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Study of Blue-Green Algae and Assessment of the Microcystin in Shrimp Aquaculture Farms in Sarawak

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ABSTRACT

Blue-green algae blooms can cause severe water quality deterioration including scum formation and toxin production. A total of 17 shrimp farms in Sarawak were assessed from February to July 2018 for the abundance of blue-green algae (cyanobacteria) and the levels of microcystin in the tissue of shrimps using enzyme-linked immunosorbent assay (ELISA). There was a high cell count of *Microcystis* sp. at 6.77 x 10^8 cells/ L in Muara Tebas, *Anabaena* sp. at 4.99 x 10^7 cells / L in Telaga Air and *Pseudanabaena* sp. at 1.69 x 10^8 cells/ L in Kuala Baram. Microcystin was detected in most of the shrimp samples collected from the 17 farms in Sarawak throughout the study. The highest level of microcystin was 0.448 ppb, which was detected in Selabat whereas a value below 0.15 ppb was detected in Bandar Baru Semariang, Santubong and Oya. This study demonstrated that microcystin was detected in aquaculture samples collected from shrimp farms in Sarawak. It is, therefore, necessary to further conduct an investigation on blue-green algae in shrimp farms and methods to control their growth.

Keywords: Aquaculture, blue green algae, ELISA, microcystin, shrimp

INTRODUCTION

The shrimp aquaculture is the most important aquaculture species cultured in the world. The annual production of white shrimps in Malaysia was 35,648.04 metric tonnes in 2017 valued at RM 911,568,000 of which the state of Sarawak contributed 3,196.07 metric tonnes (Department of Fisheries Statistics, 2017). There are 657 shrimp's aquaculture ponds in Sarawak.

Blue-green algae, scientifically known as cyanobacteria are microscopic single-celled organisms that grow naturally in fresh and salt waters. They are not algae (eukaryotes) but are a type of bacteria (prokaryotes). Blue-green algae are named after the blue pigment phycocyanin. Therefore, they act like plants by using sunlight to manufacture carbohydrates from carbon dioxide and water, a process known as photosynthesis. They are present in most water bodies. Blue-green algae has vesicles or gas pockets inside vacuoles within their cells that they inflate with gas, thus are able to regulate their buoyancy in response to environmental conditions. This is an advantage over other algae as they have the ability to sink and rise at their will and move to where nutrient and light levels are at their highest.

Blue-green algae blooms can cause severe water quality deterioration including scum formation, toxin production, hypoxia, foul odours and tastes (Anderson *et al*., 1993; Chorus & Bartram, 1999; Paerl & Huisman, 2009: Guzman-Guillen *et al*., 2013). Sometimes they grow to large populations known as blooms which are mostly harmful due to the fact that certain species are capable of producing toxins (Ye *et al*., 2009, Qiao *et al*., 2013). Microcystins are among the most common and dangerous cyanotoxins (Singh *et al*., 2012), produced by some cyanobacteria genera, including *Microcystis*, *Anabaena* and *Planktothrix* (DeFigueiredo *et al*., 2004). Microcystin enters the fish body through gills, diet and food chain (Poste, 2011; Schmidt, 2013), destroys the liver tissues and causes fish death (Falconer, 2008). Besides, this cyanotoxin also can accumulate in fish/shrimp tissues and pose a health risk to the human when consumed (Peng, 2010). In addition, some cyanobacteria are also capable to synthesise two highly odorous compounds called geosmin and 2-methylisoborneal (MIB) that can cause earthy-musty taste on fish (Wnorowski, 1992; Tucker, 2000). Blue-green algae are also important as a potential resource for renewable energy and natural products (Beck *et al*., 2012).

The majority of the microcystin producing blooms are dominated by *Microcystis* (Dai *et al*., 2008; Xu *et al*., 2008). These cyanotoxins (hepatotoxins and neurotoxins) have been the cause of liver failure and even death (Carmichael *et al*., 2001) as well as chronic effects in humans that are subjected even to low-level exposure (Chorus *et al*., 2001; Sivonen & Jones, 1999). The production of cyanotoxins in blue-green algae is the primary concern because it can pose lethal and sub-lethal effects in both humans, animals, and fishes (Landsberg, 2002; Sangolkar *et al*., 2009; Chen *et al*., 2014). Toxic blue-green algae poisonings have been reported in animals such as birds, cattle, and sheep (Carmichael *et al*., 2001) and have caused over 350 cases of suspected or confirmed poisonings or deaths in the U.S. between the 1920s and 2012 (Backer *et al*., 2013).

Excessive growth of blue-green algae is a common concern in aquaculture ponds (Rodgers, 2008). This phenomenon also causes depletion of oxygen level in the water column of aquaculture ponds and subsequently leads to the mortality of aquatic species (Boyd, 1998). Harmful algal blooms (HABs) in the aquaculture industry can cause serious economic losses.

A preliminary study on the effect of the bloom to the United States economy reported that the country lost more than USD 40 million per year and at least USD 1 billion per decade (Landsberg, 2002). In order to minimise the unnecessary losses in the aquaculture sector, an effective approach for aquaculture monitoring must be developed. To tackle this issue, it is very important to draw attention to the root cause which is the environment where the aquatic organisms thrive.

The occurrence of blue-green algae blooms is becoming more frequent worldwide. In recent years, the incidence has increased globally in frequency, severity, and duration (Chorus & Bartram, 1999). The succession of toxic cyanobacterial species and fluctuation in biomass, which is influenced by seasonal changes in various environmental factors including nutrients, grazing, light and temperature, is believed to affect the concentration of microcystin in the field (Janse *et al*., 2005; Gobler *et al*., 2007).

Hence, the research on water quality dynamics and practical management of water quality problems in aquaculture ponds is very important and has provided tangible benefits to fish and shrimp producers (Tucker *et al*., 2008).

Studies evaluating the diversity and dynamics of blue-green algae have recently been conducted in Japan (Kazuhiro *et al*., 2006; Mitsuhiro *et al*., 2007), Finland (Vaitomaa *et al*., 2003), Germany (Kurmayer *et al*., 2003), Canada and the United States (Rinta- Kanto & Wilhelm, 2006; Gobler *et al*., 2007). Currently, more than 65 countries worldwide including Thailand, Vietnam, Philippine, and Singapore have recorded the detection of toxic cyanobacteria in the water environment, with Malaysia confirming presence of toxic blue-green algae mostly in lakes in 2015 (Sinang *et al*., 2015; Mohamad *et al*., 2016) and toxin-producing *Microcystis* successfully isolated from Ayer Itam reservoir, Penang by Sim Yi Jing (Sim, 2015). In Malaysia, there is very little monitoring and assessment of these blooms in the aquaculture industry. Previous studies were conducted on freshwater fish only by Nasarudin & Ruhana (2007; 2011) and Mosleh *et al*., (2011). There is no study about the levels of microcystin and species of blue-green algae in shrimp aquaculture farms in Sarawak.

Therefore, this study was conducted to determine the abundance of blue-green algae in shrimp aquaculture farms in Sarawak. This study also aims to identify the relationship between environmental factors (e.g. temperature, pH and nutrients) with the relative abundance of blue-green algae in shrimp aquaculture farms by assessing their relationships in 17 different locations. This research is crucial for aquaculture monitoring, particularly in the Malaysia aquaculture system. Besides, this study is also important for public health risk protection to ensure safe fish supply to be delivered to consumers.

MATERIALS AND METHODS

Location and Description of Aquaculture Farms

Seventeen aquaculture farms in Sarawak, Malaysia were selected in this study. The farms were located in Bandar Baru Semariang, Santubong, Selabat, Muara Tebas, Rambungan, Telaga Air, Selumit, Mukah, Oya, Kuala Baram and Sempadi (Figure 1). All of the chosen aquaculture ponds comprised of earth ponds in use for shrimp production business. The water source was obtained from nearby natural water bodies.

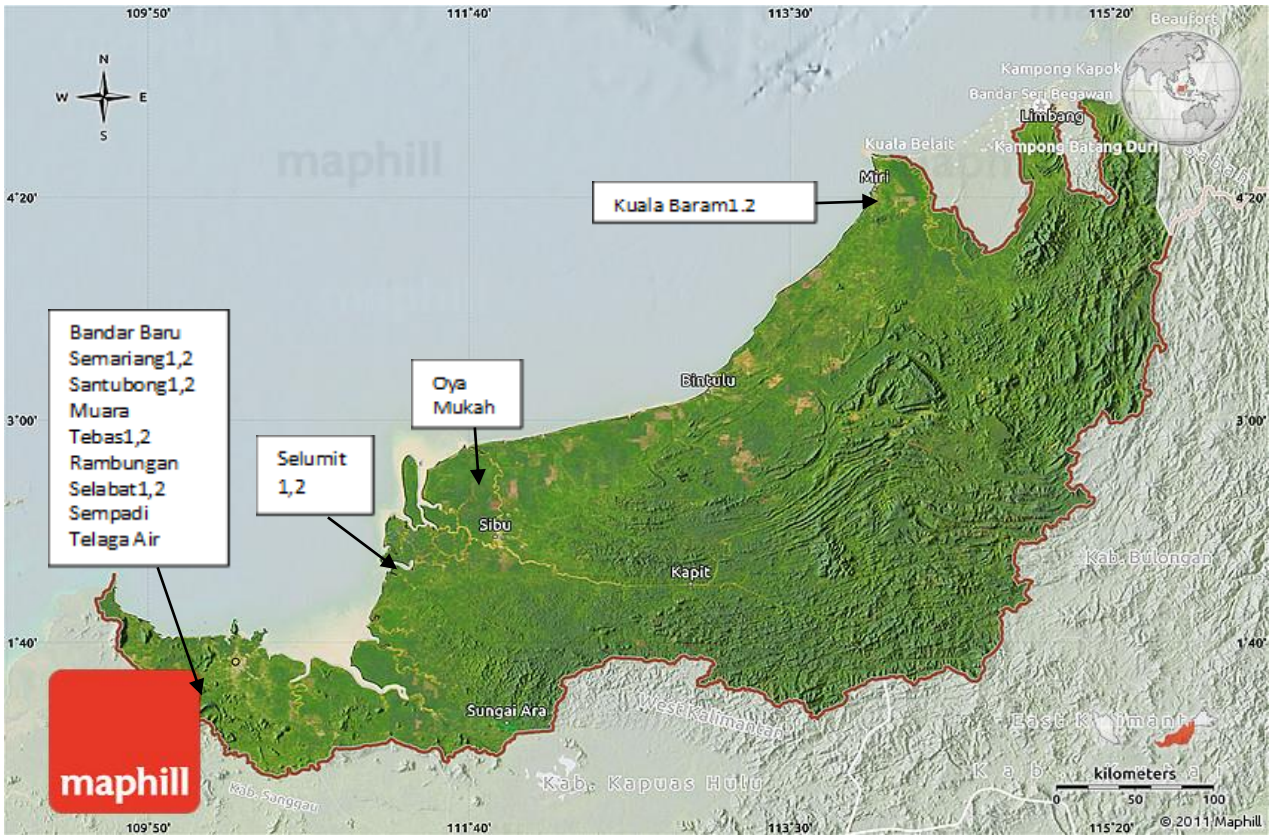

Figure 1. Map of the location of 17 shrimp aquaculture ponds in Sarawak

Water Sampling

Sampling was carried out in each aquaculture farms between February to July 2018. For each farm, a total of three areas of the pond were sampled. Water temperature, dissolved oxygen (DO) and pH were measured on-site with a portable probe (Horiba) at a depth of 0.5 m from the water surface. The water sample was then grabbed from 0.15 m below the water surface. Approximately 100 mL of the grabbed water sample was placed and stored immediately into High-Density Polyethylene (HDPE) bottle. The sample bottles were placed in a cooler container containing ice in order to maintain the freshness of the sample as well as to protect the samples from sunlight. Water samples were brought back to the laboratory for subsequent analysis.

Nutrients Analysis

Nutrients were analysed within two days after the sample collection (Jackson, 2000). Four types of nutrients were quantified in this study: nitrate, phosphate, nitrite, and ammonia. Water samples were pre-filtered through 0.45 µm membrane filter prior to analysis. Ultra-pure water (Sartorius Stedim Biotech) was used throughout this study and ion analysis was carried out according to Jackson (2000). The results were compared against ion standards. The concentration of phosphate, nitrate, nitrite, and ammonia were also determined using the HACH 2800 spectrophotometer. For the measurement of phosphate, nitrate, nitrite, and ammonia in the water, the samples were filtered through 0.45-mm-pore size filters (Millipore, USA).

Microcystin Analysis

For the analysis of microcystin, samples were collected on GF/F (pore size, 0.7 mm; Whatman) filters. The analysis of the microcystin was done following the methods and procedures of the Abraxis ELISA kit. Microcystin concentration was measured and screened using the ELISA reader. Only positive results above 1 ppb (Minimum Permissible level, MPL 1 ppb) were confirmed using High-Performance Liquid Chromatography (HPLC) (Hitachi, Japan).

Total Blue-Green Algae Abundance

The total blue-green algae in each sample were determined by direct microscopical count method

using the Inverted Light Microscope (Olympus, Japan IX70). The cell counting microscopic slide used known as Sedgewick-Rafter chamber. Both morphotypes are capable of producing structure visible to the naked eye, such as pinhead or larger, spherical, or irregular colonies (*Microcystis* sp.) and bundles of filaments (*Anabaena* sp.) like sawdust in shape and size. The species of blue-green algae were identified following the description and photos shown in the reference book entitled Marine Phytoplankton of the Western Pacific.

RESULTS

Occurrence and Abundance of Blue-green algae Biomass in Aquaculture Systems

In this study, blue-green algae were observed in the aquaculture farms during the period of the year which is warm. The studied blue-green algae were dominated by three species: *Microcystis* sp., *Anabaena* sp. and *Pseudanabaena* sp. In this study, most of the ponds, 16/17 farms, (94%) were experiencing blue-green algae as the cell count was above the level of 0.3×10^3 cells/L. As for the toxic blue-green algae, *Microcystis* sp., only four farms (23.5%) were positive. The highest concentration was detected in a pond in Muara Tebas which was 6.77×10^8 cells/L, followed by Santubong 1 (4.49×10^7 cells/L) and Santubong 2 (1.69×10^7 cells/L). The lowest biomass of *Microcystis* sp.was observed in Sempadi at 4.525×10^6 cells/L. Most of the sampled ponds (9/17 farms), 52.9% were having *Anabaena* sp. of concentrations above 0.3×10^3 cells/L (Table 1). The highest abundance of *Anabaena* sp. was found in Telaga Air at 4.99×10^7 cells/L whereas the lowest abundance of 0.486×10^3 cells/L in Selumit. No *Anabaena* sp was found in Selabat, Muara Tebas, Rambungan, Selumit 1, Oya, Muara Tebas 2 and Sempadi. The occurrence of *Pseudanabaena* sp. were found in all the farms except 3 farms, (14/17 farms), 82.3%. The highest cell count was found in Kuala Baram at 1.69×10^8 cells/L while the lowest cell count of 0.302×10^3 cells/L was found in Oya. No *Pseudanabaena* sp. was discovered in three farms in Rambungan, Santubong and Sempadi.

Physical Characteristics of the Study Aquaculture Ponds

Physical characteristics of water samples varied between locations and ponds as shown by the physical water quality chart (Figure 2). The temperature ranged between 25.4 to 31.2 °C and most ponds were having temperature below 31 °C. Water temperature between 25 to 32 °C was reported to be optimum for the growth of shrimp species (Boyd, 1998; MWQS-DOE, 2019). The water pH during sampling ranged from 6.62 to 10.21. Aquaculture water with pH 7.00 to 9.00 is categorised as an ideal pH for the growth of fish as well as crustaceans while pH 9 to 11 can cause slow development to aquatic species (Boyd, 1998; MWQS-DOE, 2019). The turbidity of the water parameter in the aquaculture water ranged from 5 to 391 mg/L in this study. The salinity recorded was 5.2 ppt to 30.1 ppt.

Nutrient Analysis of the Shrimp Aquaculture Farms

Among all of the analysed dissolved inorganic nutrients in the water samples collected, nitrate were in the range of 0.3-3.5 mg/L which is within the desired concentration (0.2-10 mg/L) (Boyd, 1998; MWQS-DOE, 2019). Nitrite concentration was recorded at between 0.003-0.532 mg/L and in the preferred concentration of less than 1 mg/L. Some of the collected water samples have recorded ammonia (0–3.6 mg/L) and phosphate (0.05–1.44 mg/L), which are within the acceptable ranges as shown by the water nutrient analysis chart (Figure 3).

Relationship Between Physical Parameters of Water with Blue-Green Algae Biomass

A significant correlation ($p<0.05$) was found between temperature and pH with blue-green algae biomass (Table 2). However, there is no relationship detected between any of the nutrients with blue-green algae intensity in the water column. Besides, there was a low level of phosphate, nitrate, nitrite and ammonia analysed in the water sample.

Detection of Microcystin

Microcystin was detected in the tissue of the shrimps in most of the farms 12/17 (75%) as shown in Figure 4. The highest level of microcystin was detected in Selabat which was 0.448 ppb, followed by Kuala Baram (0.346 ppb) and Telaga Air (0.265 ppb). The lowest level of below 0.15 ppb was observed in Bandar Baru Semariang, Santubong 1;2 and Oya. However, no microcystin (5/17 farms, 29%) were detected in Rambungan, Mukah, Muara Tebas, Kuala Baram and Selumit.

Table 1. Table of cyanobacterial biomass in the 17 shrimp aquaculture ponds of Sarawak

Cyanobacteria / Locations	*Anabaena* sp. (cell/L)	*Pseudanabaena* sp. (cell/L)	*Microcystis* sp. (cell/L)
Bandar Baru Semariang	0	$5.52x10^{6}$	0
Santubong	$1.50x10^{3}$	$7.86x10^{5}$	$4.49x10^{7}$
Selabat	0	$3.32x10^{7}$	0
Muara Tebas	0	$4.07x10^{7}$	$6.77x10^{8}$
Rambungan	0	0	0
Telaga Air	$4.99x10^{7}$	$5.35x10^{7}$	0
Selumit	0	$2.8x10^{5}$	0
Mukah	$8.05x10^{3}$	$2.533x10^{3}$	0
Oya	0	$0.302x10^{3}$	0
Kuala Baram	$6.63x10^{6}$	$1.69x10^{8}$	0
Bandar Baru Semariang2	$5.15x10^{6}$	$2.66x10^{7}$	0
Santubong2	$9.53x10^{5}$	0	$1.69x10^{7}$
Selabat2	$7.08x10^{4}$	$6.208x10^{7}$	0
Muara Tebas2	0	$5.8x10^{4}$	0
Sempadi	0	0	$4.525x10^{6}$
Selumit2	$0.486x10^{3}$	$9.325x10^{3}$	0
Kuala Baram2	$2.95x10^{3}$	$1.083x10^{4}$	0

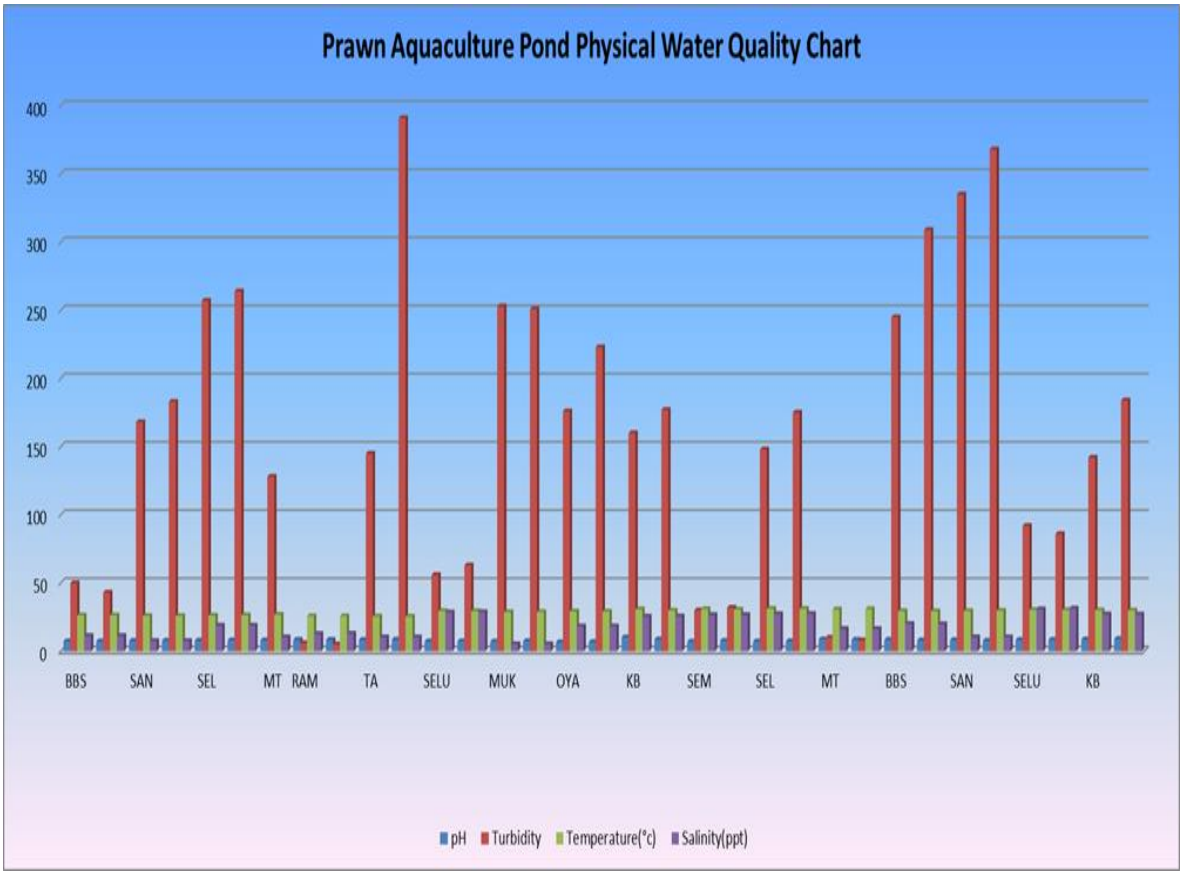

Figure 2 Physical water quality chart of the 17 shrimp aquaculture ponds of Sarawak.

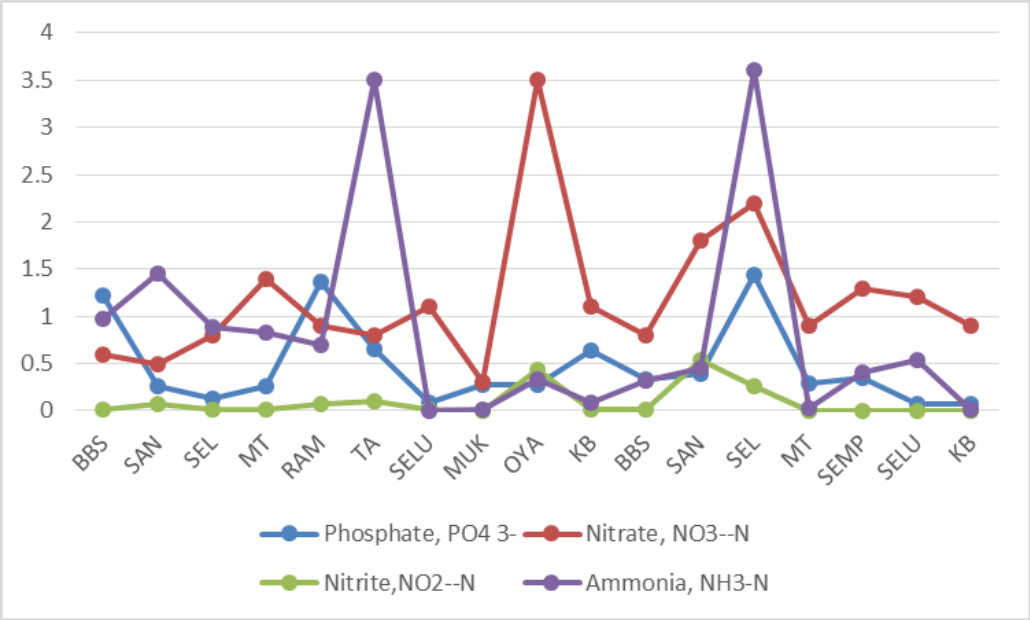

Figure 3 Water nutrient analysis chart of the 17 shrimp aquaculture farms

Table 2. Cyanobacterial biomass in the 17 shrimp aquaculture ponds of Sarawak

Parameter	Cyanobacteria Biomass
Temperature	0.419*
pH	0.426*
Salinity	-0.112
Turbidity	-0.145
Nutrients	
Nitrate	-0.239
Nitrite	-0.126
Phosphate	-0.138
Ammonia	-0.118

Note: *p<0.05; “p<0.01; - = no correlation

DISCUSSION

The blue-green algae were able to thrive when the conditions of temperature, light, and nutrient status are conducive and therefore the water surface host increased growth of blue-green algae.

The water temperature in all sampled farms was quite high, and most of them were around 30 °C during the sampling period. Most species of cyanobacteria attained optimum growth rate within the temperature range of 25 to 35 °C (Kumar *et al*., 2011; Lürling *et al*., 2012). Other studies reported that some species of cyanobacteria such as *Microcystis* sp. can out-compete other phytoplanktons at 30 °C and above (Fujimoto *et al*., 1997). This suggests that cyanobacteria may be able to thrive to a certain extent of high temperature in aquaculture farms. A similar finding was also reported by a research conducted in Penang whereby a significant correlation was observed between cyanobacterial cell density with temperature when the range of temperature was between 27.10 to 32.30 °C (Nasarudin & Ruhana, 2007). A similar result was also reported by a research conducted on *Tor tambroides* ponds in in Serian, Sarawak (Nasarudin & Ruhana, 2007).

Water pH was detected to have a positive correlation with cyanobacterial biomass in this study. Cyanobacteria activity and intensity in the water column were reported to be influenced by the water pH (You *et al*., 2007). During intense increasing population, photosynthetic activities of phytoplanktons increase and cause depletion of free carbon dioxide. This subsequently leads to an increase in pH value which favours the dominance of cyanobacteria (Dokulil & Teubner, 2000).

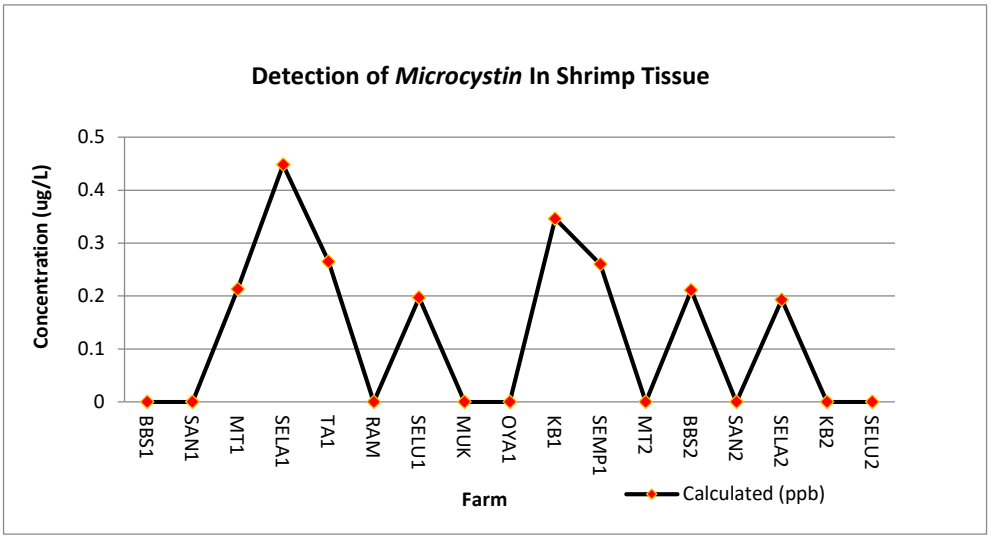

Figure 4 Detection of microcystin in shrimp tissue in 17 shrimp aquaculture farms of Sarawak

Many scientific literatures stated that cyanobacterial proliferation is closely related to nutrient concentration in water bodies (Chorus & Bartram, 1999). This phenomenon was also reported in aquaculture ponds (Kankaanpää *et al*., 2005). Therefore, many literatures stated that cyanobacterial abundance in aquaculture ponds was affected by a combination of multiple environmental factors and nutrients. In this study, we found the four water nutrients and physical parameters such as temperature and pH allowed the growth of blue-green algae proliferation in aquaculture ponds as well as its toxicity. All the four nutrients analysed were within their permitted levels in the water samples. The main reason for the analysed nutrient to be in the permitted levels could probably be due to minimal input of phosphorus into the aquaculture water body since the concentration of phosphorus in most fish feeds are relatively quite low. Phosphorus is one of the 20 inorganic minerals which comprised about 1.0-2.5% of the fish diet (Pandey, 2013). Nitrite concentration in aquaculture water was also reported to be very low and the acceptable range is below 1 mg/L. A similar result was obtained and compared with the water analysis conducted in freshwater fish (*Tor tombroides*) ponds in Sarawak which detected between 0.001 to 0.007 mg/L nitrite in the water sample throughout the study period and reported that this parameter has no significant relationship with both cyanobacteria cell density in aquaculture water body (Nasarudin & Ruhana, 2007).

To gain a better understanding of the potential toxin-producing blue-green algae, ELISA was performed to screen the blue-green algae population. In this study, we found the presence of microcystin at a level of less than 1 ppb [Maximum Acceptable Value, MAV of 1 ppb in Ministry of Health] in the tissue of the shrimps. Therefore, oral exposure route is one of the important routes for fish or shrimps accumulation of cyanotoxins (Ernst *et al*., 2001) through the food web. Accumulation of cyanotoxins in fish through the food web could bring a potential threat to human food safety. Harmful cyanobacterial blooms and cyanobacterial toxins (cyanotoxins) have occurred in many eutrophicated freshwaters including lakes, reservoirs and aquaculture ponds around the world (Paerl & Huisman, 2009). The blooms of these cyanobacteria and their cyanotoxins are harmful to aquatic biota (Landsberg, 2002). Cyanotoxins can also accumulate in fish tissues via direct feeding on phytoplankton or uptaking of dissolved toxins through epithelium (gills, skin) (Ibelings & Chorus, 2007). In the aquatic environment, the concentration of microcystin exists in surface water during periods of cyanobacterial blooms. In humans, microcystins have been identified for the first time in the serum (average 0.228 ng/mL) of a chronically exposed human population (fishermen at Lake Chaohu, China), together with an indication of hepatocellular damage (Chen *et al*., 2009).

There have been public health concerns about

the occurrence of cyanotoxins in aquaculture farms in many countries. The hazard to fish populations has only recently been taken into consideration with the increasing occurrence of blooms and a better knowledge of the cyanotoxins needs to be studied further (Stone & Bress, 2007).

CONCLUSION

The results of this study provided an important baseline for the contamination status of the aquatic environment in Sarawak's aquaculture industries and the presence of blue-green algae species. Their presence must be avoided as they lead to the production of microcystins which are threats to human food safety. It is important to educate the farmers and aquaculturists on the risks and consequences associated with microcystins in the shrimps.

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