ANALYSIS OF TOXIC CYANOBACTERIAL ABUNDANCE IN SELECTED AQUACULTURE SYSTEMS AND ITS EFFECT ON *Oreochromis* spp.

ANN ANAK SINDEN

UNIVERSITI PENDIDIKAN SULTAN IDRIS

2016

ANALYSIS OF TOXIC CYANOBACTERIAL ABUNDANCE IN SELECTED AQUACULTURE SYSTEMS AND ITS EFFECT ON *Oreochromis* spp.

ANN ANAK SINDEN

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

THIS THESIS IS SUBMITTED IN FULFILLMENT OF THE REQUIREMENT
FOR THE DEGREE OF MASTER OF SCIENCE
(RESEARCH MODE)

FACULTY OF SCIENCE AND MATHEMATICS
UNIVERSITI PENDIDIKAN SULTAN IDRIS

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

2016

ABSTRACT

This study aims to analyse the abundance of toxic cyanobacteria in selected aquaculture systems and its effect on *Oreochromis* spp. In this study, a total of forty freshwater fish aquaculture ponds were sampled from ten different locations in Perak, Malaysia. To analyse the effects of naturally-occurring microcystins concentration in Perak aquaculture environments on fish, *Oreochromis* spp. fingerlings were cultured in water treated with cyanobacterial extracts in the laboratory. Study results revealed that the most commonly found cyanobacterial taxa in Perak aquaculture systems was *Microcystis* spp. During the sampling periods, the majority of the sampled ponds water were under cyanobacterial bloom and contained unsafe concentration of microcystins exceeding 20 µg/L. A combination of temperature and pH was correlated to the proliferation of cyanobacteria and its toxicity in the selected aquaculture ponds. Microcystins accumulated in fish tissues were dependent on the concentration of microcystins in the surrounding water. Despite high microcystins bioaccumulation, this study discovered that microcystin concentrations did not give impacts to the survival and growth of *Oreochromis* spp. These findings illustrated the potential health risk of toxic cyanobacteria through fish consumption in Malaysia which can be two to three orders of magnitude higher than the tolerable daily intake guideline (0.04 µg MC-LR / kg body weight per day) recommended by World Health Organization. In conclusion, the abundance of toxic cyanobacteria in Malaysia aquaculture systems may cause accumulation by fish at a harmful level. As an implication, this study can serve as a guide on the occurrence of toxic cyanobacteria in our freshwater systems particularly in aquaculture ponds, as well as its potential bioaccumulation in aquatic organisms which may lead to significant health threat to human through food web.

ANALISIS KELIMPAHAN ALGA BIRU-HIJAU TOKSIK DALAM SISTEM AKUAKULTUR TERPILIH DAN KESANNYA TERHADAP *Oreochromis* spp.

ABSTRAK

Kajian ini bertujuan menganalisis kelimpahan alga biru-hijau toksik dalam sistem akuakultur terpilih dan kesannya terhadap *Oreochromis* spp. Dalam kajian ini, sebanyak empat puluh buah kolam akuakultur ikan air tawar telah disampel daripada sepuluh lokasi yang berbeza di Perak, Malaysia. Bagi menganalisis kesan kepekatan semulajadi mikrosistin dalam persekitaran akuakultur di Perak terhadap ikan, benih *Oreochromis* spp. dikulturkan dalam air yang dirawat dengan ekstrak alga biru-hijau di makmal. Dapatan kajian menunjukkan bahawa taksa alga biru-hijau yang paling kerap ditemui dalam sistem akuakultur di Perak adalah *Microcystis* spp. Semasa tempoh persampelan, majoriti daripada air kolam tersebut berada di bawah paras *bloom* serta mengandungi kepekatan mikrosistin yang tidak selamat melebihi 20 µg/L. Gabungan suhu dan pH didapati bertindak sebagai pemboleh ubah alam sekitar utama yang mencetuskan percambahan alga biru-hijau, serta ketoksikan di dalam kolam akuakultur terpilih. Mikrosistin yang terkumpul dalam tisu ikan adalah bergantung kepada kepekatan mikrosistin yang berada dalam air di sekitarnya. Meskipun pengumpulan biologi mikrosistin yang tinggi, kajian ini mendapati bahawa kepekatan mikrosistin tidak memberi kesan kepada kelangsungan hidup serta pertumbuhan *Oreochromis* spp. Penemuan kajian ini menggambarkan potensi risiko kesihatan yang berkaitan dengan alga biru-hijau toksik melalui pengambilan ikan akuakultur di Malaysia yang berkemungkinan dua hingga tiga kuasa magnitud lebih tinggi daripada garis panduan pengambilan harian boleh diterima (0.04 µg MC-LR / kg berat badan per hari) yang disyorkan oleh Pertubuhan Kesihatan Sedunia. Kesimpulannya, kelimpahan alga biru-hijau dalam sistem akuakultur di Malaysia berkemungkinan menyebabkan pengumpulan oleh ikan pada tahap yang memudaratkan. Implikasinya, kajian ini boleh dijadikan panduan berkaitan kejadian alga biru-hijau toksik dalam sistem air tawar terutamanya dalam kolam akuakultur, serta potensi pengumpulan biologinya dalam organisma akuatik yang boleh membawa kepada ancaman kesihatan yang serius kepada manusia melalui rantai makanan.

CONTENTS

CHAPTER 3 METHODOLOGY

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
APHA	American Public Health Association
CB	Cyanobacterial biomass
chl-*a*	Chlorophyll-*a*
DO	Dissolved oxygen
DoF	Department of Fisheries
EDI	Estimated daily intake
EPA	United States Environmental Protection Agency
eq.	Equivalent
FAO	Food and Agriculture Organisation
F_T	Instantaneous chlorophyll fluorescence
GPS	Global Positioning System
HAB	Harmful Algal Bloom
HDPE	High-density polyethylene
HPLC	High performance liquid chromatography
IFRPC	Indigenous Fisheries Research and Production Centre
Ks	Half-saturation constants
MC	Microcystin
MC-LR	Microcystin-LR
MIB	2-Methylisoborneal
NAHRIM	National Hydraulic Research Institute of Malaysia

PDA	Photodiode array
RM	Ringgit Malaysia
rpm	Rotation per minute
r.u	Relative unit
SPE	Solid phase extraction
SRP	Soluble reactive phosphate
TDI	Tolerable Daily Intake
TFA	Trifluoroacetic acid
TN	Total nitrogen
TP	Total phosphorus
UNEP	United Nation Environmental Protection
USD	U.S. Dollar
UV	Ultra-violet
v/v	Volume per volume
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cyanobacteria, also known as blue-green algae are prokaryotic organisms possessing photosynthetic pigments and can proliferate in water bodies such as ponds, lakes, reservoirs and slow moving streams (Butler, Carlisle, Linville, & Washburn, 2009; Chorus & Bartram, 1999). Just like other phytoplanktons, cyanobacteria is part of the microbial community and acts as the primary producer for aquatic organisms (Palmeri, Barausse, & Erik, 2013). Other than being important in the aquatic food chain, cyanobacteria also assimilate ammonia as its nitrogen source for growth, hence minimising the accumulation of this toxic compound in water systems (Paerl & Tucker, 1995).

In aquaculture ponds, large amount of nitrogen is introduced into the water systems as most of the manufactured fish pellets contain about 32 to 45% of protein (Pandey, 2013). Nearby human activities such as rapid urbanisation, industrialisation and intensifying agriculture also contributing to nutrient inputs into the aquaculture water bodies and result in eutrophication (Yang, Wu, Hao, & He, 2008).

Eutrophication is an excessive nutrient enrichment in water bodies (Kaufman & Franz, 2000). Phosphorus and nitrogen that are available in human sewage and livestock excrement, as well as synthetic fertilisers are believed to be the main contributors to eutrophication (Schindler, 2012). Eutrophication has been considered as a rapidly growing environmental crisis in freshwater and marine systems worldwide (Selman & Greenhalgh, 2009). According to United Nation Environmental Protection (UNEP), about 30 to 40% of lakes and water reservoirs all over the world have been affected by eutrophication (Yang et al., 2008). Eutrophication is also a critical issue in Malaysia. The preliminary desktop study on the status of lake eutrophication in Malaysia indicated that more than 60% of the lakes reviewed out of 90 lakes in Malaysia were eutrophicated (National Hydraulic Research Institute of Malaysia [NAHRIM], 2005; Zati & Salmah, 2008).

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

Eutrophic water body causes excessive growth of phytoplanktons which usually leading to the dominance of cyanobacteria (Havens, 2008). The dominance of cyanobacteria over other phytoplanktons in water bodies are mainly due to its buoyancy characteristic that enable this species to compete for nutrients (Bellinger & Sigee, 2010). The overgrowth of cyanobacteria disturbs the natural balance of the

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

aquatic ecosystem and ultimately result in cyanobacterial bloom (Selman & Greenhalgh, 2009).

Cyanobacterial bloom is a common issue in aquaculture industry (Rodgers, 2008). This phenomenon causes depletion of oxygen in water column of aquaculture ponds leading to mortality of aquatic species (Snyder, Goodwin, & Freeman, 2002), a condition known as hypoxia (“Health and Ecological Effects,” 2015). Cyanobacterial bloom can cause severe economic losses (Landsberg, 2002; Rodgers, 2008). Preliminary study conducted in United States revealed that the country lost more than USD 40 millions per year and at least USD 1 billion per decade due to harmful algal blooms (HABs) in aquaculture sector (Landsberg, 2002; Rodgers, 2008). In Malaysia, lossess of not less than RM 20 millions were reported in relation to massive fish kills at finfish farms in Penang due to prolonged HAB event from 2005 to 2006 (Sin Chew Daily, 2005 as cited in Lim, Gires, & Leaw, 2012).

Some species of cyanobacteria such as *Oscillatoria* spp., *Anabaena* spp., and *Microcystis* spp are capable of synthesising two highly odorous compounds called geosmin and 2-methylisoborneal (MIB) that can cause earthy-musty taste on fish (Paerl & Tucker, 1995; Tucker, 2000; Schrader & Dennis, 2005; Zhong et al., 2011). Despite being non-toxic to human, these compounds are nuisance to the public (“Health and Ecological Effects,” 2015) as they can alter the natural taste of aquatic products. The production of off-flavour compounds are more common in freshwater aquaculture systems as compared to marine and brackish water due to acceptable salinity range as well as nutrients abundance (Paerl & Tucker, 1995). This problem usually adds about 10 to 20% to the production cost of aquaculture practise (Keenum

& Waldrop., 1988; Paerl & Tucker, 1995) which can sum up to USD 60 millions annually (Tucker, 2000).

In addition, several species of cyanobacteria are also capable to produce secondary metabolites known as cyanotoxin. Cyanotoxin can be classified into three categories based on the mode of action: hepatotoxin, neurotoxin and dermatotoxin (Rodgers, 2008). Among all, microcystin is the most commonly found cyanobacterial toxin in freshwater system (Poste, Hecky, & Guildford, 2011; Schmidt et al., 2013).

Microcystin falls under the group of hepatotoxin and its contamination in aquaculture industry has long been reported in many past literatures (Barros, de Souza, Tavares, & Amaral, 2010; Peng et al., 2010). This toxin enters fish body via the gills, diet and food chain (Poste et al., 2011; Schmidt et al., 2013), destroys the liver tissues and leads to fish death (Hudnell, 2008; Schmidt et al., 2013). Besides, microcystin can also accumulate in fish tissues and pose health risk to human through fish consumption (Peng et al., 2010; Poste et al., 2011).

Malaysia is located in tropical region with an average temperature of 26 to 28 °C throughout the year (Malaysian Meteorological Department, 2015). Hot climate in this country is expected to induce the growth of cyanobacteria and promotes the persistence of toxic blooms (Ferrão-Filho & Kozlowsky-Suzuki, 2011). Hence, there is a possibility for the toxic cyanobacterial biomass to be present in majority of water bodies in Malaysia (Sinang, 2012b) including the aquaculture systems.

1.2 Problem Statements

Malaysia produces large amount of fish through aquaculture practices (Department of Fisheries Malaysia [DoF Malaysia], 2013a) in order to cope with the demand of increasing human population (The World Bank, 2015). As mentioned earlier, excessive growth of cyanobacteria disturbs the water quality of aquaculture ponds and leads to fish death (Zimba, Khoo, Gaunt, Brittain, & Carmichael, 2001; Jewel, Affan, & Khan, 2003). The survived fish, however, may have accumulated cyanotoxin, particularly microcystin which can be dangerous enough to pose health threat to human (Peng et al., 2010; Poste et al., 2011 ; Schmidt et al., 2013).

Due to the potential health risk of microcystin contamination, World Health Organisation (WHO) has established the provisional guidelines for Microcystin-LR which are 1.0 µg/L for drinking water and 0.04 µg/kg body weight per day for tolerable daily intake (TDI) (Chorus & Bartram, 1999). However, the information on the risk associated with the consumption of aquatic products from eutrophicated water system is still lacking (Peng et al., 2010). Most of the previous studies conducted on toxin accumulation in aquatic species focused on toxicological concern with the key objectives of determining the target organ of cyanotoxin (Peng et al., 2010; Zhang, Xie, Liu, & Qiu, 2009).

In Malaysia, a number of research has been conducted on cyanobacteria, however, the assessment of this noxious species in our freshwater aquaculture system is still limited. The evaluation of cyanobacteria compositions in Sarawak aquaculture systems reported the presence of *Anabaena* spp., *Chamaesiphon* spp., *Lynbya* spp., *Microcystis* spp., *Oscillatoria* spp., and *Spirulina* spp. (Mohd. Nasarudin & Ruhana, 2011b; Ramlah, 2005 as cited in Mohd. Nasarudin & Ruhana, 2011b). Among all of the detected genera, *Microcystis* spp., the primary producer of hepatotoxic microcystin (Mioni et al., 2011), was found at the most abundant in earth aquaculture ponds (Mohd. Nasarudin & Ruhana, 2011b). Since cyanobacteria community varies on spatial scales (Sinang, 2012a), more research is needed to assess the types of cyanobacteria present in water column of aquaculture especially the occurrence of potentially toxic strains.

Additionaly, the toxicity of cyanobacteria in Malaysia freshwater system is also rarely studied. Sinang et al. (2015) reported the presence of microcystins in all of the water samples collected from freshwater lakes in Selangor. Connecting that fact, it is possible that microcystin is present in Malaysia aquaculture system. Jasmina, Samsur and Ruhana (2010) assessed the toxicity of cyanobacteria, however, the study was only carried out on the laboratory-cultured sample. Due to limited study on cyanobacterial toxicity particularly the assessment of environmental sample, more research focusing on cyanobacterial toxin in actual aquaculture system is urgently needed.

Since there is lack of scientific studies on cyanobacterial bloom and cyanobacterial toxicity carried out in Malaysia (Lim, Leaw, & Usup, 2003; Sinang et al., 2015), it is not exaggerating to say that our present knowledge on the potential health risk of cyanobacterial toxin especially on aquatic products is still inadequate. To address the issue, this study was undertaken to establish a profile for cyanobacterial diversity, abundance, and toxicity in selected fish aquaculture systems in Perak, Malaysia. Apart from that, this study also aimed to investigate the bioaccumulation of microcystin on Red Tilapia (*Oreochromis sp.*) tissues and its impact on the survival and growth of fish.

1.3 Research Questions

This study was carried out based on research questions as below:

1. Does potentially toxic cyanobacterial genera present in the selected Perak aquaculture systems?
2. How abundant is the occurrence of toxic cyanobacteria in water column of aquaculture ponds in terms of biomass and microcystin produced?
3. What triggers the occurrence of toxic cyanobacterial bloom in aquaculture ponds?
4. Does microcystin bioaccumulation in fish tissues affects the survival and growth of fish?

1.4 Research Objectives

The research aimed to investigate the presence and abundance of toxic cyanobacteria in aquaculture systems, as well as its toxic accumulation in fish tissues. In more specific, this study aimed to:

1. Determine the presence of potentially toxic cyanobacterial genera in the selected Perak aquaculture systems.

2. Quantify the cyanobacterial biomass and microcystin concentration available in water body of aquaculture ponds.
3. Identify the main trigger of toxic cyanobacterial bloom in aquaculture ponds in Perak.
4. Analyse the bioaccumulation of microcystin in *Oreochromis* spp. tissues and its effect on fish survival and growth.

1.5 Significance of Study

Since there is a lack of cyanobacteria research in Malaysia, this study is important to enhance our present knowledge on the occurrence of toxic cyanobacteria, particularly in Malaysia aquaculture systems. This research is also essential for public health risk protection. Through microcystin bioaccumulation experiment, this study revealed how dangerous is the naturally-occurring microcystin concentration available in our aquaculture water, especially to the Red tilapia (*Oreochromis* spp.) consumers. Understanding of the relationships between cyanobacterial biomass, microcystin production, and environmental parameters, as well as the information on the potential microcystin accumulation in aquatic products, will assist fish farmers in aquaculture monitoring. The application of these knowledges certainly will give positive impacts to both fish yield and the farmer's income.

1.6 Scopes and Limitations of Study

This study was carried out within the scopes and limitations as below:

1. Water samples were collected from commercialised freshwater fish aquaculture ponds in Perak, Malaysia.
2. Potentially toxic cyanobacteria identified were those that capable of producing microcystin according to Sivonen & Jones (1999) namely *Microcystis* spp., *Anabaena* spp., *Anabaenopsis* spp., *Oscillatoria* spp., *Planktothrix* spp., and *Nostoc* spp.
3. Total chlorophyll-*a* was used as a proxy to estimate the cyanobacterial biomass.
4. Selected environmental parameters were temperature, pH, dissolved oxygen, nitrate, phosphate, nitrite, magnesium, calcium, and ammonium.
5. Microcystin is the only cyanotoxin quantified in this study and the concentration was expressed in microcystin-LR (MC-LR) equivalents.
6. Microcystin concentrations quantified from the aquaculture water samples were only extracted from the intracellular cells.
7. The cultured fishes were exposed to the microcystin through immersion technique and the accumulation was studied only on *Oreochromis* spp. tissues. The source of microcystin was from crude cyanobacterial extracts dominant with *Microcystis* spp.