INFLUENCE OF TEMPERATURE AND RAINFALL ON CYANOBACTERIA ABUNDANCE AND MICROCYSTINS PERSISTENCE IN THE SELECTED PUBLIC WATER SUPPLY SYSTEM

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ABSTRACT

Cyanobacterial blooms pose increasing threats to water safety due to their ability to produce toxic secondary metabolites such as microcystins. This study aimed to assess the abundance of cyanobacteria and the occurrence of microcystins contamination in Malaysia's water supply system, particularly under local climatic conditions. The Sungai Labu Off-River Storage (ORS) was selected as the study site to investigate the presence of microcystin-producing cyanobacteria and their correlation with environmental factors. Cyanobacterial abundance was determined using photosynthetic pigment measurements (chlorophyll-a and phycocyanin) via portable sensors, and molecular identification was conducted using quantitative polymerase chain reaction (qPCR) targeting the *mcy*E gene. Microcystins concentrations were quantified using Enzyme-Linked Immunosorbent Assay (ELISA). The relationship between variables was analysed using Pearson correlation, and an artificial neural network (ANN) model was developed to predict microcystins levels based on biological and climatic data. Findings revealed that the highest cyanobacterial abundance and microcystins levels were recorded at 83,521.33 cells/mL and 2.629 µg/L, respectively at the reservoir (Point 2), where *Microcystis* spp. dominated. Microcystins were consistently detected across all water supply points, including post-treatment stages, though remaining below 1.0 µg/L. Phycocyanin showed a significant correlation with both *mcy*E gene copies (r = 0.553, p<0.05) and microcystins concentration (r = 0.548, p<0.01), while weekly rainfall significantly influenced phycocyanin (r = 0.471, p<0.05) and *Microcystis* abundance (r = 0.438, p<0.05). The ANN model for Point 2 demonstrated high predictive accuracy for microcystins concentrations with R^2=0.894. This study confirms the spatial persistence of cyanotoxins within the water supply chain, even after treatment processes. Phycocyanin proved to be a reliable indicator of measuring toxic cyanobacterial abundance under tropical climates. This study demonstrates how ANN model and phycocyanin pigment sensors assist water authorities in predicting microcystin contamination risks, improving early warning systems and optimizing treatment strategies against cyanotoxin contamination.

PENGARUH SUHU DAN HUJAN TERHADAP KELIMPAHAN SIANOBAKTERIA DAN KETERUSAN MIKROSISTIN DALAM SISTEM BEKALAN AIR AWAM TERPILIH

ABSTRAK

Pertumbuhan berlebihan sianobakteria semakin mengancam keselamatan air kerana keupayaannya menghasilkan metabolit sekunder toksik seperti mikrosistin. Kajian ini bertujuan menilai kelimpahan sianobakteria dan kejadian pencemaran mikrosistin dalam sistem bekalan air Malaysia, khususnya dalam keadaan iklim tempatan. Kolam takungan hiliran (ORS) Sungai Labu telah dipilih sebagai tapak kajian untuk mengenal pasti kehadiran sianobakteria pengeluar mikrosistin dan hubungannya dengan faktor persekitaran. Kelimpahan sianobakteria ditentukan menggunakan pengukuran pigmen fotosintetik (klorofil-*a* dan fikosianin) melalui sensor mudah alih, manakala pengesanan molekul dilakukan melalui reaksi berantai polimerase kuantitatif (qPCR) terhadap gen *mcy*E. Kepekatan mikrosistin dianalisis menggunakan *Enzyme-Linked Immunosorbent Assay* (ELISA). Hubungan antara parameter dianalisis menggunakan korelasi Pearson dan model rangkaian neural buatan (ANN) dibangunkan bagi meramal kepekatan mikrosistin berdasarkan data biologi dan iklim. Dapatan menunjukkan kelimpahan sianobakteria dan kepekatan mikrosistin tertinggi masing-masing direkodkan pada 83,521.33 cells/mL dan 2.629 µg/L, di kolam takungan (Poin 2) yang didominasi oleh *Microcystis* spp. Mikrosistin dikesan secara konsisten di semua lokasi rangkaian bekalan air termasuk selepas proses rawatan, namun kekal di bawah paras 1.0 µg/L. Fikosianin menunjukkan korelasi signifikan dengan salinan gen *mcy*E (r = 0.553, p<0.05) dan kepekatan mikrosistin (r = 0.548, p<0.01), manakala taburan hujan mingguan mempengaruhi fikosianin (r = 0.471, p<0.05) dan kelimpahan *Microcystis* (r = 0.438, p<0.05). Model ANN bagi Poin 2 menunjukkan ketepatan yang tinggi dalam meramal kepekatan mikrosistin dengan R^2=0.894. Kajian ini membuktikan kehadiran mikrosistin secara berterusan dalam sistem bekalan air walaupun selepas proses rawatan. Fikosianin dikenalpasti sebagai penunjuk yang boleh dipercayai bagi mengukur kelimpahan toksik sianobakteria dalam iklim tropika. Kajian ini menunjukkan bagaimana model ANN dan sensor pigmen fikosianin membantu pihak pengurusan air meramalkan risiko pencemaran mikrosistin, menambah baik sistem amaran awal dan mengoptimumkan strategi rawatan terhadap pencemaran sianotoksin.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

32P	Phosphorus-32
Adda	Constituent C_{20}β-amino acid in microcystins and nodularins
AI	Artificial Intelligence
ALF	Alert Level Framework
ANN	Artificial Neural Network
ARIMA	Autoregressive integrated moving average
ASM	Akademi Sains Malaysia
BLAST	Basic Local Alignment Search Tool
Chl-*a*	Chlorophyll-*a*
Ct	Threshold cycle in qPCR
D-Glu	D-Glutamate
DAF	Dissolved air flotation
DBPs	Disinfection byproducts
DNA	Deoxyribonucleic acid
DWTP	Drinking water treatment plant
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
GAC	Granular activated carbon
GV	Guideline value

HAAs	Haloacetic acids
HOCl	Hypochlorous acid
KLIA	Kuala Lumpur International Airport
KMAM	Drinking Water Quality Surveillance Programme
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LUAS	Lembaga Urus Air Selangor
MC-Htyr	Microcystin-homotyrosine
MC-LR	Microcystin- leucine arginine
MC-XZ	Microcystins variants with abbreviation
mcy	Microcystin
*mcy*A-J	Genes encoding microcystin synthetase subunits
MC	Microcystin
MOH	Ministry of Health Malaysia
NAFLD	Non-alcoholic fatty liver disease
NAHRIM	National Water Research Institute of Malaysia
NCBI	National Centre for Biotechnology Information
NPRS	Non-ribosomal peptide synthetase
NWQS	National Water Quality Standard
OATPs	Organic anion-transporting polypeptides
ORS	Off-river storage
PAC	Powdered activated carbon
PAR	Photosynthetically active radiation
PB	Phosphate buffers
PCR	Polymerase chain reaction
PKS	Polyketide synthase

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PPIA	Protein phosphatase inhibition assay
PROTRCH	Phytoplankton Responses to Environmental Change
qPCR	Quantitative real-time PCR
R^2	Coefficient of determination
RE	Relative error
RMSE	Root mean squared error
sp./spp.	Species
SPSS	Statistical Package for Social Sciences
SSE	Sum of squares error
THMs	Trihalomethanes
TP	Total phosphorus
TSI	Trophic state index
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
WHO	World Health Organization
WN	When necessary

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

This chapter provides an overview of this study. This chapter elaborates on the background of this study, problem statement, research objectives, research significance, and limitations of this study.

1.1 Research background

Water is a necessary resource to sustain life and the environment. Two major sources of water are groundwater and surface water. Water bodies that form above the ground are considered as surface water, which includes creeks, rivers, lakes, reservoirs, wetlands, streams, and the ocean (Mgbenu & Egbueri, 2019). Most of the surface water provides ecosystem services including irrigation, energy production, recreational activities, fishery and as well as drinking water sources (Sterner et al., 2020).

In Malaysia, surface water is considered as the most reliable water source compared to groundwater due to its accessibility. Therefore, Malaysia is primarily dependent on surface water from rivers, reservoirs, and lakes for safe and clean water supply to residential areas, industries, and agriculture fields (Hossain & Mahmud, 2019).

Water quality is very crucial particularly for public health, aquatic ecosystems, and other purposes including drinking, recreation, and agriculture. On a larger scale, particularly in the economically developing countries, substantial population and economic growth lead to an increase in water demand, a decline in water supplies, and an increase in water pollution (Boretti & Rosa, 2019). Anthropogenic activities resulting in the release of household wastes and sewage, industrial wastes, oil, and agricultural wastes are known as major causes of water quality degradation (Saravanan et al., 2021). In Malaysia for instance, water pollution is significantly related to patterns of land uses. It has been reported that up to 87% of urban land for development is a major source of water pollution due to runoff and soil erosion (Camara, Jamil, & Abdullah, 2019). Meanwhile, 82% of agricultural land and 77% of degraded forest land contribute to water pollution physically and chemically (Camara et al., 2019). Recent progression in the severity of water pollution will certainly harm humans, animals, and other life beings.

Water pollutants can be classified into organic and inorganic pollutants, biological pollutants, and radioactive pollutants (Sharma, Sauraj, & Negi, 2020). Accumulation of pollutants in the water bodies, especially lakes, rivers and reservoirs, will degrade water quality. Since lakes, rivers and reservoirs are sources of clean water, it’s crucial to identify water pollutants that are present before supplying it to the consumers. Biological pollutants refer to the existence of microorganisms including algae, bacteria, viruses, or protozoan in the water bodies. Several of these microorganisms produce toxins or metabolites which are harmful to humans and aquatic organisms. For instance, the abundance of algae such as cyanobacteria (blue-green algae) in the freshwater ecosystems will negatively impact on human's health and the aquatic environment due to its ability to produce cyanotoxins (Zhang et al., 2022; Metcalf & Codd, 2020).

Cyanobacteria are a diverse group of prokaryotic microorganisms that are commonly present in water ecosystems. Excessive cyanobacterial proliferation in the freshwater ecosystems is commonly associated with environmental factors. Three primary environmental factors that predominantly regulate cyanobacterial growth are high temperature, excellent light, and nutrient enrichment (Napiórkowska-Krzebietke, Kalinowska, Bogacka-Kapusta, Stawecki, & Traczuk, 2021). The optimum temperature for cyanobacteria to grow is 25℃ and above whereby at these temperatures, cyanobacteria are able to grow even in the minimum light just enough to support photosynthesis (Guo et al., 2023). On top of that, increase of nitrogen and phosphorus in aquatic ecosystems lead to eutrophication in lakes, rivers, and estuaries. High nitrogen loading and nitrogen to phosphorus ratio might change the distribution of cyanobacterial species in water bodies Huisman et al. (2018). For instance, the domination of *Microcystis aeruginosa* in cyanobacterial bloom is highly correlated with additional of ammonium, meanwhile, *Planktothrix agardhii* dominated blooms in presence of nitrate, urea, and ammonium (Yang et al., 2023; Chaffin & Bridgeman, 2014).

Other climatic factors such as precipitation also indirectly contribute to high cyanobacterial proliferation. Water temperature, light availability and nutrient loads in water bodies are influenced by local weather and hydrologic circumstances (Vinçon-Leite & Casenave, 2019). Kitan and Sinang (2020) had reported the correlation of local seasonal rainfall in Slim River Lake, Malaysia with water temperature which can trigger the dominance and diversity of cyanobacteria species. Consequently, high intensity of rainfall accelerates the nutrients loading into the water bodies as a result of surface runoff within the watershed (Yao, Dai, Gao, Gan, & Yi, 2021). Thus, cyanobacteria will proliferate in water bodies due to high concentration of nutrients in water bodies during the heavy rainfall.

In relation to water safety issues, cyanobacteria are capable of producing toxigenic pollutants known as cyanotoxins. In freshwater ecosystems, cyanotoxins are produced by several cyanobacteria genera including *Microcystis*, *Planktothrix*, *Anabaena*, *Nostoc*, *Dolichospermum*, *Leptolyngbya*, *Phormidium*, and *Geitlerinema* (Du et al., 2019; Salmaso et al., 2016). The cyclic heptapeptide microcystins is the most common and well known cyanotoxins compared to anatoxins and saxitoxins and it is synthesized non-ribosomally. To date, more than 279 microcystins congeners have been reported and one of them, MC-LR has been classified as the most frequent and potent variant that is carcinogenic to humans (Bouaïcha et al., 2019).

Microcystins contamination in humans is well-known through oral exposure in which by consuming contaminated drinking water, inhaling aerosolized toxins, and direct contact with polluted water (Lad et al., 2022). Several observable symptoms may be initiated when humans are exposed to microcystins including vomiting, diarrhea, abdominal discomfort, liver failure, and fatal in severe cases (Annadotter et al., 2001; Carmichael, 2001; Pouria et al., 1998). Hepatotoxic microcystins have been linked to the occurrence of liver related diseases. Microcystins are known to promote liver tumors which lead to liver failure in humans due to inhibition of protein phosphatase 1 and 2 A (PP1 and PP2A) activity (Shi et al., 2021; Jia et al., 2019; He et al., 2018; Li et al., 2017). The inhibitory effect of microcystins will activate the cell proliferation pathway and thus promote the excessive proliferation of hepatocytes (Shi et al., 2021). In addition to its carcinogenic effects on liver, microcystins have been reported to affect cardiovascular system, reproductive system, respiratory system, renal system, nervous system, digestive system, and excretory system based on laboratory animal and in vitro experiments (Xu et al., 2021; Xu et al., 2020; Cao, Massey, Feng, & Yang, 2019; Yan, Li, Li, & Zhao, 2017; Zhou, Xu, Yu, & Yu, 2017; Wang et al., 2016).

Consuming drinking water contaminated with microcystins will have an impact on human health. The World Health Organization (WHO) has established a guideline for drinking water quality for water safety in support of public health due to potential of water contamination by microcystins especially in drinking water. The maximum value of microcystins in drinking water has been set to 1.0 µg/L by the Ministry of Health Malaysia following the recommendation from WHO (Ministry of Health Malaysia, 2004). In Malaysia, this parameter is not regularly monitored in the drinking water sources by water authorities. Other water parameters including physical and chemical properties are regularly monitored, however, microcystins concentration is only monitored when it is necessary. Therefore, it is essential to investigate the presence of hepatotoxic microcystins in water supply systems before being distributed to the end users. Moreover, it is also significant to understand the dynamics of cyanobacteria and microcystin occurrence in water supply sources as it is crucial for water safety management and public health protection.

1.2 Problem statements

Two years of lockdown due to COVID-19 negatively impacts the world's economic and social disruption. However, this pandemic also affects the environment, specifically water supply from the reservoirs. There is a significant correlation between algae blooming in reservoirs due to COVID-19 lockdown as reported by Alcantara et al. (2021). High concentrations of chlorophyll-*a* and phycocyanin was detected in Brazil's reservoirs due to high inflows of wastewater from the residential area surrounding the watersheds. Similarly, ORS Sungai Labu experienced cyanobacterial blooms due to closure of water treatment plant during the pandemic. Massive cyanobacterial blooms in reservoirs will affect the odor and taste of drinking water (WHO, 2015). However, the most concerning issue on cyanobacterial blooms is the presence of microcystins in the water supply system.

Under the specific favourable environmental conditions including nutrient enrichment, high temperature, light availability, and variability of rainfall intensity (Napiórkowska-Krzebietke et al., 2021; Yao et al., 2021), the cyanobacterial species has high chances to proliferate rapidly and form high biomass concentrations, known as blooms. These environmental factors are further intensified by the impacts of climate change. The impacts of high cyanobacterial abundance in water bodies contribute to a degradation in the water quality in which a noticeable green colour mat forming on the water surface and foul odours emitted from the blooms. The foul odours emit as an earthy and musty odour in the water bodies which are produced by certain cyanobacterial species such as *Planktothrix* spp. and *Pseudanabaena* spp., in which the compounds are known as geosmin and 2-methylisoborneol (Manganelli, Testai, Tazart, Scardala, & Codd, 2023; Su et al., 2023). Importantly, the secondary metabolites synthesize by cyanobacteria, known as cyanotoxins, are recognized as an emerging health hazard due to its negative impacts on human health as well as aquatic environments (Metcalf & Codd, 2020).

Hepatotoxic microcystins is the most common toxin found in contaminated water due to cyanobacterial blooms. Reports on cyanobacteria blooms in the water supply reservoirs have been extensively published worldwide including Brazil (Alcantara et al., 2021), Australia (Golshan et al., 2020), Africa (Habtemariam, Kifle, Leta, Beekman, & Lürling, 2021), Ghana (Addico, Hardege, Kohoutek, Degraft-Johnson, & Babica, 2017) and United Kingdom (Cantwell, 2021). In Malaysia, only few studies have reported on cyanobacterial occurrence in the water supply sources. Omar et al. (2017) had reported the dominance of cyanobacteria species in Semberong Barat Dam, Johor which contributes to high reading of chlorophyll-*a*. High concentrations of chlorophyll-*a* recorded was 50 µg/L which is greater than the acceptable limit by WHO (2003). Rohaslinda also reported the presence of cyanobacteria in Air Itam Dam, Penang in 2017. Even so, no reports on the biomass quantification were recorded. These local studies indicate the potential risk of cyanotoxin contamination in our water supply system. Though there have been several reports on cyanobacteria contamination in local water supply systems, to date there is no data reported on quantification of microcystins in water supply sources.

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After all, microcystins parameters should be listed as a parameter that requires regular monitoring in raw and treated water in order to understand the principle of cyanobacteria and microcystins dynamics in our water supply system. At present, the temporal dynamics of cyanobacteria abundance and microcystins concentration in water supply systems are largely neglected. The unavailability of data on the dynamics of cyanobacteria abundance in water supply sources and microcystins concentration in the water supply system in Malaysia needs in-depth study to ensure raw water and treated water supply is safe to be consumed.

Various factors influence cyanobacteria blooms in water bodies as reported in previous studies including nutrients (nitrogen and phosphorus) and climatic factors (rainfall and temperature) (A Rahman et al., 2022; Ho & Michalak, 2020; Reichwaldt & Ghadouani, 2012). However, the role of environmental variables tends to be site-specific (Sinang, Reichwaldt, & Ghadouani, 2013). The significant factors that influence cyanobacteria and microcystins dynamics are temperature and rainfall as described by Zhang et al. (2017) and Lehman, Kurobe, & Teh (2020). However, these factors are not being determined under local climatic conditions. Hence, these knowledge gaps become challenges in determining the risk of microcystins contamination and cyanobacterial abundance in Malaysia's water supply system due to local climatic factors.

Malaysia experiences tropical climate following its annual rainfall which is 80% and its climate is influenced by the winds blowing from the Indian Ocean and the South China Sea (Department of Information Malaysia, 2016). This variability of local temperature and rainfall highly influences the formation of cyanobacterial bloom in water supply sources. A statistical model will be developed to evaluate the persistence of microcystins in water supply system based on local climatic factors (temperature and rainfall) and cyanobacteria abundance which can predict the period of likely high risk of microcystin contamination in the water supply system using readily available temperature and rainfall data. In this study, a new finding will be generated from the local data set. A statistical model will be developed to facilitate the risk assessment for microcystins contamination in our water supply system.

1.3 Research objectives

The purpose of this study is to determine the influence of climatic factors on abundance of cyanobacteria producing microcystins in Off-River Storage Sungai Labu (ORS Sungai Labu). Using the molecular and statistical analysis approaches, the current status of cyanobacterial abundance and microcystins concentrations in ORS Sungai Labu and its distribution network can be assured to accommodate the following objectives:

1. To examine the spatial and temporal variabilities of cyanobacterial abundance in ORS Sungai Labu.
2. To quantify microcystins concentrations at various points of the water supply system.
3. To evaluate the influence of cyanobacterial abundance dynamics in ORS Sungai Labu with microcystins concentrations at various points of the water supply system.

4. To correlate the variability of temperature and rainfall with cyanobacterial abundance in ORS Sungai Labu.
5. To develop a statistical model in predicting microcystins persistence at various water sampling points based on temperature, rainfall, and cyanobacterial abundance in ORS Sungai Labu.

The questions to be addressed in this study are:

1. Do cyanobacterial abundances in ORS Sungai Labu vary on spatial and temporal basis?
2. What are the levels of microcystins concentrations at various points of the water supply system?
3. What is the relationship between the dynamics of cyanobacterial abundance and microcystins concentrations at various points in ORS Sungai Labu?
4. How does the diversification of temperature and rainfall influence cyanobacterial abundance in the reservoir?
5. How to predict microcystins persistence at various water sampling points based on temperature, rainfall, and cyanobacteria abundance in the water supply system?

The hypotheses of this study include:

1. The high abundance of cyanobacteria in the ORS Sungai Labu reservoir significantly contributes to elevated microcystin concentrations throughout the water supply system.

2. Elevated temperature and increased rainfall are positively associated with higher cyanobacterial abundance and enhanced persistence of microcystins in the water supply system.

1.4 Significance of the study

The significance of this study lies in the investigation of microcystin presence at various points of the water supply system in ORS Sungai Labu, Selangor. This study will be the first report on detecting microcystins in Malaysia's reservoir. In fact, the Malaysian population is dependent on clean water which is distributed from the reservoirs; hence, it is important to ensure water supply from the reservoir is safe to be consumed. In this study, rainfall and temperature are the main factors that influence the abundance of cyanobacteria in reservoirs. Thus, this study will provide the fundamental knowledge to better understand how Malaysia's climate will impact the cyanobacteria abundance and microcystins persistent in the water supply system.

Furthermore, a statistical model will be developed to interpret the data of rainfall, temperature, and cyanobacteria abundance in association with microcystin persistence. It is crucial to develop a predictive model particularly in the context of global warming. Elevated temperatures, shifts in precipitation patterns, and more frequent extreme weather events associated with global warming are likely to intensify the proliferation and toxin production of cyanobacteria. Therefore, a reliable predictive model can serve as a valuable tool for forecasting bloom occurrences and support timely intervention and water quality monitoring.

In summary, this research will be beneficial to society through safe drinking and water supply assurance. The statistical model developed from this study will eventually benefit the authority notably water operators and water industry authority in managing and operating water supply systems. Precautionary measures can be taken beforehand to avoid any contaminants to the water supply which could negatively impact on human health.

1.5 Limitations of the study

This study highlighted several limitations. Firstly, the sampling area is limited to Sepang district where ORS Sungai Labu is located with the duration of sampling is 12 months. In this study, ORS Sungai Labu in Sepang, Selangor was selected by Lembaga Urus Air Selangor (LUAS) authority for water sampling location. This reservoir supplies water specifically to Kuala Lumpur International Airports (KLIA) as well as nearby areas and this studied ORS was reported to have algal blooms proliferation.

Besides, this study is only focusing on the *mcy*E gene which is responsible for microcystins production. Based on previous studies, other cyanotoxins including anatoxins, saxitoxins, and cylindrospermopsis are also contributing to water contamination which has adverse impact on human's health. On top of that, the scope of this study only emphasizes on the most toxigenic microcystins variants, which is microcystin-LR (MC-LR). To date, 279 variants of microcystins have been reported, however, MC-LR is the most potent in term of its acute toxicity.

This study also limits cyanobacteria species producing microcystins which are *Microcystis* spp., *Anabaena* spp., and *Planktothrix* spp. Though these species are well known as microcystins producers, other species such as *Nostoc* spp. and *Oscillatoria* spp. too have the ability to produce microcystins based on previous studies. Other limitation is nutrients availability such as nitrogen and phosphorus which was not assessed in this study. Nutrient enrichment is known in contributing to cyanobacterial blooms. Since this reservoir is assumed in a relative stable or controlled nutrient level where nutrient is regularly monitored, the nutrient inputs might not significantly vary. Therefore, this study focuses specifically on the climatic variables to better understand how future climate change will impact the risk of cyanobacterial bloom even when nutrient inputs are managed. Even so, future study can integrate nutrient availability to fully capture all the key environmental drivers influencing cyanobacterial dynamics of the studied reservoir.