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First Report on Microcystins Contamination in Giant Freshwater Prawn (*Macrobrachium rosenbergii*) and Nile Tilapia (*Tilapia nilotica*) Cultured in Earthen Ponds

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ABSTRACT

Phytoplankton including cyanobacterial blooms frequently occurred in aquaculture ponds. Some cyanobacteria produced toxins that may accumulate in the food web and eventually to aquaculture products. The aim of this study was to investigate the incidence of cyanobacteria and the contamination of microcystins in giant freshwater prawn (*Macrobrachium rosenbergii*) and Nile tilapia (*Tilapia nilotica*) cultured in earthen ponds. This study was carried out in green water system of 4 prawn and 6 fish ponds during April 2006 - February 2007. Cyanobacterial composition was identified by microscopic method and microcystins were analyzed by ELISA technique. It was shown that the amount of cyanobacteria especially *Microcystis aeruginosa* Kützing (n.d.-45,000 cells/L) and microcystins (n.d.-3.20 µg/kg d.w.) in the prawn ponds was higher than that in fish ponds (n.d.- 983 cells/L & n.d.-0.84 µg/kg d.w.). Both prawn and fish contained concentrations of microcystins close to or above the recommended limit for human consumption (0.04 µg/kg day TDI guidelines set by the WHO). This result implied that aquaculture products especially giant freshwater prawns cultured in earthen ponds with green water system are likely to be contaminated with microcystins. The finding is useful for aquaculture in term of food safety in Thailand.

Key Words: Microcystins; *Microcystis aeruginosa*

INTRODUCTION

The occurrence of cyanobacterial bloom creates a significant water quality problem (Srisuksomwong *et al*., 2011), as certain species of cyanobacteria are capable of producing toxins. Some members of genera such as *Microcystis*, *Anabaena*, *Nostoc* and *Aphanizomenon* produce a wide range of potent toxins including a group of hepatotoxins called microcystins in freshwater (Harada, 1996; Chorus & Bartram, 1999; Codd *et al*., 2005).

Microcystins can accumulate in the fish tissue (Tencalla *et al*., 1994; Magalhaes *et al.*, 2001; Soares *et al*., 2004), mussels (Vasconcelos, 1995; Amorim & Vasconcelos, 1999) and aquatic macrophytes (Pflugmacher *et al*., 1998). They also can be transfer from crab larva to salmons through food chain (Williams *et al*., 1997). Accumulation of these toxins in the fish could be a threat to human food safety (Magalhaes *et al.*, 2001). The World Health Organization (WHO) defined in the Drinking Water Guidelines for microcystin-LR in drinking water should not exceed 1 µg/L (WHO, 2006) and a provisional tolerable daily intake (TDI) of 0.04 µg/kg bw per day for microcystin-LR was proposed.

There are many aquaculture ponds throughout Thailand. In the traditional cultivation of Nile tilapia (*Tilapia nilotica*) and giant freshwater prawn (*Macrobrachium rosenbergii*), farmers usually establish green water by adding organic or inorganic fertilizer or loading of nutrients from fish waste to promote phytoplankton growth. These ponds typically experience cyanobacterial bloom (Whangchai *et al*., 2008). An occurrence of *Microcystis aeruginosa* and microcystins in prawn ponds had been studied by Prommana *et al.* (2006). They found high population of *M. aeruginosa* and 3.0-11.5 µg/L of microcystins in water. However, the accumulation of microcystins in prawn was not studied. Therefore, the incidence of toxic cyanobacteria especially *M. aeruginosa* and microcystins in aquatic farms were investigated. The data would be useful for food safety aspect and public health to avoid the damaging effect of cyanobacteria and their toxins.

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MATERIALS AND METHODS

Distribution of *Microcystis aeruginosa* and Microcystins in Prawn and Fish Ponds

Study sites: Ten aquaculture ponds (Table I) of similar size were randomly selected. Four prawn ponds were located in Thoeng District, Chiang Rai Province. Four fish ponds were located in Pan District, Chiang Rai Province and two other fish ponds were located at Sansai District, Chiang Mai Province. Sampling was carried out during April 2006 to February 2007.

Sampling of water, prawn and fish for analysis: Twenty liters of water samples from each pond were taken through plankton net (mesh size 10 µm). Phytoplankton were then collected and preserved by adding 0.7 mL of Lugol's solution to 100 mL of sample (Greenberg *et al.*, 1992). One liter of water sample from each pond was analyzed for microcystins. Three tilapias and giant freshwater prawns of marketable size were collected from each pond and stored in ice box during transportation.

Determination of some physico-chemical properties of water: Some water quality parameters, i.e. temperature, pH, conductivity, dissolved oxygen, nitrate-nitrogen, ammonium nitrogen and soluble reactive phosphorus were measured in the field and laboratory according to Greenberg *et al*. (1992).

Identification and counting of cyanobacteria: The identification of cyanobacterial species was carried out using related texts such as Komárek and Komáková-Legnerová (2002a & b) and Komárek and Anagnostidis (2005). *Microcystis aeruginosa* cells were counted with a haemacytometer and calculated as number of cells.ml^{-1}. Biovolume of cyanobacteria was evaluated according to Rott (1981).

Analysis of Microcystins

Extraction of microcystins: Microcystins were extracted after Kankaanpää *et al.* (2005) with modification. Fish and prawn tissues were dissected and freeze-dried (-20°C) before extraction and ELISA analysis. One mL of 100% methanol was added into 2-5 g fish and prawn tissues for extraction overnight. The extracts were centrifuged at 12,000 rpm for 30 min and the supernatants were concentrated to 150 µL with a heat block (50°C, overnight), and centrifuged at 12,000 rpm for 30 min before ELISA analysis.

Microcystin analysis by ELISA assay:ELISA Microcystin Plate Kit (Catalog No. EP022), ENVIROLOGIX INC© was used and performed in accordance with the manufacturer's instructions. A standard curve was constructed using three calibrators (0.16, 0.5 & 2.5 µg/L, respectively) supplied with the kit. The absorbance at 450 nm was measured with a microplate reader (Spectra MR, DYNEX Technologies). The microcystin concentration in each extract was expressed as MC-LR equivalent.

Data evaluation: The water quality was classified into trophic level according to the criteria of Wetzel (2001) Lorraine and Vollenweider (1981) and Peerapornpisal *et al*. (2007) by considering some physico-chemical parameters and dominant species of phytoplankton. The computer statistical package, SPSS for Windows version 10.0 was used to perform statistical analysis of the results.

RESULTS

Identification and enumeration of *M. aeruginosa* and phytoplankton: The dominant species of phytoplankton which were found in prawn and fish ponds were green algae, dinoflagellates and euglenoids, whereas cyanobacteria were found in lower amount in fish pond. High amount of *Microcystis* spp. especially, *Microcystis aeruginosa* Kützing was found in prawn ponds (Fig. 1). Only 36% of fish ponds which were found *M. aeruginosa* whereas, 84% of prawn ponds were contaminated with this cyanobacterium. Not only *M. aeruginosa* but also other microcystin producing cyanobacteria were found in this investigation. They were *Anabaena* sp., *Cylindrospermopsis curvispora* Watanabe, *M. wesenbergii* Komárek, *M. ichthyoblabe* Kützing, *M. flos-aquae* (Wittrock) Kirchner ex Forti and *Oscillatoria* sp. Six species of them were found in fish ponds, whereas five species of them were found in prawn ponds. Total biovolume (µm^3/L) of these cyanobacteria in prawn ponds water was higher than those in fish ponds water (Table II).

Analysis of microcystins: Total microcystins were analyzed by ELISA Microcystin Plate Kit. Both prawn and fish samples were found to be contaminated with microcystins at the concentration of n.d.-3.20 µg/kg d.w. and n.d.-0.84 µg/kg d.w., respectively (Fig. 2). The amount of microcystins in the prawn samples was higher than fish samples. Although it seemed to be correlated with cell counts of *M. aeruginosa*, there was no correlation between *Microcystis* cell number and microcystins in prawn and fish (r^2 = -0.0409).

Analysis of some physico-chemical properties of water: Water quality was classified to mesotrophic to meso-eutrophic status. It seemed that the water quality in prawn pond was slightly better than that in fish pond because the amount of nutrients in prawn pond was lower than in fish pond (Table III). However, the nutrients in both ponds decreased slightly in July 2006, because it was rainy season.

DISCUSSION

This study found that the highest amounts of *M. aeruginosa* were found in July 2006 but no relationships between the cyanobacterium and environmental parameters. Generally, either nitrogen or phosphorus is the limiting nutrient in aquatic systems. Enrichment of waters with one or both of these nutrients stimulates algal growth

(Oliver & Ganf, 2000). Cyanobacterial blooms often develop in the eutrophic lakes; it is assumed that they require high phosphorus and nitrogen concentrations, even though cyanobacterial blooms often occur when the concentration of dissolved phosphate is low (Mur *et al.*, 1999). However, research is on the correlation between nutrients, phytoplankton growth and relative abundance of cyanobacteria, did not find the relationship between cyanobacterial cells and nutrients (Oliver & Ganf, 2000). Similarly in this study, statistical analysis showed negative correlation between the occurrence of toxic cyanobacteria and water quality.

Table II: Biovolume (µm^3/L) of microcystin producing cyanobacteria which were found in prawn and fish ponds during April 2006 – February 2007

Sample Cyanobacteria	Giant freshwater prawn	Nile Tilapia
Anabaena sp.	41	8,408
Cylindrospermopsis curvispora	0	32,357
Microcsytis aeruginosa	858,801	10,795
M. wesenbergii	581,218	4,451
M. icthyoblabe	1,182	0
M. flos-aquae	3,548	0
Oscillatoria sp.	357,917	4,783
Total	1,799,159	60,794

Table III: Some physico-chemical properties of water in fish and prawn ponds

Parameters	Mean±S.E.	
	Giant freshwater prawn pond water (n=13)	Tilapia pond water (n=14)
pH	7.68±0.19	7.36±0.12
DO (mg/L)	5.61±0.53	5.89±0.21
Temperature (°C)	29.88±0.54	27.63±1.05
Conductivity (µS/cm)	369.75±34.07	197.31±14.23
NH_4-N (mg/L)	0.29±0.04	0.17±0.03
NO_3-N (mg/L)	0.61±0.12	0.91±0.15
PO_4-P (mg/L)	0.07±0.02	0.20±0.07

more than three weeks (Sivonen & Jones, 1999). Similar to Edwards *et al.* (2008) found that rate of degradation of microcystin-LR, LF and nodularin in water samples ranges from a half-life of four to eighteen days depending on the water body, climatic condition, and the concentration of dissolved microcystins and in some cases, the previous bloom history of a water reservoir. Similar to this study, the information from the prawn farm's owner shown that the bloom of *M. aeruginosa* had happened before.

Fig. 1: *Microcystis aeruginosa* count (cells/mL) in the fish (T) and prawn ponds (P) during 2006-2007

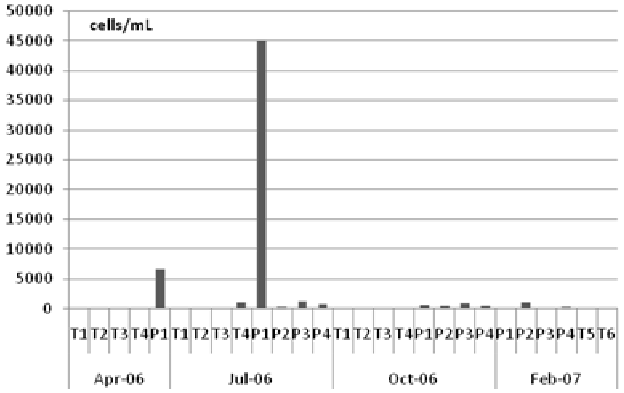

Fig. 2: Microcystins (µg/kg d.w.) in fish (T) and prawn (P) meat from various ponds during 2006-2007

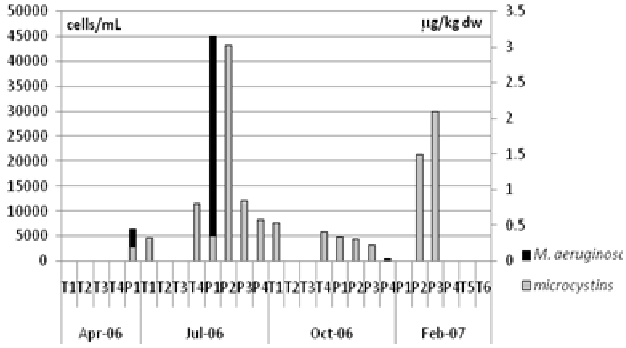

Table I: Location, size and type of feed of experimental ponds

Animals	Number of Ponds	Location	Pond size (m^2)	Type of feed
Giant freshwater prawn	4	Thoeng District, Chiang Rai (P1, P2, P3, P4)	4,800	Commercial feed
Nile tilapia	6	Pan District, Chiang Rai (T1, T2, T3, T4) and Sansai District, Chiang Mai (T5, T6)	3,200	Commercial feed

The amount of toxin in tilapia and prawn has been found to reach levels as high as 0.84 and 3.20 µg/kg, respectively so that in a typical meal an adult could be exposed to 1 and 75 of times the seasonal TDI. In the ponds that *M. aeruginosa* was formerly present; microcystins were detected in spite of no *Microcystis* cells were found at the sampling date. It means that if toxic *Microcystis* occurred in the water bodies, microcystins might be found. Microcystin molecules are found to be very stable (Falconer, 2005). Although microcystins are susceptible to breakdown by aquatic bacteria found naturally in river and reservoirs, degradation of microcystin can be as short as two days or

In conclusion, in this is the first report on contamination in prawn and fish samples from aquaculture ponds in Thailand. Therefore, regular monitoring of toxic cyanobacteria in aquaculture ponds should be carried out to prevent and solve the community health problems which may be caused by toxic cyanobacteria and their toxins. Pond management is essential for a productive aquaculture farm.

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