Cyanobacterial Composition and Microcystin Accumulation in Catfish Pond

Suneerat Ruangsomboon[a], Wichien Yongmanitchai [b], Paveena Taveekijakarn [a] and Monthon Ganmanee [a]

[a] Program in Fisheries Science, Faculty of Agricultural Technology, King Mongkut’s Institute of Technology Ladkrabang, Bangkok 10520, Thailand.
[b] Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

*Author for correspondence; e-mail: krsuneer@kmitl.ac.th

Received: 7 December 2012
Accepted: 22 April 2013

ABSTRACT

Cyanobacterial blooms frequently occur in catfish ponds, but the potential effects of its secondary metabolites on cultured animals in ponds and human beings remain largely unknown. In the present study, high chlorophyll-a levels (1.28-2.06 mg/L) indicated massive phytoplankton blooms in the studied ponds, with a maximum density of $52 \times 10^6$ cells/L of potentially toxic cyanobacteria, composed mainly of *Microcystis aeruginosa*, *Oscillatoria* spp., *Lyngbya* sp., *Pseudanabaena* spp., *Anabaena* spp., *Anabaenopsis* sp., and *Cylindrospermopsis* sp. However, microcystins were not detected by liquid chromatography-mass spectrometry in samples of water and fish. Instead, microcystin-RR in phytoplankton was estimated to be between 0.014 and 0.202, and correlated positively with the density of *Anabaena* spp. ($r = 0.33$, $p < 0.05$), alkalinity ($r = 0.37$, $p < 0.05$), and ammonia ($r = 0.48$, $p < 0.01$). Microcystin was not detected in the fish in this study, but microcystin accumulation was found in 43% of analyzed phytoplankton samples. Therefore, long-term monitoring is recommended in order to fully understand microcystin accumulation patterns at all degrees of phytoplankton bloom.

Keywords: catfish, fish pond, microcystin, phytoplankton bloom, toxic cyanobacteria

1. INTRODUCTION

Aquaculture is one of the fastest growing animal food sectors around the world, and cultured fish supplies a portion of the animal protein intake for the human population [1]. In Thailand, carnivorous catfish species (*Clarias macrocephalus* vs. *C. gariepinus*) have become major commercial breeding varieties and are mainly cultured in soil ponds for a long period of time. Especially in the last decade (2000-2010), their farming has grown intensively and rapidly, with a 237% overall increase in fish production [2].

As a tropical region of Southeast Asia with high temperatures, Thailand is favorable for cyanobacteria growth, since rapid cyanobacteria growth has been shown to be associated with such temperatures. For example, Wang et al. [3] reported that the bloom of toxic cyanobacteria in freshwater bodies is associated with high levels of
nutrients, light intensity, temperature, and pH. Also, the conditions of high fish-stocking densities and feeding rates during catfish culture are suitable for the prolific development of cyanobacteria. It is known that cyanobacteria can produce a number of secondary metabolites and compounds. Among these, microcystin is considered to be one of the most dangerous toxins, as it is known to be a potent hepatotoxin that may cause liver damage in humans, fish, and other organisms, and may promote tumor growth [4].

A study has shown that microcystin production by cyanobacteria increased proportionally to the amount of nutrients (e.g., total phosphorus) [3]. Microcystin is released from cyanobacterial cell lysis; hence, a high concentration of dissolved microcystin may occur in water immediately after the collapse of cyanobacterial blooms. Microcystin contents in fish may vary according to both their exposure time and bloom duration. Therefore, the degree of risk of human exposure to microcystin via consumption of fish may vitally depend on the accumulation of microcystin in fish [5]. Smith et al. [6] revealed that about 47% of the studies investigating microcystin accumulation in edible tissues of aquatic animals exceeded the Tolerable Daily Intake of 0.04 mg/kg/bw/d, and suggested that human intoxication is possible through both intensive and extensive aquaculture.

It is a general view that microcystin concentration in carnivorous fish is lower than that of phytoplanktivorous fish [7] because the latter directly ingest cyanobacteria, whereas the former accumulate microcystin via accidental ingestion, passive assimilation through gills [8], and transdermal adsorption [9]. Although there are extensive studies on microcystin bioaccumulation in fish, most of these focused on phytoplanktivorous fish, notably tilapia and carp [10-12]. By comparison, less information was produced for carnivorous fish, such as catfish [8, 13]. To date, there is still no published information regarding both the occurrence of toxin-producing cyanobacteria and the quantities of toxins found in catfish-culturing systems [13]. Moreover, cyanobacterial toxins in pond water and farmed animals are occasionally investigated after a die-off event [8], but are rarely regularly monitored. Regular, frequent monitoring could provide accurate information about cyanobacterial toxins that could prevent fish mortality, as ambient toxin concentrations can vary over a season and this accumulation can occur at sub-lethal concentrations [14].

Thus, our concern is whether massive cyanobacterial blooms in catfish ponds may produce microcystins during the culturing process, and if so, how they may affect human health. Thus, the goals of this study are four-fold: 1) to investigate species composition and abundance of cyanobacteria; 2) to measure the concentration of microcystins (MC-LR, -RR, -YR) in three levels of phytoplankton, water, and fish; 3) to assess the potential risk to humans from microcystin contamination; and 4) to detect the key environmental factors that regulate the occurrence of microcystin in catfish ponds.

2. MATERIALS AND METHODS

2.1 Site Description

There were 3 catfish ponds (Clarias macrocephalus vs. C. gariepinus) used in this study. All are 60 × 80 × 1.5 m in size. These ponds are located in Klong Suan Sub-district, Bang Bo District, Samut Prakan Province, central Thailand. At this location, the average temperature, relative humidity, and rainfall were 22-42°C, 73% and 1743 mm/y, respectively. The locations of ponds were: pond 1, 13°41′52.08″N, 100°50′53.34″E;
They are all soil ponds and are managed by private owners. Their water supplies are mainly from the influent of the Klong Pravatebureerom Canal. During the study period, all ponds were isolated (no extra water entering each of these ponds). The culturing period was from April (summer, the time of cyanobacteria bloom) to October (pond 1-rainy season) and November (pond 2, 3-winter season). The total culturing periods for Pond 1, 2, and 3 were 24, 28, and 32 weeks, respectively, based on the situation of fish size and market demand.

2.2 Sampling
Phytoplankton, water, and fish were sampled every two weeks during the culturing period. Water samples were collected from five locations per pond at about 30 cm water depth and 1 m off the shores [15]. The volume of water collected for microcystins analysis was one liter per sample. All samples were preserved on ice and then stored at -20°C in the laboratory.

Water quality parameters were determined to describe the environmental conditions of the sampling area. Measurements of all parameters were carried out every two weeks (30 cm depth; 8 a.m.). Surface water temperature and transparency were measured using a Celsius thermometer and a Secchi disc. pH and conductivity were measured using a digital meter. Dissolved oxygen (DO), biological oxygen demand (BOD), nitrate nitrogen (NO$_3^-$), and nitrite nitrogen (NO$_2^-$) were determined following the methodology of APHA [15]. Total alkalinity, hardness, nitrogen (NH$_3$), orthophosphate (PO$_4^{3-}$), and total suspended solids (TSS) were analyzed following Stirling [16]. Chlorophyll and dry weight were determined following Becker [17].

2.3 Phytoplankton Sampling and Identification
A 15 mm-mesh plankton net was used to collect phytoplankton. At each location, a volume of 20 L of surface (depth 0-30 cm) water was passed through the net and finally concentrated into a 100 ml sample. A 50 ml subsample was taken and preserved in 4% formaldehyde. In the laboratory, species identification were performed by using a Nikon (E 200) microscope at magnifications of 200X and 400X. After identification, the cell counting of filamentous cyanobacteria (Oscillatoria, Cylindrospermopsis, Lyngbya, Arthrospira, Raphidiopsis) was enumerated filament-by-filament, and estimated by the average number of cells per filament. For colonial cyanobacteria (Microcystis), cell numbers were estimated by colony size and average number of cells per colony.

A volume of 100 L of surface water was passed through plankton net and finally a concentrated sample was stored at -20°C in the laboratory.

2.4 Fish Sampling
Fifteen fish of each pond were captured with multi-mesh gillnets every two weeks. These fish were measured, weighed and sacrificed immediately, then dissected in the field into muscle and liver and immediately frozen at -20°C condition.

2.5 Microcystin Extraction
Water: A water sample (1 L) was filtered through Whatman TM GF/C filter paper (0.22 mm pore size; Millipore).

Phytoplankton: Freeze dried cells (~ 0.1 g dw) were homogenized and extracted three times with 2 ml of 75% methanol for 15 min using ultrasound. The extract was centrifuged at 5,000 rpm and the supernatant was
evaporated and re-dissolved with 20% methanol.

Fish: Dried samples of fish, liver, and muscle (~ 0.5 g dw for each tissue) were homogenized and extracted three times with 10 mL of 70% methanol with the addition of 1% v/v trifluoroacetic acid for 5 min using ultrasound. The extract was centrifuged at 5,000 rpm and the supernatant was evaporated and re-dissolved with 20% methanol.

The water sample and solution of phytoplankton and fish extracted were directly passed through a C18 cartridge (Waters sep-pak Millipore), which had been preconditioned by washing with 3 mL of 100% methanol and 3 mL of distilled water. The column was washed with 1 mL of distilled water and 1 mL of 20% methanol. The final step column was washed with 0.4 mL of 80% methanol and 0.6 mL of distilled water and used for the final detection and identification of microcystins by liquid chromatography-mass spectrometry (LC-MS).

2.6 Microcystins Analysis by LC-MS

Qualitative and quantitative analysis of microcystins were performed using LC-MS (Shimadzu, Kyoto, Japan) system equipped with an auto sampler (SIL-HT), a LC pump (LC-10AD), a photo-diode array detector (SPD-M10A) and MS (2010A) using Zorbax Eclipse XDB-C18 column (2.1x150 mm, Agilent, USA) and 55% methanol in 0.1% formic acid as mobile phase at a flow rate of 0.2 mL/min with 10 μL injection. The identification and quantification of the toxin are determined by comparison with an authentic toxin standard (Kanto Chemical, Co. Ltd., Tokyo, Japan). The quality control in each analytical batch were reagent blank spike recovery and cross-check toxin standard. The limit of detection for the microcystin was 0.25 μg/L. Standards of pure microcystins (MC-LR, -YR, and -RR) were purchased from Pure Chemical Industries (Cica-Reagent).

2.7 Statistics

All values are expressed as mean ± standard deviation. Significant differences of fish growth were determined using analysis of variance (ANOVA) with 95% confidence. A Pearson correlation test was used to establish association between different variables. Significance was accepted for \( p < 0.05 \).

3. RESULTS AND DISCUSSION

3.1 Fish Growth and Water Quality

The growth of catfish was not significantly different among the three ponds (Figure 1), with average weights of 305.0 ± 10.6, 312.6 ± 14.6, and 331.2 ± 17.4 g/body for ponds 1, 2, and 3, respectively; however, the water quality parameters revealed significant pond variation (Table 1).

There was no mass mortality during the culturing period, a result indicating that cyanobacterial blooms did not significantly affect the survival of fish. Water quality also did not affect survival. Even though the levels of ammonia, pH, and DO (Table 1) in the ponds were not optimal at all times, the fish still survived intact. The optimum concentration of ammonia for aquatic animals did not exceed 0.2 mg/L; the optimum pH values for fish production is between 6 and 9, with DO not lower than 5.0 mg/L [18].
Figure 1. Average weight of catfish in various culturing ponds containing dense phytoplankton bloom.

Table 1. Minimum, maximum and mean of physico-chemical variables of water from catfish pond.

<table>
<thead>
<tr>
<th></th>
<th>pH (°C)</th>
<th>T (°C)</th>
<th>Cond (ms/cm²)</th>
<th>DO (mg/L)</th>
<th>BOD (mg/L)</th>
<th>Alk (mg/L)</th>
<th>Hard (mg/L)</th>
<th>TSS (g/L)</th>
<th>Tran (cm)</th>
<th>NH₃ (mg/L)</th>
<th>NO₂⁻ (mg/L)</th>
<th>NO₃⁻ (mg/L)</th>
<th>PO₄³⁻ (mg/L)</th>
<th>Ch-a (mg/L)</th>
<th>DW (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond 1</td>
<td>minimum</td>
<td>7.0-</td>
<td>32.8-</td>
<td>0.97-</td>
<td>6.1-</td>
<td>13.3-</td>
<td>32.7-</td>
<td>96.7-</td>
<td>0.54-</td>
<td>4.0-</td>
<td>1.27-</td>
<td>0.01-</td>
<td>0.12-</td>
<td>0.08-</td>
<td>0.38-</td>
</tr>
<tr>
<td></td>
<td>maximum</td>
<td>9.1</td>
<td>38.3</td>
<td>2.76</td>
<td>27.8</td>
<td>173.3</td>
<td>205.0</td>
<td>170.3</td>
<td>2.62</td>
<td>19.7</td>
<td>8.48</td>
<td>0.53</td>
<td>2.35</td>
<td>2.24</td>
<td>3.57</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>8.1</td>
<td>35.0</td>
<td>1.56</td>
<td>17.2</td>
<td>59.6</td>
<td>137.5</td>
<td>122.0</td>
<td>1.58</td>
<td>11.3</td>
<td>4.98</td>
<td>0.17</td>
<td>1.06</td>
<td>0.99</td>
<td>2.06</td>
</tr>
<tr>
<td>Pond 2</td>
<td>minimum</td>
<td>5.3-</td>
<td>26.0-</td>
<td>1.33-</td>
<td>2.8-</td>
<td>8.3-</td>
<td>80.0-</td>
<td>193.3</td>
<td>13.2-</td>
<td>28.3</td>
<td>7.34</td>
<td>0.26</td>
<td>1.56</td>
<td>1.55</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>maximum</td>
<td>8.2</td>
<td>40.1</td>
<td>2.96</td>
<td>24.2</td>
<td>174.7</td>
<td>198.3</td>
<td>193.3</td>
<td>3.12</td>
<td>28.3</td>
<td>7.34</td>
<td>0.26</td>
<td>2.52</td>
<td>2.02</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>6.9</td>
<td>34.4</td>
<td>2.19</td>
<td>8.0</td>
<td>39.5</td>
<td>92.6</td>
<td>163.0</td>
<td>1.96</td>
<td>13.4</td>
<td>2.60</td>
<td>0.12</td>
<td>1.02</td>
<td>0.22</td>
<td>1.28</td>
</tr>
<tr>
<td>Pond 3</td>
<td>minimum</td>
<td>3.6-</td>
<td>25.0-</td>
<td>1.94-</td>
<td>1.6-</td>
<td>1.5-</td>
<td>53.3-</td>
<td>118.7</td>
<td>1.13-</td>
<td>6.7-</td>
<td>7.47</td>
<td>0.01-</td>
<td>0.11-</td>
<td>0.03-</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>maximum</td>
<td>7.4</td>
<td>38.7</td>
<td>4.66</td>
<td>14.4</td>
<td>172.0</td>
<td>170.3</td>
<td>217.7</td>
<td>3.46</td>
<td>31.7</td>
<td>21.82</td>
<td>0.35</td>
<td>3.95</td>
<td>1.78</td>
<td>3.61</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>6.4</td>
<td>33.6</td>
<td>2.79</td>
<td>5.1</td>
<td>42.7</td>
<td>111.6</td>
<td>167.9</td>
<td>2.08</td>
<td>13.5</td>
<td>11.77</td>
<td>0.15</td>
<td>1.18</td>
<td>0.33</td>
<td>1.68</td>
</tr>
</tbody>
</table>

(T = water temperature, Cond = conductivity, Alk = alkalinity, Hard = hardness, Tran = transparency, Ch-a = chlorophyll-a, DW = dry weight)

3.2 Species Diversity and Abundance of Phytoplankton

Chlorophyll-a levels (1.28-2.06 mg/L) were higher than those of intensive aquaculture reported (0.075 mg/L, sometimes 0.3-1mg/L) [6], indicating dense phytoplankton blooms in the studied catfish ponds. Among these ponds, pond 1 had the highest chlorophyll-a level, followed by ponds 3 and 2 (Table 1, Figure 2a), corresponding to the density of total cyanobacteria (Table 2, Figure 2b). The cyanobacterial blooms were generally dominated by Microcystis aeruginosa, Arthrospira platensis, Pseudanabaena spp., and Oscillatoria spp. Thirteen cyanobacterial genera were identified in catfish ponds, of which seven genera (Oscillatoria, Microcystis, Lyngbya,
Pseudanabaena, Anabaena, Anabaenopsis, and Cylindrospermopsis) can produce a variety of toxins of strain-dependent microcysts [14]. The maximum densities of potentially toxic cyanobacteria in ponds 1, 2, and 3 were $53 \times 10^6$, $2.8 \times 10^6$, and $25 \times 10^6$ cells/L, respectively (Figure 2c).

In all fish ponds, the maximum densities of *M. aeruginosa*, Pseudanabaena spp., Oscillatoria spp., and *Anabaena* spp. occurred mostly at the 12th-16th week of the studied period (Figure 2d-g). The maximum density of *M. aeruginosa* ($49.5 \times 10^6$ cells/L) in the fish pond was higher than those reported by Kankaanpaa et al. [14] in a prawn pond, where the maximum density of *M. aeruginosa* was $3.5 \times 10^4$ cells/L [14].

There were differences among the three fish ponds in terms of both the species composition of cyanobacteria found and their relative abundance. It was surprising to find that not only non-fixing cyanobacteria, but also fixing cyanobacteria, strongly depended on an adequate amount of ammonia and orthophosphate in order to survive (Tables 2 and 3). This assumption was supported by the result that the maximum densities of *Anabaena* spp. and *M. aeruginosa* were found in pond 1, which had the highest concentration of ammonia and orthophosphate, but not in pond 2, which had the lowest (Table 1).

### Table 2. Average density of cyanobacteria (cells/L) in catfish pond.

<table>
<thead>
<tr>
<th>Cyanobacteria</th>
<th>Pond 1</th>
<th>Pond 2</th>
<th>Pond 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chroococcus spp.</td>
<td>213</td>
<td>569</td>
<td>346</td>
</tr>
<tr>
<td>Cyanosarcina sp.</td>
<td>412</td>
<td>678</td>
<td>310</td>
</tr>
<tr>
<td>Gloeocapsa pleurocapsoides</td>
<td>9,089</td>
<td>4,767</td>
<td>110</td>
</tr>
<tr>
<td>Merismopedia spp.</td>
<td>779,510</td>
<td>39,574</td>
<td>27,086</td>
</tr>
<tr>
<td>Microcystis aeruginosa*</td>
<td>9,715,337</td>
<td>-</td>
<td>4,189,560</td>
</tr>
<tr>
<td>Anabaena spp.*</td>
<td>20,850</td>
<td>-</td>
<td>4,135</td>
</tr>
<tr>
<td>Anabaenopsis sp.*</td>
<td>-</td>
<td>2500</td>
<td>-</td>
</tr>
<tr>
<td>Cylindrospermopsis sp.*</td>
<td>587</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raphidiopsis spp.</td>
<td>833</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lyngbya sp.*</td>
<td>6,121</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oscillatoria spp.*</td>
<td>1,330,959</td>
<td>827,090</td>
<td>587,678</td>
</tr>
<tr>
<td>Pseudanabaena spp.*</td>
<td>167,934</td>
<td>14,374</td>
<td>961,135</td>
</tr>
<tr>
<td>Arthrospira platensis total potentially toxic cyanobacteria</td>
<td>6,281,386</td>
<td>3,600,002</td>
<td>733,857</td>
</tr>
<tr>
<td>cyanobacteria</td>
<td>11,241,788</td>
<td>843,964</td>
<td>5,742,508</td>
</tr>
<tr>
<td>total cyanobacteria</td>
<td>18,313,231</td>
<td>4,489,554</td>
<td>6,504,217</td>
</tr>
</tbody>
</table>

- not found   * potentially toxic cyanobacteria
Figure 2. Variation of chlorophyll-a (a), total cyanobacteria (b), potentially toxic cyanobacteria (c), *Microcystis aeruginosa* (d), *Pseudanabaena* spp. (e), *Oscillatoria* spp. (f), and *Anabaena* spp. (g) in catfish pond.

Table 3. Pearson correlation between microcystin content in phytoplankton, total cyanobacteria, cyanobacteria produced microcystin and nutrient.

<table>
<thead>
<tr>
<th></th>
<th>MC</th>
<th>Cyan</th>
<th>Os</th>
<th>Micro</th>
<th>Lyng</th>
<th>Cyan</th>
<th>Ana</th>
<th>Pseuda</th>
<th>NH$_3$</th>
<th>NO$_2$</th>
<th>NO$_3$</th>
<th>PO$_4$</th>
<th>tox cyan</th>
</tr>
</thead>
</table>
| Correlation is significant at the 0.05 level
| 0.06 | 0.33* | 0.48* | 0.56** | 0.92** | 0.06 | 0.92** | 0.56** | 0.99** | -0.05 | -0.09 | -0.18 | 0.27 | 0.06 |

*Correlation is significant at the 0.05 level**Correlation is significant at the 0.01 level (MC = microcystin, Cyan = total cyanobacteria, Os = *Oscillatoria*, Micro = *Microcystis*, Lyng = *Lyngbya*, Cylin = *Cylindrospermopsis*, Ana = *Anabaena*, Pseuda = *Pseudanabaena*, tox cyan = potentially toxic cyanobacteria)
3.3 Microcystins in Water, Fish, and Phytoplankton

Microcystins were not detected throughout the farming period in the samples of water and fish (muscle and liver) from all catfish ponds. These results contrast with those from ponds with massive blooms in other regions. For example, Zimba and Grimm [13] reported that approximately 50% of channel catfish ponds in the southeastern United States had a detectable level of microcystin in the water that was beyond the level (1 ng/mL) recommended by the World Health Organization as safe for human consumption [1].

Although we did not detect microcystin in these waters, the water quality indicated that the studied ponds were of eutrophic conditions (Table 1), which should be favorable for toxic cyanobacteria blooms. This is possibly due to the low density of toxic cyanobacteria in the studied ponds. For example, catfish ponds from Zaria of Nigeria, where the microcystins in the water were 0.6-5.89 mg/L [19], contained 12.5 to 25 times more toxic cyanobacteria than found in our studied ponds.

The concentrations of microcystin were detected only in phytoplankton, with microcystin-RR values of 0.014-0.202 mg/g dw for pond 1, 0.024-0.141 mg/g dw for pond 3, but no detection in pond 2 (Figure 3). It is probable that both *M. aeruginosa* and *Anabaena* spp. could contribute to the production of microcystins (ponds 1 and 3), since they did not occur in pond 2 (Figure 2d, g). Although there were a higher densities of *M. aeruginosa* and *Anabaena* spp. in the present study than those reported by Kankaanpaa et al. [14] in a prawn pond, the microcystin levels in this study were lower (0.5-1.2 mg/kg dw) [14], indicating that microcystin concentration depends not only on cyanobacterial density, but also on cyanobacterial strain.

**Figure 3.** Variation of intracellular microcystin in phytoplankton of catfish pond.
The results of this study show that only microcystin-RR (MC-RR) was detected in phytoplankton; MC-LR and MC-YR were not detected. Different microcystins have different toxicity profiles. Among these, MC-RR has the lowest toxicity [20]; however, standard criteria of MC-RR for human consumption have not been proposed by the World Health Organization.

The highest concentrations of MC-RR in ponds 1 and 3 were at the 24th and 32nd week of the farming period, respectively, and MC-RR was not detected in pond 2 throughout the sampling period (Figure 3). These variations could be a consequence of different environmental conditions, as well as changes in the dominance of cyanobacterial species and strain composition among sampling periods [7, 21].

The time period of the maximum density of potentially toxic cyanobacteria (Figure 2c) did not simultaneously correspond to the maximum microcystin content (Figure 3). This is possibly due to the fact that a small portion of secondary metabolites are lost during the maximum cyanobacterial (exponential) growth [22].

The bloom of toxic cyanobacteria in other regions of Thailand has been reported. For example, a toxic cyanobacterium, *M. Aeruginosa*, with $5.86 \times 10^4$ g/g microcystin concentration was present in a channel in the Northeast region [23]. Also present in a reservoir in the same region were *Cylindrospermopsis* sp., *Microcystis* sp., *Oscillatoria* sp., and *Pseudanabaena* sp., with microcystin concentrations in water of 1.09-1.23 g/L [24]. In the Northern region, the toxic cyanobacteria *Microcystis* spp. were dominant in prawn ponds, with a microcystin concentration of 0.44 g/kg dw [25]. In duck-husbandry ponds and the water of a dam in the central region, *M. aeruginosa* was dominant, with microcystin concentrations of 0.7-0.8 mg/g dw [26].

### 3.4 Correlation Analysis

Microcystin content in phytoplankton was highly correlated with the density of *Anabaena* spp. ($r=0.33, p<0.05$) (Table 3). This may indicate that *Anabaena* spp. was the major potentially cyanobacteria-producing microcystin in the studied ponds. Normally with mixed cyanobacterial blooms in eutrophic water, it is uncommon to identify only one species as a microcystin producer; however, Vareli et al. [27] reported that only one genus, *Microcystis* sp., from mixed cyanobacterial blooms was responsible for all the microcystin production in a lake.

Densities of *M. aeruginosa*, *Lyngbya* sp., and *Anabaena* spp. were significantly correlated with orthophosphate ($r=0.35, p<0.05; r=0.44, p<0.01$; and $r=0.39, p<0.01$, respectively), whereas the density of *Cylindrospermopsis* sp. correlated with nitrite ($r=0.39, p<0.05$) (Table 3). The microcystin content in phytoplankton was positively correlated with ammonia ($r=0.48, p<0.01$) and alkalinity ($r=0.37, p<0.05$), and negatively correlated with TSS ($r=-0.36, p<0.05$) and transparency ($r=-0.35, p<0.05$). No correlation with nitrate or orthophosphate was observed.

It has been reported that many environmental factors, such as nitrogen, phosphorus, iron, pH, light intensity, and temperature, may affect microcystin production [28, 29]. In this study, we found that alkalinity and ammonia correlated with the amount of microcystin in phytoplankton (Tables 3 and 4). This result supports the hypothesis that nitrogen can promote growth and microcystin production of toxic cyanobacteria [30], since ammonia plays a major role as a nitrogen source for microcystin synthesis in cyanobacteria of catfish ponds.

Kankaanpaa et al. [14] reported that the peak of the cyanobacterial bloom was
obviously boosted by high nutrient content, especially phosphate availability. We found a similar result, as the density of potentially toxic cyanobacteria was significantly correlated with orthophosphate (Table 3). Although it is common to associate the massive development of cyanobacteria with increases in nutrients, it has not always been possible to establish a clear relationship between nutrient levels and toxicity. Some authors even claim that the production of toxins is not necessarily connected with eutrophication [31].

In this study, microcystin was detected only in phytoplankton, so catfish might get microcystin only via ingested toxic cyanobacteria. However, there was no detection of microcystin in fish muscle and liver at all, even during the intensive catfish production in which the cyanobacteria were blooming for more than 60-90% of the culturing period. It is unlikely that the catfish did not ingest the toxic cyanobacteria at all, but it might be that they did not ingest enough, and for a long enough time, for detection.

The results of this study showed that there was no accumulation of microcystin in fish cultivated in these ponds, which were fed and managed by private owners. The situation is very different for fish feeding in natural ecosystems. In the ponds, catfish were fed with commercial feed; thus, they could partially avoid ingesting the toxic cyanobacteria. In contrast to these ponds, catfish in a natural habitat that has toxic cyanobacteria blooms are more likely to accumulate more microcystin because they need to continually feed on available food. Therefore, the chance of accidentally taking in toxic cyanobacteria is higher than that for the fish in our ponds.

It seems that carnivorous catfish that live in bodies of water with toxic cyanobacterial blooms may accumulate lower microcystin than phytoplanktivorous fish, and high microcystin accumulation in phytoplanktivorous fish has been previously reported. For example, microcystin concentration was highest in the muscle of omnivorous fish, followed by phytoplanktivorous fish, and was lowest in carnivorous fish [7]. Nyakairu et al. [12] reported that microcystin accumulation in herbivorous fish (*Oreochromis niloticus*) in Lake Mburo was higher than in omnivorous fish (*Lates niloticus*), and suggested that omnivorous fish have more alternative sources of food.

In this study, after catfish were harvested, the water with dense, toxic cyanobacterial bloom was drained into a canal that was also used for plant (mimosa, banana, mango, coconut, etc.) farms. After cyanobacterial cell lysis, microcystin can be immediately released and could accumulate in plant tissues [32]. This is of special concern, and consideration should be taken, since some treatment may be needed before draining the water from catfish farms.

4. CONCLUSIONS

This study was an attempt to continuously monitor the distribution of microcystin in catfish ponds in Thailand. The microcystins in water and catfish (muscle and liver) were not sufficient to pose a risk to humans; however, the degree of bloom toxicity may vary, and accumulation may still reach a toxic level. Therefore, long-term monitoring is recommended in order to understand microcystin accumulation patterns at all degrees of cyanobacterial bloom throughout the country. The consequences of the production and potential liberation into the water of intracellular microcystin must be taken into consideration, along with other attempts to elucidate their influence in the final concentration of dissolved microcystin in water and to
ascertain the potential effects on other carnivorous fish.

ACKNOWLEDGEMENTS

This work was supported by grants from the King Mongkut’s Institute of Technology Ladkrabang.

REFERENCES


