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Accumulation of microcystins in water and economic fish in Phayao Lake, and fish ponds along the Ing River tributary in Chiang Rai, Thailand

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ABSTRACT

This study determined the levels of microcystins in water and fish from Phayao Lake, Phayao Province and selected fish ponds along the Ing River tributary in Chiang Rai Province. Samples were collected monthly for 8 months (January to August 2011 for Phayao Lake, and November 2008 to June 2009 for fish ponds) and were analyzed by HPLC. The highest total microcystin-LR levels in water and fish in Phayao Lake were recorded in April 2010 at 2.60 ± 2.48 μg·L^{-1} and 0.20 ± 0.03 μg·kg^{-1} dry weight, respectively. Microcystis aeruginosa Kütz were the dominant species (271.6 ± 72.4 mm^3/m^3) in the lake. Colony number of Microcystis spp showed a positive correlation with soluble orthophosphate (r^2 = 0.77). Similarly, Nile tilapia ponds surveyed along the tributary in Chiang Rai were contaminated with microcystins as well. The highest concentration detected in water was in March 2009 (0.58 ± 0.24 μg·L^{-1}), whilst the maximum concentration in fish was recorded in April 2009 (2.68 ± 0.51 μg·kg^{-1} dry weight). Microcystis spp. dominated the pond waters and was positively correlated with chlorophyll a (r^2=0.80) and soluble nitrate (r^2=0.71). The highest concentration of the cyanobacteria was recorded in February 2009 at 4272.5 ± 62.3 mm^3/m^3. Results showed that total microcystin-LR concentration in fish in Chiang Rai ponds were higher than in Phayao Lake. This study suggested the possible health risks associated with the bioaccumulation of microcystins in fish (Nile tilapia) cultivated in fish ponds along the tributary in Chiang Rai and in Phayao Lake.

Keywords: Microcystins; Phayao Lake; Water; Nile tilapia

1. INTRODUCTION

Thailand is one of the significant exporters of fish fillet in the United States, along with Taiwan, Mainland China and Indonesia. Other key fillet markets include Japan and Italy [1]. Tilapia is mainly derived from fish farming in cages and ponds either through monoculture or polyculture with other economic fishes such as hybrid catfish. The consumption of Nile tilapia (*Oreochromis niloticus*) in Thailand is very popular, especially in the northern part where freshwater consumption rate was reported to be up to 32 kg per person per year [2] The Chiang Mai Aquacultural Cooperative (CMA Co-op) also reported that demand for freshwater fish in Chiang Mai amounts to 40,000 kg/day [3]. The largest producer of tilapia (approximately 17.71 tons/day of fish products) is in the Upper North, including the Phan District, Chiang Rai Province, where tilapia production is primarily semi-intensive based on natural foods derived from fertilizers or animal manure [4,5]. The fish are generally raised along with livestock, especially chicken and pigs, to reduce production costs. However, integrated fish farming often face water quality problems caused by nutrients (nitrogen and phosphorus) from animal wastes. Because the volume of waste discharged into the fish pond is excessive, it results in a rapid growth of phytoplankton (algal bloom) especially during summer, which mostly often rendered the fish with musty off-flavor, or worst,

contaminated with toxic microcystins.

Microcystin is a hepatoxin produced by blue-green algae such as *Anabaena* spp., *Oscillatoria* spp., and *Microcystis* spp. with *Microcystis aeruginosa*, having the most number of toxic species known. Microcystins are cyclic peptides made from seven amino acids [6,7], considered as the most common and one of the most dangerous groups of cyanotoxins found in water [8]. Microcystins are toxic pollutants in the water that cause harm to humans by inducing diseases of the digestive system. These toxins affect the liver by inhibiting protein phosphatase and cause liver cancer in rats [9]. Magalhaes *et al.* [10] reported the accumulation of microcystin in fish used for human consumption, from the lake Jacarepagua in Brazil.

Kwan Phayao is one the largest artificial lakes in northern Thailand, located in the province of Phayao. It covers an area of 2.3 km^2, with a mean depth of 1.7 m and located at an altitude of 380 meters above sea level. It is situated at the southern tips of two mountains, Doi San Klang and Doi Huai Nam Khao. The lake is fed by the Ing River, which empties to the north. To the south and west of the lakes are rice paddies, at the mouth of the Ing River is marsh area, which is a significant residence for water birds [11]. Kwan Phayao is the main source of raw water for domestic, drinking and agricultural use of Phayao residents. However, its water quality is deteriorating due to anthropogenic and farming activities, with livestock effluent from the basin and sewage discharges from Phayao City are being the major pollution sources. These significant sources of nutrients, especially nitrogen and phosphorus, contribute to the eutrophication of the lake which leads to algal blooms and subsequently to microcystin production, contaminating the water and aquatic animals.

Therefore, the purpose of this research is to monitor the levels of toxic microcystins and phytoplankton diversity in Phayao Lake, Phayao province and in selected aquaculture ponds in Chiang Rai province, Thailand.

2. MATERIALS AND METHODS

2.1. Sample Collection

The present study was carried out in Phayao Lake due to its geographical significance as a tourist destination in Phayao province, and in selected fish ponds in the province of Chiang Rai, along the Ing River tributary. The period of sampling (8 months) was from January to August 2011 (Phayao Lake) and from November 2008 to June 2009 (Chang Rai fish ponds). Two-liter samples of water were collected in polyethylene containers for physicchemical analysis and 1-L samples were collected for microcystin analysis, from both lake and ponds. Samples from Phayao Lake were collected from three fixed sampling points as shown in **Figure 1**. Samples were preserved on ice, transported to the laboratory and stored at -20℃.

Fish samples (Nile tilapia) were collected at the same time as the water samples, with the aid of fishing net. A total of 24 and 48 samples of Nile tilapia were sampled from Phayao Lake and from 6 fishponds in Chiang Rai, respectively during the period of study. Samples were immediately kept in ice and transported to the laboratory. Fish samples were weighed, filleted and stored in the freezer at -20℃ until analysis.

Phytoplankton was sampled by filtration of pond water (with a net of 10-µm mesh. Samples were concentrated in a 30-mL bottle and preserved with Lugol's solution.

2.2. Microcystin in Water

Microcystin in water was measured following a method adapted from Prommana *et al*. [12] with modification. Two-hundred-milliliter water sample was placed in a 500-mL beaker and boiled for 1 hour. The sample was then filtered thru a 0.45 µm GF/C glass filter disc, and the pH was adjusted to pH 7.0 using a solution of 0.01 M NaOH or 0.01 M HCl. Microcystins (MCs) in the water sample was extracted by solid phase extraction (SPE) using a Strata-X 33 µm polymeric reversed phase (500 mg/6 mL) SPE cartridge. After conditioning the SPE cartridge with 5 mL of 100% methanol followed by 10 mL of of Milli-Q water, the water sample was applied to the cartridge at a flow-rate of 20 $mL \cdot min^{-1}$. After the cartridge was washed with 20 mL of 10%methanol, residue of MCs was eluted twice with 5 mL of 10% methanol at a flow-rate of 5 $mL \cdot min^{-1}$.

The MC-containing fraction was evaporated to dryness. This fraction was dissolved in 5 mL of 100% methanol and was filtered through 0.45 µm Millipore filters. One-hundred microliters of the methanolic extract was then injected into the high performance liquid chromatograph (HPLC) for the detection and quantification of MCs.

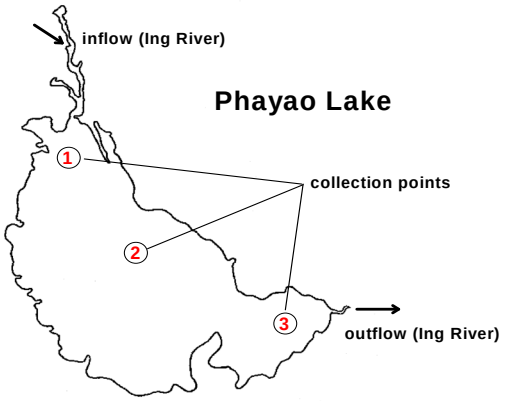


Figure 1. Sketch map of Phayao Lake showing the sampling points.

2.3. Microcystin Analysis in Fish

Deep-frozen fish fillets were thawed and then 10-g samples were freeze-dried before extraction. One-gram freeze dried sample was homogenized in a mortar and then extracted three times with 10-mL 80% methanol, mixed well with a vortex mixer for 10 minutes and was left overnight. The extract was centrifuged at 5,000 rpm for 10 minutes. The supernatant was transferred in a separatory funnel, 15 mL of hexane was added, the mixture was shaken for 10 minutes and then the methanol fraction was collected. This was repeated two more times prior to HPLC analysis.

2.4. Water Quality and Nutrient Analysis

Temperature, pH, dissolved oxygen and turbidity were measured in situ, using a multimeter (TOA DKK WQC-22A Model, Japan). Alkalinity, hardness, total ammonia-nitrogen, nitrate-nitrogen, nitrite-nitrogen, orthophosphate-phosphorus and chlorophyll a were determined in the laboratory by standard methods [13].

2.5. Cyanobacterial Identification and Counting

The identification of *Microcystis* colonies and other cyanobacteria was carried out using a microscope. Cyanobacterial cells were counted with a haemacytometer and calculated as mm^3/m^3.

3. RESULTS

Microcystin-LR (MC-LR) was detected in 29% (7 out of 24) of water sampled from Phayao Lake throughout the survey period. The amount of total MC-LR in water ranged from, "not detected" (ND) - 7.56 $\mu g \cdot L^{-1}$ with an average concentration of 0.69±0.28 $\mu g \cdot L^{-1}$ (**Figure 2**). On the other hand, Nile tilapia from the lake which were collected at the same sampling date as with the contaminated water samples, were likewise found to be tainted with toxic MC-LR, which ranged from ND - 0.26 $\mu g \cdot kg^{-1}$ dry weight. The average concentration in fish was 0.06 ± 0.02 $\mu g \cdot kg^{-1}$ dry weight. The highest total MC-LR contaminations in both water and Nile tilapia in Phayao Lake were recorded in April 2010 (**Figure 2**).

Majority of water samples (67%) from the 6 fish ponds in Chiang Rai province were contaminated with the hepatoxin. The thirty-two MC-LR contaminated samples of water had an average concentration of 0.22 ± 0.28 $\mu g \cdot L^{-1}$, where the highest concentration was detected in March 2009 (**Figure 3**). Out of the 48 samples of Nile tilapia, 23 were found positive for toxic MC-LR. Detected concentrations ranged from ND - 5.91 $\mu g \cdot kg^{-1}$ dry weight with an average of 1.22 ± 0.48 $\mu g \cdot kg^{-1}$ dry weight. The highest concentration recorded in fish was in April 2009

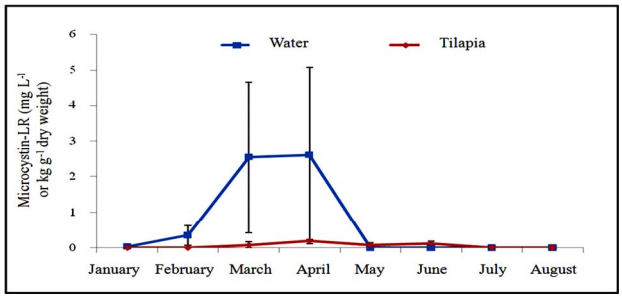


Figure 2. Concentration of MC-LR in lake water and fish (Nile tilapia) sampled between January to August 2011.

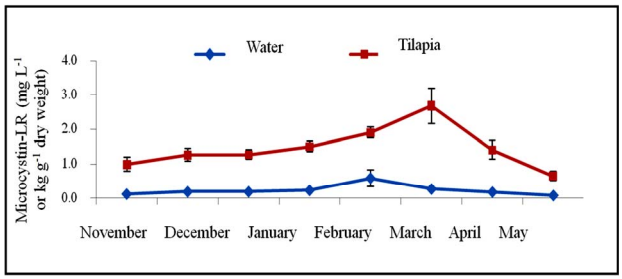


Figure 3. Concentration of MC-LR in pond water and fish (Nile tilapia) sampled between November 2008 and June 2009.

(**Figure 3**).

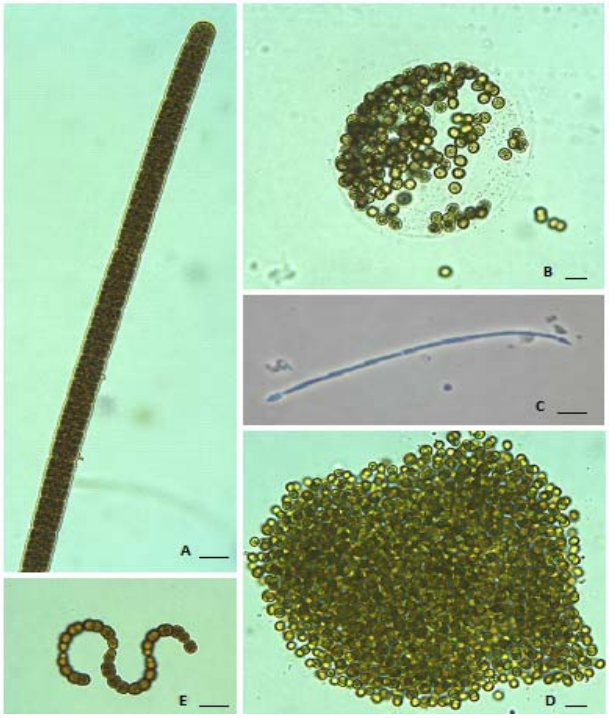
In Phayao Lake, *Microcystis aeruginosa* Kütz. (271.6±72.4 mm^3/m^3) dominated the water. Other microcystin-producing cyanobacteria identified include, *Microcystis wesenbergii* Kom. (120.8 ± 14.0 mm^3/m^3), *Anabaena* spp. (1.0 ± 0.2 mm^3/m^3), *Oscillatoria* spp. (74.0 ± 41.9 mm^3/m^3) and *Cylindrospermopsis raciborskii* (Wolosz.) Seenayya & Subba (5.5 ± 2.5 mm^3/m^3) (**Figure 4**). Correlation analysis showed that *Microcystis* species (*Microcystis aeruginosa* Kütz. and *Microcystis wesenbergii* Kom.), had a positive relationship with soluble orthophosphate ($r^2 = 0.77$).

Likewise, *Microcystis* spp. dominated the Chiang Rai ponds throughout the survey period. *Anabaena* spp. and *Oscillatoria* spp. were also present in the ponds (**Figure 4**). Biovolume of *Microcystis* spp. was positively correlated with chlorophyll a (r^2=0.80) and soluble nitrate (r^2=0.71). Highest concentration of the cyanobacteria was recorded in February 2009 at 4272.5±62.3 mm^3/m^3.

4. DISCUSSIONS

This study shows another compelling evidence of the pervasive contamination of microcystins in eutrophic surface waters and the bioaccumulation of these toxins in fish tissues. The results, where 67% of water and 48%of Nile tilapia samples from Chiang Rai freshwater ponds; and 29% of water and Nile tilapia samples from Phayao Lake, were contaminated with MC-LR, corroborate the

findings of previous studies conducted in freshwater



Scale bar = 10 µm

Figure 4. Microcystin-producing cyanobacteria identified in Phayao Lake and in Chiang Rai ponds. (A) *Oscillatoria* spp. (B) *Microcystis wesenbergii* Kom. (C) *Cylindrospermopsis raciborskii* (Wolosz.) Seenayya & Subba (D) *Microcystis aeruginosa* Kütz. (E) *Anabaena* spp.

prawn and tilapia ponds in northern Thailand [12,14]. Due to the dominance of the genus *Microcystis* spp., (*Microcystis aeruginosa* Kütz. and *Microcystis wesenbergii* Kom) in Phayao Lake and in Chiang Rai ponds, their presence in these surface waters could be implicated for the production of the microcystins detected in this study. This confirms the findings of Prommana [15] that *M. wesenbergii* were found to be the dominant species in Kwan Phayao reservoir (Phayao Lake). Similarly, Peerapornpisal *et al.*[16] reported that *Microcystis,* which include *M. aeruginosa* and *M. wesenbergii,* were the main species found in water resources in Thailand whilst Ruangrit *et al.* [14] reported that the dominant *Microcystis* species in giant freshwater prawn ponds and in tilapia ponds in northern Thailand were *M. aeruginosa* and *M. wesenbergii*. Moreover, the presence of *Microcystis* spp. in these bodies of water may be indicative of constant nutrient enrichment, as shown by its established positive correlation with soluble orthophosphate (r^2 = 0.77) in Phayao Lake, and with soluble nitrate (r^2 = 0.71) in Chiang Rai ponds. These species are known not to tolerate nutrient poor conditions in aquatic ecosystems [17]. Potential microcystin-producing genera such as *Anabaena* spp. and *Oscillatoria* spp. and *Cylindrospermopsis raciborskii* were also identified in this study. However, they were only present in small amounts and could not be significant contributors to microcystin production in the lake and ponds [12].

The mean level of MC-LR in Nile tilapia was higher in ponds compared with that in the lake (**Table 1**). Detected levels of MC-LR in the fish were below the recommended guideline value for a tolerable daily intake (TDI) of microcystin at 0.04 µg kg^{-1} body weight day^{-1}, except for some fish pond samples which have exceeded the allowed value. The apparent presence of microcystin in Nile tilapia from both Phayao Lake and aquaculture ponds in Chiang Rai could have a serious negative implication on people that use the fish from these waters as a source of meat. The toxins contained within fish tissues may pose an alternative route of exposure to humans. This is because there is sufficient published data that implicate microcystins of bioaccumulation in fish tissues [14,18,19].

As a planktivorous fish, Nile tilapia consumes cyanobacteria particularly *Microcystis* and other filamentous species. Aside from getting contaminated with dissolve microcystins from the water through the gills, direct ingestion of these toxic cyanobacteria, which through the intestinal tract, could be another possible route of uptake of microcystin. Zhao *et al*. [19] showed that microcystin accumulation rates in muscle and liver tissues are directly proportional to ingestion rates for Nile tilapia. Furthermore, the contamination of fish with microcystins also affects negatively the former's growth and productivity [20], thus pose problems as well in the standpoint of aquaculture production and fish quality.

The extent of cyanobacterial contamination of Phayao Lake is one significant issue in this study. The lake is the main source of raw water for domestic, agricultural and drinking purposes as well as a source of food and livelihood for local fishermen. Although the MC-LR values detected were below the safety limits, the combined risks from MC-LR contamination of water and fish pose a possible health hazard to the residents and cannot be overlooked. Similarly, aside from possible health risks to humans, the presence of these toxins in Nile tilapia production ponds could negatively affect fish growth and productivity, and therefore, effective pond management should be adopted to minimize these problems in aquaculture.

Table 1. Mean levels of Microcystin-LR in water and fish.

Source	Microcystin-LR	
	Water (µg·L^{-1})	Fish (µg·kg^{-1} dw)
Phayao Lake	0.69±0.28	0.06±0.02
Fish ponds	0.22±0.09	1.22±0.48

5. CONCLUSIONS

The assessment on the levels of microcystins in water and Nile tilapia in Phayao lake caused by the presence of cyanobacteria i.e. *Microcystis*, due to nutrient enrichment (eutrophication) should, therefore, be of great concern. The communities normally use water from Phayao Lake as raw water for household consumption, agricultural and industrial use and the lake as source of food and livelihood for local fishermen. The present study has also suggested that *Microcystis* species and microcystins in cultivation ponds in Chiang Rai, may pose a possible hazard to aquatic organisms and to humans through the food web. Consumers that feed on fish from these aquaculture ponds may be at the risk of continuous microcystins poisoning. It is therefore recommended that fish farmers should develop management strategies to control cyanobacterial growth and minimize the incidence of bioaccumulation of microcystin in cultured fish.

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