.073	0.066	5	2	2	0.053	0.154	0.202
MC-YR	Lognorm	0.047	0.071	4	2	1	0.026	0.103	0.154
	Expon	0.042	0.039	2	1	4	0.030	0.093	0.120
	Loglogistic	0.081	-	1	4	6	0.026	0.124	0.216
MC-LR	Gamma	0.076	0.070	1	1	2	0.055	0.168	0.216
	Expon	0.075	0.073	2	2	1	0.053	0.170	0.221

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Table 5(on next page)

Estimated value of the quantile for overall sample in different theoretical distributions

Projects	Pollutant	Real value(µg/L)	Types	Predicted value (µg/L)	Relative difference of real value (%)
P50	MC-RR	0.058	Gamma	0.056	3.01
			Invgauss	0.053	7.81
			Lognorm	0.026	8.82
	MC-YR	0.024	Expon	0.030	25.17
			Loglogistc	0.026	10.03
	MC-LR	0.057	Gamma	0.055	2.02
			Expon	0.053	6.10
P75	MC-RR	0.011	Gamma	0.099	13.00
			Invgauss	0.095	16.91
			Lognorm	0.053	13.88
	MC-YR	0.062	Expon	0.057	8.17
			Loglogistc	0.056	9.41
	MC-LR	0.112	Gamma	0.104	6.79
			Expon	0.104	7.46
P90	MC-RR	0.136	Gamma	0.154	12.93
			Invgauss	0.154	13.09
	MC-YR	0.108	Lognorm	0.103	4.31
			Expon	0.093	14.10

			Loglogistc	0.124	15.12
	MC-LR	0.166	Gamma	0.168	1.09
			Expon	0.170	2.46
	MC-RR	0.167	Gamma	0.194	16.26
			Invgauss	0.202	21.06
			Lognorm	0.154	29.08
P95	MC-YR	0.119	Expon	0.120	0.58
			Loglogistc	0.216	80.78
	MC-LR	0.215	Gamma	0.216	0.26
			Expon	0.221	2.56
	MC-RR	0.378	Gamma	0.285	24.71
			Invgauss	0.321	15.02
			Lognorm	0.325	127.11
P99	MC-YR	0.143	Expon	0.183	27.56
			Loglogistc	0.735	412.77
	MC-LR	0.266	Gamma	0.326	22.62
			Expon	0.338	27.03

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Table 6(on next page)

Daily exposure to MCs intake through drinking water (μg/kg/d)

Projects	MC-RR		MC-YR		MC-LR	
	Adult	Child	Adult	Child	Adult	Child
Mean	0.002	0.005	0.001	0.003	0.002	0.005
P50	0.002	0.004	0.001	0.001	0.002	0.004
P85	0.004	0.008	0.003	0.006	0.004	0.009
P90	0.004	0.009	0.003	0.007	0.005	0.010
P95	0.005	0.010	0.003	0.007	0.006	0.013

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Table 7(on next page)

Carcinogenic exposure of MCs from source water

Projects	MC-RR		MC-YR		MC-LR	
	Adult	Child	Adult	Child	Adult	Child
Mean	1.27×10^{-5}	1.37×10^{-5}	3.34×10^{-6}	5.76×10^{-6}	5.20×10^{-6}	8.10×10^{-6}
P50	1.40×10^{-5}	1.43×10^{-5}	2.21×10^{-6}	4.40×10^{-6}	4.69×10^{-6}	8.30×10^{-6}
P85	5.43×10^{-5}	1.13×10^{-4}	6.93×10^{-6}	1.09×10^{-5}	9.18×10^{-6}	1.28×10^{-5}
P90	5.47×10^{-5}	1.45×10^{-4}	7.60×10^{-6}	1.16×10^{-5}	9.85×10^{-6}	1.32×10^{-5}
P95	8.63×10^{-5}	1.83×10^{-4}	8.11×10^{-6}	1.20×10^{-5}	1.11×10^{-5}	1.38×10^{-5}

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Table 8(on next page)

Maximal acceptable level and negligible level recommended by different institutions

Institutions	Maximum risk level	Ignore the level of risk	Remarks
USEPA	1×10^{-4}	--	Radiation
ICRP	5×10^{-5}	--	Radiation
Royal Association of England	1×10^{-6}	1×10^{-7}	--
Holland Environmental Protection Agency	1×10^{-6}	1×10^{-8}	Chemical contaminants
Swedish Environmental Protection Agency	1×10^{-6}	--	Chemical contaminants

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Table 9(on next page)

Non-carcinogenic exposure risk of MCs using source water

Projects	MC-RR		MC-YR		MC-LR	
	Adult	Child	Adult	Child	Adult	Child
Mean	0.005190	0.011352	0.030260	0.066190	0.054490	0.119200
P50	0.004123	0.009019	0.017090	0.037380	0.040400	0.088380
P85	0.009157	0.020031	0.067480	0.147610	0.104530	0.228660
P90	0.009722	0.021267	0.077190	0.168840	0.118790	0.259860
P95	0.011903	0.026038	0.085150	0.186270	0.153860	0.336580

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Figure 1

Sampling sites in the Yongjiang River
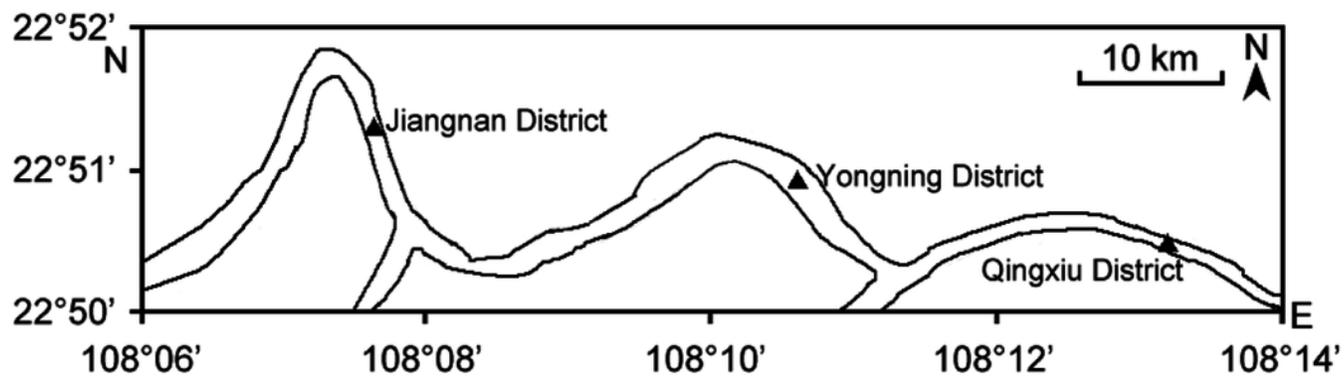
Figure 2

HPLC chromatogram of MCs

(A) HPLC chromatogram of MCs standards, 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). (B) HPLC chromatogram of MCs water samples, the same time represents the same substance, like A.
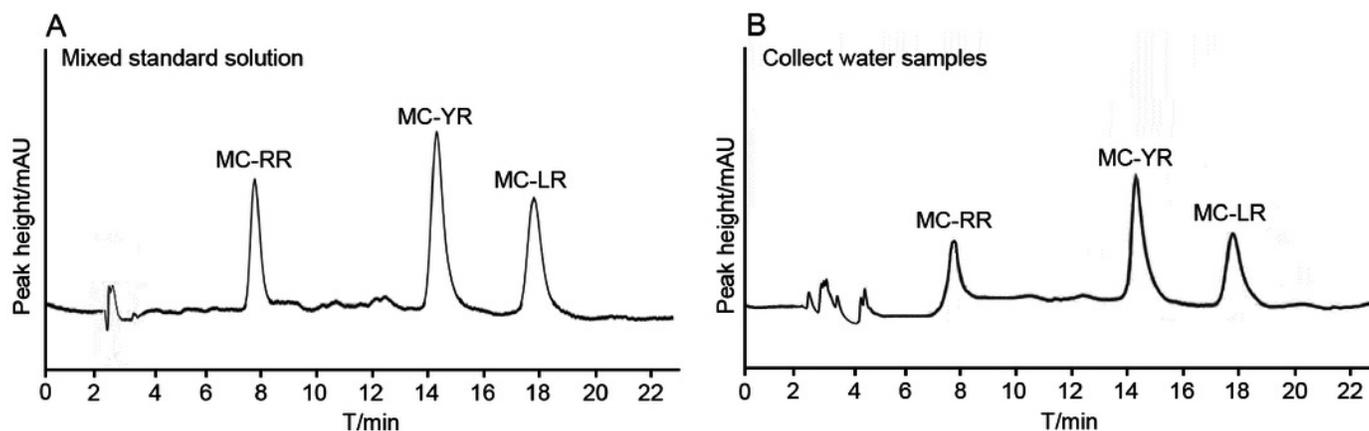
Figure 3

Concentration of MCs in source water in various seasons

TMCs is the sum of the total concentration (intracellular and extracellular) of each individual microcystin detected. This means, that TMCs=TMC-LR+TMC-RR+TMC-YR.
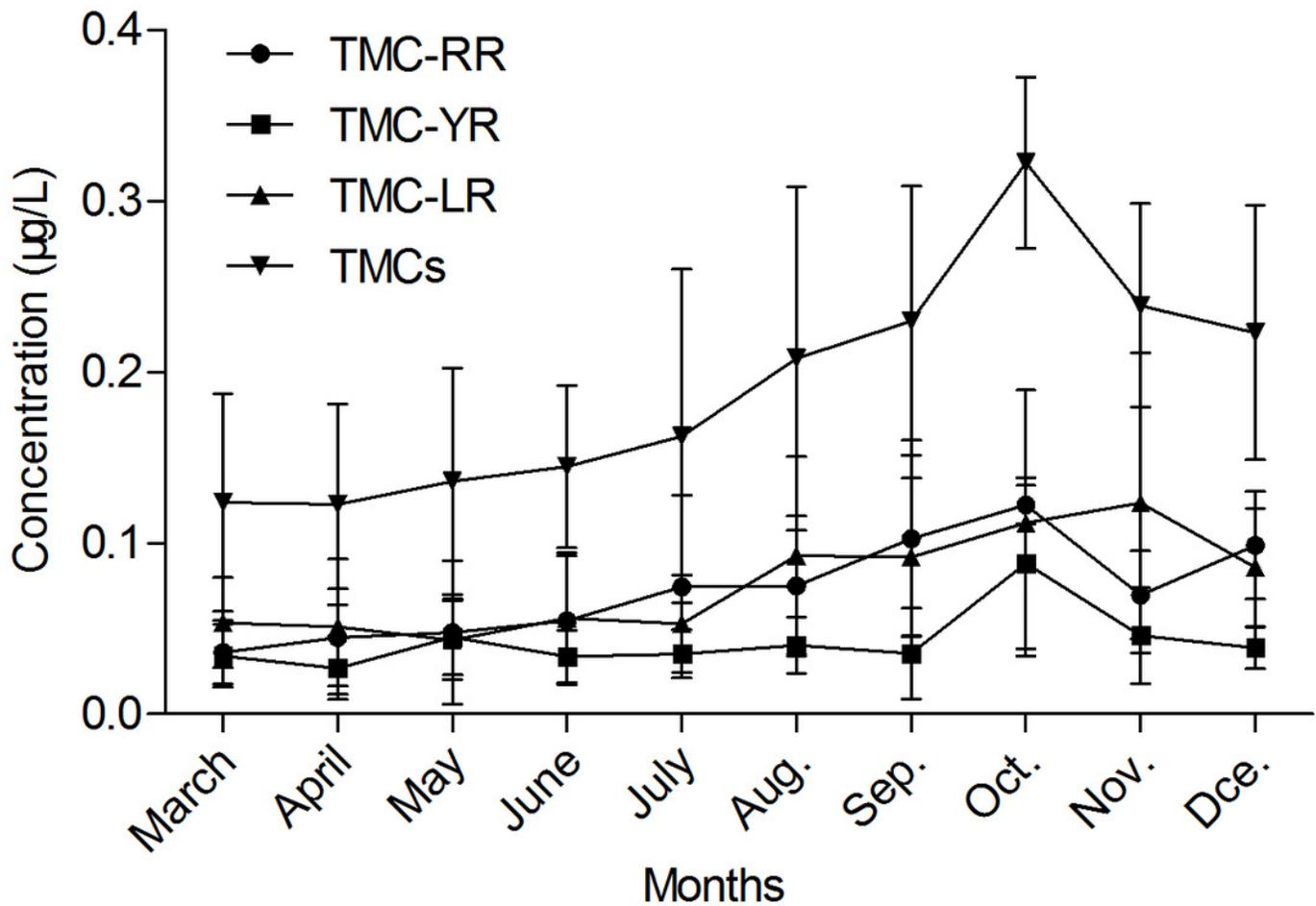
Figure 4

Probability distribution graph after fitting of MC-RR in source water (μg/L)

The data comparison revealed that the optimal fitting distribution of the most suitable concentration of MC-RR in source water was Gamma (μ 0.073, σ 0.061) (first number μ as the position parameter and the second number s as the scale parameter). The abscissa in Figure 4 represents the concentrations of MC-RR; the concentrations are partitioned, the length of each interval is the group distance, the rectangular area is the frequency of the group, and the ratio of the total sample utilized, and the vertical axis is the frequency divided by the group distance obtained. The fitting results can be visually observed from the coincidence of the blue rectangular shape (93.9%) and the area under the red curve (91.7%).
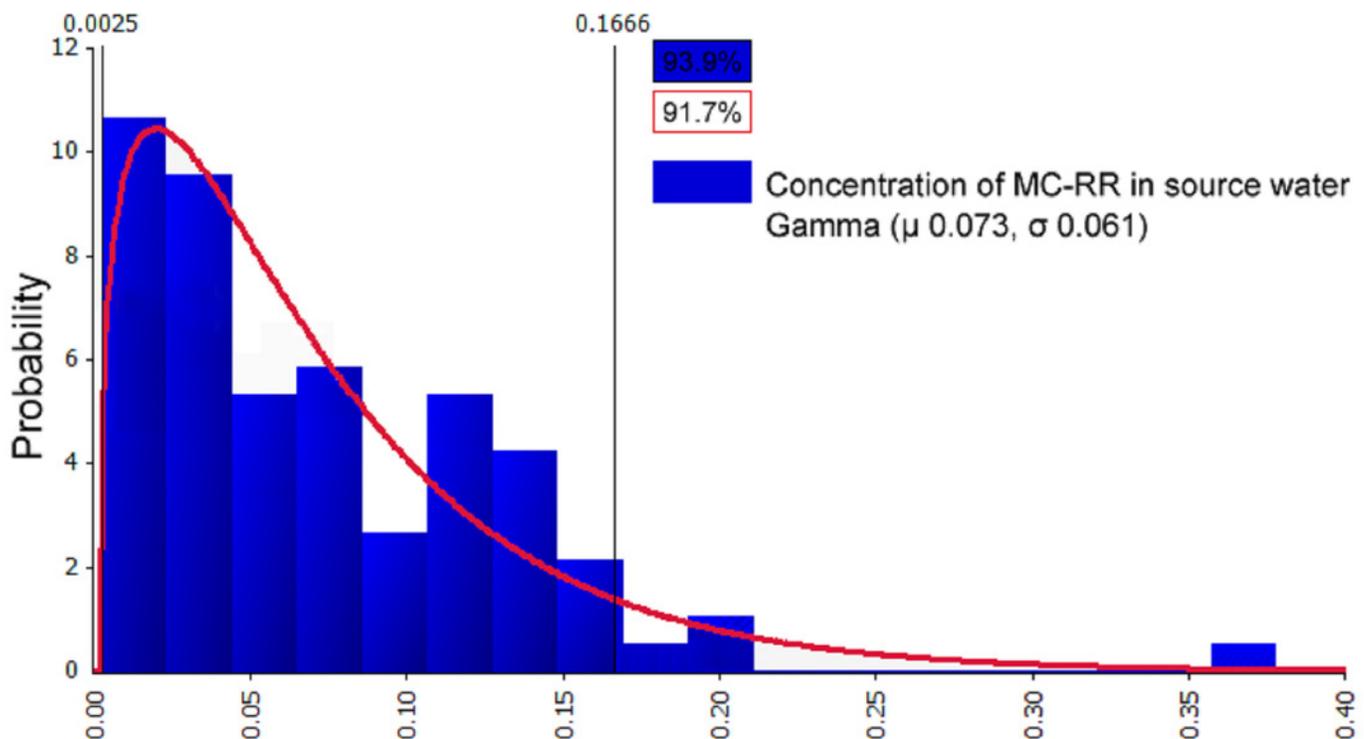
Figure 5

Probability distribution graph after fitting of MC-LR in source water (μg/L)

The data comparison showed that the best-fitted distribution of MC-YR concentration was Lognorm (μ 0.047,σ 0.071). The abscissa in Figure 5 represents the concentrations of MC-YR; the concentrations are partitioned, the length of each interval is the group distance, the rectangular area is the frequency of the group, and the ratio of the total sample utilized, and the vertical axis is the frequency divided by the group distance obtained. The fitting results can be visually observed from the coincidence of the blue rectangular shape (90.0%) and the area under the red curve (88.9%).
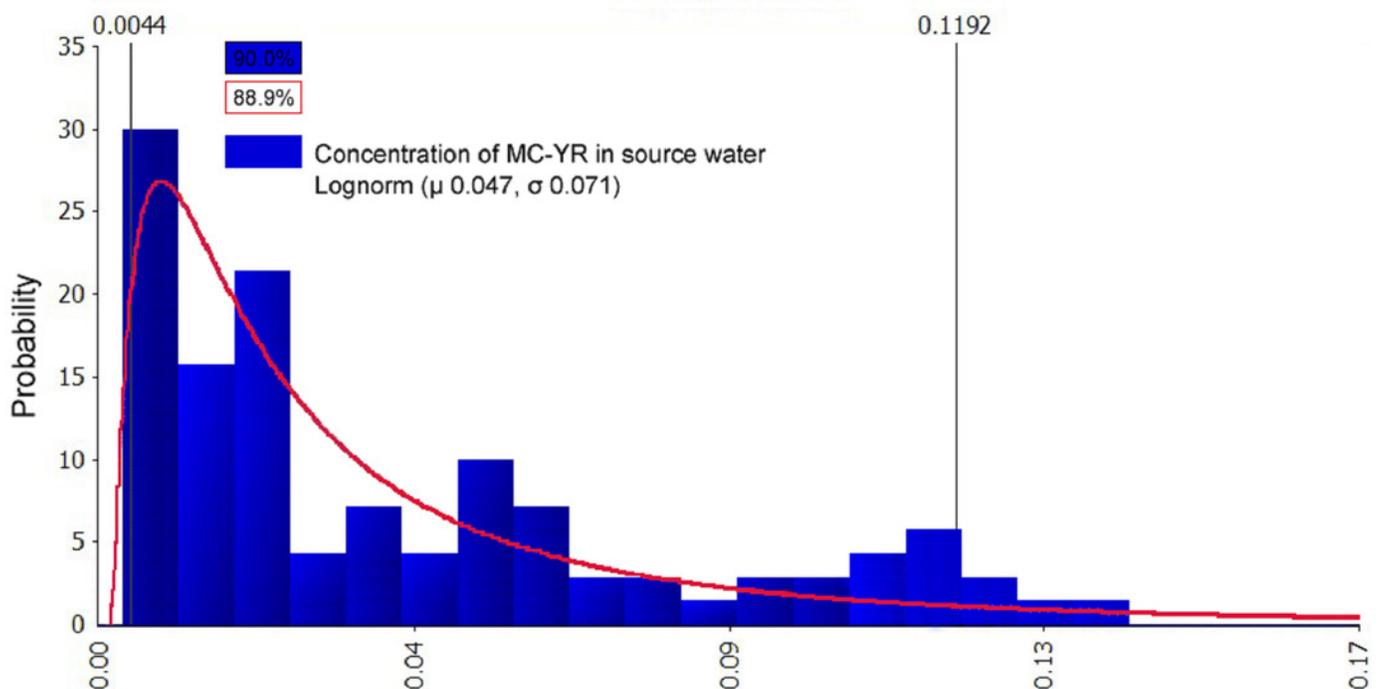
Figure 6

Probability distribution graph after fitting of MC-LR in source water (μg/L).

The data comparison showed that the best-fitted distribution of MC-LR concentration was Gamma (μ 0.076, σ 0.070). The abscissa in Figure 6 represents the concentrations of MC-LR; the concentrations are partitioned, the length of each interval is the group distance, the rectangular area is the frequency of the group, and the ratio of the total sample utilized, and the vertical axis is the frequency divided by the group distance obtained. The fitting results can be visually observed from the coincidence of the blue rectangular shape (90.0%) and the area under the red curve (88.0%).