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INFLUENCE OF RAINFALL, AIR TEMPERATURE, PHYSICAL AND CHEMICAL WATER PROFILES ON CYANOBACTERIA BIOVOLUME IN SLIM RIVER LAKE, PERAK, PENINSULAR MALAYSIA

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ABSTRACT

This study aimed to identify the influence of rainfall, air temperature, physical and chemical water profiles on cyanobacteria biovolume in Slim River Lake, Perak, Peninsular Malaysia. Water samples were collected twice per month for 12 months in five sampling stations (S1-S5) to represent the lake's overall condition. Samplings were consistently conducted at the same time in the morning to reduce the influence of surrounding air temperature variations. The physical, chemical, and biological parameters were analysed according to American Public Health Association (APHA) standard methods. Rainfall and air temperature data were obtained from the Malaysian Meteorological Department. Data were successfully collected and the results indicated that Slim River Lake's physical, chemical, and biological water profiles were highly variable on a temporal scale. Total phosphorus, total nitrogen, chemical oxygen demand, chlorophyll-*a* and biovolumes of *Microcystis* spp. were recorded as 0.20-1.96 mg/L, 1.0-4.2 mg/L, 9.50-82.0 mg/L, 2.08-132.05 µg/L, 6.13×10^{6}- 1.65×10^{8} μm^3 /mL, respectively. These parameters were recorded at levels exceeding the recommended limit values by National Lake Water Quality Criteria and Standards (NLWQS). Temporal fluctuations in physical, chemical, and biological profiles cannot be generalized into similar patterns. Rainfall and air temperature ranged as 36.3-642.5mm and 30.71-35.0^{o}C, respectively. Rainfall and air temperature explained up to 72.3% and 77.3% variation in lake water quality. Multivariate Principal Component Analysis (PCA) suggested that 71.5% of chlorophyll-*a* variation and 70.2% of *Microcystis* spp. biovolumes variation can be explained by changes in rainfall, air temperature, physical and chemical water parameters. In conclusion, this study successfully showed that the physical, chemical and biological profiles in the Slim River Lake are variable and influenced by the variability of local rainfall and air temperature. Moreover, parameters included in the PCA analysis can largely explain temporal changes of cyanobacteria biovolume. In implication, the study outputs are useful for lake quality assessment and rehabilitation strategy formulation to mitigate the impact of changes in physical, chemical and biological profiles.

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PENGARUH HUJAN, SUHU UDARA, PROFIL FIZIKAL DAN KIMIA AIR TERHADAP ISIPADU BIO SIANOBAKTERIA DI TASIK SLIM RIVER, PERAK, SEMENANJUNG MALAYSIA

ABSTRAK

Kajian ini bertujuan untuk mengenal pasti pengaruh hujan, suhu udara, profil fizikal dan kimia air terhadap isipadu bio sianobakteria di Tasik Slim River, Perak, Semenanjung Malaysia. Sampel air dikutip dua kali sebulan selama 12 bulan di lima stesen pensampelan (S1-S5) bagi mewakili keadaan keseluruhan tasik. Persampelan dijalankan secara konsisten pada masa yang sama pada waktu pagi untuk mengurangkan pengaruh variasi suhu udara di sekeliling. Parameter fizikal, kimia dan biologi telah dianalisis mengikut kaedah standard Persatuan Kesihatan Awam Amerika (APHA). Data hujan dan suhu udara diperoleh daripada Jabatan Meteorologi Malaysia. Data telah berjaya dikutip dan hasilnya menunjukkan bahawa profil fizikal, kimia dan biologi air Tasik Slim River berubah-ubah pada skala temporal. Jumlah fosforus, jumlah nitrogen, permintaan oksigen kimia, klorofil-*a* dan isipadu bio *Microcystis* spp. direkodkan masing-masing sebagai 0.20-1.96 mg/L, 1.0-4.2 mg/L, 9.50-82.0 mg/L, 2.08-132.05 µg/L, 6.13×10^{6}- 1.65×10^{8} μm^3 /mL. Parameter ini direkodkan pada tahap melebihi nilai had yang disyorkan oleh Kriteria dan Piawaian Kualiti Air Tasik Kebangsaan (NLWQS). Perubahan profil fizikal, kimia dan biologi pada skala temporal tidak boleh digeneralisasikan kepada corak yang serupa. Hujan dan suhu udara masing-masing berjulat 36.3-642.5mm dan 30.71-35.0^{o}C. Hujan dan suhu udara menjelaskan sehingga 72.3% dan 77.3% variasi dalam kualiti air tasik. Analisis multivariat *Principal Component Analysis (PCA)* mencadangkan bahawa 71.5% variasi klorofil-*a* dan 70.2% variasi isipadu bio *Microcystis* spp. boleh dijelaskan oleh perubahan hujan, suhu udara, parameter fizikal dan kimia air. Kesimpulannya, kajian ini berjaya menunjukkan bahawa profil fizikal, kimia dan biologi di Tasik Slim River adalah berubah-ubah dan dipengaruhi oleh faktor hujan dan suhu udara. Tambahan pula, parameter dalam analisis PCA sebahagian besarnya boleh menjelaskan perubahan temporal isipadu bio sianobakteria. Implikasinya, hasil kajian ini berguna untuk penilaian kualiti tasik dan penghasilan strategi pemulihan untuk mengurangkan kesan perubahan dalam profil fizikal, kimia dan biologi.

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LIST OF ABBREVIATION

ANOVA	Analysis of Variance
GLM	Generalised Linear Multivariate
GLM-AED	General Lake Multivariate- Aquatic Eco - Dynamics
BRT	Boosted Regression Tree (Method)
EPA	Environmental Protection Agency
SPSS	Statistical Package for the Social Sciences
IR LED	Infrared Light Emitting Diode
MPF	Maximum Principle Flux
MSL	Mean Sea Level
NGS	Next Generation Sequencing
NIS	Nikon Imaging Software
NLWQS	National Lake Water Quality Standards
PCA	Principle Component Analysis
SE	Standard Error
TLI	Trophic Level Index
TSI	Trophic State Index
UKM	University Kebangsaan Malaysia
USA	United States of America

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USEPA United States Environmental Protection Agency

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CHAPTER 1

INTRODUCTION

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1.1 Study Background

Lake is a confined water body completely encircled by land with no immediate connection to the sea except by a stream or river that sustains or drains the lake (Luo et al., 2020). In addition to wetlands and ponds, lakes are lenticular or standing water system with a long water retention period and complex population (Huang et al., 2015). Lakes are categorized into natural lakes and human made lakes (Huang et al., 2015). Lake has various services,

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including water source, drainage, food supply, flood control, hydroelectricity generation, transportation, recreational, and eco-tourism (Huang et al., 2015; Kertész et al., 2019). To date, deterioration of the lake's water quality has been an alarming problem in most developing tropical countries. Lakes receive various pollutants, primarily from land use changes, domestic activities, and farming (Kertész et al., 2019). Due to the detrimental impact on environmental, economic, and health aspects, lake degradation has been a significant concern recently and was addressed as a serious issue both locally and globally.

Agricultural intensity and dramatic changes to biogeochemical cycles have profoundly impacted anthropogenic inputs of nutrients on the Earth's surface (Elsworth et al., 2020). Furthermore, between 30 to 50 percent of the Earth's surface has been transformed, typically resulting in more efficient transport pathways for excess nutrients to reach surface waters (Baulch et al., 2019). Consequently, a phenomenon mainly triggered by the excess nutrients in surface water termed eutrophication, has become a growing concern for lake degradation.

Eutrophication is a process by which primary productivity increases within a water body to the point where the ground surface has built up. Eutrophication can be divided into two categories; natural and cultural. The process of natural eutrophication happens gradually and very slowly in geological time. Meanwhile, anthropogenic activities can dramatically accelerate it, commonly known as human-made, cultural, and industrial eutrophication (Bhagowati & Ahamad, 2018). In particular, industrial and domestic waste

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discharge in lakes causes hypereutrophic state characterised by excessive nutrient availability, low light penetrability, and frequent algal blooms (Du et al., 2019).

In eutrophic lakes, their natural ecological balances are often disturbed by the changes in the phytoplankton community. Phytoplankton is often dominated by cyanobacteria. Cyanobacterial bloom is a term used to describe a lake ecosystem condition where its phytoplankton community is mainly dominated by cyanobacteria (Shan et al., 2019). Cyanobacteria (also referred to as blue-green algae) is a photosynthetic bacteria present in most lake water columns. Cyanobacteria can quickly proliferate to form large populations on the water, forming blooms (Josué et al., 2019). Cyanobacterial blooms in a freshwater lake ecosystem primarily consisted of *Microcystis* spp, *Anabaena* spp or *Cylindrospermopsis* spp. In contrast, in the marine ecosystem, *Lyngbya* spp, *Synechococcus* spp. and *Trichodesmium* spp. were often dominated (MohaMaD et al., 2016). Cyanobacterial blooms affect human health worldwide due to some cyanobacteria's ability to release toxins (Krztoń et al., 2019). Exposure to cyanobacteria toxins such as microcystin, presents a severe health hazard, including poisoning, liver failure, tumours, and death to livestock, wildlife, and humans (Preece et al., 2017). In Malaysia, concerns about the effects of lake eutrophication have increased in recent years, requiring the formulation and implementation of laws and programs for its prevention (Tang, 2019).

At present, significant efforts have been made to investigate the factors affecting the lake's eutrophication. These activities were taken to prevent further lake degradation and ensure adequate management and remediation efforts to restore its stability (Sharip et

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al., 2014; Vogt et al., 2018). Nevertheless, complex and inter connected impacts of natural factors (i.e., rainfall, climate change), urbanisation, and industrialisation, have become the key challenges and obstacles in maintaining the lake's water quality and ecosystems (Shahabudin & Musa, 2018). Moreover, the complexities of eutrophication within the ecosystem has presented challenges in developing the best eutrophication management strategy (Descy et al., 2016).

Local weather and hydrologic conditions are among the factors that may influence the eutrophication responses as they can alter nutrient loads into the lake water (Vinçon Leite & Casenave, 2019). Malaysia's weather is influenced by the equatorial climate, characterised by hot and humid weather all year round. The annual weather variability is closely associated with the southwest and the northeast monsoons, which occurred between April to September and October to March, respectively (Tang, 2019). The Southwest monsoon has drier weather with lower rainfall relative to the northeast monsoon. To date, the impacts of local rainfall and air temperature variabilities on water quality, eutrophication progression, and cyanobacteria are still scarce and needs furtherance. The local rainfall and air temperature refer to rainfall and air temperature associated with a particular locality or area of concern. In this study, the local rainfall and air temperature were obtained from Felda Sungai Behrang, a weather monitoring station in Slim River, Perak.

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1.2 Problem Statement

Eutrophication and cyanobacteria in freshwater lakes can cause profound implications, mainly foul odours, water column deoxygenation, toxicity, destruction of aquatics life, and disturbance to food chain stability (Sinang et al., 2015). It has been documented in the literature that high nutrient availability and temperatures tend to mutually enhance eutrophication symptoms, such as the dominance of cyanobacteria, floating plants, and massive loss of underwater flora and fauna (Scholz et al., 2017).

The existing knowledge on eutrophication suggests that locally unique or site specific environmental factors may also influence eutrophication progression. The variability of site-specific environmental factors composed of physical, chemical and biological water profiles may be more profound on a temporal basis in a small lake and a spatial basis in a large lake ecosystem. Understanding the changes or variability in lake water column's physical, chemical, and biological profiles is crucial in understanding lake eutrophication progression. This information is important for lake eutrophication management. Slim River Lake, a small shallow lake known as an iconic landmark to Muallim district, is chosen for this study. This is due to the need for the local authority to establish a eutrophication management plan for this lake. However, a recent and comprehensive study on Slim River Lake's physical, chemical and biological water profiles is unavailable. Slim River Lake's water quality assessment was performed in 2012 (Nayan et al., 2012), but the study was undertaken primarily to determine its suitability for recreational purposes. Thus, inadequate data regarding Slim River Lake's physical, chemical, and biological water

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profiles and how it changes over a temporal basis have caused difficulties in enforcing its management plan.

On the other issue, the physical, chemical, and biological water profiles in a lake may largely depend on the local rainfall and air temperature patterns. The local rainfall and air temperature refer to rainfall and air temperature associated with a particular locality or area of concern. According to the previous study, rainfall distribution and frequency significantly affect eutrophication (Deng et al., 2019). Extreme rainfall caused an increase in nutrient loadings due to surface runoff and consequently led to the water column's desertification. According to a study conducted in Lake Falemprise, Wallonia, the dominance of phytoplankton genera during eutrophication was highly influenced by weather variability (Descy et al., 2016). During warm and stable local weather, cyanobacteria such as *Aphanizomenon flos-aquae* dominated the lake and other phytoplankton groups (chlorophytes, diatoms, chrysophytes, and euglenophytes) due to the stratification of the water column and increased light intensity (Descy et al., 2016). Nevertheless, the lake was dominated by *Planktothrix agardhii* during cold and windy weather due to vertical mixing and phosphorus release from sediment. In addition, it was also reported that the influence of climatic factors in different seasons might surpass the effect of nutrient availability in the water bodies (Deng et al., 2019).

Despite numerous studies conducted in four-season countries, the influence of local weather patterns on a lake's eutrophication symptoms has never been discussed locally in Malaysia, including Slim River Lake. Additionally, there is an inadequate assessment of cyanobacteria in eutrophic lakes, especially in lakes used for recreational purposes such as

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Slim River Lake. Previous studies on eutrophication-related issues in Malaysian lakes mainly focus on nutrients, phytoplankton, and macrophyte variabilities (Othman et al., 2014; Sharip et al., 2016; Rizhinashvili, 2017). Due to the current information gap, the temporal variability of eutrophication symptoms, mainly cyanobacteria, remain widely unknown. As a result, the period of high cyanobacteria biomass, which imply a possible period of high cyanobacteria toxin, cannot be explained.

In terms of the proposed mathematical analysis, many previous studies have also proposed mathematical analysis to study climatic factors' potential impact on cyanobacteria succession in eutrophic lakes (Ho et al., 2019; Stauffer et al., 2019). In general, these studies' analysis show that an increase in cyanobacteria's relative biovolumes was related to increased water temperature (Elliott, 2012; Reid et al., 2019; Mantzouki et al., 2016). However, their proposed analyses did not use local air temperature or rainfall parameters. Therefore, a multivariate regression analysis that includes local rainfall and air temperature is needed to explain the variability of cyanobacteria and phytoplankton in an aquatic ecosystem, especially in our local setting.

Thus, to address the gaps mentioned above, this study was conducted to establish the physical, chemical and biological profiles and examine its temporal variability in the Slim River Lake. The temporal variability of physical, chemical and biological profiles was examined against the variability of local rainfall and air temperature patterns. Furthermore, cyanobacteria's succession in the study lake was against the local rainfall, air

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temperatures, physical and chemical profiles. This study also assesses phytoplankton and cyanobacteria diversity through microscopic analyses. Besides fulfilling the future lake management need, a study conducted in Slim River Lake also serves as a proxy better to understand the response of a small shallow lake to the changes in local rainfall and air temperature patterns.

1.3 Study Objectives

The ultimate aim of this study was to characterize the influence of rainfall, air temperature, physical and chemical water profiles on cyanobacteria succession in a small shallow lake ecosystem. Specifically, the objectives of this study are as follows:

1. To determine the temporal variability of lake's water physical, chemical and biological profiles in Slim River Lake area.

2. To determine the influence of rainfall and air temperature on the lake's water physical, chemical, and biological profiles.

3. To develop a multivariate principle component analysis (PCA) to explain the cyanobacteria biovolume based on rainfall and air temperature patterns, in lake chemical, and physical profiles.

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1.4 Study Questions

This study was designed to answer the following research questions:

1. What are the temporal variabilities of lake's water physical, chemical and biological profiles in Slim River Lake area?

2. How does the rainfall and air temperature influence variabilities of physical, chemical and biological profiles in Slim River Lake area?

3. How much of the cyanobacteria biovolume can be explained from rainfall and air temperature patterns, in lake chemical and physical profiles, based on the multivariate principle component analysis (PCA).

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1.5 Study Hypotheses

1. Lake's water physical, chemical and biological profiles in Slim River Lake are highly variable on a temporal basis.
2. The lake's water physical, chemical and biological profiles in Slim River are influence by local rainfall and air temperature.
3. The biovolume of cyanobacteria can be explained by the multivariate principle component analysis (PCA) based on rainfall and air temperature patterns, in lake chemical and physical profiles in the lake.

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1.6 Significance of the Study

This study elucidated the influence of rainfall and air temperature variability on the water quality physical, chemical and biological profiles of a lake regarding contaminations exposure and cyanobacteria growth. Data and information obtained in this study provided a clear picture of the significant role of rainfall and air temperature factors and their relationship with other water parameters that may potentially impact the lake's condition and aquatic life diversity. It thus enables certain integrated lake management systems and maintenance to be planned during the rainy-wet and sunny-dry seasons (Ben Eledo et al., 2017). Therefore, the deterioration and degradation of the lake's water quality and ecosystem can be prevented. The study significantly contributes to preventing and reducing exposure to toxic cyanobacteria in the lake, especially at Slim River Lake. This lake is an essential site for recreational activities, including fishing and drinking water sources for nearby livestock farms. In addition, this study is essential as there is a call for more scientific data on cyanobacterial bloom, diversity, and toxicity to draw more awareness on this disturbing problem from the Malaysian scientific community, specifically and globally (Jung et al., 2015). The period of toxic cyanobacteria blooms and toxins production can pose a great threat to the animals and humans who contact the contaminated lake water. Therefore, explaining the most probable period associated with high toxic cyanobacterial biovolume can reduce the risk of exposure through appropriate awareness-raising and advice to the local communities. Moreover, the statistical multivariate regression analysis obtained can explain the occurrence of cyanobacterial bloom in other similar eutrophic lakes in Malaysia or other countries with a similar climate, such as Indonesia and Thailand. Slim River lake is a human made recreational lake built from the former ore mining sites. It has

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been extensively used for fishing, leisure, sport, and social gathering for the community of Muallim District, Perak. It receives high loads of nutrients primarily from surface run-off and discharges from the nearby farm, contributing to its eutrophication problem.

This study contributes to the knowledge and data regarding the temporal variability of this lake's physical, chemical, and biological profiles. This reflects its health status and contamination of the ecosystem. Besides, data on the genetic community obtained through Next Generation Sequencing (NGS) revealed the presence of cyanobacteria this lake, which justify the need for a proper eutrophication control and health risk assessment by the local authority.

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1.7 Scopes and Limitation

This study only focused on the evaluation of water quality (physical, chemical, and biological properties) and cyanobacteria diversity and biovolumes in Slim River Lake, Perak state, Malaysia (3º 49' 26.688" N; 101º 24' 30.6216" E). So, in this thesis, this lake will only be referred to as Slim River Lake. This man-made lake is chosen as a proxy for this study as it is a small shallow lake and highly used by locals for recreational and leisure fishing activities. The lake is surrounded by agricultural lands, mainly oil palm and a cow farm. From the previous study conducted by Aeriyanie et al. (2021), it was revealed that the Slim River Lake has a high level of nutrients with occurrence of high total phytoplankton and cyanobacteria biomass. Therefore, this lake is very suitable for this study.

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In this study, the local rainfall and air temperature refer to the measurements at the closest weather monitoring station which located in Ladang Sungai Behrang. The distance of this weather monitoring station to the study lake is approximately 10 km. The rainfall and air temperature data were obtained from the Department of Meteorology in Perak state.

There are several limitations are identified and described as follows:

i. The water quality analysis is performed in one lake with five sampling sites to represent the lake's overall condition and is limited to the sampling duration of 12 months.
ii. Due to certain limitations during the study, data collection in S4 and S5 was started later than S1, S2 and S3.
iii. The physical, chemical and biological profiles analysed refer to water temperature, turbidity, pH, total nitrogen (TN), total phosphorus (TP), dissolved oxygen (DO), chemical oxygen demand (COD).
iv. The biological parameters were included chlorophyll-*a* and biovolumes of *Microcystis* spp., because they represented most abundance genera of cyanobacteria in Slim River Lake.

1.8 Thesis Organisation

The thesis is organised into six chapters, as summarised in Figure 1.1. Chapter 1 commences with an introduction to the background of the study. This chapter includes the problem

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statement, study objectives, study questions, and significance of the study. Moreover, this chapter also explains the scope and limitations of the study.

Next, Chapter 2 portrays a literature review of previous studies from relevant sources that have contributed to the intellectual advancement of the current issues, findings, theories, and debates related to the influence of local rainfall and air temperature on the lake's water quality and cyanobacteria of the present study. This chapter offers a conceptual description, organisational pattern, and critical assessment of these previous works concerning the current study being investigated.

The methodology used in this study is presented in Chapter 3. The materials and methods utilised are described in this chapter. Chapter 4 contains all the results and findings of this study. Chapter 4 is divided into three sections based on the research objectives; i. Temporal variability of physical, chemical and biological profiles, in Slim River Lake, ii. Influence of local rainfall and air temperature on the lake's physical, chemical, and biological profiles, and iii. A multivairate principle component analysis (PCA) to explain the biovolumes of cyanobacteria from local rainfall and air temperature patterns in lake chemical and physical profiles. Chapter 5 focuses on the discussion of results according to the stated objectives. In the end, Chapter 6 concludes the thesis and provides recommendations for future study.

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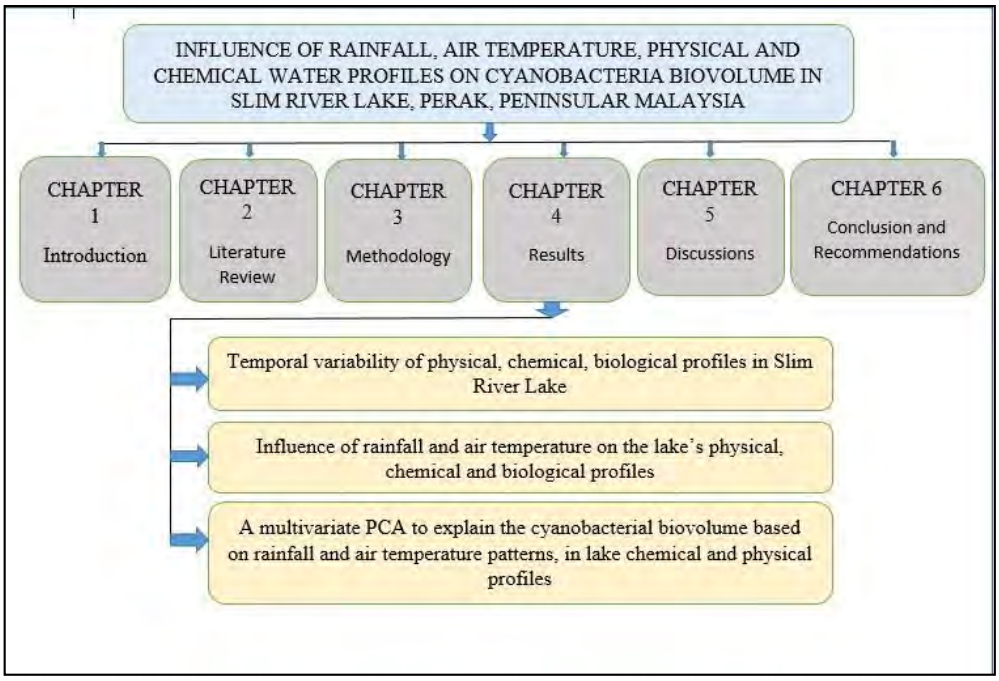


Figure 1.1. Thesis organisation

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CHAPTER 2

LITERATURE REVIEW

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2.1 Introduction to Lake Eutrophication

Eutrophication is a process of lake transition from one trophic state to a higher trophic state due to the changes in biochemical properties of water induced by excess nutrients, such as nitrogen and phosphorus (Cunha et al., 2013). It is also a result of combined actions of illumination, heat, and hydrodynamics, which contributed to a collection of inter-related biological, chemical, and physical processes (Kılıç & Yücel, 2019). The rapid growth of phytoplankton and other microorganisms lead to algae bloom, as well as degradation of water quality is often linked to lake eutrophication, both of which endanger the aquatic

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ecosystem and its normal function (Le Moal et al., 2019). To date, eutrophication in the lake has been one of the world's significant environmental concerns due to the decline of water quality and ecosystem disruption (Bhagowati & Ahamad, 2019). An example of eutrophic lake is shown in Figure 2.1.



Figure 2.1. Eutrophic lake shows the state of water quality degradation due to high algae biovolume

Lakes serve a vital role in continental watersheds' biogeochemical and hydrologica l cycles since fluxes are continuously traded between the water body, atmosphere, and lake's bottom (Vinçon Leite & Casenave, 2019). In addition, the lake also performs various functions, including water source, irrigation, generation of hydropower, flood control, food supply, medium of transportation, tourism, reclamation, and silt retention (Shahabudin &

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Musa, 2018). Unfortunately, eutrophication caused severe lake destruction and restricted the aforementioned lake's function. Therefore, the comprehension of the mechanism involved triggered the lake's eutrophication, which will help tackle these problems through competent management policies. In Malaysia, more than 60% of the 90 lakes surveyed were known to encounter the eutrophication problem (Pin et al., 2019). Lake water in Malaysia is characterised as surface water and evaluated according to the Water Quality Index guideline enrolled by the Department of Environment of Malaysia. Other than that, the National Lake Water Quality Criteria and Standard (NLWQS) had been established by National Hydraulic Research Institute of Malaysia (NAHRIM). NLWQS aimed to provide information and aid in decision making regarding the fitness of the lake, reservoir, dam, pond, or any impoundments for any usage or purposes. This guideline also provides a guide for lake water quality monitoring to determine its eutrophication status.

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2.2 Indicators of Lake Eutrophication

The signs of eutrophication in the lake are different due to the variation of biotic and abiotic components. The indicators of lake eutrophication are summarised in Table 2.1. (Hutchins & Hitt, 2019).

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Table 2.1

Indicators of lake eutrophication based on biotic and abiotic factors

Indicator	Parameter
Nutrients	Nutrient load
	Nutrient outflow
Chemical status indicator	Phosphorus content (Total phosphorus, ortho-phosphate)
	Nitrogen content (Total nitrogen, nitrate, nitrite, ammonia)
Impact indicator	Ecological and environmental status
	Dissolved oxygen
	Secchi depth (transparency)
	Phytoplankton (population, chlorophyll-*a*, biovolume, toxic algae)
	Macrophytes (population, depth of lower growth)
	Macrozoobenthos (population, biomass)

In general, eutrophication indicators can be categorised into pressure, chemical status, and impact indicators (Hutchins & Hitt, 2019). Pressure and chemical status indicators correspond to the detection and measurement of the nutrient's capacity and condensation in the lake. On the other hand, impact indicators focus on the biological factors and responses of living organisms, such as phytoplankton, macrophytes, macrozoobenthos, and eutrophication stress (Le Moal et al., 2019).

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2.3 Causes ofLake Eutrophication

As discussed in the earlier section, lake eutrophication is perceived as collective consequences caused by natural processes and numerous abuses of lake water bodies, resulting in a loss and diminishing of the functions they usually support. In general, causes of eutrophication can be categorised into natural and cultural eutrophication.

Natural eutrophication refers to a phenomenon of slowly lake ageing to become mature and more productive (Wen, 2020). Natural eutrophication is a prolonged process and requires roughly thousands of years to develop. The lakes' lifespan was limited by their depth, runoff, and watershed characteristics (Wen, 2020). Shallow lakes aged faster than the deep lakes produced by Earth's crust movement. The lakes were gradually sedimented and indeed became shallow enough to be dominated by rooted plants. This caused the lake to evolve naturally into the pond, wetland, swamps, and marshland until the dry land was formed (Jeffrey et al., 2019).

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Figure 2.2. Dominance of rooted plants and sedimentation in the lake.

Meanwhile, cultural eutrophication involved an anthropogenic input of phosphorus and nitrogen to the water bodies. It originates from point and non-point sources of pollution, which include changes in land practice, such as agricultural, animal feedlots, factories, and urban areas development (Orihel et al., 2017). In the coming decades, the human population's growth, land use shifts, and nutrient level in the water bodies are expected to elevate continuously (Haas et al., 2019). These factors significantly enrich the inland and coastal waters with anthropogenic nitrogen and phosphorus inputs from internal and external sources (Smith et al., 2016).

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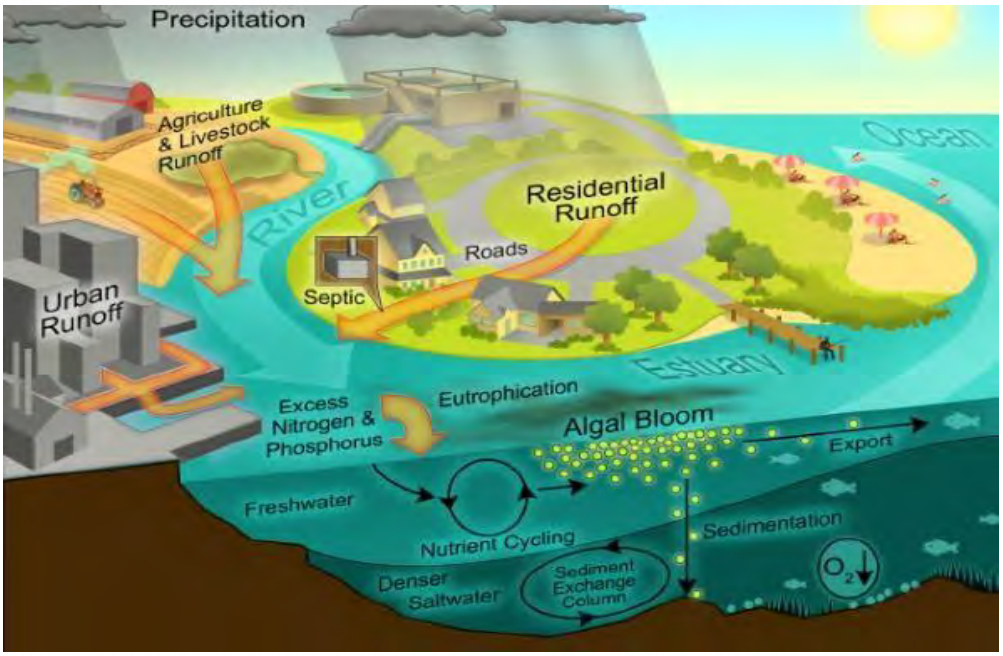


Figure 2.3. Sources of cultural eutrophication source: (www. projecteutrophication. weebly. com)

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Nitrogen fixation and decomposition of the dead organism are among the internal sources of nutrients. Meanwhile, the external nutrients load was derived from anthropogenic inputs, including detergents, fertilisers, organic waste, and sewage effluent (Othman et al., 2014). To date, many studies have been carried out to confirm the connections of physical, chemical, and ecological processes to the implications of eutrophication (Li et al., 2020). While many considerable signs of progress have been made in the comprehension of eutrophication, its known causes are strongly interconnected with each other, and the influences of one factor to another are not yet fully explained (Kies et al., 2020). In fact, each factor impact varies even on the same ecosystem due to the difference in seasonal and inter-annual responses (Gobler et al., 2017).

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2.4 Effects of Lake Eutrophication

2.4.1 Degradation of Lake

Lakes can be classified according to their productivities, represented by the nutrients' concentration and the growth of flora and fauna they can sustain. The Carlson Trophic State Index recommended a system based on the Trophic Level Index (TLI) (Pin et al., 2019) to classify the lakes according to their nutrition levels (Wang et al., 2019). Phosphorus and nitrogen are the primary nutrients responsible for categorising lakes according to their trophic levels. Eutrophication generally changes the lake's trophic level from better to worse.

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The lake's trophic condition is the major issue in its management because it reflects the interaction between a lake's nutrient status and organic matter decomposition. The terms oligotrophic, mesotrophic, eutrophic, and hypertrophic explain a water body's nutrient status. The properties of oligotrophic, mesotrophic, eutrophic, and hypertrophic states are provided in Table 2.2. The nutrient availability of each trophic level was denoted by total phosphorus and total nitrogen concentrations. Meanwhile, its abundance of aquatic life can be inferred from the concentration of chlorophyll-*a* , chemical oxygen demand, and water transparency (Secchi disk depth) of the water bodies.

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Table 2.2

Properties of oligotrophic, mesotrophic, eutrophic, and hypertrophic states in lake ecosystem

Properties	Trophic state			
	Oligotrophic	Mesotrophic	Eutrophic	Hypertrophic
Total phosphorus (µg/L)	5 – 10	10 – 30	30 – 100	> 100
Total nitrogen (µg/L)	250 – 600	500 – 1100	1000 – 2000	> 2000
Chlorophyll-*a* (mg/m_3)	0 – 1	1 – 10	10 – 65	65 – 100
COD (mg/L)	0 – 0.3	0.3 – 4	4 – 10	10 – 25

Adapted from (Bhagowati & Ahamad, 2019; Du et al., 2019).

The oligotrophic lake has a low nutrient concentration (TP: 5 – 10 µg/L; TN: 250 - 600 µg/L) and is considered to have low productivity. The oligotrophic lake's water body is transparent and consists of low microphytobenthos and benthic plant growth (Pérez Ruzafa et al., 2019). Since the oligotrophic lake has less phytoplankton growth, light can penetrate deeper, and there has been less decomposition that could decrease the oxygen dissolved (Schulhof et al., 2019). In addition, water from the oligotrophic lake is suitable for drinking purposes (Bhagowati & Ahamad, 2019). However, the lake's trophic status

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can be shifted from oligotrophic to eutrophic due to the incremental rise in nutrient inputs during eutrophication.

Eutrophic lakes are considered high productivity and are characterised by loading nutrient concentrations (TP: 20 – 100 μg/L; TN: 1000 - 2000 μg/L) and a high population of aquatic animal and plant life (Du et al., 2019). Eutrophic lakes are usually found in the area where the soils were fertile and used as farmland. Since eutrophic lakes have high densities of aquatic life, a lot of decomposition occurs at the lake's bottom, causing it to become anoxic due to the reduction of dissolved oxygen (Huang et al., 2015). Based on Lake Erie's surveillance data in North America, the average oxygen consumption rate for water underneath the thermocline was commensurate to the cumulative phosphorus concentration recorded in the earlier year (Rucinski et al., 2010). A similar outcome was also documented in the eutrophic Chesapeake Bay, United States (Kleinman et al., 2019). Moreover, the range of dissolved oxygen concentrations during day and night in the eutrophic lake was wider than in the oligotrophic lake (Ansari & Gill, 2014). In the eutrophic lake, the oxygen level is higher during the day due to photosynthesis by a high phytoplankton population. However, the dissolved oxygen decreases significantly at night due to increases in respiration and microbial oxygen consumption.

On the other hand, the mesotrophic lake has properties between eutrophic and oligotrophic lakes and has an intermediate productivity value. In contrast, the hypertrophic lake is a condition where extremely high concentrations of nutrients are present (TP: > 100 μg/L; TN: > 2000 μg/L), causing severe water quality problems (Bhagowati & Ahamad, 2019). Under this trophic state, the frequency of algae bloom was higher, water became

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less visible, and aquatic life was absent at deeper depth due to the lack of dissolved oxygen (Yuan & Jones, 2020).

2.4.2 Loss of Biodiversity and Disruption of Ecosystems Stability

Eutrophication contributed to a very unwelcome modification of the lake ecosystem's structure and stability (Rathore et al., 2016). This includes several ecosystem changes, such as an increased frequency and period of hypoxia (Scavia et al., 2014). The anoxic condition resulting from the decomposition of dead phytoplankton severely impacted other species' resilience that continues to survive in the absence of oxygen. The anoxic area was dominated by an anaerobic microorganism that released toxic compounds, such as ammonia and hydrogen sulfide, as a result of organic matter decomposition. These toxic compounds were deadly to sensitive species and promoted the loss of biodiversity in the lake. It is emphasised that the lake ecosystem's stability was profoundly affected by the loss of its biodiversity (Monchamp et al., 2018). Despite a fundamental ecological concept that emphasised only diverse ecosystems are balanced, the effect of eutrophication upon lake ecosystem diversity and stability has not gained much interest.

Moreover, eutrophication has often contributed to the loss of aquatic vegetation. Lake ecosystem that was previously dominated by macrophytes was gradually transformed into murky water consisting of algae (Qin et al., 2013). This ecosystem transition resulted from macrophytes and phytoplankton's different nutrient consumption efficiency (Qin et al., 2013). The extinction of submerged plants in the lake ecosystem was widely reported in other countries (Seto et al., 2013). For instance, since the 1970s, the macrophyte

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coverage of submerged plants has been declined significantly in Lake Kasumigaura, Japan (Qin et al., 2013). Moreover, a study performed in the Chesapeake Bay also indicated that eutrophication progressively reduced the number of submerged plants in the bay's upper and middle areas (Orth et al., 2019). Also, through the eutrophication cycle in Lake Taihu, China, the macrophyte species richness and coverage area reduced (Qin et al., 2013). According to (Dubey & Dutta, 2020), the immersed plant richness of 60 lakes in the United Kingdom and Poland was decreased due to the elevation of nitrogen and phosphorus concentrations in the lake.

In addition, eutrophication also endangered other aquatic species and increased the rate of fish mortality. The occurrence of eutrophication decreased biodiversity across the entire lake ecosystem, simplified the biotic population structure, and degraded ecosystem stability (Qin et al., 2013). The lake ecosystem's stability was disturbed by the replacement of sensitive species with ones with more tolerance to the stress caused by eutrophication (Siuda et al., 2020). This led to the changes in the food chain and predation activity of thelake's organism. According to (Stauffer et al., 2019), the food web was interrupted since some microzooplankton and mesozooplankton members are unable to consume toxin- producing algae species. Furthermore, increased fish death was observed due to the high

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concentration of cyanotoxin released by cyanobacteria bloom and hypoxic conditions, resulting from the bloom respiration and senescence (Benayache et al., 2019).



Figure 2.4. Cyanobacteria bloom, cyanotoxin released and hypoxic, increased fish death (Benayache et al., 2019).

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2.4.3 Animal and Public Health Issues

Eutrophication's impact includes concern regarding public health deterioration (Nazari Sharabian et al., 2018). Water authorities worldwide have paid extra attention to eutrophication problems because it poses health risks to humans and the animals receiving direct or indirect services from lakes (Sinang et al., 2015). Nevertheless, eutrophication prevention and maintenance are still onerous and challenging, given this recognised fact (Kimambo et al., 2019). Toxin poisonings related to eutrophication have been reported in

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pets, livestock, and wildlife (Preece et al., 2017). Toxins are elevated in the lake water as soon as the harmful algae population increases. Humans can be affected by various routes, including consumption of polluted water and seafood, inhalation of dried algae biovolume through aerosol or air particles, and direct lake water exposure through skin contact (Bajard et al., 2018). However, the symptom of poisoning varies in human, including skin irritation, paralysis, gastrointestinal, and neurological effects (Kimambo et al., 2019).

Additionally, eutrophication threatens public health, not only by its foul odour, but also by mobilising pollutants, such as arsenic, methylated Hg, Fe, and methane (Tang et al., 2019). According to (Tang et al., 2019), anoxia induced by algae biovolume degradation promoted arsenic release from the sediment to lake water and atmosphere. Therefore, algae-induced anoxia in arsenic-polluted freshwaters may pose greater health risks to the residents nearby, tourists, fishermen, and wild animals through respiratory exposure.

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2.4.4 Effects on Economic

In the last two decades, attempts have been made to determine eutrophication's monetary effect (Le Moal et al., 2019). The effects of eutrophication mentioned previously are accountable for eutrophication's direct and indirect impact on the economy. Lots of money has been spent on removing algae and dead fish from the lake to prevent secondary environmental pollution. In addition, a considerable cost was invested in reducing and monitoring lake degradation. The United States Environmental Protection Agency

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estimated a cost of up to $27.70 per m^3 for eutrophication control, primarily for nitrogen and phosphorus removal purposes (Wurtsbaugh et al., 2019).

Eutrophication reduced the lake's functional and aesthetic values for recreational and eco-tourism (Nasir et al., 2014). Therefore, local communities involved in tourism activities, such as boat operators, tourist guides, craft sellers, and food and hotel industries, were severely affected by eutrophication due to a massive shortage of visitors (Sharip et al., 2016). In addition, fishing industries faced potential economic problems and revenue loss due to the toxins produced by harmful algal blooms. This caused the lake's food sources to be unsuitable for human consumption. Furthermore, there was also an increase in the cost of medical treatment for eutrophication-induced human disease.

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2.4.5 Increase of Phytoplankton Population Resulting in Algae Bloom

The most apparent effect of eutrophication is a rise in the abundance of phytoplankton and the frequency of algal blooms (Tang et al., 2019). In recent decades, continuous eutrophication has been linked with an intensification of toxic algae bloom with fatal and destructive impacts (Stauffer et al., 2019). Toxic algae blooms are often composed of cyanobacteria, which are also known as blue-green algae (Weirich & Miller, 2014). As reported in the literature, the analysis of phytoplankton community from 26 lakes in western Uganda found that, in both dry and rainy seasons, most of the lakes were dominated by cyanobacteria 78% of total cell counts, followed by green algae 17%, and diatoms 4% (Nankabirwa et al., 2019).

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Nitrogen and phosphorus are essential nutrients required for phytoplankton growth. Therefore, a lake with high nitrogen and phosphorus concentration provides adequate nutrients for the proliferation of the phytoplankton population, which results in a rise of organic aggregates in the water column. When the lake water is utilised for raw water sources, phytoplankton can be hard to eliminate and, therefore, add to the water supplied some unpleasant tastes. In addition, eutrophic conditions are shown to contribute to an increment dominance of cyanobacteria that caused a direct threat to human health and lake ecosystems by the production of deadly cyanobacterial toxins (Matthews et al., 2014).

2.5 Cyanobacteria

2.5.1 Cyanobacterial Bloom

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Cyanobacterial bloom can be described as a massive cyanobacteria growth that eventually floats and covers a large water surface area (Pick, 2016)

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Figure 2.5. Scum of cyanobacteria biomass covering the surface of water body (De Santi et al., 2019).

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During the period of increased air temperature, the cyanobacteria population was often dominated by *Dolichospermum circinale*, *Phormidium* spp. *Microcystis* spp., *Oscillatoria* spp., *Sphaerospermopsis* spp., *Cuspidothrix* spp., *Aphanocapsa* spp., *Aphanizomenon* spp. and *Anabaena* spp. (Malazarte et al., 2017).

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Figure 2.6. Common cyanobacteria genera in freshwater ecosystem. (I). Chroococcales: a *Chroococcus subnudus* spp., b *Ch. Limneticus* spp., c *Cyanothece aeruginosa* spp., d *Snowella litoralis* spp., e *Microcystis aeruginosa* spp. (II). Pleurocapsales: f *Pleurocapsa minor* spp. (III). Oscillatoriales: g *Planktothrix agardhii* spp., h *Limnothrix redekei* spp., i *Arthrospira jenneri* spp., j *Johanseninema constricum* spp., k *Phormidium* spp., l, m *Oscillatoria* spp., n *Schizothrix* spp., o T*olypothrix* spp., p *Katagnymene accurate* spp., (IV). Nostocales: q *Dolichospermum planctonicum* spp., r *Dolichospermum* spp., s *Nostoc* spp., t *Nodularia moravica* spp. (V). Stigonematales: u, v *Stigonema* spp.

The diversity of cyanobacterial communities in the cyanobacterial bloom had been previously investigated at the molecular level using polymerase chain reaction (PCR) (Lee et al., 2015) and next-generation sequencing (NGS) of 16S rRNA region (Woodhouse et al., 2018). NGS use of cyanobacteria's target genes, such as 16S rRNA, is of great importance for detecting cyanobacterial functions and assessing bacterial water quality source (Fondi & Liò, 2015). Next-Generation Sequencing is also used to examine cyanobacterial compositions and their environmental activities, such as spreading

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antibiotic genes and pollutant degradation in lake water, which increases potential biological risk factors in water quality and management (Tan et al., 2015).

In this study, the temporal variability of cyanobacterial biovolumes is investigated. This is because the main goal for cyanobacteria bloom monitoring in aquatic environments is to reveal the period of high cyanobacterial biovolume, which could cause significant health risks. In addition, eutrophic conditions are shown to contribute to an increment dominance of cyanobacteria that caused a direct threat to human health and lake ecosystems by the production of deadly cyanobacterial toxins (Matthews et al., 2014). Therefore, it is wise to consider investigating the variabilities of cyanobacterial biovolumes and its associated environmental influences, particularly nutrients.

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2.5.2 Effects of Cyanobacteria Blooms in the Aquatic Ecosystem

Harmful algal blooms are broadly defined as high-biovolume producers that negatively affect the health of wildlife, aquatic ecosystems, and human populations through the release of toxins or by causing hypoxia and anoxia (Brown et al., 2020). Harmful algal blooms often produce toxins, causing nausea, vomiting, diarrhoea, muscular paralysis, respiratory paralysis, and death in humans (Glibert et al., 2018). A common type of cyanobacterial toxins is shown in Figure 2.7.

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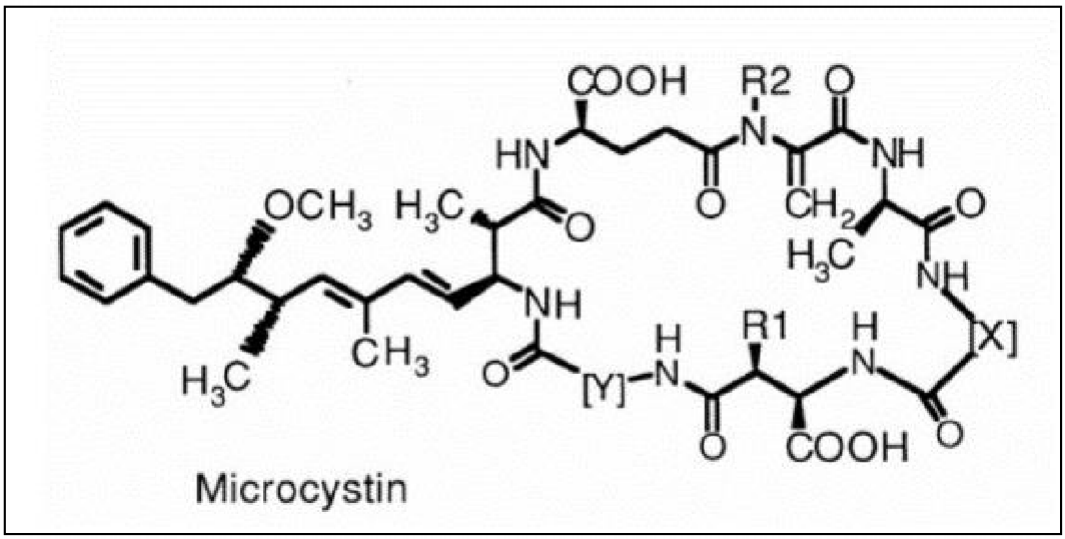


Figure 2.7. Molecular structure of the most common cyanobacteria toxin microsystin

Bioaccumulation of harmful algal blooms related toxins in fish and shellfish resulted in nearly 2000 reported cases of human poisoning (15% mortality) globally (Sarkar, 2018). Cyanobacteria can produce cyanotoxins in the forms of hepatotoxins, neurotoxins, and dermatotoxins (Woodhouse et al., 2014). (Merel et al., 2013) pointed out: (1) not all cyanobacteria strains produce cyanotoxins, (2) cyanotoxins are generally released under certain stressed conditions, which have yet to be fully understood (Wang et al., 2019).

Wildlife can be poisoned by consuming contaminated fish or zooplankton (Sayers et al., 2019). Harmful algal blooms can cause massive killings of farmed and wild fish, negatively impacting the economy and biodiversity (Sakamoto et al., 2020). Freshwater harmful algal blooms have an estimated annual cost of 2.2 to 4.6 billion dollars to the North American economy, and marine harmful algal blooms have annually cost at least 82 million

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dollars for the North American economy (He et al., 2020). The prevalence of harmful algal blooms has been increasing globally. Within tropical areas, nearly every country experiences harmful algal blooms, and 300 coastal regions have been impacted (Zhu et al., 2014).

2.5.3 Influence of Climate on the Cyanobacteria

Two primary drivers have been identified to increase cyanobacterial harmful algal blooms: climate change and eutrophication (O'neil et al., 2012). Generally speaking, cyanobacteria prefer warmer temperatures (~25ºC and above), though other factors associated with climate change are essential to cyanobacteria growth (Alghanmi et al., 2018). For instance, changes in global rainfall patterns from climate change will affect the chemical and hydrological conditions of the receiving water bodies, affecting cyanobacteria in ways that have yet to be fully explored (Yang et al., 2016).

Global annual rainfall is expected to increase, though not as rapidly as rainfall intensity (Shawul et al., 2020). This implies longer dry periods separating more intense rainfall events. Many lakes and reservoirs with a high nutrient loading experience the proliferation of cyanobacteria in their water during the summer months.

Cyanobacteria are the most common phytoplankton in lakes and reservoirs, and their occurrence is natural. However, when they grow to high levels and bloom, they can cause aesthetics, emit an odour if they wash up on shore and die, and occasionally be toxic

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to aquatic organisms. Before undertaking costly remediation projects or evaluating the effectiveness of nutrient reduction strategies, resource managers need to understand how local weather and climate affect cyanobacteria blooms (Havens et al., 2019). Regardless the link between the increase in exogenous nitrogen and phosphorus inputs and the augmented incidence of eutrophication, climate changes represent additional factors influencing the frequencies and magnitudes of cyanobacteria blooms. The impact of climate change on water resources includes the warming of air and water temperature, changes in abundance, increased storm intensity, and rising of salinity levels in water bodies. The rise of greenhouse gases from man's activity represents one of the leading causes of global warming (Jain, 2015).

It could be concluded based on the literature that the local climatic factors, particularly rainfall and air temperature, might significantly affect the eutrophication in lakes and the variability in abundance of cyanobacterial biomass. However, the effects can be unique and site-specific. At present, the influence of local climatic factors on cyanobacteria blooms and the occurrence of eutrophication in Slim River lake have never been assessed.

2.6 Physical and Chemical Properties of Lake and Their Influences on Cyanobacteria

2.6.1 Water Temperature

Lake or reservoir forms density layers, and the water body is thermally stratified due to temperature changes (Noori et al., 2020). In a deep lake system, vertical temperature

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stratification has an essential and indirect effect on cyanobacteria distribution and growth by limiting dissolved substances' vertical transfer (Xiao et al., 2020). Stratification is characterised by the upper layer of uniformly warm, circulating, and fairly turbulent water called the epilimnion (Wang et al., 2020). The epilimnion floats upon a colder undisturbed region known as the hypolimnion. Between these two layers is located a transitional layer known as the metalimnion. Here, the thermocline, or the maximum rate of temperature decrease with respect to depth, is found (Braaten et al., 2019).

In both deep and shallow systems, there is an inverse relationship between temperature and dissolved oxygen, whereby warmer waters are less capable of retaining as much dissolved oxygen as cold water (Weinke & Biddanda, 2019). In addition, temperature also affects the conductivity of water. It has been stated that the specific conductance usually increases from 2 % to 3 % per degree Celsius (Pier & Mateo, 2020). The higher conductivity values were almost always prevalent in warmer waters during hot seasons (Schwefel et al., 2019). The summer or hot season, as expected, had the highest overall conductivity, while the winter or cold season had the lowest conductivity (Haakonsson et al., 2020). Other than that, warmer water is also associated with higher chlorophyll-*a* concentrations (Piniewski et al., 2020). This is due to the fact that with the increased photoperiod and warmer temperatures, phytoplankton populations were much greater in the reservoir (Merel et al., 2013). Despite that, the current theory attributes blooms largely to the rising global temperatures (Liu et al., 2019). The temperature might have been a contributor, but it might not be the only mechanism by which cyanobacteria find dominance in surface waters (Vergalli et al., 2020).

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Increasing mean water temperatures and temperature variability will likely play a substantial role in shaping lake phytoplankton communities in particular. Increased temperature may increase the magnitude and frequency of cyanobacterial blooms (Krinos et al., 2019). Providing the appropriate environmental conditions for an increase in cyanobacteria will contribute to the production of deadly cyanobacterial toxins. Eutrophic conditions and water temperature might lead to a direct threat to human health and lake ecosystems.

2.6.2 Turbidity

Turbidity is an essential factor in determining the water quality of a particular aquatic system (Teixeira et al., 2017). It influences the penetration of light used in photosynthetic activities by aquatic plants and is the measure of depth into which light can penetrate the water column (Shen et al., 2019). It also affects the limnological stratification with variation in its value (Milan et al., 2015). The elevation of turbidity leads to a reduction in water's transparency value (Nharingo & Moyo, 2016).

Generally, water will be most transparent during cold temperature and at its murkiest with the warm temperature as well as increased algal and cyanobacterial growth (Beaulieu et al., 2013). In addition, rainstorms also may affect water clarity (Li et al., 2019). Damage from rainfall runoff and high stream velocities may result in a higher concentration of suspended particles in the inflowing stream and, therefore, decrease water clarity (Varol et al., 2019). The presence of debris and other rapidly settable matter interferes with turbidity

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determination (Kumari & Gupta, 2020). Turbidity is inversely proportional to water transparency and is influenced by the density of suspended solid particles, cyanobacterial, bacterial, algal, and released toxins mass (Brophy, 2019).

The turbidity of water depends on the number of suspended substances, such as clay, bacteria, phytoplankton, organic and inorganic compounds, as well as algal and parasitological organisms (González et al., 2019). Light penetration in the water depends broadly on the optical properties. It is reduced in turbid water, affecting the stratification of aquatic vegetation and photosynthesis (Iachetti & Llames, 2015).

High turbidity (Perga et al., 2018) can give a negative condition for long periods due to decreased light penetration (Richardson et al., 2019). The geologic factors of water bodies and climate play an important role in environmental changes and their impact on phytoplankton and cyanobacteria communities in multiple pathways (Perga et al., 2018). In some cases, high turbidity also stimulated algal growth (Beck et al., 2019). These contrasting findings highlight the need to investigate the effects of water turbidity on cyanobacteria biovolume based on the local environmental condition in the study lake. This study determined the temporal variabilities of turbidity, potential causes of variabilit ies, and the influence of turbidity on cyanobacterial biovolume based on the observational dataset gathered from Slim River Lake.

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2.6.3 Hydrogen Ion Concentration

Hydrogen ion concentration (pH) is another critical factor in water quality (Ahuja, 2019). It is a measure of hydrogen ion activity, which essentially affects most of the chemical and biochemical reactions in the aquatic system (Shah et al., 2019). Determination of pH realizes the acid-alkali balance of water. The measure of the hydrogen ion activity in the $\log_{10}$ of the inverse of the hydrogen ion concentration in a water sample is known as pH (Zhang et al., 2019). The concentration of hydrogen ions in natural water depends on the dissociation of carbonic acid and hydroxyl ions produced during bicarbonates' hydrolysis (Capasso, 2019). Also, pH is affected by the amount of CO_2 utilised by photosynthetic organisms, such as algae and cyanobacteria, which tends to reduce CO_2 content and increase pH. Microbial activity, especially in the hypolimnion and bottom muds, generates large amounts of carbon dioxide through respiration, decreasing pH (Boyd, 2020).

The EPA sets a standard for pH at 6.5 - 9 for freshwater aquatic life (Genter et al., 1988). In natural water, free CO_2 is the primary source of acidity, which forms carbonic acid and adds to water's acidic property (Taylor et al., 2013). The decomposition and degradation of organic materials may also increase the acidity of water. However, the influx of acidic nature substances, such as organic acids, mineral acids, humic acid, tannic acids, and hydrolysing salts, contributed to the water body's acidification (Boyd, 2020). Acidified waters are generally less buffered and less productive in nature due to the unavailability of a sufficient amount of dissolved bicarbonates, which produce CO_2 for a high photosynthesis rate (Su et al., 2020). pH is one of the important variables in a

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eutrophication mathematical analysis in explaining water quality indicators and cyanobacterial density (Lopes et al., 2020). Due to the possibility of the effect of pH on the activity of microbial cells, and thus its impact on the cellular metabolism of cyanobacteria (Gonzalez Aravena et al., 2018). Controlling the pH by reducing nutrient loading and land use can be important in decreasing the potential for overgrowth of cyanobacteria and decreasing the toxins secreted from them (Liao et al., 2016). Moreover, the alkaline pH found in warmer climatic conditions may be favoured cyanobacteria blooming as it can use bicarbonate as a source of carbon (Vilar & Molica, 2020). In this study, the temporal variabilities of water pH, potential causes of variabilities, and the influence of pH on cyanobacterial biovolume were determined based on an observational dataset gathered from Slim River Lake.

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2.6.4 Nitrogen and Phosphorus

A strong relationship between cyanobacteria concentrations and major nutrients has been extensively reported (Melendez Pastor et al., 2019). Nutrient availability has been linked to several environmental concerns, including eutrophication, acidification of freshwater lakes and streams, forest decline, climate change, disturbances to ecosystems, and changing decomposition rates (Quinlan et al., 2021). Major nutrients, which include nitrogen and phosphorus enrichment, are known to accelerate water bodies' eutrophication and promotes algal blooms (Ngatia et al., 2017). Nitrogen and phosphorus from sediments are also important determinants of macrophyte invasion, whereby eutrophic ecosystems have an increased risk of macrophyte invasions. In several tropical freshwater ecosystems,

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increases in nitrogen and phosphorus levels were the leading cause of successful colonisation by free-floating macrophytes (Thomaz et al., 2015).

2.6.4.1 Nitrogen

Large amount of nitrogen entering water bodies are generally from agricultural lands that use nitrogen fertilisers (Huang et al., 2017). On top of that, animal population and associated animal wastes, atmospheric deposition (from industrial facilities and vehicle exhaust), and run-off from roadways and residential areas are other nitrogen sources (Xia et al., 2020). Rainwater runoff can include nitrogen with land uses (Jeon, Kwon, Jung, Kang & Min, 2015). The amount of nitrogen can increase with the increase in livestock farming and agricultural uses of the land, especially near the lake and in rainy seasons (Ye, Yuan, Han, Marip & Qin, 2017). Furthermore, effluents from wastewater treatment plants are essential nitrogen sources in receiving natural waters (Talang, Sirivithayapakorn & Polruang, 2020).

Total nitrogen concentrations (TN) in surface water has been under major consideration s to control water eutrophication and improve water quality. To identify at- risk surface water bodies and protect them from eutrophication, the United State Environmental Protection Agency (US EPA) developed guidelines, which state that total nitrogen concentrations should not exceed 0.3 mg/L in streams and rivers or 0.1 mg/L in lakes and reservoirs. Nitrogen pollution, leading to eutrophication of inland waters, has

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resulted in an increase in global algal biovolume and photosynthesis. Primary production is approximately 60% higher than expected background levels in lakes, streams and rivers (Xu et al., 2014). It is widely reported that in-lakes total nitrogen concentrations may fluctuate on a temporal basis due to various factors influencing factors (Pilon et al., 2019).

Dissolved organic nitrogen degradation produces low molecular weight compounds from high molecular weight compounds. Thus, the degradation makes dissolved organic nitrogen obtainable to some microorganisms, including algae, cyanobacteria, micrograzers, bacterioplankton, cyanobacterium, and phytoplankton (Svedn et al., 2016). Bioavailable dissolved organic nitrogen is crucial in receiving water bodies since it causes excessive growth of unwanted species and ultimately reduces surface waters' quality (Mackay et al., 2020). As such, it is generally known that nitrogen could promote cyanobacteria and algae growth and causes oxygen depletion resulting in fish kill and affecting the waters' commercial and recreational activities (Li et al., 2019).

Sunlight or artificial light decomposes the degradable part of dissolved organic nitrogen into fewer complex substances. Light exposure breaks down the degradable part of dissolved organic nitrogen to fewer complex substances and finally makes dissolved organic nitrogen bioavailable to water species (Simsek et al., 2013). The photochemical degradation of dissolved organic nitrogen in dissolved organic matter is an important step in producing nutritious components in water bodies (Hood et al., 2015). Cyanobacteria can mineralise biodegradable dissolved organic nitrogen once the best environment is attained (Wadhawan et al., 2014). Dissolved organic nitrogen is an important nitrogen source for

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cyanobacteria, which biomineralised it to ammonia, a preferred nitrogen source for algae and phytoplankton (Hu et al., 2016). Biodegradable dissolved organic nitrogen plays a crucial role in many chemical and biological processes in the natural ecosystem.

Total nitrogen represents the sum of the different forms of nitrogen, such as ammonium, particulate nitrogen, dissolved organic nitrogen, ammonia, nitrite, and nitrate (Wu et al., 2014). These forms are transformed among themselves by various processes such as denitrification, decomposition, nitrification, fixation, or exchange reduction of nitrogen to ammonium process (Troitsky et al., 2019). In this study, nitrogen is quantified as total nitrogen suggested in NLWQS and the US EPA guidelines. As nitrogen may be largely affected by the inflow of surface runoff, therefore it is worth investigating the temporal variabilities of total nitrogen and how it influences cyanobacterial biovolume in the study lake.

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2.6.4.2 Phosphorus

Phosphorus is an essential proliferative factor for algal and cyanobacteria cells in freshwater ecosystems (Paerl et al., 2019). Algal and cyanobacterial cells require phosphorus to build peptides and cell membranes (Aubriot, 2019). Large phosphorus concentrations contribute to accelerated eutrophication or ageing of lakes and reservoirs (Boyd, 2020). Phosphorus concentrations greater than 0.017– 0.020 mg/L indicate eutrophic conditions (Smucker et al., 2013). Meanwhile, dissolved orthophosphate level is

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commonly less than 0.005 to 0.020 mg/L and is rarely higher than 0.10 mg/L, even in highly eutrophic waters (Zhang et al., 2019).

Phosphorus enters as a major factor in ongoing reactions such as photosynthesis and respiration in ecosystems, and processes of sediment flow and rainfall contribute to these processes (Walve et al., 2021). Given the importance of phosphorus in biological reactions, it is considered one of the most important basic pollutants of water and its quality (Qiu et al., 2018). Phosphorus has an essential role in the nutrient accumulation of freshwater (Mir et al., 2019; Sinang, Poh, Shamsudin & Sinden, 2015). Hence its contribution to algal blooms and eutrophication in lakes combined with nitrogen (Bulleri et al., 2020).

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One of the main sources of phosphorus in aquatic ecosystems is stationary and non-stationary sources such as human activities represented by population and urban growth (Fang et al., 2021). The source of incoming phosphorus to the water system can be through internal and external loading. External loading occurs with the transfer of nutrients from the runoff. Thus, the eutrophication process occurs (Orihel et al., 2017). Furthermore, raising livestock near the lake can increase the amount of phosphorus in lake water during increased rainfall (Ye, Yuan, Han, Marip & Qin, 2017). As for the internal loading, it occurs due to the accumulation of nutrients in the benthic area of the lakes as a result of some chemical and physical processes (Orihel et al., 2017). During the dry season and increased temperatures, the production of phosphorus from the lake sediments to the water column increases, which significantly affects the blooming of cyanobacteria, especially

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with a decrease in the external loading of phosphorus (Tammeorg et al., 2016). Therefore, sediments' internal loading of nutrients plays an important role in releasing phosphorus into lake waters and leading to re-eutrophication (Yang, Yang, Geng, Yin & Chen, 2020). Total phosphorus is a comprehensive measure of the forms of phosphorus available in lake water, either in dissolved form or as particles. The measurement of total phosphorus in lakes is preferred because it represents orthophosphate and phosphorus in plants and animals found in lake water. As well as the fact that the fixed and stable ratios are total phosphorus and give a clear picture of the nutritional status and water quality (Yang et al., 2018).

2.6.5 Dissolved Oxygen

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Oxygen quantified in its dissolved phase is called dissolved oxygen (Tomasetti & Gobler, 2020). Dissolved oxygen is one of the greatest indicators for water quality because sufficient dissolved oxygen is crucial for the survival of fish and other aquatic life. Oxygen is dissolved into water from the atmosphere. Plants and algae in water bodies also contribute dissolved oxygen to water bodies during daylight hours through photosynthesis. Dissolved oxygen (DO) is typically measured and reported as concentrations using either mg-DO/L of water (mg/L) or percentage of saturation as the units. The state criteria for dissolved oxygen shall not be less than 5 mg/L, where daily and seasonal fluctuations above this level shall be maintained (Haile, 2019).

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The dissolved oxygen concentration depends on several factors, including temperatures, organic pollutants, salinity, and atmospheric pressure. Dissolved oxygen has an inverse relationship with water temperature. As water temperature increases, dissolved oxygen decreases. Warmer water has less capacity to hold oxygen than colder water. Therefore, dissolved oxygen is basically lower in hot water. This happens because as the water particles heat up and gain kinetic energy, they move or vibrate more quickly (Ye, Wang, Ji, Wu, Qu, Wang & Liu, 2021). Besides temperature, dissolved oxygen also relates to the amount of organic matter in water. This leads to decomposing these substances while consume the amount of oxygen dissolved in the water, reducing its levels significantly. As a result, it indicates organic pollution's occurrence inside the waterbody (Seow et al., 2019). On top of that, dissolved oxygen (DO) also has an inverse relationship with water salinity. As water salinity increases, it holds less oxygen, resulting in decreased dissolved oxygen. In contrast, dissolved oxygen drops when low atmospheric pressure (Haile, 2019). In this study, the temporal variabilities of dissolved oxygen and the influence of dissolved oxygen on cyanobacterial biovolume were determined based on an observational dataset gathered from Slim River Lake.

2.6.6 Chemical Oxygen Demand

Natural organic detritus, organic waste from industrial and agricultural run-off, wastewater treatment plants, and failing septic systems are prime sources of organic matter in water ecosystems (Ansari et al., 2020). As mentioned in the earlier section, organic matter can

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cause a complete deficiency of dissolved oxygen, causing mortality to the aquatic organisms.

Chemical Oxygen Demand (COD) refers to the oxygen quantity needed during the oxidation of organic matter content of a sample and convert it into carbon dioxide and water under aerobic conditions. It is expressed in milligram per litre (mg/L), which signifies the oxygen quantity consumed, during the oxidation of organic matter, per litre of an aqueous sample. In the oxidation process, a strong oxidant (potassium dichromate) is used in the presence of a catalyst (silver) and strong acid (sulfuric acid) to convert most of the carbon to carbon dioxide, while any hydrogen present in the organic matter is converted to water (Mirzaee et al., 2019).

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In chemical oxygen demand analysis, carbon derived from a living matter in water is combined chemically with oxygen via $Cr_2O_7^{2-}$ and $K_2Cr_2O_7$. This reaction reduces the dichromate ion ($Cr_2O_7^{2-}$) to the green chromate ion (Cr^{3+}). The amount of Cr^{3+} produced is proportional to the amount of organics present. The colourimetric method is used for determining the amount of chromium in a particular valence state. Colourimetric analysis works because dichromate ion and trivalent chromium absorb at different wavelengths. The dichromate ion absorbs at 420 nm whereas, the Cr^{3+} ion absorbs around 600-620 nm. In general, low range chemical oxygen demand (<150 mg/L) reading measures the remaining yellow Cr^{6+} while a high range chemical oxygen demand (<15,00 mg/L) reading measures the amount of green Cr^{3+} produced. The presence of chlorine in the sample

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interferes with the organic matter's oxidation, but it can be overcome by complexing chloride with mercuric sulfate ($HgSO_4$) (Stone, 2011).

The chemical oxygen demand (COD) parameter is often used to determine the amount of organic matter in aquatic environments (He et al., 2019). According to Ansari et al. (2020), increased amounts of chemical oxygen demand in water make it unfit for aquatic life. High level of chemical oxygen demand in polluted water can be caused by high level of biodegradable of organic matters as pointed by Scholz (2016). Therefore, the temporal variability of chemical oxygen demand, how it is influenced by local air temperature and rainfall and how it determines the biovolume of cyanobacterial biovolume are investigated in this study.

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2.6.7 Chlorophyll-*a*

Algal (including green cyanobacteria) mass is frequently quantified by measuring chlorophyll-*a* (Hartmann et al., 2019). It is important to note that chlorophyll-*a* is in all photosynthetic species, including green algae and diatoms (Murchie et al., 2020). All cyanobacteria are autotrophic, i.e., they can synthesise their own organic food (Wang et al., 2020). Similar to the chloroplast of the autotrophic plants, cyanobacteria cells possess green chlorophyll-containing plastids involved in the process of photosynthesis (Jiang et al., 2020). These cyanobacteria cells produce carbohydrates with the help of light energy available from the sun and purify the atmospheric air by consuming carbon dioxide (CO_2) and evolving oxygen at the same time (Larkum et al., 2020).

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Chlorophyll-*a* is an important indicator and assessment of cyanobacterial biovolume and evidence of eutrophication (Zhang, Huang & Wang, 2020). Chlorophyll-*a* level is used to measure the water quality in the freshwater ecosystem (Qi et al., 2014). Higher levels of chlorophyll-*a* in water signify poor water quality, which occurs when there is a high algal production due to high nutrients (Li et al., 2017).

Environmental changes may play a major role in the increased concentration of chlorophyll-*a*, thus the growth of phytoplankton and cyanobacteria that are highly affected by climate changes (Richardson et al., 2019). Also, it is expected that the increase or decrease of phytoplankton and cyanobacteria, which is represented by the concentration of the amount of chlorophyll-*a* due to the increase in water flow events, may depend on the time of year and not only on the amount of nutrient inputs (Richardson et al., 2019). The level of chlorophyll-*a* in the water could fluctuate over time, particularly during the rainy season, when nutrients are flushed into the water. Therefore, the temporal variability of chlorophyll-*a* is investigated to understand the temporal variability of cyanobacterial biovolume and understand how it is being influenced by local air temperature and rainfall in the study lake.

2.7 Influence of Climate (Air Temperature and Rainfall) on the Water Quality

Recent findings indicate that lakes' turbid water and cyanobacteria are likely to aggravate due to global climate change (Zhang et al., 2020). As indicated in Figure 2.8, the air temperature may stimulate phytoplankton growth, whereas warmer surface water stimulates phytoplankton growth. However, earlier onset of thermal stratification may

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lower nutrient exchange with the hypolimnion, inhibiting phytoplankton growth. On top of that, precipitation may increase stream discharge which potentially increases overall nutrient load and thus promotes phytoplankton growth.

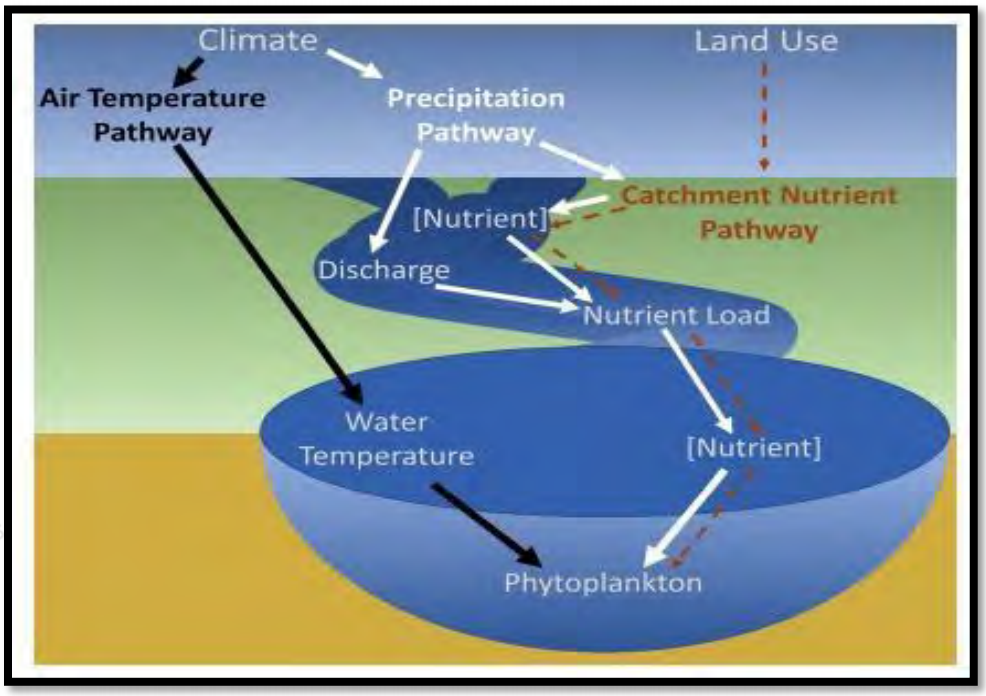


Figure 2.8. Pathways for climate and land use to affect phytoplankton (Ward et al., 2020)

Climate change is associated with shifts in mean climate conditions and more severe extreme weather conditions (Cody et al., 2015). As shown in Figure 2.8, the effects of higher water temperatures under the global warming act are related to increasing nutrients, which worsen the eutrophication problem that is already acute (Brönmark & Hansson, 2017). Rising temperatures and rainfall, increasing atmospheric carbon dioxide concentrations, increasing nutrients discharge, and hydrologic modifications are expected

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to enhance eutrophication and toxic cyanobacterial proliferation in water bodies (Visser et al., 2016). Additionally, these factors are also known to favor the development of microcystin-producing cyanobacterial species (Paerl& Otten, 2013).

In both long and short temporal scales, climatic and local weather factors are essential in understanding the patterns in the lake's physical, chemical, and biological profiles. This is because a wide range of natural physical, chemical, and biological factors may interact and vary seasonally according to differences in weather conditions, runoff volumes, and water levels. For example, increasing nutrient load during the wet season may subsequently lead to a high prevalence of toxic cyanobacteria blooms in the following short temporal scales (Huisman et al., 2018). A better understanding of eutrophication under local weather settings is one of the most significant accomplishments. The changes in global rainfall patterns from climate change affect the chemical and hydrological conditions of the receiving waterbodies (Eslamian, 2016), affecting cyanobacteria in ways that have yet to be fully explored (Romaní et al., 2016). Global annual rainfall is expected to increase, though not as rapidly as the intensity of rainfall. This implies longer dry periods separating more intense rainfall events.

Rainfall impacts many hydrological, chemical, and water quality aspects of the receiving water bodies, both directly and indirectly (Nowak et al., 2020). Direct wet deposition of rainfall may adjust the pH, temperature, and nutrient concentrations of the receiving waterbody (Zanon et al., 2020). Erosion and agricultural runoff may be induced by rainfall, transporting sediment and nutrients into surface water sources, causing

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eutrophication (KasthuriThilagam et al., 2019). Some studies have found that large rain events promoted cyanobacteria blooms in the water reservoir through increased nutrient loading and acceleration of an anoxic zone (Li et al., 2015). Increased flow rates from rainfall will induce flushing and may increase turbulence and resuspension of sediment (Li et al., 2015).

The previous study found that flushing effects from large rainfall events would reduce cyanobacteria in Malaysia's water reservoir (Nursuhayati et al., 2013). Temperature stratification of a water body, a condition where many cyanobacteria may exploit, may be temporarily disrupted in shallow lakes or shorelines of deeper lakes by rainfall events (Visser et al., 2016). (Srifa et al., 2016) found that dry periods promoted cyanobacteria growth in Lake George, Florida. Spotty rainfall in conjunction with temperatures 3-6 ºF above normal in summer months has led to short to moderate drought conditions in the Northeast, which may promote cyanobacteria growth (Srifa et al., 2016). As suggested in Bing et al. (2020), the state of deterioration in the lake water quality and the excessive growth at the highest levels of biovolume concentration, especially cyanobacteria, is due to the influence of rainfall and air temperature. This proves the significant role of local weather factors in increasing the state of trophic in water ecosystems.

Few studies have been conducted in Malaysia to investigate rainfall's effect on the increased runoff volumes and nutrients loading into lake waters (Omar et al., 2016). A study conducted by Aeriyanie et al. (2020) on Slim River Lake, which represents the current study lake, discussed the effect of local rainfall on the changes in lake's depth, total

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phosphorus, and phytoplankton abundance. However, this study did not investigate the influence of local rainfall and air temperature on the physical, chemical, and biological water quality parameters. Therefore, this study was conducted to elucidate further the contribution of local rainfall and air temperature in the lake's eutrophication symptoms.

2.8 Mathematical Analysis for Cyanobacteria Biovolumes

Due to the cyanobacterial blooms' transitory characteristics, mathematical analysis of cyanobacteria biovolume using biochemical multivariate regression analysis has been adopted as a proactive measure for algal bloom control. Key biological, physical, and chemical parameters are selected as inputs for various mathematical analysis, and the early proposed (in a time frame ranging from 1 day to 1 year) have been accomplished. Most multivariate regression analysis provided relatively promising explaintion for cyanobacteria, with a correlation coefficient over 0.85. (Table 2.3). Among those multivariate regression analysis, multivariate regression is the most accurate method since it is suitable for linear biological problems. The several multivariate regression methods are winning ground in a broad scope of scientific fields and, in particular, ecology, due to their ability to produce probabilistic-oriented inferences. The multivariate regression analysis was able to explain cyanobacterial biovolumes without scarifying accuracy. With more sophisticated multivariate regression analysis, the multivariate regression analysis accuracy is significantly improved over time (Haakonsson et al., 2020).

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In terms of the multivariate regression analysis input parameters, the water temperature has been consistently accepted as a primary factor for cyanobacterial explaintion (Huisman et al., 2018). This is inconsistent with the theory stating that increases in cyanobacterial blooms are due to global climate change (Jackson et al., 2016). Nutrients, such as nitrate and phosphate, are also considered relevant in some studies (Jackson et al., 2016). However, it is not always the case. For example, some studies found that neither nitrogen nor phosphorus concentrations was sensitive in predicting cyanobacterial biovolume since the lake was already eutrophic (Smith et al., 2016; Verhamme et al., 2016). From the studies by (Fernández et al., 2013), it is clear that with the addition of physical and chemical parameters to the multivariate regression analysis input, the results of prediction are more relevant (R^2 from 0.84 to 0.91) (Fernández et al., 2013). Many of the earlier studies (before 2010) tended to rely more on physical and chemical parameters, compared to biological parameters from traditional analysis methods (e.g., microscopic analysis for cell density and pigment extraction for chlorophyll-*a* concentration) (Abdelwahab & Amin, 2019).

On the other hand, with the introduction and prevalence of the real-time fluorometry techniques, recent studies (Bertone et al., 2018) included more relevant biological parameters, such as chlorophyll-*a* and phycocyanin concentration. With the input of real-time readings, the prediction time frame is also shortened to one day. However, a major problem with such multivariate regression analysis is the lack of the quality (or condition of being sensitive) evaluation, analysis of goodness-of-fit following multivariate regression analysis standardisation, and comprehensive analysis effectiveness (Shimoda & Arhonditsis, 2016). In addition, it should be noted that the results are highly

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site-specific, depending on the dominant cyanobacterial species in the source and raw water quality.

A previous study by Sharip et al. (2016) reported a simulation multivariate analysis for the Putrajaya Lake in the Malaysian context. The analysis aimed to simulate the lake flow patterns under the influence of different local weather variables. The mandatory wind-controlling variable drives the lake's flow field and the physical and chemical variables' spatial variants. This study used the influence of local climate factors represented by rain and air temperature. The analysis was used to assess water quality and its nutritional status by explaining cyanobacteria's dominance under the influence of rainfall and air temperature factors. Therefore, this study provides an essential dimension to previous studies that provides a useful tool for improving management.

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Table 2.3

Eutrophication multivariate analysis

Method	Predict	Variables	References
boosted regression tree (BRT) Tree-based regressionmethod	Cyanobacteria bloom	Water quality Meteorological Biological	(Descy et al., 2016)
multivariate regression analyais for	Cyanobacteria dominance	Limnological Meteorological Biological	(Persaud et al., 2015)
Simple multivariate analysis	Nutrient dynamic	N and P cycles Cyanobacteria	(Cottingham et al., 2015)
Linear mixed-effect multivariate	Cyanobacteria community	Water quality Zooplankton	
boosted regression tree (BRT)	Cyanobacteria bloomdynamic	Water quality Meteorological	(Bertani et al., 2017)
linear mixed multivariate analysis	Nutrient dynamic	Hydrological Land use	(Soranno et al., 2015)
Numerical Simulation Coupled 3d hydrodynamic-ecosystem multivariate	Spatial dynamics of water quality	Meteorological Hydrological Water quality Biological	(Sharip et al., 2016)
Aquatic multivariate system	Eutrophication and microcosm fragmentation	Landscape Nutrient	Di Carvalho & Wickham, 2019).
A coupled hydrodynamic-ecological multivariate (GLM-AED)	Toxic cyanobacteria bloom	Meteorological Hydrological	(Fadel et al., 2019)
generalised linear multivariate (GLM) Boosted Regression Trees Analysis (BRT)	Chloropyll-a as Eutrophication indicator	Changes in land used due to climate change and political change	(Shan et al., 2019)
one-dimensional dynamic thermal simulation multivariate analysis	Lake thermal and mixing dynamics	Climate change	(Butcheret al.,2015)
maximum flux principle (MPF) and self-organised map (SOM) a	Lake Eutrophication	Environmental data	(Du et al., 2019)

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2.9 National Lake Water Quality Standard and Criteria (NLWQS)

Water quality monitoring of inland water bodies such as lakes, reservoirs and ponds are important to ensure that these water bodies can be managed sustainably for their ecosystem functioning and services. The National Lake Water Quality Criteria and Standards (NLWQS) for Malaysian lake water use is an essential specification of the quality of water required for different protection uses. The criteria are intended to provide the required information in judging the fitness of lake water for human protection of recreational purposes and ecosystem health for the diversity of aquatic life protection.

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2.9.1 Parameters in NLWQS

The parameters used in the National Lake Water Quality Standards (NLWQS) are among the essential parameters nominated by experts to correspond to various categories of lakes in Malaysia due to their ease of measurement. This includes its relationship with the lakes and the availability of sample collection and analysis tools and cost. The physical parameters (temperature, water purity, and dissolved oxygen) were selected. Chemical parameters, such as total phosphorus and total nitrogen, refer to the lake's nutritional status and represent the biological parameters, such as the chemical oxygen demand and its components, the microorganisms in the water. It also includes the microbiology parameters in the water (NAHRIM, 2015)

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2.9.2 Categories in NLWQS

The national lake water quality standard categories are divided into four categories, A, B, C, and D. Category (A) is suitable for human recreational activities in contact with the body, such as swimming, and the water quality is free from diseases transmitted by lake water, as shown in Table 2.4. In this study, Slim River Lake was used for recreational purposes, such as swimming and fishing. Therefore, the results presented in the discussion were described according to Category A.

Table 2.4

Description of categories

CATEGORES	DESCRIPTION
CATEGORY A	• Lakes that are managed in which the water to be used for recreational purposes - primary body contact such as swimming, diving and kayaking.
CATEGORY B	• Lakes used for recreational purposes - secondary body contact such as boating and cruising. • Swimming is not allowed in this category of lakes
CATEGORY C	• The lakes are meant for the preservation of aquatic life and biodiversity.
CATEGORY D	• Lakes managed for the minimum preservation of good aquatic life in the lakes. • It applies good management practices of lakes.

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Table 2.5

List of parameter and criteria for category A, B, C, D

		Guideline Values			
Parameter	**Unit**	**Category A**	**Category B**	**Category C**	**Category D**
Temperature	℃	28	28	28	28
Turbidity	NTU	40	40 - 170	70	250
Ph	-	6.5-8.5	6. 5 - 8.5	6.0 - 9.0	5.5 -9.0
Dissolved oxygen (DO)	mg/L	6.3-7.8	5.5 - 8.7	4.5- 10.3	3.3 -10.3
Chemical oxygen demand (COD)	mg/L	10	25	25	50
Total Phosphorus	mg/L	0.01	0.035	0.035	0.05
Total Nitrogen	mg/L	0.35	0.35	0.35	0.35
Chlorophyll-*a*	ug/L	15	15	15	2.5
Cyanobacteria	Cells/mL	15000	15000	15000	15000

** 20-20 mg/L waterbody is moderately sensitive to acid inputs (4-8 mg/L dissolved calcium), >20 mg/L waterbody is low sensitive to acid inputs (>8 mg/L dissolved calcium).

Eutrophication is a serious public health concern and can have dramatic impacts on local and regional economies as well as aquatic and terrestrial ecosystems. The effects of eutrophication cause excessive production and biovolume of phytoplankton, shift in habitat characteristics due to alteration in the aquatic plants' aggregation, replacement of desirable fish by less desirable species, and the production of toxins by certain algae, such as toxic cyanobacteria. This toxin plays a major role in altering water quality and biodiversity, increasing the operating cost of public water supplies, including taste and odour problems, especially during periods of algal blooms, deoxygenation of water, after the breakdown of algal blooms, loss of

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recreational use of water due to slime, weed infestation, noxious odour from decaying algae, barriers to navigation due to dense weed growth and economic loss due to change in fish species. Therefore, good and proper management plans should also incorporate scientific findings related to hydrological and ecological issues. The succeeded plan should increase water quality and create the lake's aesthetic to maintain biodiversity to provide healthy urban life and offer social, cultural, and economic benefits, especially for the lakes in the residential areas.

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CHAPTER 3

METHODOLOGY

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3.1 Introduction

This chapter describes the methodology used in this study to achieve the research objectives. This study is divided into three parts, and the summary of the study workflow is shown in Figure 3.1. In the first part, water quality analysis was performed to identify the temporal variability of physical, chemical, biological profiles, and genetic diversity of phytoplankton in Slim River Lake.

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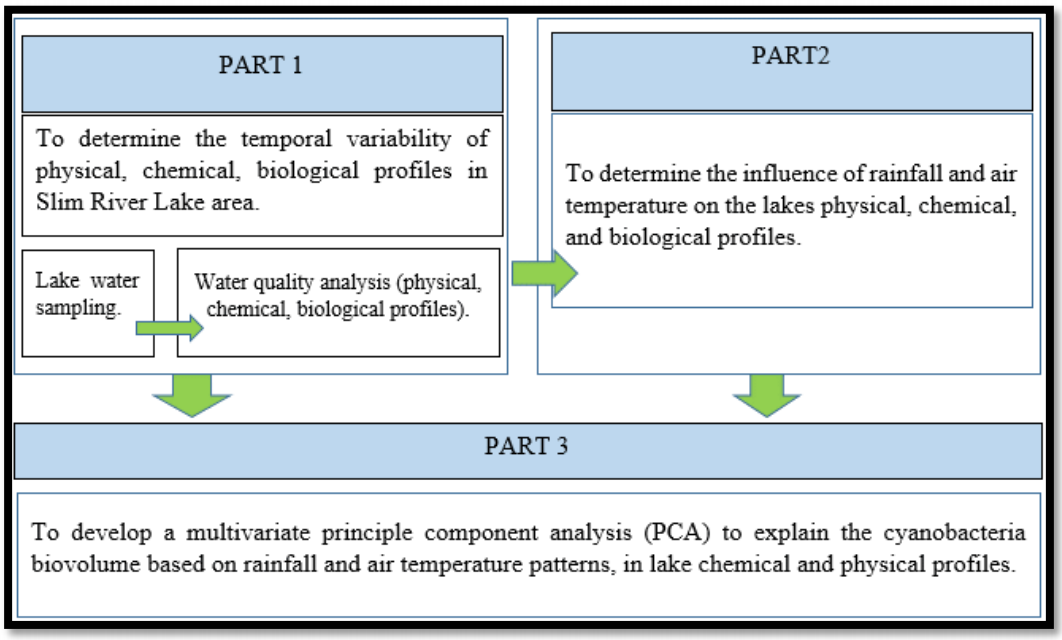


Figure 3.1. Summary of the experimental design.

In the second part, the influence of local rainfall and air temperature on the lake's physical, chemical, and biological profiles was determined using principal component analysis. The Meteorological data were obtained from the Malaysian Meteorological Department to identify the Slim River region's local rainfall and air temperature.

Furthermore, a statistical PCA for explaining cyanobacteria biovolume from local rainfall and air temperature patterns, in lake chemical and physical profiles was developed using Multivariate regression analysis, (principal component analysis).

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3.2 Study Lake Description

Slim River Lake (3º 49' 26.688" N; 101º 24' 30.6216" E), which also known as *Tasik YDP* is a human-made shallow lake (1.40-7.40m) located in the Muallim district of Perak state, Malaysia. The lake's total perimeter, total area, total water volume, and the maximum, minimum depth length and width are summarised in Table 3.1. Slim River Lake is an abandoned ore mine and is currently used for recreation, fishing, and entertainment among local communities. This lake was chosen as a study site due to its high algae and macrophyte growth.

Table 3.1

Study lake's description . Source: (Aeriyanie et al., 2020).

Description	Value
Total perimeter	1280.98 m
Total area	0.0828622 km^2
Total water volume	255168.56 m^3
Maximum depth	7.40 m
Minimum depth	1.40 m
Length	478.36 m
Width	191.31 m

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Five locations around the lake were selected as sampling sites. The location of the Slim River Lake and sampling sites are illustrated in Figure 3.2. The first sampling site (S1) is located upstream of the lake near the oil palm farm. Meanwhile, the second site (S2) is located on the lake's western side, precisely beside the highway opposite the recreational area. Moreover, the third site

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(S3) is situated on the opposite side of the first sampling site, near the livestock farm next to the Bernama river, located less than 100 m away. While the fourth site (S4) is located near the livestock breeding (south of the lake). And the fifth site (S5) is located nearby the oil palm plantation and livestock south of the lake. In general, the selection of the five sites was done according to the sources of pollution. Therefore, the selected sites were very close to pollution sources. Also, the five selected sites were located on the lake's shore due to the convenience of sampling.

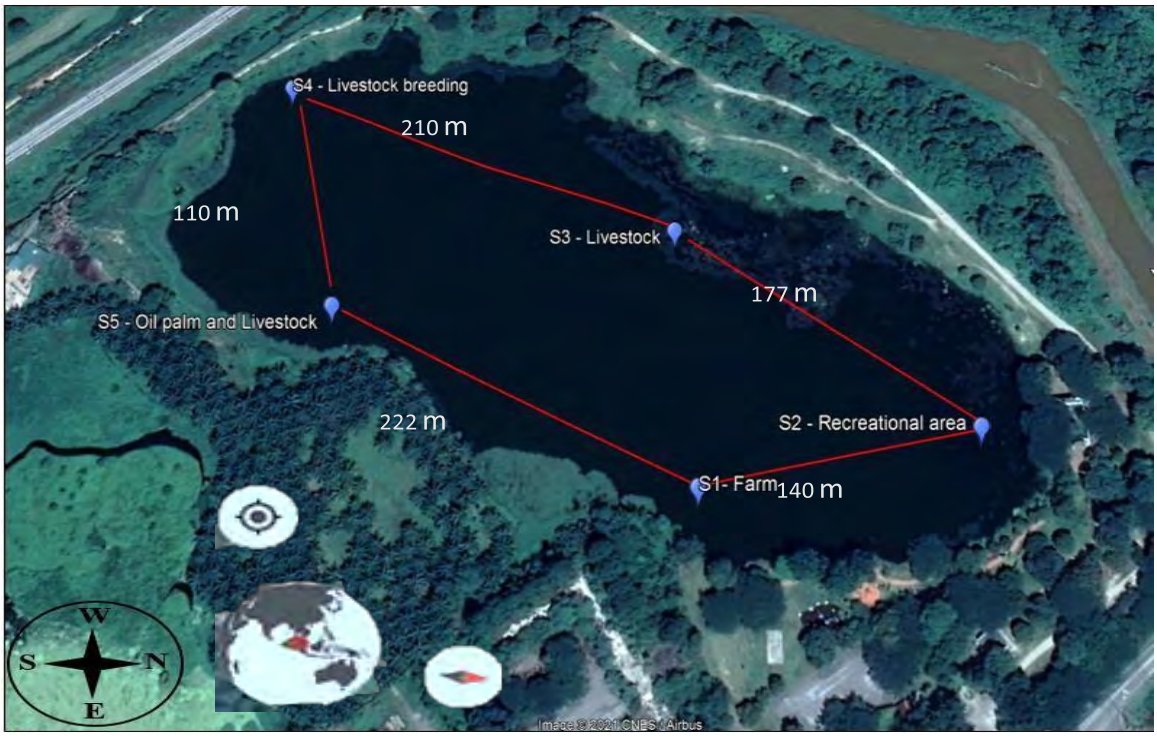


Figure 3.2. Location of the Slim River Lake and the sampling sites (S1-S5).

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Figure 3.3. View on the right side of Slim River Lake which show site 2



Figure 3.4. Oil palm plantation which located on sites 1 and 5.

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Figure 3.5. Livestock breeding which located on site 4.



Figure 3.6. The left side of the lake (playground) which located on site 3

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3.3 Overview of Data Collection, Data Analysis, and Sampling Protocol

The data obtained in this study were either measured on-site, analysed in the laboratory, or obtained from the Malaysian Meteorological Department. The summary of the data analysed in this study and the data sources are provided in Figure 3.7. On-site measurements (yellow colour) were made for the parameters affected by changes during transportation, temperature fluctuation, and other surrounding conditions.

The water quality analysis was grouped into physical, chemical, and biological parameters. Water temperature and turbidity were the physical parameters evaluated in this study. Meanwhile, the chemical parameters analysed were (pH, dissolved oxygen, chemical oxygen demand, total phosphorus, total nitrogen). On the other hand, the biological parameters consisted of analysing (total chlorophyll-*a* and cyanobacterial identification, biovolume).

The water quality analysis was conducted twice per month from (August 2017 to July 2018) in S1, S2 and S3. Meanwhile, in S4 and S5, samples were collected from May 2018 to April 2019. Through the sampling period, on-site measurements and water samples were taken from the depth of (15-20 cm) below the water surface to avoid surface scum and debris. The water samples were collected using polyethylene bottles, which were acid-washed overnight to avoid contamination. Samples were collected from 8:30 a.m. to 10:00 a.m. to reduce the influence of surrounding air temperature variations.

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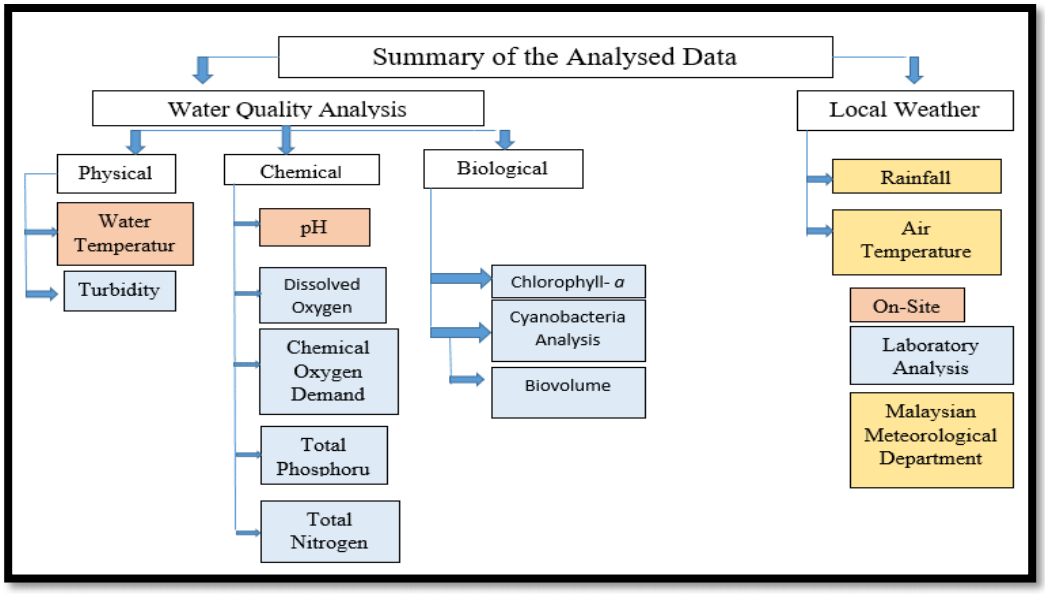


Figure 3.7. Summary of the data collection

Three replicate samples were taken at each sampling site, stored in an icebox, and transferred to the laboratory for further analysis. The samples were analysed separately, and the data were reported as the mean and standard error of the five sampling points.

3.4 Quality Control

In this study, several measures were implemented for quality control purposes. During water sampling, control measures were taken to ensure homogenised samples and low potential contamination. Polyethylene plastic bottles were acid-washed before the sampling. All

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equipment and data sheet were prepared a day before the sampling. During sampling, polyethylene plastic bottles were rinsed with the lake water. The water samples were collected at (15-20 cm) below the water surface. The water samples were then placed in the chilled container and transported to the laboratory. In addition, sampling time were fixed in the morning from (8.30 am to 10.00 am) to reduce the potential influence of rising air temperatures. In the laboratory, the collected water samples were stored and analysed within 24 hours.

3.5 Physical Properties

Three physical properties were analysed in this study, one of which was measured on-site (water temperature). The remaining (turbidity) was analysed in the laboratory under the following procedures.

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3.5.1 Water Temperature

The lake water temperature (℃) was measured on-site where a portable meter (HACH Sens ION^{+} PH_1, Spain) equipped with a temperature sensor. The portable meter was calibrated before each sampling date to ensure its accuracy. The water sampler was submerged in the lake for 5 min to acclimatise its temperature according to the lake's temperature. After that, the water sample was taken from a depth of 15 – 20 cm depth using a water sampler, and the temperature was immediately measured to minimise temperature changes.

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3.5.2 Turbidity

Turbidity is an indicator of how much the water loses its clarity because of suspended particulates. The lake water's turbidity (NTU) examined during a physical checking using a turbidity meter (Hanna Instrument LP 2000-11, Italy) and distilled water as blank. The sample was added into a cleaned glass cuvette, and the turbidity was measured in a range of 0 – 50 NTU. This instrument consists of an infrared light-emitting diode (IR LED) with a wavelength of 890 nm and a silicon photocell light detection unit to ensure light emission consistency and minimise the turbidity interference caused by the colour. The turbidity meter was calibrated before each sampling date to ensure its accuracy.

3.6 Chemical Properties

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Five chemical properties were analysed in this study, one of which was measured on-site (pH), and the remaining four parameters dissolved oxygen, chemical oxygen demand, total nitrogen, and total phosphorus were analysed in the laboratory under the following procedures.

3.6.1 pH

Its pH value can indicate the acidity or alkalinity of a water sample. The pH was measured on site with a portable pH meter (HACH SensION+ PH1, Spain). The pH was measured directly

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from the water sample that was collected using the same protocol, as described in Section 3. 5.1. The pH meter was calibrated before each sampling date to ensure its accuracy.

3.6.2 Dissolved Oxygen

Dissolved oxygen is a measurement of the concentration of oxygen necessary for the existence of all aquatic organisms in the water bodies. In this study, dissolved oxygen (mg/L) was measured using a dissolved oxygen meter (YSI incorporated 550A, USA). The dissolved oxygen probe was rinsed with distilled water prior to usage. After that, the dissolved oxygen probe was inserted into the lake water sample, and the probe was moved through the sample until the dissolved oxygen reading was stabilised. The dissolved oxygen value was measured using 0 – 20 mg/L range setting, with the accuracy of ± 0.3 mg/L per reading. The portable dissolved oxygen meter was calibrated before each sampling date to ensure its accuracy.

3.6.3 Chemical Oxygen Demand

Chemical oxygen demand (COD) is a measurement of the amount of oxygen required to completely oxidise all the organic carbons present in the water sample of CO_2 and H_2O. Chemical oxygen demand (mg/L) is measured using the USEPA Reactor Digested Method (method 8000) and High Range chemical oxygen demand Digestion Reagent (HACH, USA).

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About 100 mL of lake water sample was homogenised, and 2 mL of the sample was pipetted into the chemical oxygen demand vial by holding it at a 45° angle. The content of the vial wasmixed by inverting it several times. The chemical oxygen demand vial used in this study consisted of 2.5 mL of silver sulfate-sulfuric acid, 0.025 N potassium dichromate, and 0.03- gram mercuric sulphate.

Next, the chemical oxygen demand vial was placed in a preheated digital reactor block (HACH DRB200, USA) for 2 h at 150 °C. After the digestion, the vial was inverted several times and left to cool to room temperature. After that, the sample's chemical oxygen demand was measured using a spectrophotometer (HACH DR2500 Odyssey, USA) against a blank (chemical oxygen demand vial filled with deionised water).

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3.6.4 Total Phosphorus

Total phosphorus (TP) was analysed using Test 'N Tube Total High Range Phosphorus Reagent Set (HACH, USA) following the molybdovanadate with acid persulfate digestion method 10127. About 5 mL sample was mixed with potassium persulfate in a vial, and the mixture was heated for 30 min in a digital reactor block (HACH DRB200, USA). After cooled to room temperature, 2 mL of 1.54 N of NaOH and molybdovanadate reagent were added to the vial and mixed gently by inverting the vial. After 7 mins of the reaction period, the phosphorus

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concentration was determined using a spectrophotometer (HACH DR2500 Odyssey, USA) against a deionised water blank.

3.6.5 Total Nitrogen

Total nitrogen (TN) was quantified using Test 'N Tube High Range Total Nitrogen Reagent Set (HACH, USA) following the persulfate digestion method 10072. The vial, containing a (0.5 mL) sample and digestion reagents (nitrogen persulfate and nitrogen hydroxide), was placed into a digital reactor block (HACH DRB200, USA) and heated for 30 min digest. The digested samples were left to cool to room temperature before other nitrogen reagents were added. The nitrogen concentration was subsequently quantified using a spectrophotometer (HACH DR2500 Odyssey, USA) against a deionised water blank.

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3.7 Biological Properties

All the biological properties were analysed in the laboratories according to the following procedures.

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3.7.1 Chlorophyll-*a*

The total chlorophyll-*a* concentration (ug/L) was determined according to the standard method (APHA, 1985), a previously described protocol by (Carlson, 1977). The analysis was performed under dimmed light to prevent chlorophyll-*a* degradation. A known water samples volumes was filtered through 47 mm glass fibre filter paper (Whatman Glass Microfiber GF/C, UK). Algae-containing filter paper was covered with aluminium foil and freeze-thawed three times in a freezer (-20 ^{o}C) and then immersed in a test tube containing 10 mL of 90% acetone.

After that, the test tube and its content were placed in a water bath filled with ice for 7 min to break up the algae cell wall. The extract was then subjected to 5 min centrifugation at 5000 rpm to separate the particulate matter and remaining debris. The absorbance at 750 nm and 665 nm was measured using a spectrophotometer (PRIM-SECOMAM, France) against 90% acetone blank before and after acidification with 0.2 mL of 1% HCl. The concentration of chlorophyll- *a* (ug/L) was then calculated based on revised Lorenzen's equation as shown in the Equation 3.3.

$$\text{Chlorophyll-}a,\ \text{mg/m3} = [26.7\ (665a - 665b)\ \text{x Vl}] / [\text{V2 x L}] \quad \text{(Eq. 7)}$$

where:

Vl = volume of extract, L

V2 = volume of sample, m^3

L= light path length or width of cuvette, cm

665b and 665a = absorbance of 90 % acetone extract before and after acidification.

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3.7.2 Cyanobacteria Analysis

For cyanobacteria analysis, phytoplankton samples were collected from the Slim River Lake with a plankton net (mesh size 37 µm) following the previously described protocol (Sinang et al., 2015). The lake water was collected from 15 – 20 cm depth from the water surface and poured into the plankton net. This process was repeated several times to ensure that enough phytoplankton was collected. After that, the net was rinsed with the lake water, and the phytoplankton collected was stored in a 250 mL amber bottle. The phytoplankton sample was preserved with Lugol's solution until further analysis. Cyanobacteria identification cell count, and biovolume analysis were performed in this study according to the following procedures.

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3.7.2.1 Cyanobacteria Identification and Key Morphological Characteristics

Light microscopy was performed with an inverted microscope (Nikon Eclipse TE 2000-U, UK). The lake water samples were mixed thoroughly prior to the microscopic analysis. A simple wet mount method was used for microscopic observation, where the aliquots of each sample were placed using glass slides and covered with a coverslip. Photographs of cyanobacteria were captured at 400 times magnification using a software NIS Elements Basic Research Version 3. The cyanobacteria were then identified according to the phytoplankton taxonomic guidelines (Schuergers et al., 2016). The cyanobacteria were identified down to the genera level based on the key morphological characteristics.

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Table 3.2

Key morphological characteristics of Microcystis spp

Couplet no	Statement	Refer to couplet no.
1b	Plants microscopic or if visible to the naked eye it is normally because they are present as a mass - but still requiring microscopic observation to determine the more detailed morphology	3
3b	Cells individual or in groups that may be regular or irregular in shape but not forming a filament, strand or ribbon.	56
56a	Cell pigments not localized in chloroplasts .	57
57b	Cells occur as isolated individuals or form colonies which are not epiphytic	58
58b	. Cells not as above but individual or in spherical, ovate or irregular colonies but 3-dimensional and not a flat plate	59
59b	Cells individual or in colonies that are not hollow (at most clathrate (see glossary) with cells distributed throughout	63
63b	Mucilaginous colonies composed of many cells often in the hundreds .	66
66a	Large mucilaginous colonies. Cells approximately spherical to globular in shape	67
67a	Cells of colony densely crowded within the mucilage.....*Microcystis* spp	

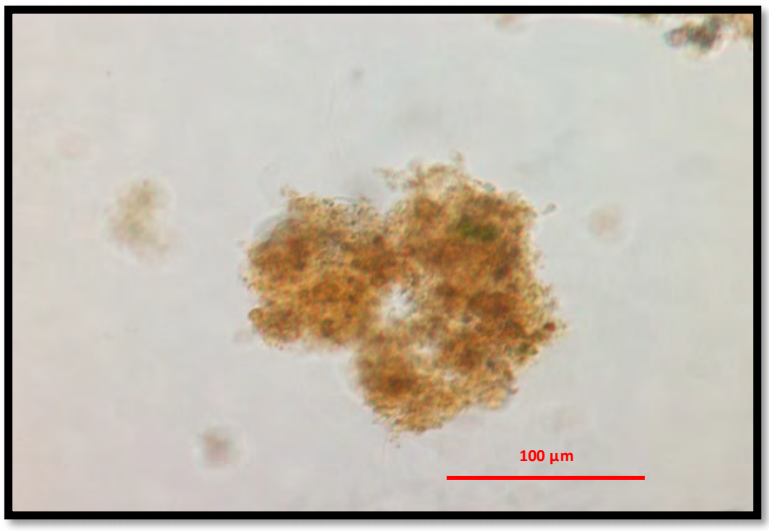


Figure 3.8. Microcystis spp

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Furthermore, a combined Next Generation Sequencing (NGS) method was used with the microscope, providing more accurate monitoring techniques for evaluating cyanobacteria's genetic in lakes water. NGS classification allows deeper analysis of society, which may be associated with more data collection techniques, such as microscopy (Newcombe, Ho & Neto, 2021). However, five litres of water samples were taken from the study lake water and sent to the Apical Scientific Sdn Bhd. The methodology's protocol may be summarised in six (6) steps: Sampling, genomic DNA extraction, genomic DNA purification, amplification, sequencing, and data analysis (Zamyadi et al., 2019).

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3.7.2.2 Cell Count

Cell count quantification (cells/mL) was performed using a light microscope (Leica DME Microscope, Germany) and a haemocytometer (QIUJING, China) with a cell depth of 0.10 mm. This haemocytometer consisted of an improved Neubauer ruling pattern with 0.0025 mm^2 square. The haemocytometer was cleaned using 70 % ethanol to remove all the dirt and grease on its surface. The coverslip was fixed until Newton's ring (rainbow-like ring) was observed to ensure that the coverslip had adhered via suction to the haemocytometer. After that, 10 µL of phytoplankton sample was pipetted on the edge of the coverslip. The haemocytometer was checked carefully to ensure that the sample was well distributed on top of the grids. Four outer

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squares with a 1.0 mm width each, as shown in Figure 3.9, were counted. To observe accuracy and consistency throughout counting, the protocol, as shown in Figure 3.6, was implemented. For each grid, only bacterial cells within the large square and those crossing the edge of two out of the four sides (top and left) were counted. The total cell count was calculated according to Equation 3.4.

$$\text{Cell count (cells/mL)} = \frac{\text{Total number of cells}}{\text{Total Number of grid}} \times 10^4 \qquad \text{(Eq 3.4)}$$

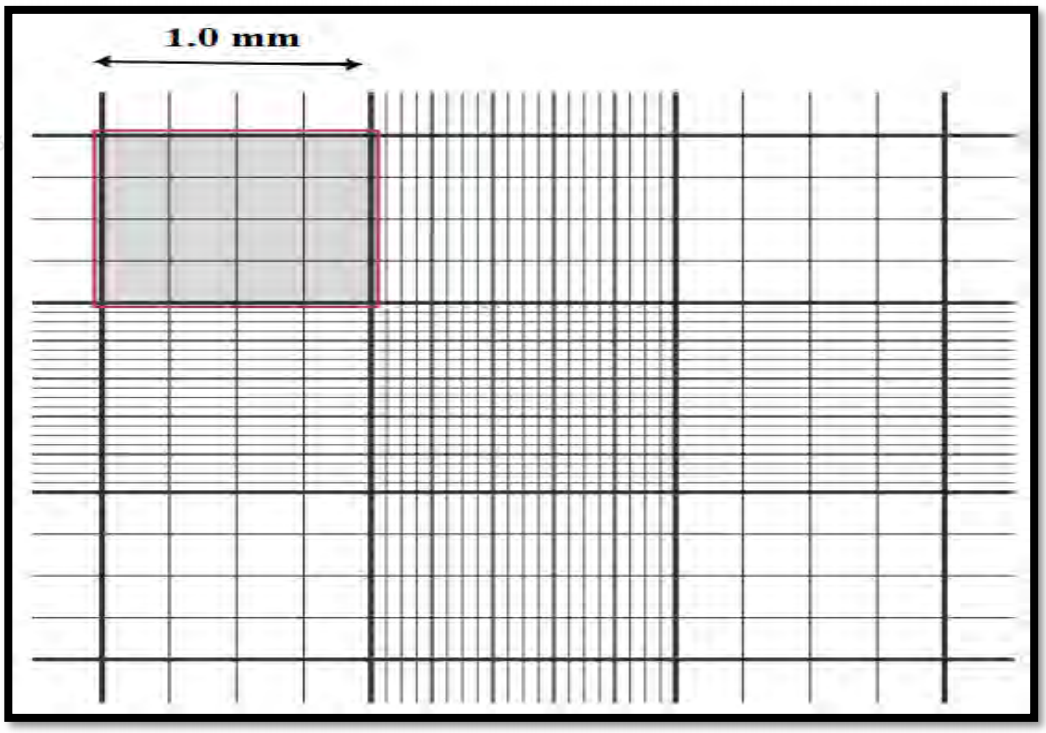


Figure 3.9. Outer square of haemocytometer

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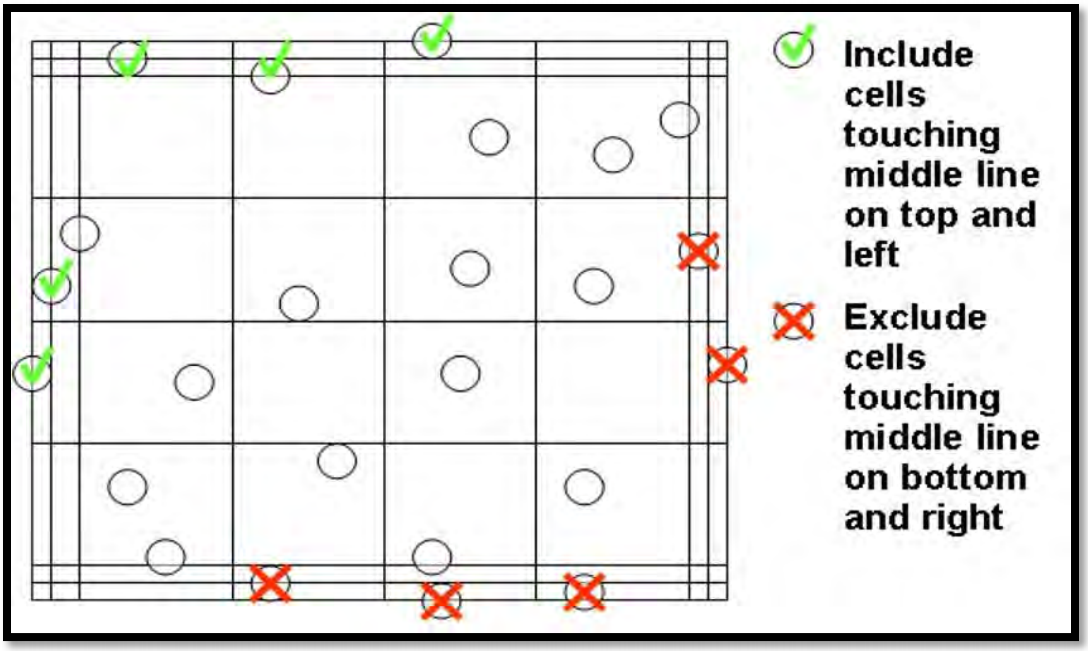


Figure 3.10. Cellcounting protocol

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3.7.2.3 Biovolumes

Biovolumes (μm^3/mL) of *Microcystis* spp. were determined based on the average cell count and cell volume. Average cell volume was determined by assuming the idealised geometric bodies for each species, as shown in Figure 3.11. The diameter (d) and height (h) were measured by an inverted microscope (Nikon Eclipse TE 2000-U, UK) and software NIS Elements Basic Research Version 3.

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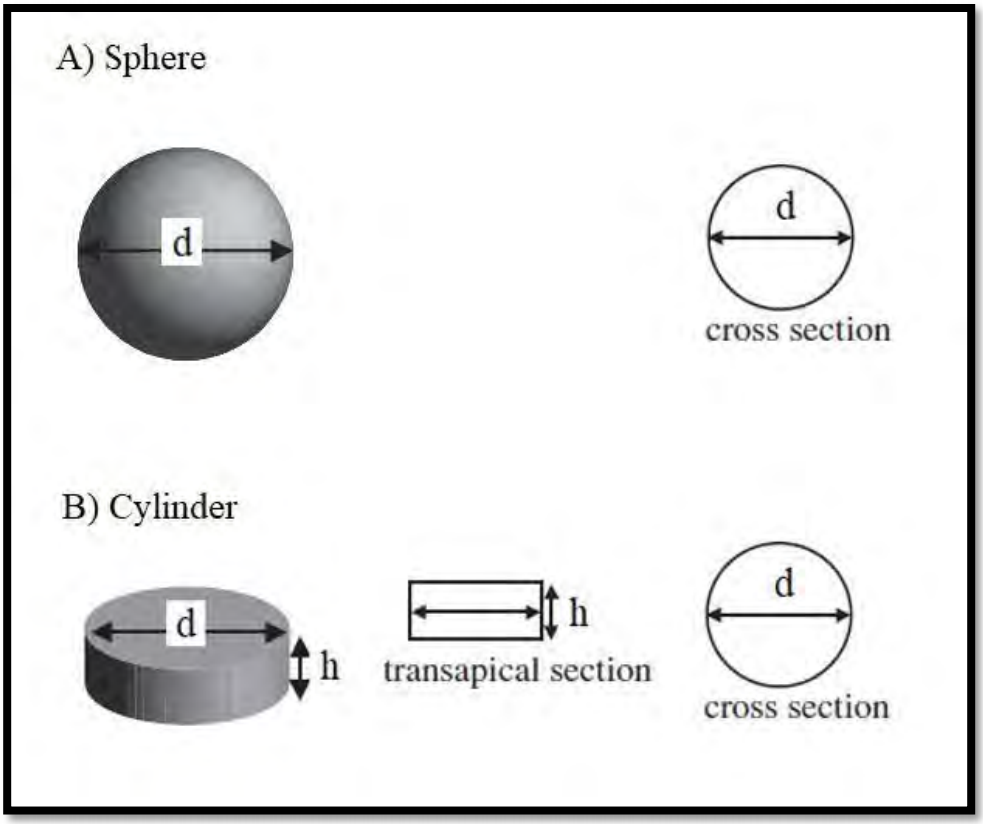


Figure 3.11. A geometric pattern for estimation of cyanobacterial biovolume (Sun, & Liu, 2003).

The average cell volume of *Microcystis* spp., was calculated according to the sphere geometric pattern, given by Equation 3.5. The biovolume (µm³/mL) of *Microcystis* spp was then calculated using Equation 3.7.

$$V_{sphere}\ (\mu m^3) = \frac{\pi}{6} \times d^3 \qquad \text{(Eq 3.5)}$$

Biovolume (µm³/mL) = Average cell volume (µm³) x total cell count (cell/mL). (3.7)

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3.8 Local Rainfall and Air Temperature

The meteorological data records of daily precipitation volume (mm) and average air temperature (°C) in the study area from August 2017 to April 2019 were requested from the Department of Meteorology, Malaysia. The data obtained were measured at Felda Sungai Behrang Meteorological Station, located at line 03° 47 'N and 101° 28' E, respectively. The height of the Meteorological Data Measurement Facility was 48 meters above mean sea level (MSL). Daily meteorological data for precipitation volume and air temperature were used to determine the total monthly mean, precipitation changes, and air temperature in this study. Since the data obtained from the Meteorological Department are reliable, it is assumed that the measurements of the rainfall volumes falling on this station also cover the area within the Slim River Lake.

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3.8.1 Rainfall

The daily rainfall volume obtained was measured as multiple rain occasions collected from 08:00 a.m. to 08:00 a.m. the next day (Malaysia time). The Malaysian Meteorological Department utilised an automatic rainfall measurement system consisted of a tipping bucket and weighing rainfall, as shown in (Figure 3.12). The tipping bucket has a receiver funnel that brought the rainwater to two collecting containers, where the receiver was designed like a see-saw. After 0.2 mm of rainwater was collected, the water's mass caused the receiver to tumble

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down, freeing up space. An electrical signal was sent every time the receiver was tilted down, allowing the amount of rainfall to be recorded over time. The maximum detectable rainfall for this device is 200 mm/h.



Figure 3.12. Automatic rainfall measurement instrument used by Malaysian Meteorological Department

3.8.2 Air Temperature

For precise air temperature measurement, the Malaysian Meteorological Department used a temperature detection system with a combination of internal circuit technology and a platinum

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resistance thermometer. Dry and wet bulb thermometers were supported vertically and placed inside the Stevenson screen. The Stevenson screen allowed the air to circulate freely and acted as an instrument shelter to protect the instrument against rainfall and direct radiation. Figure 3.13 shows the wet and dry bulb thermometer set-up on the right and left side, respectively. The wet thermometer was wrapped with a muslin cloth and placed in a tank filled with pure water. The temperature was recorded as a thermo-hydro graph, which combines the functions of these two thermometers.



Figure 3.13. Air temperature measurement instrument used by Malaysian Meteorological Department

3.9 Data Processing and Statistical Analysis

Data normality test was first performed prior to detailed statistical analysis. The normality of data was assessed based on the Shapiro -Wilk test. Data were then log-transformed to meet the

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assumptions of normality. In objectives 1 and 2, data were reported and interpreted as mean and standard error. The temporal variations of physical, chemical, and biological parameters were examined with the one-way ANOVA using Minitab 18 software and Duncan's multiple range tests (DMRT). The significance level was as $p < 0.05$. Tukey method of Post Hoc comparison was also used to compare the means and determine the significant difference on a temporal scale. Minitab 18 was also used to conduct multivaraite principle component analysis (PCA) to find the correlation and to make explain statistical between lake properties (physical, chemical, and biological) and local conditions (rainfall and air temperature) in Slim River Lake. In summary, data analyses for each study objectives are summarised in Table 3.3

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Table 3.3

Summary of data analyses for each study objective

Study Objective	Data Interpretation	Statistical Analysis
1. To identify the temporal variability o lake's water physical chemical and biologica profiles in Slim River Lake area.	Descriptive Mean and standard error	ANOVA Tukey method for post hoc comparison Significance level $p<0.05$ PCA
2. To determine the influence of rainfall and air temperature on the lake's water physical chemical, and biological profiles.	Descriptive Mean and standard error	PCA
3. To develop a PCA to explain the cyanobacteria biovolumes based on rainfall and air temperature patterns, in-lake chemical, and physical profiles.	Descriptive Mean and standard error	PCA

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CHAPTER 4

RESULTS

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This section consists of all the results obtained and analysed during the study in Slim River Lake. A detailed description of the obtained results concerning each of the research objectives is emphasized. In this study, water samples were analysed for nine parameters which were divided into three main categories (physical, chemical, and biological parameters). All data is presented according to research objectives in the following sections.

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4.1 Objective 1: To Determine the Temporal Variability of Lake's Water Physical, Chemical, and Biological Profiles in the Slim River Lake Area.

The temporal variabilities lake's water physical, chemical, and biological profiles in Slim River Lake were investigated. The water quality analysis was performed on the study lake with five sampling locations to represent the lake's overall condition. The results of each parameter studied were obtained and analysed. However, several parameters were identified in this study and are described as follows: (a) the physical profiles are analysed for water temperature and turbidity; (b) the chemical profiles are analysed for pH, dissolved oxygen, chemical oxygen demand, total nitrogen, and total phosphorus; (c) the biological profiles are analysed for total chlorophyll- *a* and biovolumes of *Microcystis* spp. In this study, only the biovolumes of *Microcystis* spp., was quantified as this genus was present in abundance and most commonly associated with cyanobacterial toxin production (Lee et al., 2015). From a scientific standpoint, lake water properties determination is needed to identify its trophic level and water classification (VishnuRadhan, Sagayadoss, Seelan, Vethamony, Shirodkar, Zainudin & Shirodkar, 2015).

4.1.1 Physical Parameters

The physical parameters that show the temporal variabilities in the Slim River Lake are presented in the results as following.

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4.1.1.1 Water Temperature

The water temperatures in this study lake are highly variable on a temporal basis in all sampling sites Figure 4.1. The water temperature ranged from 28.45-31.00°C in S1, 28.40-31.50°C in S2, 28.60-32.45°C in S3, 28.35-34.66°C in S4, and 27.00-33.85°C in S5. Overall, water temperatures recorded in this lake ranged from 27.00 – 34.66°C when data from all sites were combined. The results are shown clearly in Figure 4.1. On a temporal basis, water temperatures were significantly different when compared between months (ANOVA $F_{(11,24)} = 4.47$ in S1, $F_{(11,24)} = 6.02$ in S2, $F_{(11,24)} = 20.23$ in S3, $F_{(11,24)} = 17.32$ in S4, $F_{(11,24)} = 20.81$ in S5, $p<0.05$). The Tukey's HSD for post-hoc pairwise comparison suggests that water temperature was significantly higher in November for all sites except S5. The detailed ANOVA and post-hoc results are given as in APPENDIX (A). In general, the results indicated that the water temperatures are highly variable on temporal basis, thus supports the hypothesis set in this study.

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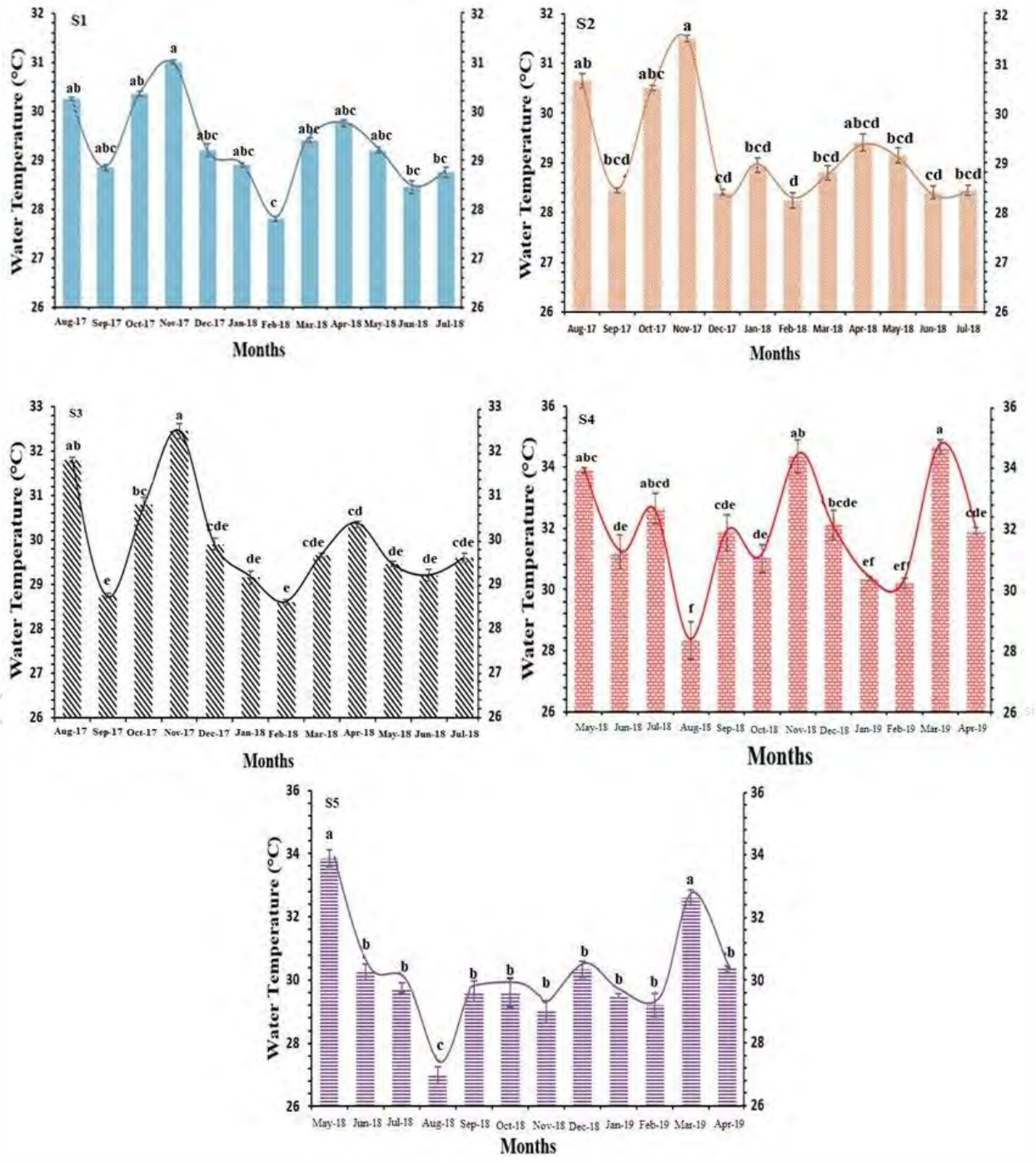


Figure 4.1. Monthly average of water temperature throughout the sampling period for five study sites (S1-S5) in the Slim River Lake. Error bars indicated standard errors. Lakes with common letters are not statistically different ($P > 0.05$).

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4.1.1.2 Turbidity

In this study, the turbidity detected in these study lakes is highly variable on a temporal basis in all sampling sites Figure 4.2. The turbidity ranged from 1.66 – 7.40 NTU in S1, 2.58 – 8.32 NTU in S2, 1.66 – 13.30 NTU in S3, 0.71 – 3.16 NTU S4, and 0.70 – 4.75 NTU in S5. Overall, water turbidity recorded in this lake ranged from 0.70 – 13.30 NTU when data from all sites were combined. The results showed clearly in Figure 4.2. On a temporal basis, turbidity was significantly different when compared between months (ANOVA $F_{(11,24)} = 767.44$ in S1, $F_{(11,24)} = 876.22$ in S2, $F_{(11,24)} = 492.35$ in S3, $F_{(11,24)} = 80.32$ in S4, $F_{(11,24)} = 302.52$ in S5, $p<0.05$). The Tukey's HSD for post-hoc pairwise comparison suggests that turbidity was significantly high in September (S1-S4) and October (S5). The detailed ANOVA and post-hoc results are given as in APPENDIX (B). In general, the results indicated that the turbidity are highly variable on temporal basis, thus supports the hypothesis set in this study.

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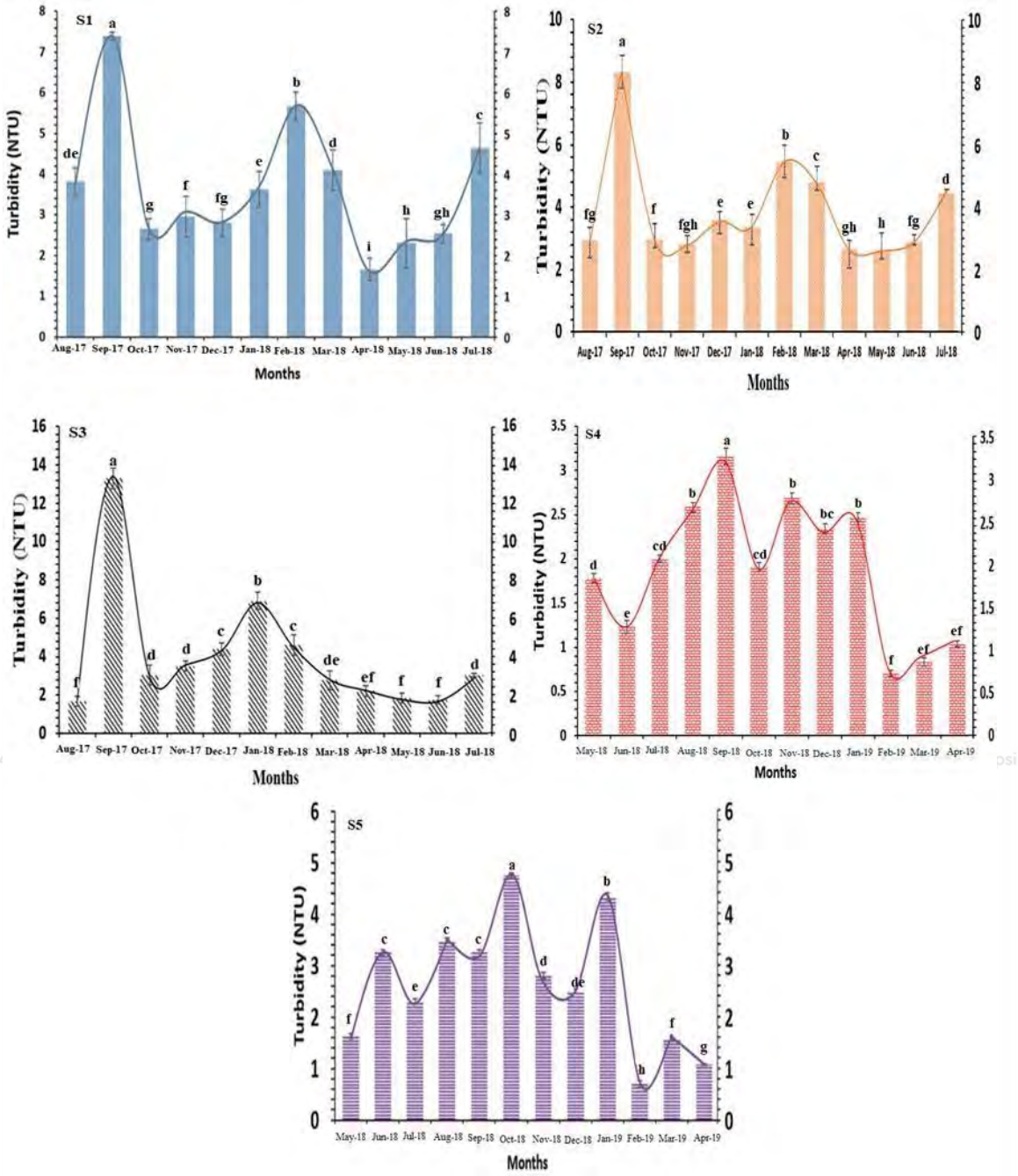


Figure 4.2. Monthly average turbidity throughout the sampling period for five different sites (five-sites S1-S5) in the Slim River Lake. Error bars indicated standard errors. Lakes with common letters are not statistically different ($P > 0.05$).

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4.1.2 Chemical Parameters

The descriptive analysis of the chemical parameters that show the temporal variabilities in the Slim River Lake are presented in the results that following. the results of the variables pH, total nitrogen, total phosphorus, chemical oxygen demand, and dissolved oxygen, were presented and elaborated.

4.1.2.1 pH

In this present study, the water pH detected in these study lakes is highly variable on a temporal basis in all sampling sites Figure 4.3. The water pH ranged from 5.98 -7.11 in S1, 6.00 – 6.73 in S2, 6.07 – 7.67 in S3, 6.12 – 7.82 in S4, and 6.00 – 8.03 in S5. Overall, pH recorded in this lake ranged from 5.98 - 8.03 when data from all sites were combined. The results showed clearly in Figure 4.3. On a temporal basis, pH was significantly different when compared between months (ANOVA $F_{(11,24)} = 54.87$ in S1, $F_{(11,24)} = 30.16$ in S2, $F_{(11,24)} = 46.95$ in S3, $F_{(11,24)} = 44.58$ in S4, $F_{(11,24)} = 66.56$ in S5, $p<0.05$). The Tukey's HSD for post-hoc pairwise comparison suggests that was significantly higher in November compared to most of other months in all sites (S1-S5). The detailed ANOVA and post-hoc results are given as in APPENDIX (C). In term of , In general, the results indicated that the pH are highly variable on temporal basis, thus supports the hypothesis set in this study.

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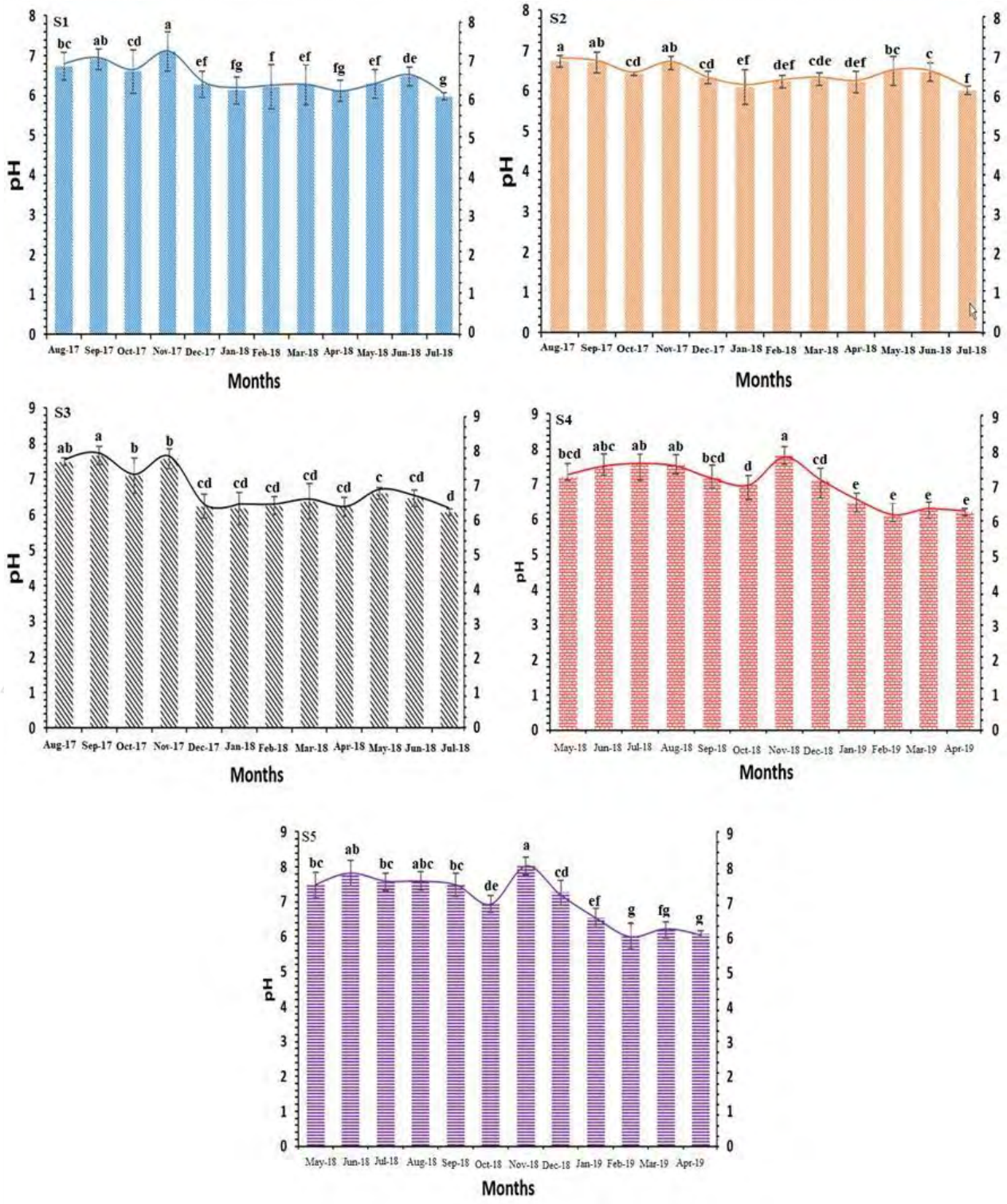


Figure 4.3. Monthly average phH throughout the sampling period for five different sites (five-sites S1-S5) in the Slim River Lake. Error bars indicated standard errors. Lakes with common letters are not statistically different ($P > 0.05$).

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4.1.2.2 Total Nitrogen

The total nitrogen level in Slim River Lake is highly variable on a temporal basis in all sampling sites Figure 4.4. The total nitrogen ranged from 1.0 – 3.5 mg/L in S1, 1.0 – 4.0 mg/L in S2, 1.0 - 4.2 mg/L in S3, 1.12 -1.58 mg/L in S4, and 1.12 – 2.27 mg/L in S5. Overall, total nitrogen recorded in this lake ranged from 1.0 – 4.2 mg/L when data from all sites were combined. The results showed clearly in Figure 4.4. On a temporal basis, total nitrogen was significantly different when compared between months (ANOVA $F_{(11,24)} = 46.24$ in S1, $F_{(11,24)} = 51.86$ in S2, $F_{(11,24)} = 3.95$ in S3, $F_{(11,24)} = 1412.85$ in S4, $F_{(11,24)} = 10.75$ in S5, $p<0.05$). The Tukey's HSD for post-hoc pairwise comparison suggests that total nitrogen is significantly lower in May (S4 and S5), September (S2), and November (S1 and S3) compared to other months. The detailed ANOVA and post-hoc results are given as in APPENDIX (D). In general, the results indicated that the total nitrogen are highly variable on temporal basis, thus supports the hypothesis set in this study.

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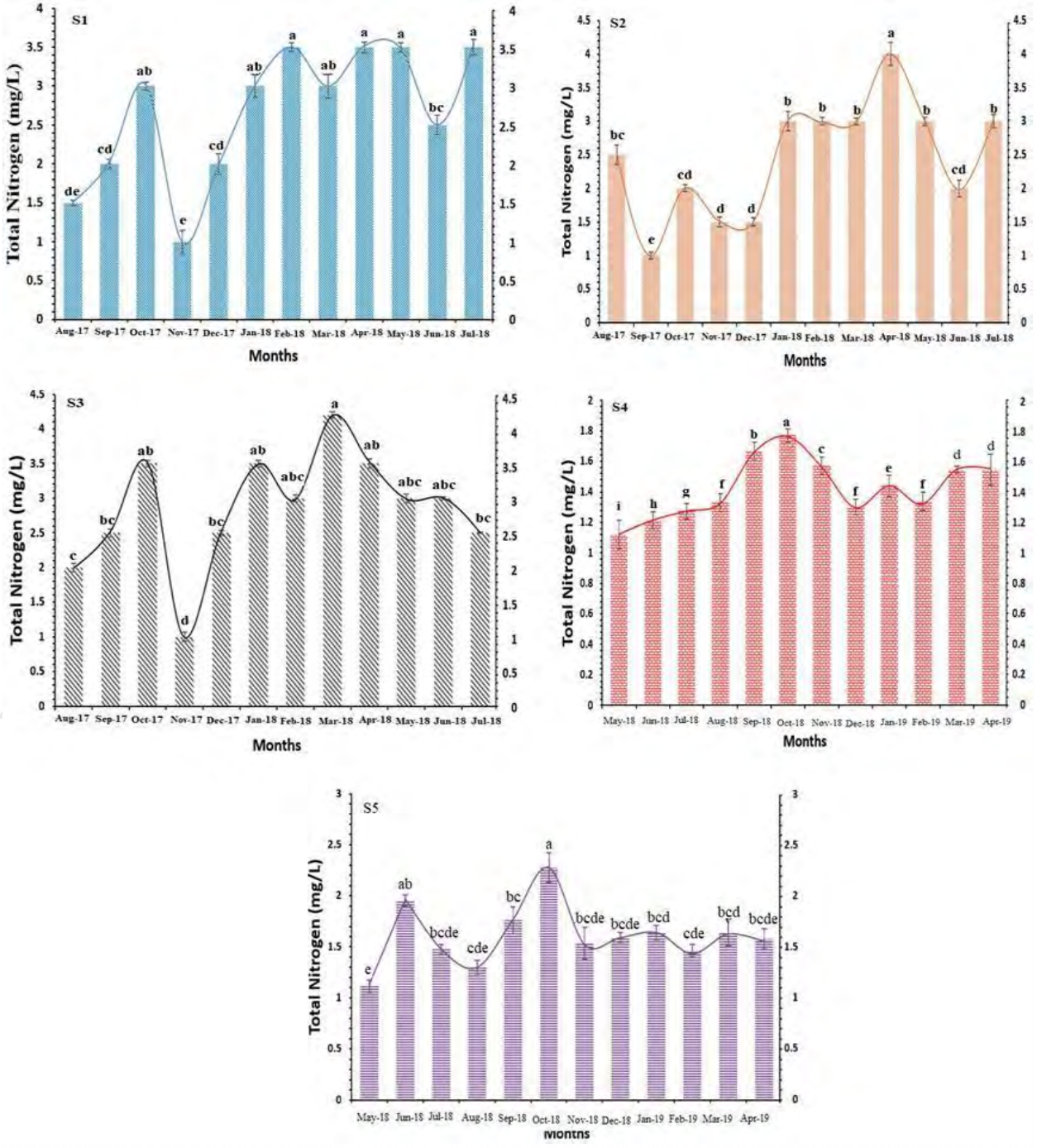


Figure 4.4. Monthly average total nitrogen (mg/L) throughout the sampling period for five different sites (five-sites S1-S5) in the Slim River Lake. Error bars indicated standard errors. Lakes with common letters are not statistically different ($P > 0.05$).

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4.1.2.3 Total Phosphorus

The total phosphorus level in Slim River Lake is highly variable on a temporal basis in all sampling sites Figure 4.5. The total phosphorus ranged from 0.3 - 1.45 mg/L S1, 0.3 - 1.75 mg/L in S2, 0.20 - 1.60 mg/L in S3, 1.12 – 1.96 mg/L in S4, and 0.78 – 1.82 mg/L in S5. Overall, total phosphorus recorded in this lake ranged from 0.20 – 1.96 mg/L when data from all sites were combined. The results are showen clearly in Figure 4.5. On a temporal basis, total phosphorus was significantly different when compared between months (ANOVA $F_{(11,24)} = 20.83$ in S1, $F_{(11,24)} = 18.21$ in S2, $F_{(11,24)} = 13.76$ in S3, $F_{(11,24)} = 15.59$ in S4, $F_{(11,24)} = 34.38$ in S5, $p<0.05$). The Tukey's HSD for post-hoc pairwise comparison suggests that total phosphorus was significantly lower in December and January in most sites except for S5. The detailed ANOVA and post-hoc results are given as in APPENDIX(E). In general,the results indicated that the total phosphorus are highly variable on temporal basis, thus supports the hypothesis set in this study.

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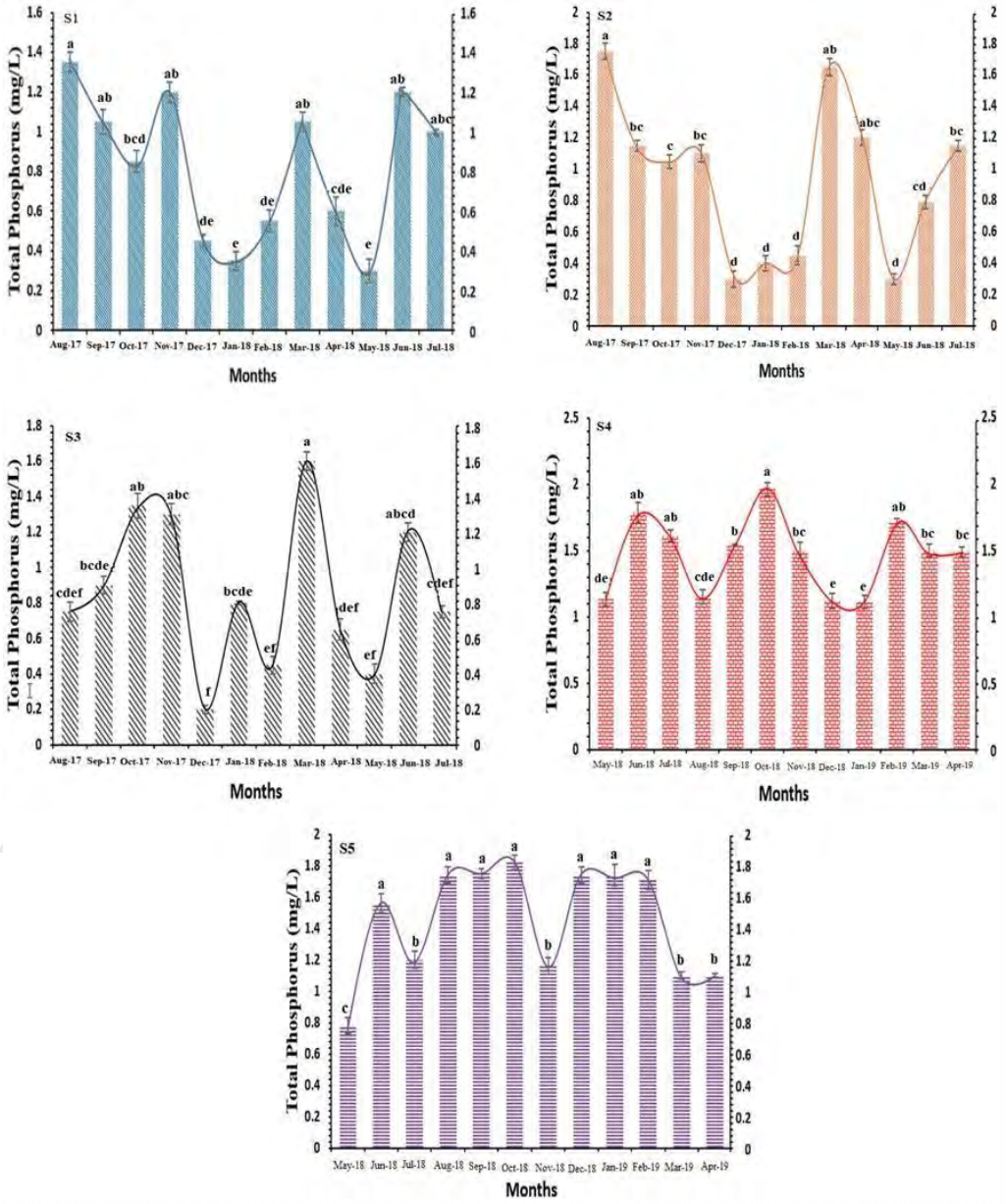


Figure 4.5. Monthly average total phosphorus (mg/L) throughout the sampling period for five different sites (five-sites S1-S5) in the Slim River Lake. Error bars indicated standard Lakes with common letters are not statistically different ($P > 0.05$).errors.

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4.1.2.4 Chemical Oxygen Demand

The Chemical Oxygen Demand level in Slim River Lake is highly variable on a temporal basis in all sampling sites. The chemical oxygen demand ranged from 13.0 – 82.0 mg/L in S1, 11.50 – 75.0 mg/L in S2, 9.50 – 78.0 mg/L in S3, 9.50 – 78.0 mg/L in S4, and 13.0 – 82.0 mg/L in S5. Overall, chemical oxygen demand recorded in this lake ranged from 9.50 – 82.0 mg/L when data from all sites were combined. The results showed clearly in Figure 4.6. On a temporal basis, chemical oxygen demand was significantly different when compared between months (ANOVA $F_{(11,24)} = 1707$ in S1, $F_{(11,24)} = 3081.83$ in S2, $F_{(11,24)} = 1780.85$ in S3, $F_{(11,24)} = 6306.99$ in S4, $F_{(11,24)} = 6396.46$ in S5, $p<0.05$). The Tukey's HSD for post-hoc pairwise comparison suggests that chemical oxygen demand was significantly higher in February in all sites (S1-S5). The detailed ANOVA and post-hoc results are given as in APPENDIX (F). In general, the results indicated that the chemical oxygen demand are highly variable on temporal basis, thus supports the hypothesis set in this study.

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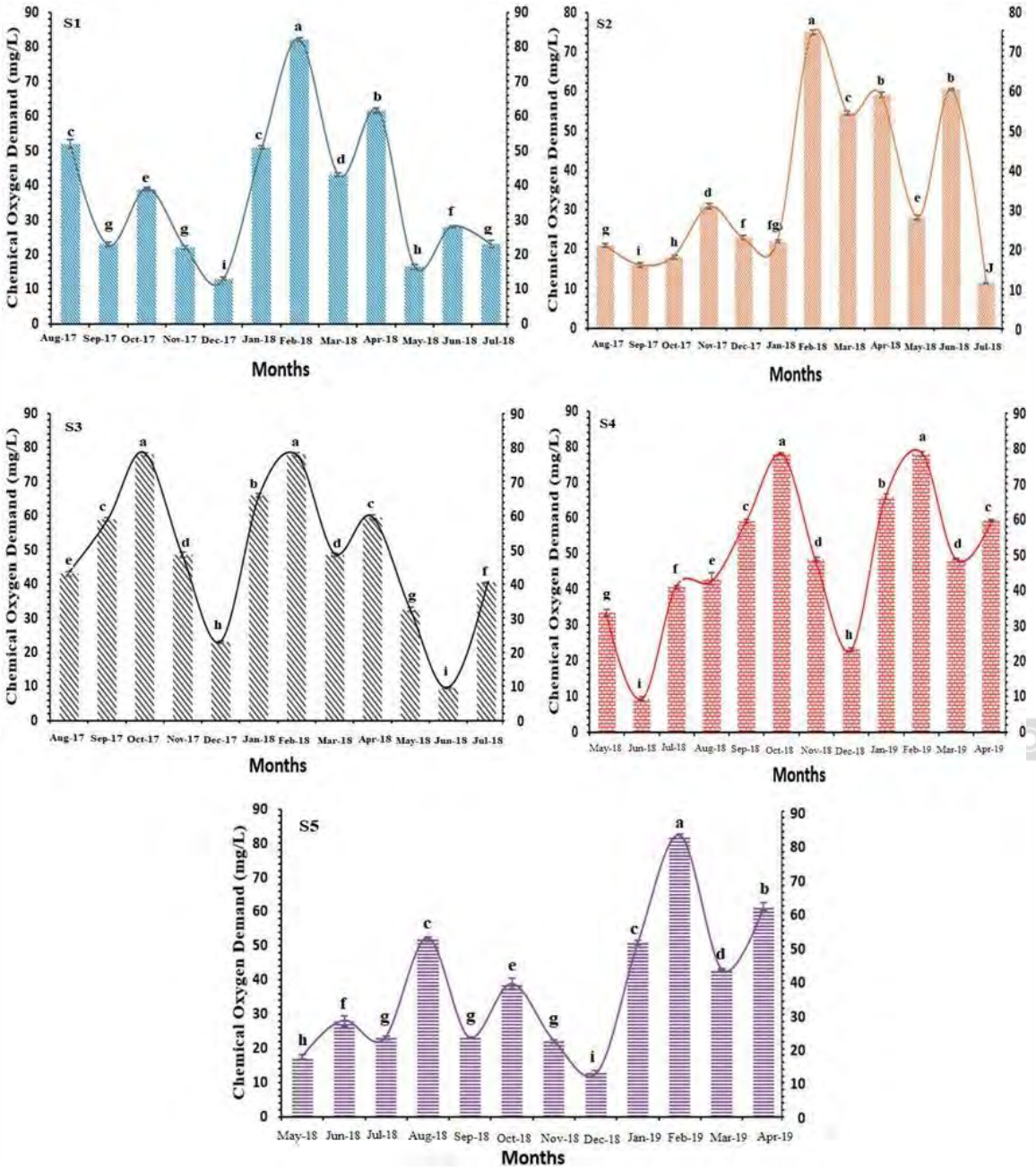


Figure 4.6. Monthly average chemical oxygen demand (mg/L) throughout the sampling period for five different sites (five-sites S1-S5) in the Slim River Lake. Error bars indicated standard errors. Lakes with common letters are not statistically different ($P > 0.05$).

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4.1.2.5 Dissolved Oxygen

The dissolved oxygen level in Slim River Lake is highly variable on a temporal basis in all sampling sites. The dissolved oxygen ranged from 5.93 – 9.44 mg/L in S1, 6.31 – 9.87 mg/L in S2, 5.37 – 9.17 mg/L in S3, 3.51 – 7.70 mg/L in S4, and 2.78 – 7.67 mg/L in S5. Overall, dissolved oxygen recorded in this lake ranged from 2.78 – 9.87 mg/L when data from all sites were combined. The results are clearly shown in Figure 4.7. On a temporal basis, dissolved oxygen was significantly different when compared between months (ANOVA $F_{(11,24)}$ = 435.19 in S1, $F_{(11,24)}$ = 171.22 in S2, $F_{(11,24)}$ = 1010.91 in S3, $F_{(11,24)}$ = 107.89 in S4, $F_{(11,24)}$ = 725.06 in S5, $p<0.05$). The Tukey's HSD for post-hoc pairwise comparison suggests that dissolved oxygen was significantly higher in June for S1-S4, and July for S5. The detailed ANOVA and post-hoc results are given as in APPENDIX (G). In general, the results indicated that the dissolved oxygen are highly variable on temporal basis, thus supports the hypothesis set in this study.

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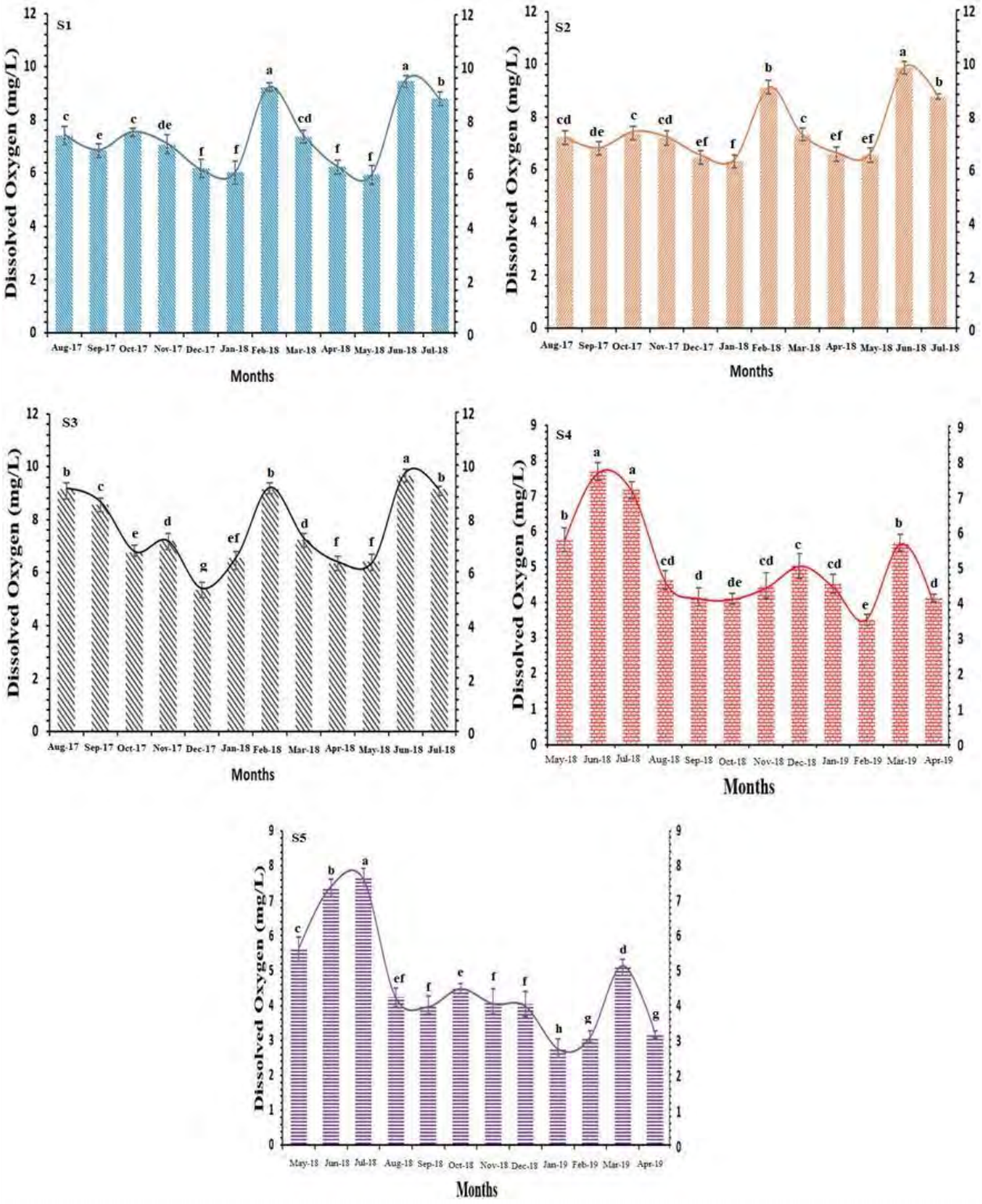


Figure 4.7. Monthly average dissolved oxygen (mg/L) throughout the sampling period for five different sites (five-sites S1-S5) in the Slim River Lake. Error bars indicated standard errors. Lakes with common letters are not statistically different ($P > 0.05$).

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4.1.3 Biological Parameters

This study's biological parameters were composed of chlorophyll-*a,* the biovolume of *Microcystis* spp.

4.1.3.1 Chlorophyll-*a*

The chlorophyll-*a* level in Slim River Lake is highly variable on a temporal basis in all sampling stations. The chlorophyll-*a* ranged from 2.08 – 40.78 ug/L in S1, 2.31 – 56.89 ug/L S2, 2.15 – 41.32 ug/L in S3, 4.95 – 107.50 ug/L in S4, and 18.69 – 132.05 ug/L in S5. Overall, chlorophyll-*a* recorded in this lake ranged from 2.08 – 132.05 ug/L when data from all sites were combined. The results are shown in Figure 4.8. On a temporal basis, chlorophyll-*a* was significantly different when compared between months (ANOVA $F_{(11,24)} = 751.39$ in S1, $F_{(11,24)} = 4366.71$ in S2, $F_{(11,24)} = 7943.47$ in S3, $F_{(11,24)} = 4975.02$ in S4, $F_{(11,24)} = 5195.29$ in S5, $p<0.05$). The Tukey's HSD for post-hoc pairwise comparison suggests that chlorophyll-*a* was significantly different between months in all sites. The chlorophyll-*a* was significantly high in February (S1), August (S2), November (S3), October (S4) and May (S5). The detailed ANOVA and post-hoc results are given as in APPENDIX(H). In general, the results indicated that the chlorophyll-*a* are highly variable on temporal basis, thus supports the hypothesis set in this study.

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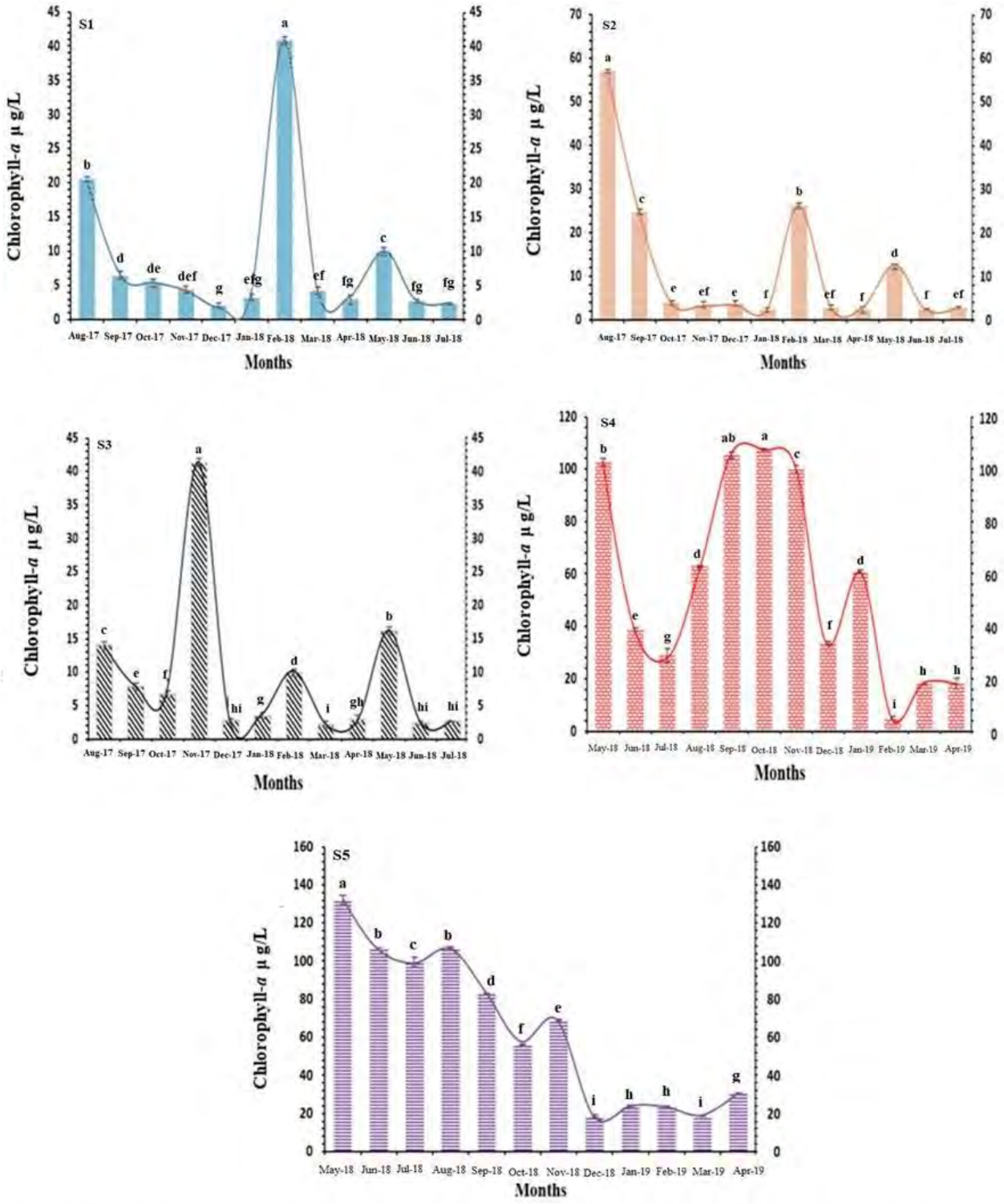


Figure 4.8. Monthly average chlorophyll-*a* level (µg/L) throughout the sampling period for five different sites (five-sites S1-S5) in the Slim River Lake. Error bars indicated standard errors. Lakes with common letters are not statistically different ($P > 0.05$).

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4.1.3.2 Cyanobacterial Biovolumes

The biovolumes of *Microcystis* spp., level in Slim River Lake is highly variable on a temporal basis in all sampling sites. The biovolumes *Microcystis* spp., ranged from 7.01 x 10^7– 1.65 x 10^8 μm^3 mL^{-1} in S1, 8.06 x 10^7 – 1.421 x 10^8 μm^3 mL^{-1} in S2, 8.06 x 10^7 – 1.63 x 10^8 μm^3 mL^{-1} in S3, 6.13 x 10^6 – 5.02 x 10^7 μm^3 mL^{-1} in S4, and 9.78 x 10^6 – 4.23 x $10^7$$\mu m^3$ mL^{-1} in S5. Overall, the biovolumes of *Microcystis* spp., recorded in this lake ranged from 6.13 x 10^6 – 1.65 x 10^8 μm^3 mL^{-1} when data from all sites were combined. The results showed clearly in Figure 4.9. On a temporal basis, the biovolumes of *Microcystis* spp., was significant ly different when compared between months (ANOVA $F_{(11,24)} = 2.83$ in S1, $F_{(11,24)} = 1.6$ in S2, $F_{(11,24)} = 1.8$ in S3, $F_{(11,24)} = 5.10$ in S4, $F_{(11,24)} = 3.44$ in S5, $p<0.05$). The Tukey's HSD for post-hoc pairwise comparison suggests that the biovolumes of *Microcystis* spp., was significantly higher in February (S1), September (S2, S4, S5), and November (S3). The detailed ANOVA and post-hoc results are given as in APPENDIX (I). In general, the results indicated that the biovolumes of *Microcystis* spp., are highly variable on temporal basis, thus supports the hypothesis set in this study.

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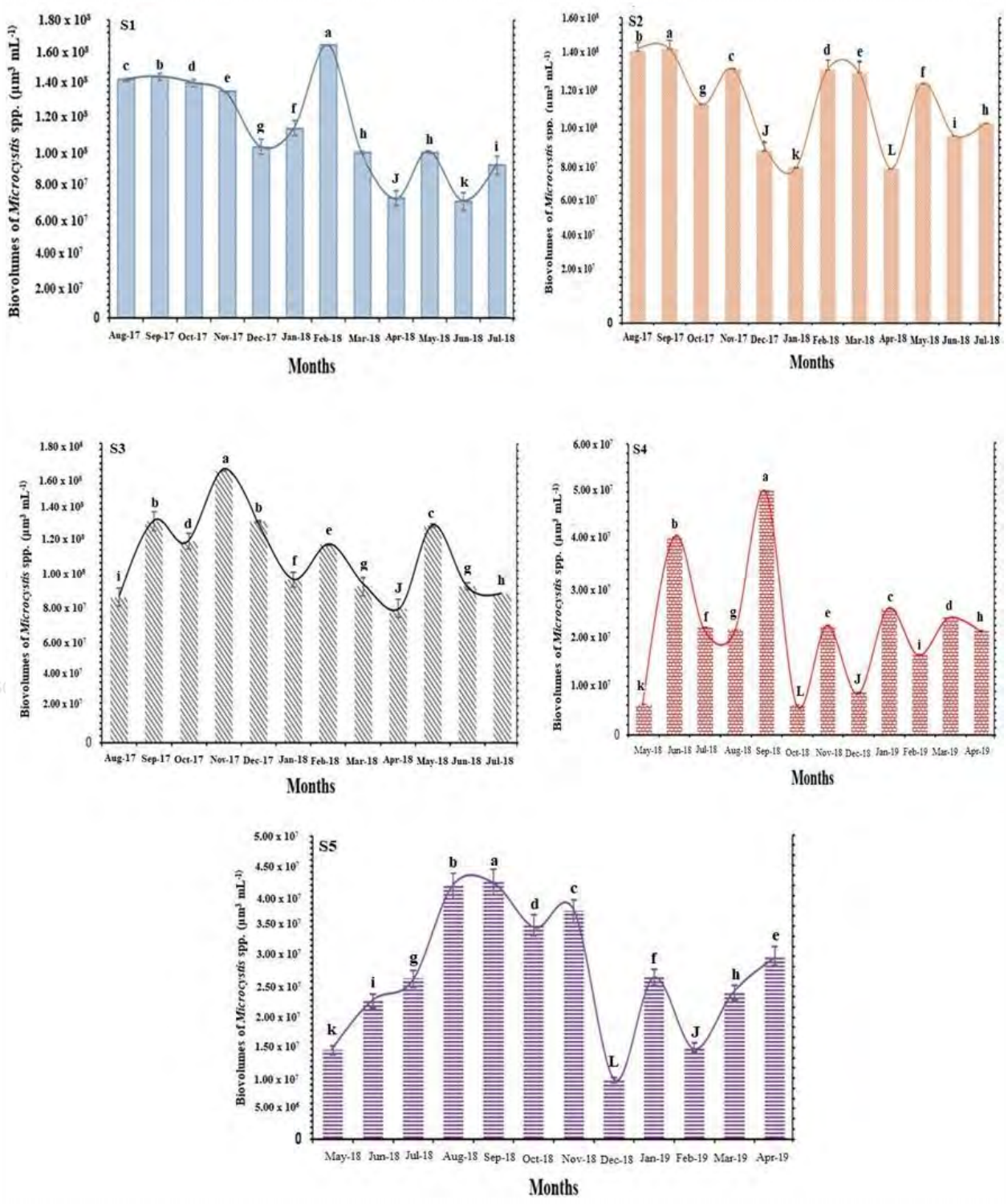


Figure 4.9. Monthly average biovolumes of *Microcystis* spp. µm³ mL⁻ throughout the sampling period for five different sites (five-sites S1-S5) in the Slim River Lake. Error bars indicated standard errors. Lakes with common letters are not statistically different ($P > 0.05$).

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4.1.3.3 Next Generation Sequencing (NGS) Analysis

The next generation sequencing (NGS) analysis was conducted to confirm the presence of cyanobacteria using the molecular approach. This analysis also revealed the genetic diversity of phytoplankton in the Slim River Lake. According to the analysis, the percentage of cyanobacteria was between 12-15% of the total phytoplankton community. This result is based on V3 and V6 regions of the 16S rRNA genes for cyanobacteria. Figure 4.10 and Figure 4.11 show the phytoplankton composition and percentages of cyanobacteria in V3 and V6 regions of the 16S rRNA.

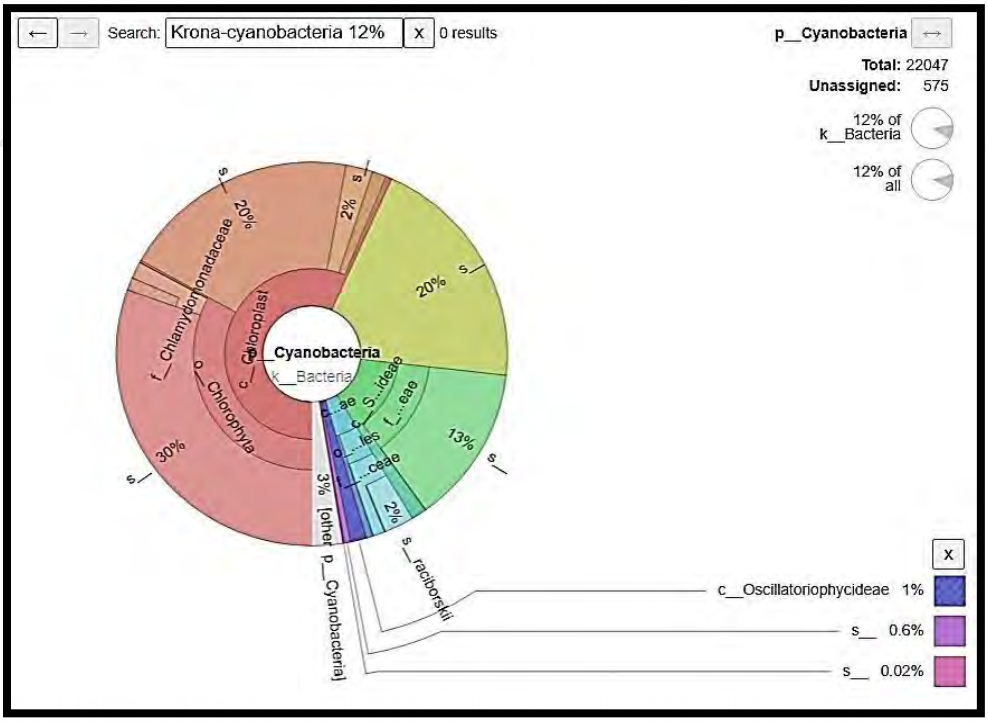


Figure 4.10. Percentage genetic diversity of cyanobacteria 12 % at the region V3.

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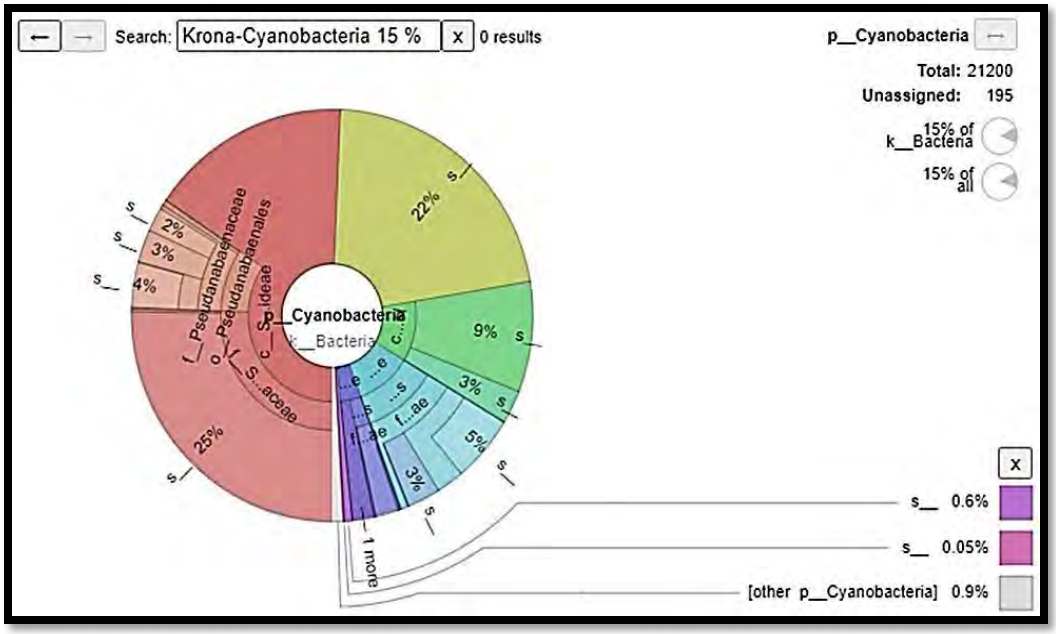


Figure 4.11. Percentage genetic diversity of cyanobacteria 15 % at the region V6

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4.1.4 Multivariate Principal Component Analysis

Multivariate Principal component analysis (PCA) has been designated to decrease the huge data matrix to a lower dimension by maintaining almost all of the data's initial variabilit y. The data matrix covariance or correlation matrix was essential for principal component analysis to compute the eigenvalues and eigenvectors so that the related components could be acquired. In this study, principal component analysis was performed to identify the independent axes or variances in physical, chemical, and biological profiles over the sampling period in Slim River Lake. The principal component analysis plot in Figure 4.10 shows the similarities and differences in temporal variabilities physical, chemical, and biological parameters. The sum of principal components of PC1 and PC2 explained 79.5 % of the variations during 12 months of the sampling period, Table 4.1, Figure 4.12, Figure

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4.13. PC1 and PC2 accounted for 48.6 % and 30.9 % of the overall variance, respectively, Table 4.1. The PC loadings in PC1 showed that the majority of parameters were positively correlated, including chemical oxygen demand, pH, water temperature, chlorophyll-*a*, and total phosphorus. On top of that, variables with negative correlations were also documented, entailing total nitrogen, dissolved oxygen, biovolumes of *Microcystis* spp., and turbidity. Months were plotted following the clockwise direction, which started in August 2017 and ended in April 2019, with no distinct grouping. These results indicated that the temporal fluctuations of these tested parameters did not show any similarity between the parameters. Therefore, in general, the hypothesis of the study for this objective has been achieved that the physical, chemical and biological water features are highly variable on a temporal basis.

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Table 4.1

Principal component analysis (PCA) of temporal variability of physical, chemical, biological profiles over 12 months in Slim River Lake.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Bio-vol Mic	-0.367	-0.268	0.173	-0.165	0.740	-0.350	0.213	0.049	-0.135
Chl-a	0.241	-0.485	-0.113	0.110	0.226	0.103	-0.754	0.195	-0.118
Water-Temperature	0.295	-0.223	-0.671	-0.582	0.030	-0.026	0.243	0.011	0.115
COD	0.409	0.244	0.236	-0.057	0.342	-0.143	-0.149	-0.191	0.719
TN	-0.148	0.408	-0.642	0.517	0.262	-0.204	-0.063	0.123	0.052
TP	0.411	-0.194	0.044	0.239	-0.232	-0.746	0.091	-0.234	-0.245
pH	0.144	-0.511	-0.028	0.541	0.087	0.342	0.481	-0.035	0.261
DO	-0.426	-0.206	-0.192	-0.002	-0.089	0.007	-0.233	-0.808	0.157
Turbidity	-0.399	-0.276	-0.002	-0.019	-0.380	-0.361	-0.083	0.447	0.531
Eigenvalue	4.3778	2.7796	0.7107	0.4521	0.2742	0.1878	0.1040	0.0800	0.0337
Variability (%)	48.6	30.9	7.9	5.0	3.0	2.1	1.2	0.9	0.4
Cumulative (%)	48.6	79.5	87.4	92.4	95.5	97.6	98.7	99.6	100.0

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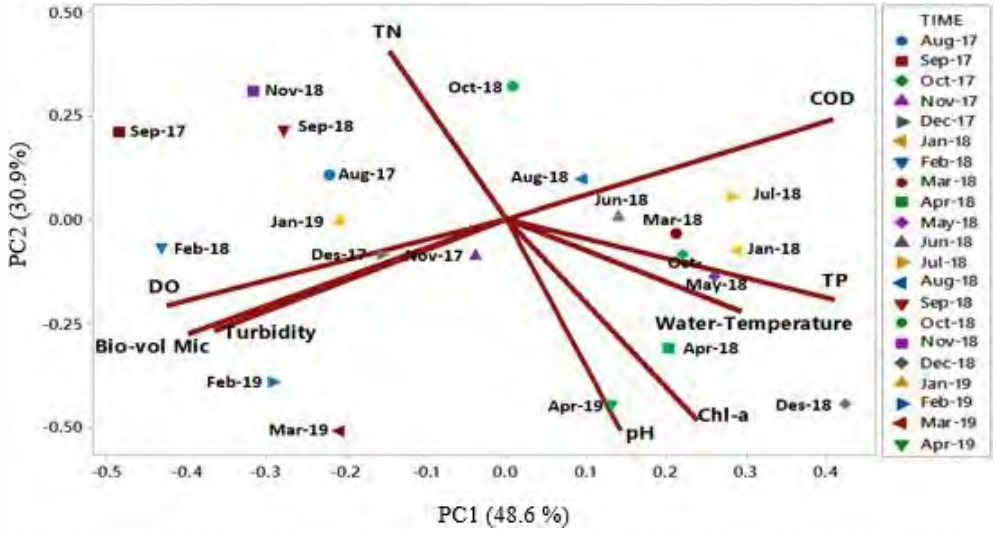


Figure 4.12. Principal component analysis (PCA) diagram of temporal variability of physical, chemical, biological profiles over 12 months in Slim River Lake.

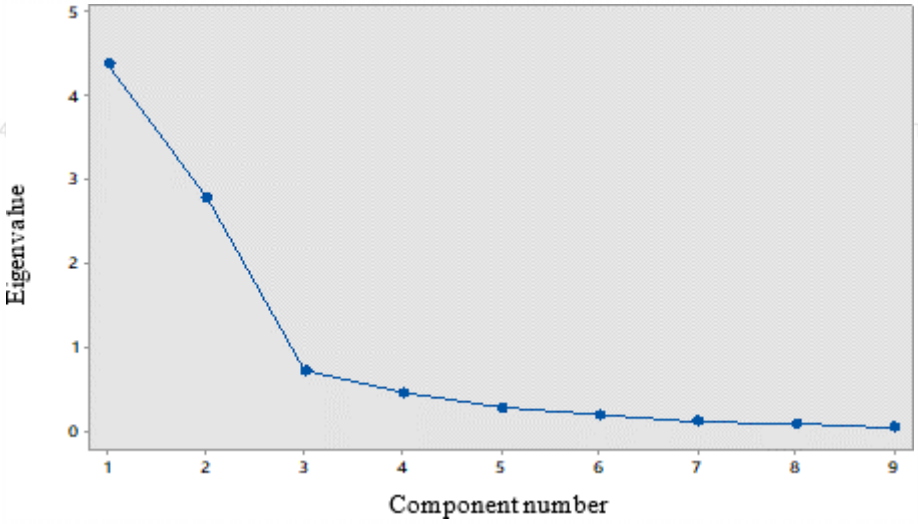


Figure 4.13. Screen plot for temporal variability of physical, chemical, biological profiles performed in Slim River Lake area.

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4.2 Objective 2: To Determine the Influence of Rainfall and Air Temperature on the Lake's Physical, Chemical, and Biological Profiles.

4.2.1 Temporal Variabilities of Local Rainfall and Air Temperatures

For precise air temperature and rainfall measurements, data were used from the Malaysian Meteorological Department to determine the influence of rainfall and air temperature on the lake's physical, chemical, and biological profiles. The highest value of rain was recorded in October 2018, with a rate of 642.5 mm. Meanwhile, the lowest value of rain was recorded in August 2018 with a rate of 36.3 mm, Figure 4.14. As for the air temperature, the highest of 35.0 °C was recorded in March 2019. Meanwhile, the lowest air temperature of 30.71°C was recorded in November 2017, Figure 4.15.

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The lowest amount of rainfall, which amounted to 36.3 mm was recorded when temperatures rose to 34.15°C. This indicates the relationship and correlation between these climatic factors, as when the rain increases, the air temperature decreases and vice versa, Figure 4.14 and Figure 4.15.

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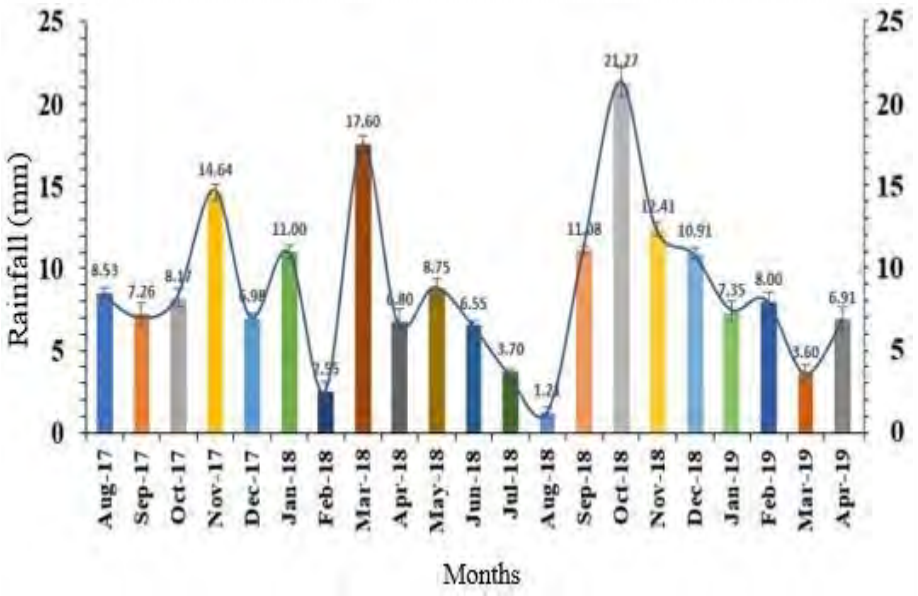


Figure 4.14 Monthly total rainfall throughout the sampling period in the Slim River Lake area. Error bars indicated standard errors.

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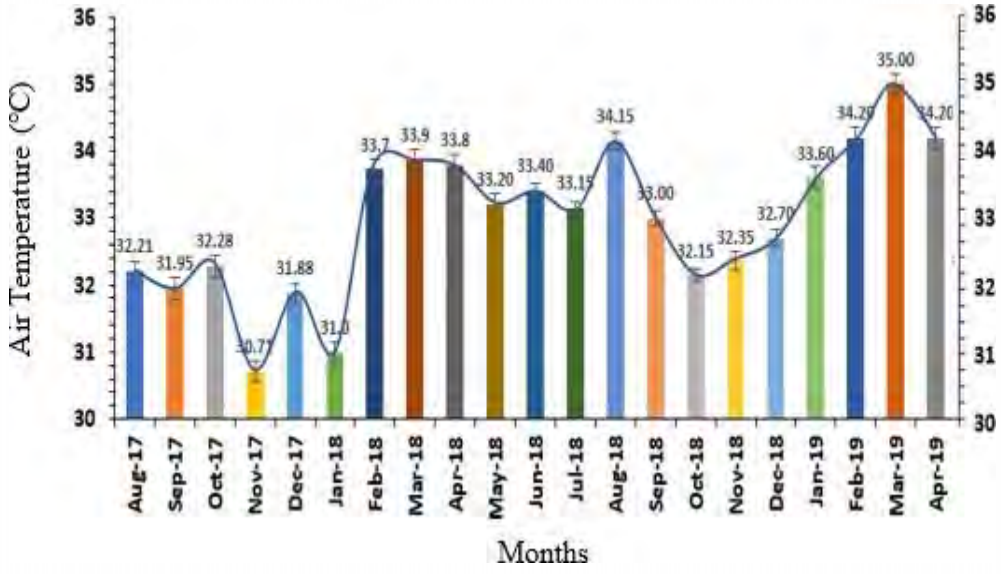


Figure 4.15. Monthly average air temperature throughout the sampling period in the Slim River Lake area. Error bars indicated standard errors.

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4.2.2 Principal Component Analysis (PCA)

Principal component analysis (PCA) was performed to estimate the relationships between rainfall and air temperature patterns and Slim River Lake's physical, chemical, and biological profiles over 12 months. The principal component analysis plots provide an overview of the similarities and differences between parameters of samples over 12 months.

Rainfall

The sum of principal components PC1 and PC2 explained 72.3 % of the variations among 12 months (Figure 4.12). PC1 and PC2 of this study accounted for 43.9% and 28.4% of the overall variance, respectively. Table 4.2, Figure 4.16, Figure 4.17. Table 4.2 presents PC loadings showing that PC1 has the majority of parameters either positively or negatively correlated with rainfall. Many parameters are positively correlated and strongly influenced by rainfall which includes chemical oxygen demand, pH, water temperature, chlorophyll-*a* , and total phosphorus, Figure 4.16. In contrast, there are also parameters which negatively correlated and strongly influenced by rainfall, including total nitrogen, dissolved oxygen, turbidity and biovolumes of *Microcystis* spp., were also documented.

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Table 4.2

Principal component analysis (PCA) of rainfall and physical, chemical, biological profiles over 12 months in Slim River Lake.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Bio-vol Mic	-0.365	-0.261	-0.152	-0.169	0.303	-0.766	0.074	-0.212	-0.042	-0.125
Chl-a	0.247	-0.474	-0.042	0.129	0.232	-0.078	-0.509	0.567	0.190	-0.139
Water-Temperature	0.305	-0.248	0.375	-0.510	0.455	0.195	0.359	-0.097	0.238	0.068
COD	0.403	0.264	-0.145	-0.073	0.072	-0.374	-0.013	0.181	-0.119	0.741
TN	-0.146	0.364	0.468	0.576	0.381	-0.160	0.138	0.117	0.302	0.002
TP	0.414	-0.186	-0.017	0.215	-0.422	-0.299	0.601	0.195	0.067	-0.276
pH	0.149	-0.494	-0.134	0.555	0.178	0.174	0.061	-0.485	-0.157	0.288
DO	-0.421	-0.221	0.090	0.019	0.105	0.179	0.360	0.529	-0.523	0.200
Turbidity	-0.393	-0.288	0.042	-0.037	-0.396	0.012	0.047	0.050	0.626	0.454
Rainfall	0.065	-0.181	0.752	-0.042	-0.345	-0.227	-0.302	-0.149	-0.327	0.070
Eigenvalue	4.3913	2.8368	1.4445	0.4535	0.3298	0.2384	0.1124	0.1018	0.0585	0.0330
Variability (%)	43.9	28.4	14.4	4.5	3.3	2.4	1.1	1.0	0.6	0.3
Cumulative (%)	43.9	72.3	86.7	91.3	94.6	96.9	98.1	99.1	99.7	100.0

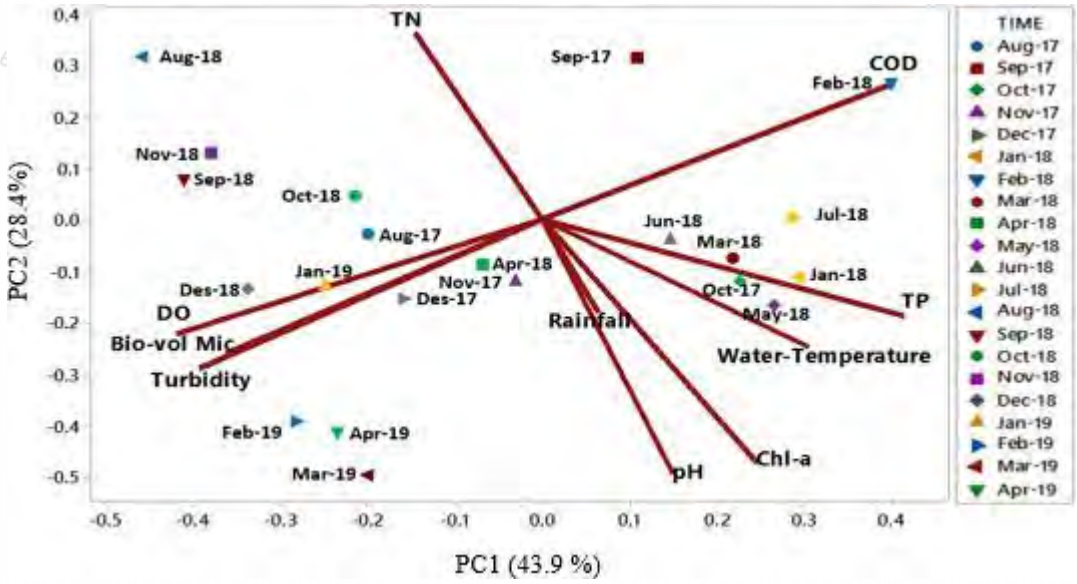


Figure 4.16. Principal component analysis (PCA) diagram of rainfall and physical, chemical, biological profiles over 12 months in Slim River Lake. the colour shows month corresponding to that in other figures.

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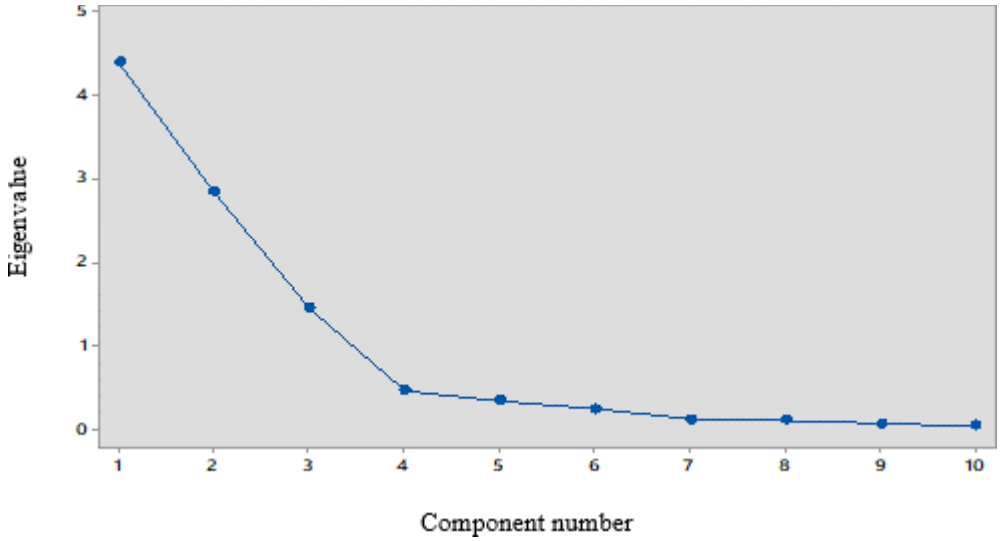


Figure 4.17. Screen plot for rainfall influence on physical, chemical, biological profiles performed in Slim River Lake.

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Air Temperature

The sum of principal components PC1 and PC2 explained 77.3 % of the variations among 12 months. PC1 and PC2 accounted for 45.6% and 31.7% of the overall variance (Table 4.3, Figure 4.18, Figure 4.19). As shown in Table 4.3, PC1 holds the majority of the parameters that are positively and negatively correlated with air temperatures. Many parameters are positively correlated and strongly influenced by air temperature, including chemical oxygen demand, water temperature, chlorophyll-*a*, and total phosphorus. On top of that, parameters that are negatively correlated and strongly influenced by air temperature include dissolved oxygen, biovolumes of *Microcystis* spp., and turbidity.

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In general, the obtained PCA results clearly indicated that the lake's water physical, chemical and biological profiles in Slim River are influence by local rainfall and air temperature, thus support the hypothesis stated for this study.

Table 4.3

Principal component analysis (PCA) of air temperature and physical, chemical, biological profiles over 12 months in Slim River Lake.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Bio-vol Mic	-0.386	0.160	-0.230	0.081	0.636	0.527	-0.126	0.212	-0.057	0.140
Chl-a	0.167	0.490	-0.040	-0.235	0.223	-0.005	0.055	-0.762	-0.165	0.117
Water-Temperature	0.239	0.300	0.663	0.098	0.396	-0.280	-0.281	0.277	-0.064	-0.093
COD	0.431	-0.144	-0.111	0.191	0.198	0.325	0.021	-0.150	0.201	-0.733
TN	-0.090	-0.397	0.482	-0.659	0.019	0.382	0.017	-0.079	-0.106	-0.058
TP	0.370	0.264	-0.060	-0.103	-0.391	0.426	-0.568	0.120	0.214	0.249
pH	0.077	0.481	-0.221	-0.524	-0.059	-0.055	0.385	0.462	0.035	-0.266
DO	-0.439	0.101	0.066	-0.164	0.044	-0.201	-0.210	-0.181	0.788	-0.160
Turbidity	-0.424	0.171	-0.060	-0.007	-0.281	-0.049	-0.453	-0.064	-0.490	-0.507
Air Temperature	0.238	-0.353	-0.449	-0.383	0.336	-0.406	-0.427	0.060	-0.078	0.042
Eigenvalue	4.5596	3.1690	0.8227	0.5481	0.3050	0.2177	0.1624	0.1034	0.0787	0.0332
Variability (%)	45.6	31.7	8.2	5.5	3.1	2.2	1.6	1.0	0.8	0.3
Cumulative (%)	45.6	77.3	85.5	91.0	94.0	96.2	97.8	98.9	99.7	100.0

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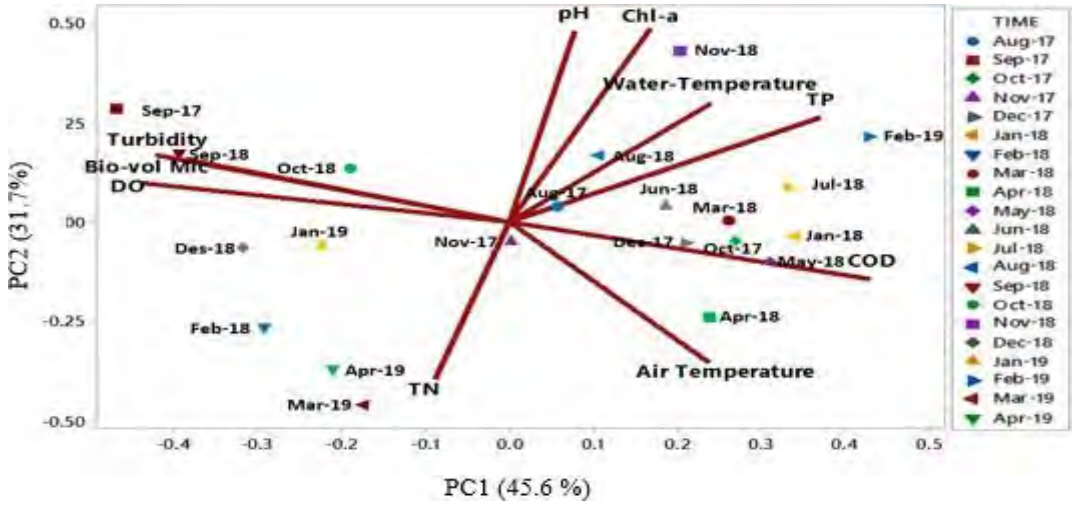


Figure 4.18. Principal component analysis (PCA) diagram of air temperature and physical, chemical, biological profiles over12 months in Slim River Lake. the colour shows month corresponding to that in other figures.

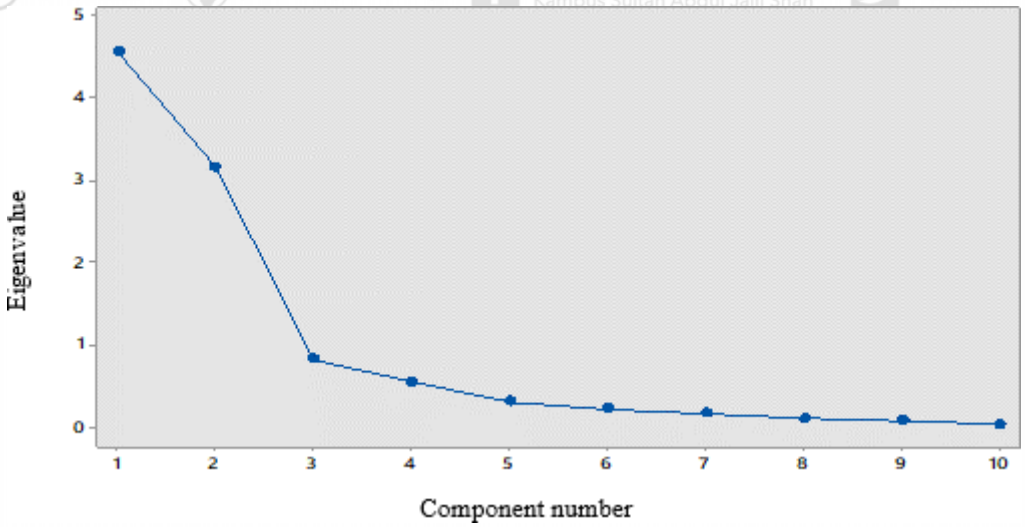


Figure 4.19. Screen plot for air temperature influence on physical, chemical, biological profiles performed in Slim River Lake.

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4.3 Objective 3: To Develop a Multivariate Principle Component Analysis (PCA) to Explain the Cyanobacterial Biovolumes Based on Rainfall and Air Temperature Patterns, in Lake Chemical, and Physical Profiles.

4.3.1 Multivariate Principal Component Analysis (PCA)

Multivariate Principal component analysis (PCA) was performed to explain the biovolume of cyanobacteria based on rainfall and air temperature patterns, in-lake chemical, and physical profiles recorded in Slim River Lake over the sampling period. The principal component analysis plots provide an overview of the similarities and differences between parameters of samples over 12 months.

Chlorophyll-*a* Level

The sum of principal components PC1 and PC2 explained 71.5 % of the variations among 12 months. The PC1 has the most extensive data, which illustrates the significant variations in the whole dataset. PC1 and PC2 of this study accounted for 39.8% and 31.7% of the overall variance, respectively (Table 4.4, Figure 4.20, Figure 4.21).

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Table 4.4

Principal component analysis (PCA) of chlorophyll-a levels and rainfall ,air temperature, physical, chemical profiles over 12 months in Slim River Lake.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Chl-a	0.290	0.411	0.114	0.221	0.207	0.068	-0.730	0.207	-0.150	0.201
Water-Temperature	0.314	0.261	-0.382	-0.287	0.556	0.267	0.376	0.113	-0.257	-0.060
COD	0.417	-0.285	0.005	-0.148	-0.061	-0.113	-0.228	0.237	0.078	-0.770
TN	-0.189	-0.299	-0.503	0.515	0.208	-0.424	-0.027	0.212	-0.297	-0.005
TP	0.443	0.145	-0.000	0.145	-0.536	-0.118	0.366	0.506	-0.058	0.260
pH	0.192	0.419	0.244	0.534	0.148	-0.245	0.308	-0.382	0.125	-0.324
DO	-0.431	0.235	0.035	0.124	0.193	0.155	0.087	0.601	0.533	-0.170
Turbidity	-0.398	0.296	0.091	0.041	-0.321	0.252	0.017	0.084	-0.647	-0.389
Rainfall	0.041	0.244	-0.707	0.116	-0.384	0.261	-0.174	-0.271	0.309	-0.103
Air Temperature	0.168	-0.440	0.138	0.496	0.060	0.708	0.082	-0.007	-0.021	0.022
Eigenvalue	3.9810	3.1716	1.4362	0.5640	0.3185	0.2101	0.1143	0.1089	0.0588	0.0366
Variability (%)	39.8	31.7	14.4	5.6	3.2	2.1	1.1	1.1	0.6	0.4
Cumulative (%)	39.8	71.5	85.9	91.5	94.7	96.8	98.0	99.0	99.6	100.0

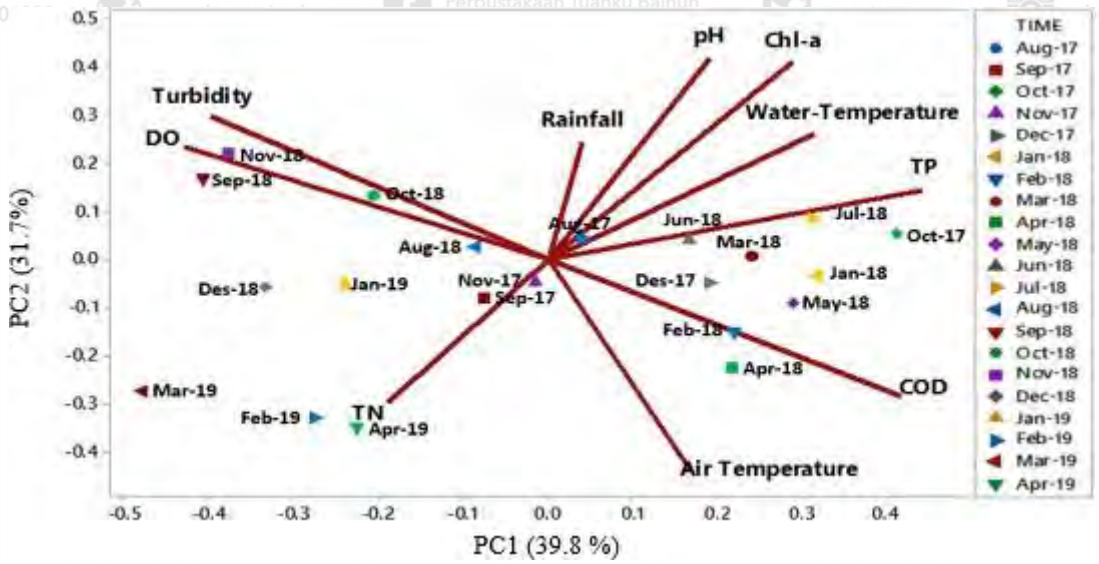


Figure 4.20. Principal component analysis (PCA) diagram of chlorophyll-*a* levels and rainfall, air temperature, physical, chemical profiles over 12 months in Slim River Lake. the colour shows month corresponding to that in other figures.

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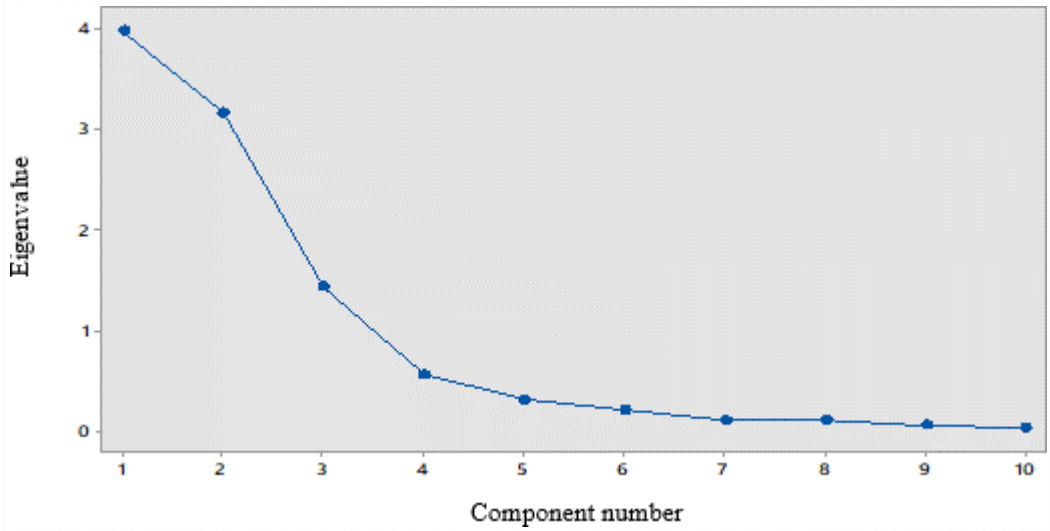


Figure 4.21. Screen plot for influence of air temperature, rainfall, water chemical and water physical patterns on chlorophyll-*a*.

As shown in Table 4.4, PC1 contains parameters that positively and negatively correlate with chlorophyll-*a*. Air temperature, water temperature, chemical oxygen demand, pH, and total phosphorus have a positive correlation and strong influence on chlorophyll-*a* . Meanwhile, chlorophyll-*a* is negatively influenced by total nitrogen, dissolved oxygen and turbidity.

Biovolumes of *Microcystis* spp.

The sum of principal components PC1 and PC2 explained 70.2 % of the variations among 12 months. PC1 has the largest data which explicates the significant variations in the whole dataset. PC1 and PC2 of this study accounted for 44.8% and 25.4% of the overall variance, respectively (Table 4.5, Figure 4.22, Figure 4.23).

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Table 4.5

Principal component analysis (PCA), biovolumes of Microcystis spp. and rainfall, air temperature, physical, chemical profiles over 12 months in Slim River Lake.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Bio-vol Mic	0.405	-0.058	-0.204	0.053	0.344	-0.774	-0.192	0.090	0.015	-0.156
Water-Temperature	-0.201	-0.446	0.292	0.293	0.599	0.038	0.302	-0.211	0.305	0.035
COD	-0.448	0.090	-0.078	0.170	0.026	-0.310	-0.215	-0.095	-0.205	0.750
TN	0.043	0.385	0.554	-0.418	0.254	0.014	-0.442	-0.181	0.269	0.053
TP	-0.337	-0.366	-0.090	-0.249	-0.370	-0.271	-0.168	-0.583	0.127	-0.296
pH	-0.016	-0.492	-0.282	-0.636	0.269	0.207	-0.157	0.288	-0.071	0.213
DO	0.451	-0.025	0.039	-0.113	0.135	0.120	0.183	-0.606	-0.566	0.168
Turbidity	0.444	-0.109	-0.029	-0.082	-0.351	-0.071	0.238	-0.084	0.589	0.495
Rainfall	0.009	-0.311	0.673	-0.134	-0.291	-0.311	0.194	0.329	-0.322	0.034
Air Temperature	-0.280	0.395	-0.134	-0.451	0.147	-0.265	0.671	-0.009	0.033	-0.040
Eigenvalue	4.4803	2.5411	1.4751	0.5348	0.3162	0.2419	0.2074	0.1077	0.0610	0.0345
Variability (%)	44.8	25.4	14.8	5.3	3.2	2.4	2.1	1.1	0.6	0.3
Cumulative (%)	44.8	70.2	85.0	90.3	93.5	95.9	98.0	99.0	99.7	100.0

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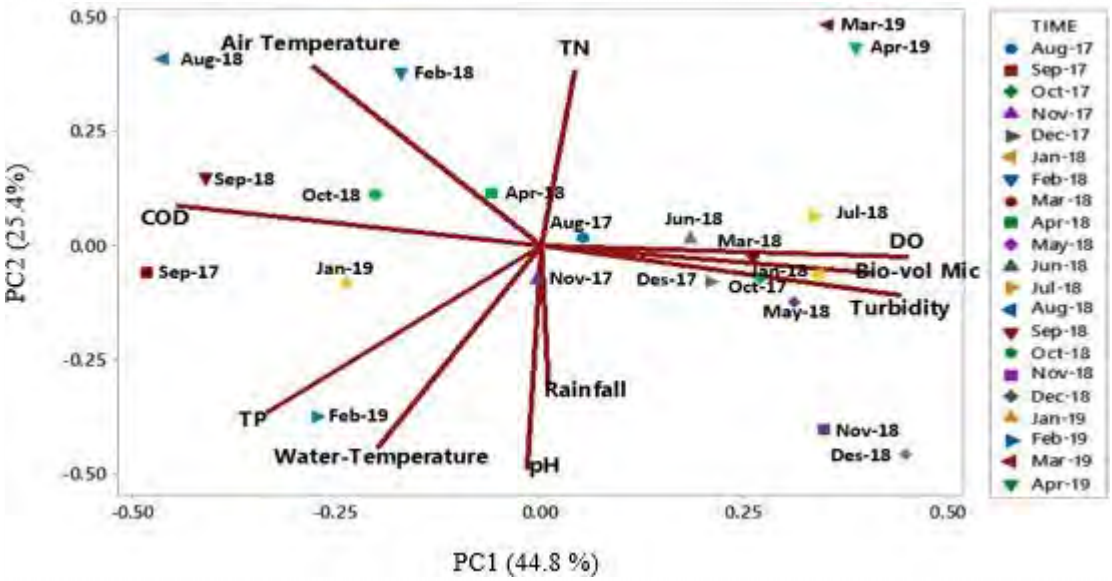


Figure 4.22. Principal component analysis (PCA) diagram, biovolumes of *Microcystis* spp. and rainfall, air temperature, physical, chemical profiles over 12 months in Slim River Lake. the colour shows month corresponding to that in other figures.

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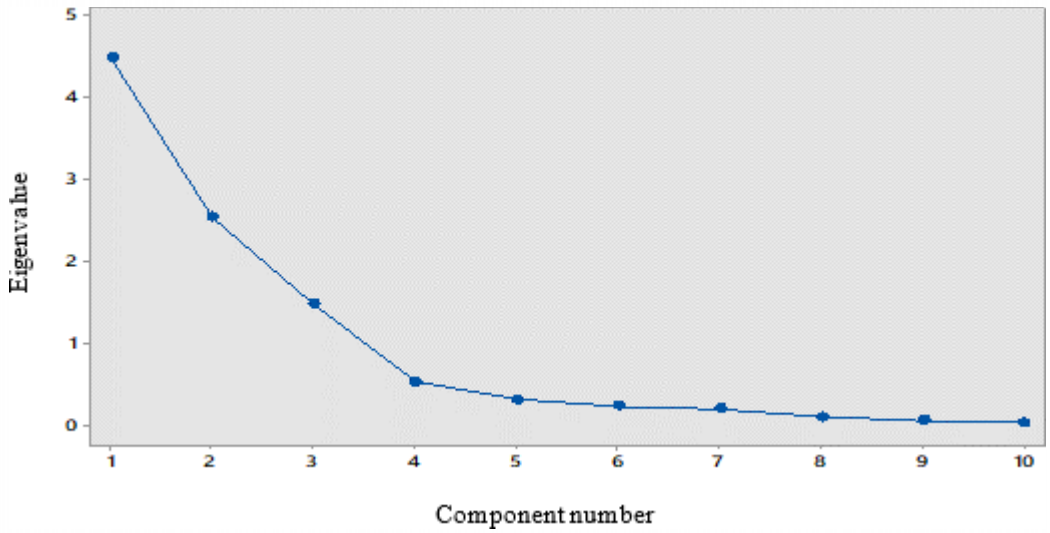


Figure 4.23. Screen plot for influence of air temperature, rainfall, water chemical and water physical patterns on biovolumes of *Microcystis* spp.

As shown in Table 4.5, PC1 has the majority of parameters that positively and negatively correlated with the biovolumes of *Microcystis* spp., in the Slim River Lake. The biovolume of *Microcystis* spp., is positively correlated and strongly influenced by dissolved oxygen and turbidity. Meanwhile, the biovolumes of *Microcystis* spp., is negatively correlated and strongly influenced by air temperature, water temperature, chemical oxygen demand, and total phosphorus.

Overall, the hypothesis setfor this study was validated and supported, whereby the PCA results obtained indicating the strength of the correlation between the increase of both chlorophyll- *a* level and cyanobacterial biovolumes (*Microcystis* spp) and water quality parameters of the study lake based on local factors, rainfall and air temperatures.

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CHAPTER 5

DISCUSSION

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The results presented in Chapter 4 are discussed in this chapter. The discussions are arranged according to the research objectives and presented in the following sections.

5.1 Objective 1: To Determine the Temporal Variability of Lake's Water Physical, Chemical and Biological Profiles in Slim River Lake Area.

The results presented in Chapter 4 suggest that Slim River Lake water's physical, chemical, and biological profiles were highly variable on a temporal basis, thus support the

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hypothesis of this study. Fluctuations in water quality during the sampling months indicate that many physical, chemical, and biological factors are involved in controlling the water quality in Slim River Lake and thus the abundance of phytoplankton. Therefore, it was important to evaluate and quantify the total of those factors.

The physical parameters of lake water investigated in this study include water temperature and turbidity. These parameters showed significant temporal variations throughout the study period in all sampling sites (S1-S5). The water temperature in Slim River Lake was higher than the other studies which reported at 28.7-28.9 °C in Aliyu et al. (2019) and Safwan Miswan et al. (2019). The water temperature in Slim River Lake is greater than 28ºC, exceeding the recommended limit of 28 ºC by NLWQS. Continuous changes in water temperature could contribute to the formation of high phytoplankton biovolume, thus increasing the primary productivity of water bodies (Jiang et al., 2019). As observed in this study, high photosynthetic rates as indicated by high chlorophyll-*a* coincide with the period of high water temperature. It has been suggested that the increase in lake temperatures will lead to an increase in the proportion of algae, which may cause the water to be deprived of oxygen (Rodgers, 2021). Lakes are expected to witness an increase in the percentage of algae that is toxic to fish and animals, and methane emissions will increase over the next decade if current rates continue as they are (Rodgers, 2021).

In general, water temperature increase following the increase of internal energy content associated with solar radiation or rising air temperature (Boyd, 2015). It has also been suggested that climatic fluctuations at a specific sampling location can result in the

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variabilities of water temperature recorded (Miswan et al., 2019). This was confirmed by this study, as it was proven that there are significant differences in the temporal variabilit ies of water temperature in the Slim River Lake area. To date, many research has revealed that climate change is contributing to a rapid increase in the temperatures of lake surfaces around the world (Woolway et al., 2020). Other than lake's productivity, high water temperature affects the sustainability and integrity of the whole ecosystem.

In Slim River Lake, the highest turbidity was observed in September and October. Turbidity measures lake water clarity (Ashraf et al., 2010). Also, turbidity is technically related to water flow and the level of total suspended solid, which occurs after heavy rainfall. The movement of water flowing towards the gradient downward may result in a decrease in the concentration of nutrients reaching the lake water, and thus reducing the turbidity rate in the lake water (Duarte et al., 2021). The turbidity can be determined based on the nature of the lake ecosystem and the volume of runoff entering the lake water (Regier et al., 2020). Besides, turbidity might also vary between wet and dry seasons. In the wet season, large amounts of runoff are transported into the lake, thus lowering the transparency (Okech et al., 2018). Even so, according to the NLWQS guideline, the turbidity in Slim River Lake is within the permissible limit of 40 NTU, suitable for supporting aquatic life, and safe for body contact activities (Hossain & Mahmud, 2019). The low turbidity value in the Slim River Lake increases light penetration and photosynthesis rate, which supports the growth of submerged aquatic organisms (Kale, 2016).

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The descriptive and inferential analysis of the chemical parameters also showed significant temporal variabilities for all tested variables, including pH, total nitrogen, total phosphorus, chemical oxygen demand, and dissolved oxygen. pH is the scale used to measure a water-based solution's acidity or alkalinity (Naubi et al., 2016; Zhang et al., 2019). In this study, the water pH ranged from 5.98 - 8.03 and significantly higher in November than most other months in all sites (S1-S5). The pH recorded in this study is similar to those reported by Islam et al. (2012) in their study of hydrological and water quality characteristics in Chini lake. High pH values or alkalinity in the environment might be due to carbon dioxide consumption during photosynthesis by photosynthetic algae (Gandaseca & Rosli, 2011; Sharip et al., 2014). In general, pH values in Slim River Lake are mostly within the acceptable limit of 6.5-8.5, as recommended in the National Lake Water Quality Criteria and Standards (NLWQS) (NAHRIM, 2015).

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The results suggest a significant variation in lake water pH regarding the temporal variability of these study parameters. Malaysia is a country with a high amount of rainfall throughout the year. Hence, the variability of pH in Slim River Lake is attributed to the amount of rain recorded over the year (Hamidi et al., 2016). Fluctuations in pH may be due to urban runoff washing the streets and agricultural lawns into the lake, thereby disrupting the lake water's pH, as Spyra (2017) reported. The pH value can change during daily and monthly periods (Tucker & D'Abramo 2008). Also, carbon dioxide can be produced from oxidizable organic matter, which changes the pH for a longer time at different values (Tucker & D'Abramo 2008). Additionally, the lake water pH changes could also be influenced by internal factors, such as photosynthetic algae activity (Naubi et al., 2016). The data for this

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study and the study reported by Aeriyanie et al. (2021) in Slim River Lake showed that exceeding the concentrations of chlorophyll-*a* gives a clear picture of the increase in photosynthesis processes thus the presence of a large biovolume of phytoplankton in this lake.

Overall, the in-lake total nitrogen concentrations in Slim River Lake showed significant temporal variabilities throughout the study period. Total nitrogen recorded in this lake ranged from 1.0 – 4.2 mg/L and highly fluctuated between months in all sampling sites. This fluctuation could be due to the inflow of fertilizer or livestock manure, which could be linked to possible runoff, directly associated with the amount of rainfall in the area (Pilon et al., 2019). Surface runoff can be considered the primary source of nitrogen pollution in Slim River Lake. Overall, total nitrogen in the Slim River Lake was higher than the permissible limit of 0.35 mg/L as recommended by NLWQS. The multifolds higher total nitrogen in Slim River lake greatly influence the growth of phytoplankton and changed the macrophyte composition. Nitrogen fixation and nitrogen stress caused by declining dissolved inorganic nitrogen also formed a different cyanobacterial dominant community in the lake (Beversdorf et al., 2013). In this case, low total nitrogen could also have been associated with the dominance of nitrogen-fixing cyanobacteria species such as *Aphanizomenon* spp. In contrast, high total nitrogen could stimulate the growth of other non-nitrogen-fixing cyanobacteria species such as *Microcystis* spp., The new nitrogen inputs into the lake potentially support the growth and prolong the algal bloom of non-nitrogen-fixing cyanobacteria (Beversdorf et al., 2013). As suggested in the literature, the size of cyanobacteria can also be affected by total nitrogen concentration in the lake (Rigosi, Carey, Ibelings & Brookes, 2014). In

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addition, increased nitrogen concentration is also known to increase toxins production by certain genera, such as *Planktothrix* spp. and *Aphanizomenon* spp. (Jankowiak et al., 2019).

The total phosphorus in Slim River Lake ranged from 0.20 – 1.96 mg/L and varied significantly on a temporal basis in all sampling sites. The total phosphorus concentration in Slim River Lake was relatively close compared to an ex-mining lakes in urban areas Puteri and Puchong Lakes (Sulaiman et al., 2018). In other comparison, the total phosphorus recorded in the Slim River Lake is generally higher than in other Malaysian lakes such as Cempaka and Chini lake was recorded from 0.24 to 0.56 mg/L (Gasim et al., 2015), this is potentially due to the increased use of land around the Slim River Lake. Overall, the total phosphorus level in this lake had consistently exceeded the recommended limit of 0.01mg/L by NLWQS.

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High total phosphorus concentration might be caused by fertilizer, livestock manure that can be found around the Slim River Lake. Total phosphorus exists naturally during natural processes, such as weathering (Mekonnen & Hoekstra, 2018; Bouwman et al., 2013). Besides, the total phosphorus level in freshwater could result from various anthropogenic activities, such as runoff containing fertilizer from agricultural land (Yan et al., 2016). The excessive total phosphorus level in freshwater could distort the ecosystem balance by negatively affecting the zooplankton population and positively promoting the phytoplankton community (Van Donk et al., 2020). Accumulated phosphorus higher than 0.01mg/L could potentially increase phytoplankton growth in a lake ecosystem (Downing et al., 2001; González et al., 2005). As described in Chapter 4, total chlorophyll-*a* which illustrate the

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total biovolume potential of phytoplankton was positively influenced by the total phosphorus. This study data also implies that Slim River Lake is a hypereutrophic lake throughout the sampling period. The persistent eutrophication in the lake will disturb the habitat diversity and lead to ecological loss caused by natural and sexual selection among aquatic populations (Alexander et al., 2017).

Chemical oxygen demand is an important tool for water quality testing specifically in freshwater ecosystems (Alprol et al., 2021). The chemical oxygen demand level varied significantly on a temporal basis in all sampling sites. The chemical oxygen demand ranged from 9.50 – 82.0 mg/L, and significantly higher values were recorded in February. The chemical oxygen demand level in Slim River Lake has consistently exceeded the standard limit of 10 mg/L, as set in the NLWQS. There are many reasons that cause high chemical oxygen demand in water. A high level of chemical oxygen demand can be attributed to a high level of oxidizable organic matters, as pointed by Scholz (2016). Oxidizable organic matters may be coming from many sources, including farm waste, oil palm waste and urban runoff. Also, the lake margin has a high density of plants and various land use such as recreational areas, oil palm plantation and farms, can contribute to a high amount of organic matter. Therefore, all those reasons can elevate chemical oxygen demand in the lake. On top of that, the increase in air temperatures in February coincides with the increase in chemical oxygen demand in the water. This observation suggests a strong relationship between the chemical oxygen demand with temperature. This is because the decomposition process is dependent on temperature (Scholz, 2016).

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Dissolved oxygen in water bodies has been widely used to measure water quality (Rahaman et al., 2016). Oxygen enters the water through the air-water interface and photosynthesis of aquatic plants (Rameshkumar et al., 2019). Dissolved oxygen in lakes could range from 1 mg/L to more than 20 mg/L due to diffusion, aeration, respiration, photosynthesis, or organic matter decomposition (Hossain & Mahmud, 2019). In Slim River lake, the dissolved oxygen level is significantly varied on a temporal basis in all sampling sites. High dissolved oxygen values were observed in June and July. The obtained result indicated that in most sampling, especially in S1-S3, the dissolved oxygen level is within the permissible limit of 6.3-7.8 mg/L as suggested in the NLWQS. Dissolved oxygen lower than 5mg/L recorded in several sampling events, especially in S4 and S5 might highlight the period of degraded water quality in the lake.

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According to WHO (2004), dissolved oxygen in the water column should be at least 5 mg/L to sustain the variability of aquatic life (Worako, 2015). The fluctuation of dissolved oxygen could be linked to the consumption of oxygen by bacteria during organic matter decomposition (Tian & Dong, 2020). Also, the dissolved oxygen level can vary due to the variability of biological productivity, atmospheric pressure, and water flow through various temporal variability in Slim River Lake (Alcántara et al., 2018). The reported dissolved oxygen in this study can effectively support various organisms' physiological activities, especially planktons.

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The biological parameters measured in this study include chlorophyll-*a and* the biovolume of *Microcystis* spp. *Microcystis* spp., is one of the well-known and common genera for the spread of cyanobacteria and an important source of microcystin dominance, so it is considered a prominent evidence for the presence of cyanobacteria in aquatic ecosystems (Dong, Zeng, Bai, Li & He, 2016).

Total chlorophyll-*a* is an important and widely adopted parameter for determining phytoplankton and the degree of eutrophication in aquatic ecosystems (Arab et al., 2019). The chlorophyll-*a* recorded in Slim River lake ranged from 2.08 – 132.05 µg/L throughout the study period. As shown in figure 4.8, the chlorophyll-*a* was significantly varied on the temporal basis with no apparent pattern across the sampling sites. The total chlorophyll-*a* concentrations also constantly exceeded the permissible limit of 15 µg/L stated in NLWQS. As a result, Slim River Lake is considered hypereutrophic with high phytoplankton biovolume throughout the sampling year. The chlorophyll-*a* level in Slim River Lake was recorded within the range of hypereutrophic based on the trophic state index (TSI) (Carlson, 1977; NAHRIM, 2015). The level indicates the presence of algae and productivity in the lake. Higher levels of chlorophyll-*a* in water signify poor water quality, which occurs when there is high algal production due to high nutrients (Li et al., 2017). Hypereutrophic level indicates bloom-forming cyanobacteria and increased nutrient concentrations (Zhong et al., 2019). The chlorophyll-*a* level in the lake justified the presence of nutrients in the lake, as reported earlier in this section. The harmful proliferation of planktonic algae is a leading agent for disrupting the aquatic environment (Harke et al., 2016).

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High productivity in several freshwater ecosystems has occurred in many lakes worldwide due to an uncontrolled influx of nutrients in the water (Berry et al., 2017; Mallin et al., 2016). In the studied lake, the chlorophyll-*a* level in the water could fluctuate over time, particularly during the rainy season, when nutrients are flushed into the water. In this study, total chlorophyll-*a* concentration is positively influenced by the total phosphorus concentration. Similarly, some studies suggest that total chlorophyll-*a* increases with increasing phosphorus concentration (Filstrup & Downing, 2017). In addition, the increment in total chlorophyll-*a* in the lake correlated with the development of the catchment area (Sharip et al. 2014). As such, the Slim River Lake potentially has high total chlorophyll-*a* due to the different land uses surrounding the lake.

On top of the total chlorophyll-*a*, cyanobacterial biovolumes were calculated every month throughout the sampling period. *Microcystis* spp., was the most dominant genera based on the calculated biovolumes in Slim River Lake, which has also been reported in Sinang et al. (2019). The *Microcystis* spp., biovolume fluctuates throughout the sampling period, ranging from 6.13 x 10^6 to 1.65 x 10^6 μm^3 mL^{-1}. The highest biovolumes for different sampling sites were observed at different months, thus the temporal variations of the biovolumes cannot be generalized. The *Microcystis* spp., biovolumes in the Slim River Lake was also found to be higher than in previous studies, which only reported about 4.28x 10^6 μm^3 mL^{-1} in lakes (Sinang et al., 2015). Cyanobacteria communities in this Slim River Lake are known as toxin-producing cyanobacteria (Aeriyanie et al., 2021). Thus, high cyanobacterial biovolumes in the Slim River Lake might be associated with microcystin concentration. *Microcystis* spp., is the common species that produce microcystin (Pham &

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Utsumi, 2018) and *Microcystis* spp., is known as the primary contributor of microcystin in freshwater lakes (Preece et al., 2017). For example, high *Microcystis* spp., in the previous study correlated with high microcystin concentration in the water column (Sinang et al., 2015). On top of toxin, cyanobacteria biovolume also increase turbidity and limit the light entering the lake (Paerl & Paul, 2011). Consequently, this will reduce the growth of aquatic macrophytes and affect underwater habitats (Jeppesen et al., 2007; Scheffer et al., 2008). In addition, cyanobacteria can also cause oxygen depletion that will affect the lake's flora and fauna (García & Johnstone, 2006).

In order to discover and evaluate phytoplankton diversity, Next Generation Analysis employs cyanobacteria target genes in the V3 and V6 areas (Fondi & Liò, 2015). The Next Generation Analysis enables simultaneous evaluation and is crucial for monitoring of cyanobacteria (Sinclair et al., 2015). In this study, cyanobacteria present in the lake have been proven by the presence of 12–15% of cyanobacteria from the overall phytoplankton.

On the other hand, principal component analysis was conducted to identify the temporal differences in physical, chemical and biological parameters within lake waters. The results suggest that the most physicochemical and biological parameters were positively and negatively correlated, the sum of principal components explained 79.5 % of the variations during the study period. The positive correlation, including chemical oxygen demand, pH, water temperature, chlorophyll-*a* , and total phosphorus. While, the negative correlations, including total nitrogen, dissolved oxygen, and biovolumes of *Microcystis* spp., and

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turbidity, have been documented through the temporal scale. The temporal fluctuations of these tested parameters did not show any similarity between parameters. Therefore, in general, the physical, chemical, and biological water profiles are highly variable on a temporal basis (Maznah & Makhlough, 2015; Goshtasbi, Atazadeh, Fathi & Movafeghi, 2021).

To conclude, some physical, chemical, and biological parameters examined in this study indicate degraded lake ecosystems with significant eutrophic conditions. The results obtained are of great importance in assessing the lake's health status, and can be used for future lake rehabilitation program. Furthermore, the relationship between physical, chemical and biological conditions is an important issue in establishing a framework for water management

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5.2 Objective 2: To Determine the Influence of Rainfall and Air Temperature on the Lake's Water Physical, Chemical, and Biological Profiles.

Malaysia's climate is characterised by a uniform temperature, high humidity, and abundant rainfall year-round. The whole of Malaysia has a classic equatorial climate, with high temperatures and wet months throughout the year. Temperature plays a significant role in biological and chemical processes within the lake ecosystem. Temperature influences the growth of aquatic organisms, dissolved oxygen content, aquatic plants photosynthetic rate, aquatic organism metabolic rates, and organism susceptibility to toxic or disease (Bhateria & Jain, 2016;

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Sajitha & Vijayamma, 2016). Moreover, temperature caused lake stratification, which different layers between upper and lower layers (Juahir, Ghazali, Ismail & Mohamad, 2019).

The influence of rainfall and air temperature on lake's water physical, chemical, and biological profiles was predicted through principal component analysis.

The principal component analysis was conducted to identify the correlations between rainfall and air temperature patterns and physical, chemical, and biological profiles in Slim River Lake over 12 months. Principal component analysis results for rainfall indicate that the effect of rain on most of the physical, chemical, and biological variables, were either negatively or positively correlated. Many parameters are positively correlated and strongly influenced by rainfall, including chemical oxygen demand, pH, water temperature, chlorophyll-*a*, and total phosphorus. In contrast, there are also parameters that are negatively correlated and strongly influenced by rainfall, including total nitrogen, dissolved oxygen, turbidity, and the biovolume of *Microcystis* spp.

The principal component analysis results indicate that the pH values fall within the standard ranges during the sampling periods at Slim River Lake. The higher pH values may be attributed to the higher photosynthesis and carbon dioxide consumption. Accordingly, increased chlorophyll-*a* concentrations are produced with higher rainfall levels, especially in October and November. pH values gradually decrease as an inverse correlation with

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precipitation in most of the sampling months (Sharip et al., 2014). The current study results showed that the water temperature in Slim River Lake is higher than the water temperatures in some studies was reported by Aliyu et al. (2019). It is also known that higher chlorophyll-*a* concentrations correlate more closely with warmer waters (Piniewski et al., 2020). Similarly, a strong and positive relationship between the increase in total phosphorus with chlorophyll-*a* was also suggested in earlier study (Filstrup & Downing, 2017). High lake water temperatures may be associated with further release of phosphorus from sediments (Kowalczewska Madura, Dondajewska, Gołdyn, Rosińska & Podsiadłowski, 2019; Magee & Wu, 2017). The highest total phosphorus was recorded in October, November, and March, which may be due to rainfall intensity in these months. This increase is attributed to the surface runoff reaching the lake and thus the deterioration of water quality (Chaves et al., 2019). Furthermore. The inverse relationship between rainfall and total nitrogen, with the effect of dilution and rinsing of increased precipitation, may affect the decrease in the total nitrogen concentration in the lake (Hennemann & Petrucio, 2016). At the same time, the denitrification and nitrification processes increase sedimentary phosphorus release into the lake (Zhang, Song, Zhou, Cao & Zhou, 2019). Also, the results indicated that the total phosphorus load that was negatively correlated with biovolumes of *Microcystis* spp., as suggested that the total phosphorus was positively correlated with chlorophyll-*a*. This case may be permissible with a low concentration of total phosphorus (Bullerjahn et al., 2016). Similarly, Christensen, Mackie & Kisling (2013) and Yan et al. (2019) also agreed that cyanobacterial growth was blooming with low total phosphorus loading and enhancement of phosphorus in sediments. Maybe the reason for the negative relationship between cyanobacterial volume and total phosphorus is due to other potential environmental factors

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controlling cyanobacterial blooming in lake waters (Silva, Vincon Leite, et al., 2019). With an increase in total phosphorus due to heavy rainfall, and thus an increase in the proportion of cyanobacteria, its percentage may decrease after severe events, which reduces the percentage of cyanobacteria (Richardson et al., 2019). Also, there may be an adverse effect of heavy rainfall on diluting the number of cyanobacteria, reducing illumination, washout of cyanobacteria, or mixing the water column (Zhou, Zhao, Bi & Hu, 2012).The previous study also shows a strong positive relationship between rainwater runoff within the lake water and the chemical oxygen demand due to increased organic waste from agricultural activities (Ansari et al., 2020). The chemical oxygen demand often represents the amount of organic matter within the lake water (He et al., 2019). Dissolved oxygen is inversely related to the amount of organic matter in the water. As a result of the decomposition of organic matter, the percentage of dissolved oxygen decreases significantly and maybe depleted significantly, attributed to the occurrence of pollution within the lake water (Seow et al., 2019). The results indicate that turbidity is negatively related to rainfall. This may be attributed to the technical association of turbidity with the gradual flow of water from top to bottom and the level of total suspended solids. This flow decreases the concentration of nutrients coming into the lake water, thus reducing turbidity (Duarte et al., 2021). The important role of ecosystem and runoff volume is important in determining turbidity (Regier et al., 2020). Low turbidity in this lake may be due to the absence of bare land in the lake's surrounding area (Sutadian et al., 2016). A low turbidity value contributes to an increase in the penetration of light into the

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study lake, an increase in photosynthesis and, accordingly, an increase in the growth of aquatic organisms (Kale, 2016).

The principal component analysis results for air temperature results indicate that the effect of air temperature on most of the physical, chemical and biological variables were negative and positive correlated. The sum of principal components explained 77.3 % of the variations during the study period. Many parameters are positively correlated and strongly influenced by air temperature, which includes chemical oxygen demand, water temperature, chlorophyll-*a*, and total phosphorus. In contrast, there are also parameters which negatively correlated and strongly influenced by air temperature, including dissolved oxygen, biovolume of *Microcystis* spp., and turbidity.

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Air temperature and solar radiation directly affect and correlate positively with water temperature and internal energy content (Boyd, 2015). During the period of increased air temperature, the amount of dissolved oxygen in lake water is affected by temperature (Juahir et al., 2019; Xu & Xu, 2016). The percentage of dissolved oxygen decreases with increasing temperature (Imanhomayoonnezhad, Arjmandi & Lahijanian, 2019; Juahir et al., 2019). In the shallow lake system, there is an inverse relationship between temperature and dissolved oxygen, which is attributed to less retention of dissolved oxygen in warm waters (Weinke & Biddanda, 2019). In addition, the percentage of dissolved oxygen decreases with what is known as hypoxia with increased respiration of aquatic organisms and the decomposition

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processes of organic matter (Juahir et al., 2019). It was also reported by Zheng et al. (2019) that increased chlorophyll- *a* was more affected by the high water temperature. Some studies have suggested that there is a correlation between a higher concentration of chlorophyll-*a* with an increase in the total phosphorus (Filstrup & Downing, 2017). Environmental changes such as air temperature changes may play a significant role in increasing the concentration of chlorophyll-*a*, which is strongly affected by climatic changes. Where the concentration of chlorophyll-*a* fluctuates with time, especially with higher temperatures and increased nutrient loading, especially phosphorus within the lake water (Richardson et al., 2019). Although the current theory suggests that cyanobacteria blooms increase with increasing global temperatures (Liu et al., 2019), and temperature can be considered an important factor, it may not be the only mechanism that reduces the volume of cyanobacteria in surface waters (Vergalli et al., 2020).The inverse relationship between total phosphorus and cyanobacterial biovolume indicates the influence of other possible environmental factors in controlling the amount of cyanobacteria in lake water (Silva, Vincon Leite, et al., 2019). Principal component analysis results suggest a strong correlation between the chemical oxygen demand and chlorophyll-*a* , in a study on Lake Hamana, Japan, whereby a strong association between an increase in chemical oxygen demand with a higher concentration of chlorophyll-*a* was reported (Kubo, Yoshida & Suzuki, 2021). As there is a strong positive correlation between the chemical oxygen demand and the lake's water temperature, the decomposition process increases with increasing temperature (Scholz, 2016). The results indicate an inverse relationship between turbidity and chlorophyll-*a* concentration. This may be attributed to the low turbidity having an important role in increasing the penetration of light rays into lake

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water and thus, increasing the concentration of chlorophyll-*a* where the photosynthesis processes are at their peak. (Kale, 2016). On the other hand, turbidity can be affected by changes in air temperature, as it can affect water runoff and thus pollutant movement while reducing water turbidity (Frau et al., 2021).

In conclusion, the results for this study objective focused on the main influences of rainfall and air temperature on the physical and chemical parameters of lake water, as well as the biovolumes of cyanobacteria. The findings highlighted the need for continuous monitoring as the local weather patterns can largely influence the physical, chemical and biological profiles in the study lake. Therefore, under increased temperature and rainfall, the lake can be further degraded by the presence of high algae biovolumes due to the increased phosphorus load. On the other side, the results significantly prove the validity of the hypothesis of the current objective of the study in terms of the effect of both rainfall and air temperatures on the water quality parameters of Slim River Lake.

5.3 Objective 3: To Develop a Multivariate Principle Component Analysis (PCA) to Explain the Cyanobacteria Biovolume Based on Rainfall and Air Temperature Patterns, in Lake Chemical, and Physical Profiles.

Multivariate principal component analysis were developed to explain cyanobacteria (biovolumes of *Microcystis* spp., and chlorophyll-*a*) from meteorology profiles and physical

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and chemical variables. Multivariate principal component analysis have a significant role in explaining cyanobacteria biovolume, which links with the changes in the water quality profiles related to the local rainfall and air temperature factors over 12 months. The PCA have successfully explained the predominant cyanobacteria responsible for the significant deterioration in water quality and their potential sources in the study period.

The multivariat principal component analysis for chlorophyll-*a* suggests that most of the physical and chemical variables were either have negative or positive influences. The principal component analysis explained 71.5 % of the variations for chlorophyll-*a* during the study period. Many parameters are positively correlated and strongly influenced by chlorophyll-*a* which include, air temperature, water temperature, chemical oxygen demand, pH, and total phosphorus. In contrast, total nitrogen, dissolved oxygen, and turbidity were negatively correlated to chlorophyll-*a*.

Climate change can increase air temperature, and in the future, will have a significant impact on increasing water temperature in freshwater and lakes (Li et al., 2015). It is also known that warmer water is associated with higher levels of chlorophyll-*a* (Piniewski et al., 2020). This is attributed to the fact that there is a positive relationship between an increase in photoperiod and warmer temperatures, with much larger numbers of phytoplankton in lake waters (Merel et al., 2013). Liu et al. (2019), highlights that the current theory attributes higher blooming to a large number of global warming. Fluctuations and increasing water

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temperatures are likely to play a major role in increasing phytoplankton in the lake water. There is also a strong positive relationship between increased cyanobacterial proliferation (higher chlorophyll-*a*) and higher temperatures (Krinos et al., 2019). The results also highlighted a strong positive relationship between higher pH and increased growth of algae and cyanobacteria, which tend to reduce the carbon dioxide content of lake water and increase the pH during photosynthesis (Boyd, 2020). Determining nutrient inputs and land use can be an important factor in controlling pH, reducing the number of cyanobacteria, and reducing their toxins (Liao et al., 2016). Furthermore, the alkaline pH present in warm conditions may be suitable for increasing the cyanobacteria population as they can utilize bicarbonate as a carbon source (Vilar & Molica, 2020). On the other hand, higher chemical oxygen demand and nutrient loading led to higher chlorophyll-a levels in the water, which occurs when there is a high phytoplankton abundance due to higher nutrient levels (Li et al., 2017). Chemical oxygen demand an organic matter can add a higher concentration of phosphorus to lake water (Kowalczewska Madura, Dundajowska & Goldin, 2010). Cyanobacteria can bloom significantly with a high concentration of phosphorus in lake water (Ma et al., 2015; Mayer, Gerrity, Rittmann, Reisinger & Brandt Williams, 2013).

The density of nitrogen is lower compared to that of phosphorus, which is due to the ability of nitrogen to adsorb inorganic molecules and affects the reduction of nitrogen diffusion rates in water (Jarosiewicz, 2009). Furthermore, the decomposition of organic matter in the sediment determines the nitrogen concentration. Therefore, the movement and reaction of nitrogen are very slow in lake water (Jarosiewicz, 2009). This may be attributed

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to nitrogen-fixing cyanobacteria, which mix nitrogen into organic matter or release nitrogen as a gas into the atmosphere. As such, nitrogen levels appear low in lake water (Jarosiewicz, 2009). In addition, there is a direct relationship between the decrease in dissolved oxygen and the denitrification processes, which affects the decrease in nitrogen concentrations in the lake water (Ding et al., 2018; Wang et al., 2019). The decrease in dissolved oxygen occurs due to the consumption of oxygen during the decomposition of organic matter by bacteria. Increasing temperatures also affect the amount of dissolved oxygen negatively (Zhang et al., 2018). The principal component analysis in this study indicates an inverse relationship between turbidity and chlorophyll-*a* concentration. This may be attributed to a decrease in turbidity and an increase in water transparency, which plays an important role in increasing the penetration of light rays into the lake water and thus increasing the concentration of chlorophyll-*a* where the photosynthesis processes are at their peak (Kale, 2016). Van den Berg et al. (1999) highlighted, that lakes that contain vegetation cover of macrophytes, even with an increase in the amount of phosphorus, have high transparency, unlike lakes that lack macrophytes in their water, which reduces the turbidity in them. This supports the study reported by Sinang et al. (2019) on Slim River Lake, which confirmed the presence of many species of macrophytes in the lake's water column.

In terms of cyanobacteria biovolumes, the multivariate principal component analysis (PCA) suggests that 70.2% variations of *Microcystis* spp. biovolumes can be explained by the parameters included in the PCA. The PCA indicates that the biovolumes are positively correlated and strongly influenced by dissolved oxygen and turbidity. In contrast, the

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biovolumes of *Microcystis* spp., are negatively correlated and strongly influenced by air temperature, water temperature, chemical oxygen demand, and total phosphorus. A strong positive correlation between the biovolumes of *Microcystis* spp., and the dissolved oxygen in lake maybe resulted from the photosynthetic activity of the biomass. It has been reported that oxygen enters the water through the air water interface and photosynthesis of aquatic plants (Rameshkumar et al., 2019). In addition, the concentration of dissolved oxygen in water is affected by temperature. The multivariate principal component analysis (PCA) also suggested that the negative correlation between the biovolumes of *Microcystis* spp., and temperatures. It was similar to the study by Haakonsson et al. (2017) which that there are some differences in cyanobacterial biovolumes with decreasing temperatures where the cyanobacterial biovolumes increased with that decrease in water temperatures. A global lake survey reported in Richardson et al. (2017), suggested an increase in cyanobacteria at the end of winter in response to a slight increase in temperatures. Additionally, Zohri et al. (2021) showed in their study that cyanobacteria can respond quickly to the availability of nutrients in the water column and not due to higher seasonal temperatures, as the temperature factor did not have a major role in the abundance of cyanobacterial species. On the other hand, the multivariate principal component analysis suggested that the biovolumes of *Microcystis* spp., exhibited a negative correlation with total phosphorus trend and chemical oxygen demand. These results were similar to the study conducted in Hongze Lake, which showed a high biovolumes of cyanobacteria with a low concentration of total phosphorus. The results also in line with a study conducted in Meiliang Lake Taihu Bay where the biovolume of *Microcystis* spp., tends to inversely with decreasing total phosphorus concentrations (Liu et

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al. 2011). This is due to the fact that changes in the properties of the lake water exchange, where some environmental factors do not appear as the total phosphorus concentration, any statistical differences with the biovolume of cyanobacteria. In general, the biovolume of cyanobacteria, especially *Microcystis* spp., was related to the stable water column, which caused a decrease in total phosphorus concentrations (Liu et al. 2011; Sheng et al. 2012; Ren et al. 2014). Kolmonen et al. (2004) also found that some types of cyanobacteria were abundant in lakes, especially where the amount of nutrients such as phosphorus and organic matter are low. This condition urges a gradual increase in eutrophication with time, as the increase in the number of cyanobacteria was negatively correlated with the amount of phosphorus and the chemical oxygen demand inside the lake.

On the other hand, Chen et al. (2018) highlighted that aquatic plants can absorb nutrients and chemicals from lake water, where these plant species adapt to the rates of photosynthesis, climatic factors, oxygen transfer, and the purification of the lake water from organic pollutants and its resistance, thus their concentrations decrease significantly. It is these plants, *Lemna minor* spp., and *Spirodela punctata* spp., where they have the ability to integrate nutrients such as phosphorus and chemicals in the lake within the structures of chlorophyll and some parts of the plant, which significantly reduces phosphorus concentrations and chemical oxygen demand within the lake (Abbasi Hassan Abadi et al., 2021). This goes in line with the study conducted on the study lake by Sinang et al. (2019).

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As indicated by the principal component analysis model, there is a strong positive relationship between the increase in cyanobacterial biovolumes and turbidity in Slim River Lake. Where turbidity is positively proportional to the volumes of phytoplankton and cyanobacteria, through the density of cyanobacteria biovolume and the amount of toxins released (Liu, Ma, Kang, Wei, He, Hu & Li, 2019). The cyanobacteria algal bloom will increase turbidity and limit light entering into the lake (Paerl & Paul, 2011). Moreover, it has been reported that higher turbidity may favour the development of *Microcystis* spp. (Chaffin, Kane, Stanislawczyk & Parker, 2018).

In conclude, multivariate principle component analysis (PCA) emphasized the response of cyanobacteria biovolumes, as indicated by chlorophyll-*a* and biovolumes of *Microcystis* spp., based on rainfall, air temperatures, lake's physical and chemical profiles. The principal component analysis explained that the increase in nutrients such as total phosphorus and organic matter had a significant role and a strong relationship with the increase in chlorophyll-*a* and cyanobacteria, influenced by an increase in temperature. These findings hint to increase the biovolumes of algae throughout the year, which warns of the significant eutrophication progression in this lake. Accordingly, the results referred to in the current objective have achieved a remarkable achievement of the study hypothesis in Slim River Lake.

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CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

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6.1 Introduction

This study was conducted to investigate the influence of local rainfall, air temperature, physical and chemical water profiles on cyanobacteria biovolumes in a shallow lake ecosystem. In the study lake, the phenomenon of eutrophication was due to the spread of cyanobacteria led to placing the lake within the highest level (hypereutrophic) and elevated physical and chemical water profiles. This is because cyanobacteria appeared in the Lake Slim River through the eutrophication cascade. The conclusions and recommendations derived from the results of this study are documented in this chapter.

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6.2 Conclusions for the Study

The physical, chemical and biological profiles in Slim River Lake generally varied on the temporal basis throughout the study period. As shown by the multivariate principal component analysis results presented in the objective1, the fluctuations physical, chemical, and biological profiles are varied over temporal scales, and their fluctuations cannot be generalized into similar groups. Rainfall can play a major role in controlling physical profiles and nutrients entering the lake water from agricultural land through surface runoff to lake water. pollutants can be transferred from agricultural land through surface runoff to lake water. Changes in some of these chemical parameters might greatly influence the genetics of cyanobacteria, abundance, and distribution of the study lake's phytoplankton. The genetic (16S rRNA) of cyanobacteria showed a variation of 12-15%. The current study indicates extremely high nutrients and cyanobacteria bloom. The current study indicates extremely high nutrients and cyanobacteria bloom. The results obtained and principal component analyses prove that this lake is at a hypereutrophic level.

In depth understanding the role of rainfall and air temperature on the variability of physical, chemical, and biological profiles in the study lake was also successfully obtained through the second study objective. Local rainfall variability within the study lake region may affect the physical and chemical parameters, affecting the cyanobacterial distribution and lake diversity. The principal component analysis results of rainfall and air temperature

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suggested that rainfall and air temperature can significantly impact the blooming of cyanobacteria. Consequently, the introduction of nutrients into the lake and its effect on cyanobacteria biovolume play a significant role.

The results further emphasize that eutrophication caused by human activities and warmer temperatures contributes to increased cyanobacteria, Based on the principal component analysis produced in the third research objective, a clear insight into how cyanobacteria biovolume responds to changes in rainfall, air temperature, and physical and chemical water profiles was illustrated. The multivariate principal component analysis showed the significant contribution of total phosphorous and pH to the cyanobacterial blooming represented by the increase of chlorophyll-*a*, in Slim River Lake. The biovolumes of these genera may change the lake's biological characteristics and make it unfit for human use and other recreational activities, due to their great effect on releasing various toxins. The lake's chemical oxygen demand level indicates a high level of oxidizable organic matter due to agricultural runoff. Besides, the rainfall factor plays a major role in determining the biovolumes of cyanobacteria in Slim River Lake, as well as the land used near the lake.

The multivariate principal component analysis (PCA) has a significant role in explaining algae bloom, which links with the changes in the water quality profiles related to the local rainfall and air temperature factors. There were differences in the physical and chemical parameters, mainly due to the increase of degradable organic matter. Additionally,

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the results obtained during the study period suggest a tremendous biovolumes of cyanobacteria and high levels of nutrients, especially total phosphorus, total nitrogen and chemical oxygen demand as a result of increased agricultural and human activities close to the lake area. Consequently, it degraded the lake's water quality, increasing the trophic levels in it.

6.3 Recommendation for Future Study

In this study, all of the desired objectives were achieved. The results of the multivariate principal component analysis in this study showed the importance of the current information obtained, which lasted one year through monitoring and experimental stages. It provides an important base for those in charge of water management to develop and improve water quality. However, some recommendations are suggested to improve and diagnose the causes of the deterioration of the Slim River Lake water quality and can be circulated to nearby lakes , the future research must be conducted to discover other parameters that were not addressed in this study like heavy metals. Heavy metals can contaminate through groundwater movement, surface water intrusion, and runoff. Consequently, the lake water and the aquatic organisms in it are polluted.

In addition, the level of environmental awareness of the risks of exposure to polluted water must be raised among the local population. As such it is also important to develop a plan to deal with any increase in water pollution. Therefore, the future research also need to

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put in place a proper framework or management plan to rehabilitate the lake. Moreover, the future study should also include with a comparison with other shallow lakes ecosystems, which would give more insight to support these findings.

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APPENDIX (A)

Water Temperature

1- One-way ANOVA Post Hoc: S1 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	26.35	2.3951	4.47	0.001
Error	24	12.85	0.5356		
Total	35	39.20			

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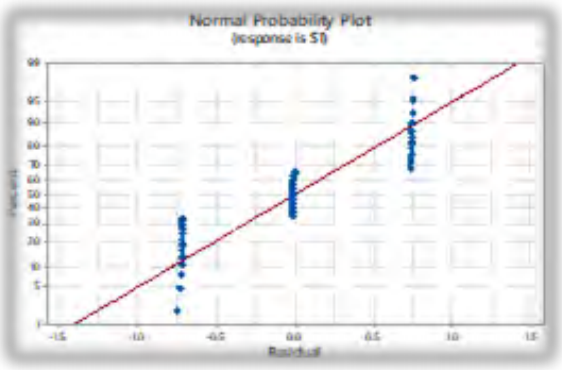
Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.731856	67.21%	52.18%	26.22%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping		
Nov-17	3	31.020	A		
Oct-17	3	30.367	A	B	
Aug-17	3	30.267	A	B	
Apr-18	3	29.767	A	B	C
Mar-18	3	29.420	A	B	C
Dec-17	3	29.220	A	B	C
May-18	3	29.213	A	B	C
Jan-18	3	28.920	A	B	C
Sep-17	3	28.867	A	B	C
Jul-18	3	28.770		B	C
Jun-18	3	28.450		B	C
Feb-18	3	27.820			C



Means that do not share a letter are significantly different.

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2- One-way ANOVA Post Hoc: S2 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	38.16	3.4690	6.02	0.000
Error	24	13.84	0.5765		
Total	35	52.00			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.759298	73.39%	61.19%	40.12%

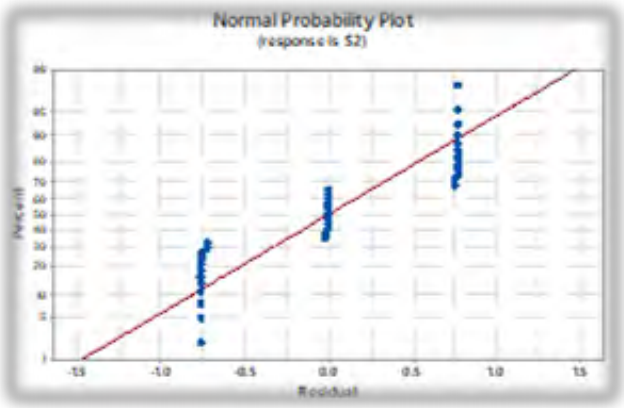
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Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping			
Nov-17	3	31.523	A			
Aug-17	3	30.653	A	B		
Oct-17	3	30.503	A	B	C	
Apr-18	3	29.423	A	B	C	D
May-18	3	29.153		B	C	D
Jan-18	3	28.953		B	C	D
Mar-18	3	28.803		B	C	D
Sep-17	3	28.457		B	C	D
Jul-18	3	28.453		B	C	D
Jun-18	3	28.403			C	D
Dec-17	3	28.403			C	D
Feb-18	3	28.257				D

Means that do not share a letter are significantly different.



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3- One-way ANOVA Post Hoc: S3 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	46.206	4.2005	20.23	0.000
Error	24	4.983	0.2076		
Total	35	51.189			

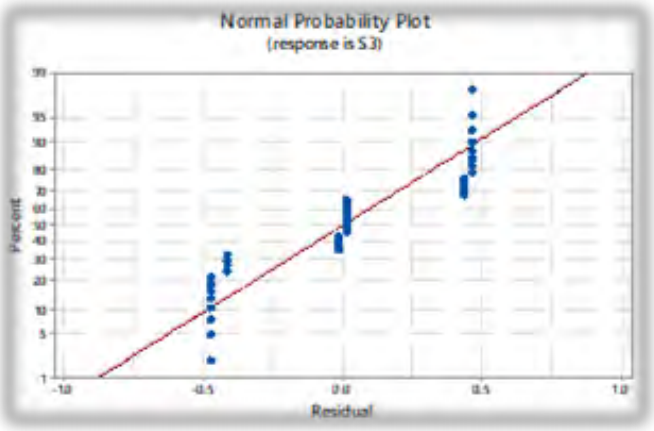
Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.455668	90.27%	85.80%	78.10%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping			
Nov-17	3	32.437	A			
Aug-17	3	31.787	A	B		
Oct-17	3	30.817		B	C	
Apr-18	3	30.337			C	D
Dec-17	3	29.887			C	D E
Mar-18	3	29.637			C	D E
Jul-18	3	29.587			C	D E
May-18	3	29.467				D E
Jun-18	3	29.217				D E
Jan-18	3	29.167				D E
Sep-17	3	28.737				E



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Feb-18	3	28.587	E

Means that do not share a letter are significantly different.

4- One-way ANOVA Post Hoc: S4 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	112.00	10.1816	17.32	0.000
Error	24	14.11	0.5877		
Total	35	126.10			

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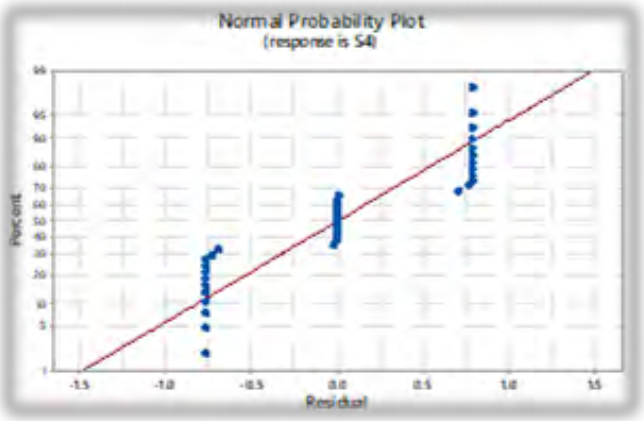
Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.766632	88.81%	83.69%	74.83%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping				
Mar-19	3	34.670	A				
Nov-18	3	34.360	A	B			
May-18	3	33.900	A	B	C		
Jul-18	3	32.660	A	B	C	D	
Dec-18	3	32.110		B	C	D	E
Apr-19	3	31.920			C	D	E
Sep-18	3	31.860			C	D	E
Jun-18	3	31.235				D	E
Oct-18	3	31.020				D	E
Jan-19	3	30.380					E F
Feb-19	3	30.210					E F



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Aug-18	3	28.350	F

Means that do not share a letter are significantly different.

5- One-way ANOVA Post Hoc: S5 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	99.18	9.0160	20.81	0.000
Error	24	10.40	0.4333		
Total	35	109.58			

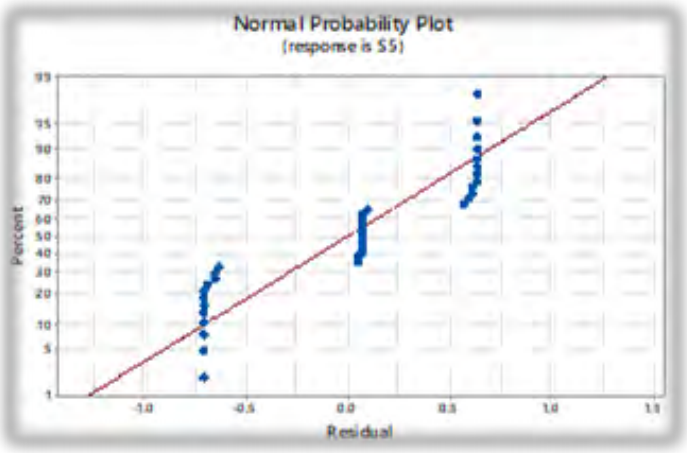
Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.658281	90.51%	86.16%	78.64%

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping
May-18	3	33.777	A
Mar-19	3	32.537	A
Apr-19	3	30.277	B
Dec-18	3	30.267	B
Jun-18	3	30.178	B
Jul-18	3	29.677	B
Sep-18	3	29.567	B
Oct-18	3	29.553	B
Jan-19	3	29.453	B
Feb-19	3	29.107	B



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Nov-18	3	28.927	B	
Aug-18	3	26.977		C

Means that do not share a letter are significantly different.

APPENDIX (B)

Turbidity

One-way ANOVA Post Hoc: S1 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	85.5442	7.77674	767.44	0.000
Error	24	0.2432	0.01013		
Total	35	85.7874			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.100664	99.72%	99.59%	99.36%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	3.8067	0.355	(3.6867, 3.9266)
Sep-17	3	7.3817	0.109	(7.2617, 7.5016)
Oct-17	3	2.6367	0.263	(2.5167, 2.7566)
Nov-17	3	2.9467	0.522	(2.8267, 3.0666)
Dec-17	3	2.7917	0.331	(2.6717, 2.9116)
Jan-18	3	3.6117	0.447	(3.4917, 3.7316)
Feb-18	3	5.6517	0.363	(5.5317, 5.7716)
Mar-18	3	4.0817	0.523	(3.9617, 4.2016)
Apr-18	3	1.6417	0.271	(1.5217, 1.7616)

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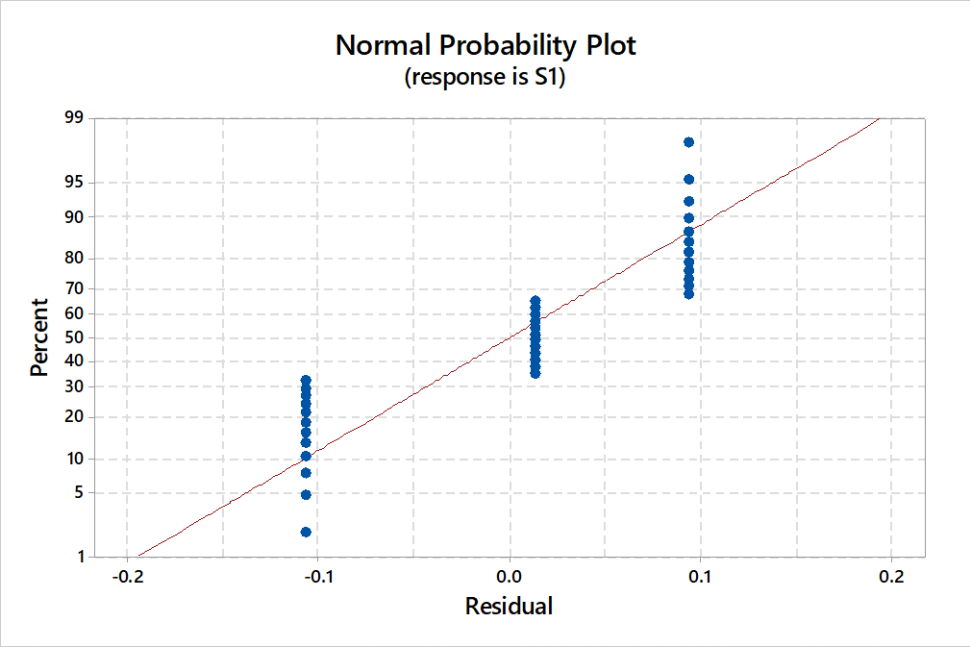
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May-18	3	2.2917	0.601	(2.1717, 2.4116)
Jun-18	3	2.5217	0.230	(2.4017, 2.6416)
Jul-18	3	4.6317	0.615	(4.5117, 4.7516)

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping							
Sep-17	3	7.3817	A							
Feb-18	3	5.6517		B						
Jul-18	3	4.6317			C					
Mar-18	3	4.0817				D				
Aug-17	3	3.8067				D	E			
Jan-18	3	3.6117					E			
Nov-17	3	2.9467						F		
Dec-17	3	2.7917						F	G	
Oct-17	3	2.6367							G	
Jun-18	3	2.5217							G	H
May-18	3	2.2917								H
Apr-18	3	1.6417								I

Means that do not share a letter are significantly different.



Normplot of Residuals for S1

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One-way ANOVA Post Hoc: S2 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	92.3143	8.39221	876.22	0.000
Error	24	0.2299	0.00958		
Total	35	92.5441			

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Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0978661	99.75%	99.64%	99.44%

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

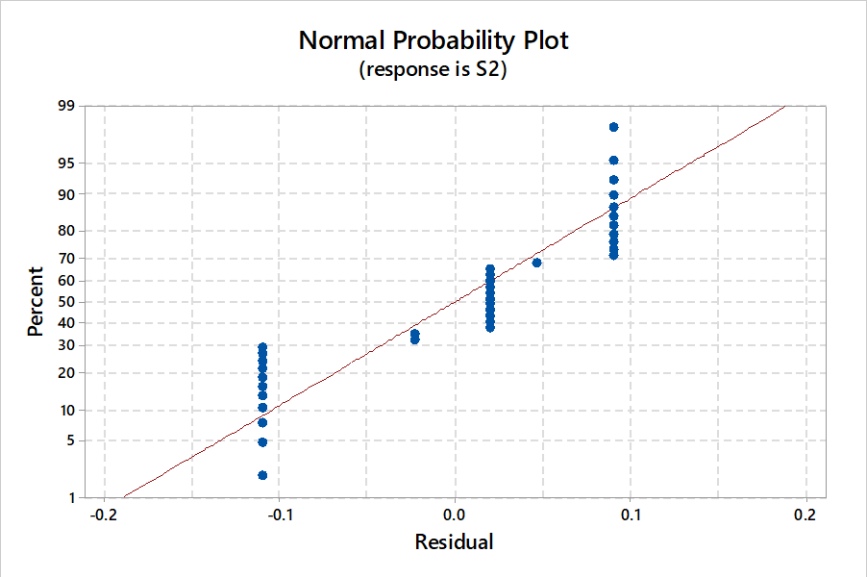
TIME	N	Mean	Grouping				
Sep-17	3	8.3000	A				
Feb-18	3	5.4250		B			
Mar-18	3	4.7750			C		
Jul-18	3	4.4783				D	
Dec-17	3	3.5800					E
Jan-18	3	3.3200					E
Oct-17	3	2.9500						F
Aug-17	3	2.9100					F	G
Jun-18	3	2.8700					F	G
Nov-17	3	2.8000					F	G	H
Apr-18	3	2.6400						G	H

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May-18	3	2.5550	H

Means that do not share a letter are significantly different.



Normplot of Residuals for S2

One-way ANOVA Post Hoc: S3 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	351.535	31.9577	492.35	0.000
Error	24	1.558	0.0649		
Total	35	353.093			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.254771	99.56%	99.36%	99.01%

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Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	1.722	0.261	(1.418, 2.025)
Sep-17	3	13.352	0.550	(13.048, 13.655)
Oct-17	3	3.102	0.500	(2.798, 3.405)
Nov-17	3	3.572	0.270	(3.268, 3.875)
Dec-17	3	4.447	0.330	(4.143, 4.750)
Jan-18	3	6.962	0.440	(6.658, 7.265)
Feb-18	3	4.657	0.550	(4.353, 4.960)
Mar-18	3	2.832	0.500	(2.528, 3.135)
Apr-18	3	2.267	0.270	(1.963, 2.570)
May-18	3	1.902	0.260	(1.598, 2.205)
Jun-18	3	1.812	0.230	(1.508, 2.115)
Jul-18	3	3.092	0.100	(2.788, 3.395)

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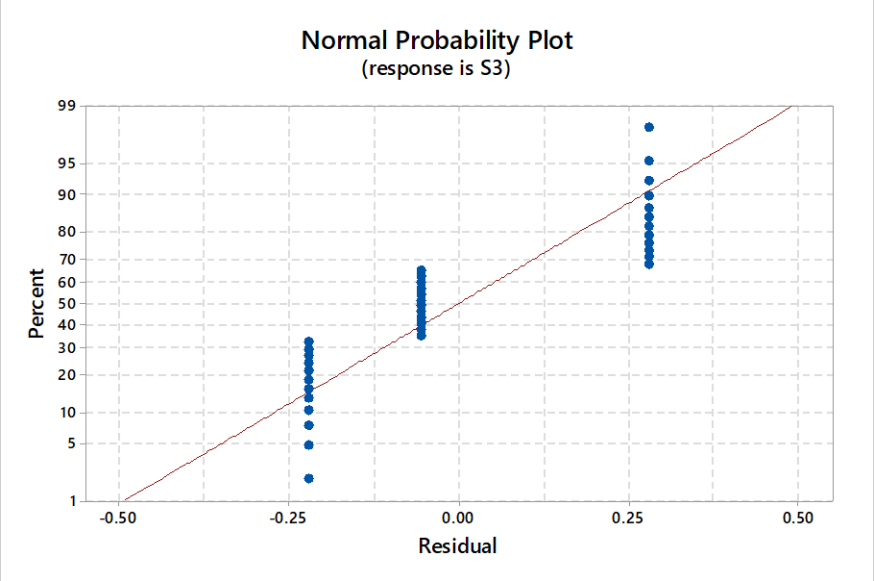
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping				
Sep-17	3	13.352	A				
Jan-18	3	6.962		B			
Feb-18	3	4.657			C		
Dec-17	3	4.447			C		
Nov-17	3	3.572				D	
Oct-17	3	3.102				D	
Jul-18	3	3.092				D	
Mar-18	3	2.832				D	E
Apr-18	3	2.267					E F
May-18	3	1.902					F
Jun-18	3	1.812					F
Aug-17	3	1.722					F

Means that do not share a letter are significantly different.

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Normplot of Residuals for S3

One-way ANOVA Post Hoc: S4 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	20.9392	1.90356	80.32	0.000
Error	24	0.5688	0.02370		
Total	35	21.5080			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.153948	97.36%	96.14%	94.05%

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Means

TIME	N	Mean	Std Error	95% CI
May-18	3	1.8250	0.050	(1.6416, 2.0084)
Jun-18	3	1.2700	0.073	(1.0866, 1.4534)
Jul-18	3	2.0400	0.044	(1.8566, 2.2234)
Aug-18	3	2.6250	0.055	(2.4416, 2.8084)
Sep-18	3	3.2000	0.090	(3.0166, 3.3834)
Oct-18	3	1.9500	0.050	(1.7666, 2.1334)
Nov-18	3	2.7250	0.061	(2.5416, 2.9084)
Dec-18	3	2.3800	0.055	(2.1966, 2.5634)
Jan-19	3	2.5150	0.050	(2.3316, 2.6984)
Feb-19	3	0.7450	0.033	(0.5616, 0.9284)
Mar-19	3	0.8750	0.044	(0.6916, 1.0584)
Apr-19	3	1.0750	0.033	(0.8916, 1.2584)

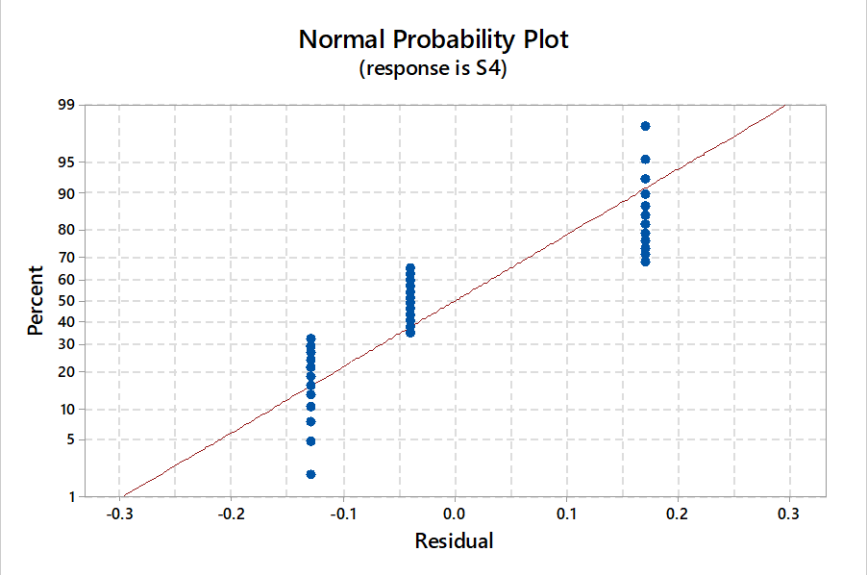
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping				
Sep-18	3	3.2000	A				
Nov-18	3	2.7250		B			
Aug-18	3	2.6250		B			
Jan-19	3	2.5150		B			
Dec-18	3	2.3800		B	C		
Jul-18	3	2.0400			C	D	
Oct-18	3	1.9500			C	D	
May-18	3	1.8250				D	
Jun-18	3	1.2700					E
Apr-19	3	1.0750					E F
Mar-19	3	0.8750					E F
Feb-19	3	0.7450					F

Means that do not share a letter are significantly different.

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Normplot of Residuals for S4

One-way ANOVA Post Hoc: S5 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	52.0234	4.72940	302.52	0.000
Error	24	0.3752	0.01563		
Total	35	52.3986			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.125033	99.28%	98.96%	98.39%

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Means

TIME	N	Mean	Std Error	95% CI
May-18	3	1.6267	0.050	(1.4777, 1.7757)
Jun-18	3	3.2467	0.061	(3.0977, 3.3957)
Jul-18	3	2.3017	0.055	(2.1527, 2.4507)
Aug-18	3	3.4817	0.050	(3.3327, 3.6307)
Sep-18	3	3.2617	0.033	(3.1127, 3.4107)
Oct-18	3	4.7467	0.044	(4.5977, 4.8957)
Nov-18	3	2.8067	0.055	(2.6577, 2.9557)
Dec-18	3	2.5017	0.050	(2.3527, 2.6507)
Jan-19	3	4.3417	0.070	(4.1927, 4.4907)
Feb-19	3	0.6967	0.060	(0.5477, 0.8457)
Mar-19	3	1.5817	0.023	(1.4327, 1.7307)
Apr-19	3	1.0717	0.010	(0.9227, 1.2207)

Tukey Pairwise Comparisons

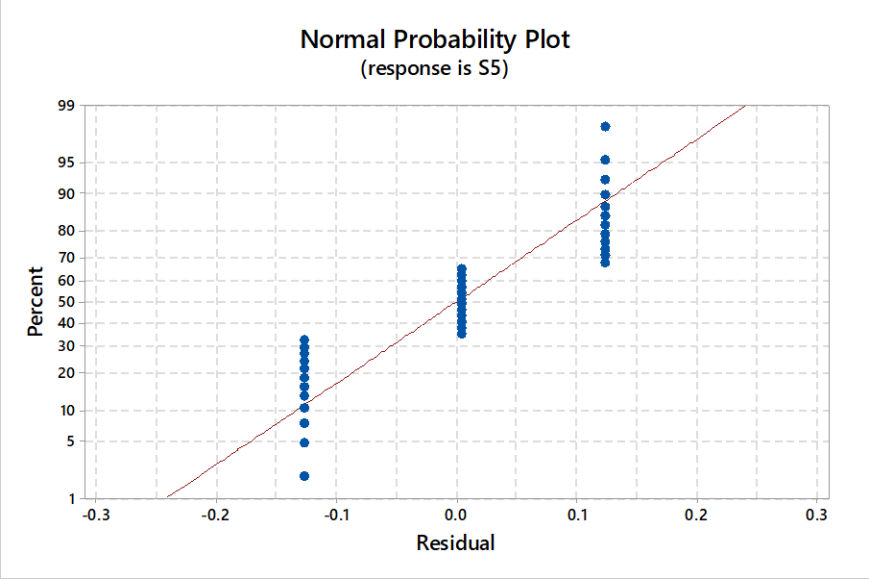
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping
Oct-18	3	4.7467	A
Jan-19	3	4.3417	B
Aug-18	3	3.4817	C
Sep-18	3	3.2617	C
Jun-18	3	3.2467	C
Nov-18	3	2.8067	D
Dec-18	3	2.5017	D E
Jul-18	3	2.3017	E
May-18	3	1.6267	F
Mar-19	3	1.5817	F
Apr-19	3	1.0717	G
Feb-19	3	0.6967	H

Means that do not share a letter are significantly different.

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Normplot of Residuals for S5

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APPENDIX (C)

pH

One-way ANOVA Post Hoc: S1 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	3.9434	0.358491	54.87	0.000
Error	24	0.1568	0.006533		
Total	35	4.1002			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0808290	96.18%	94.42%	91.40%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	6.6933	0.350	(6.5970, 6.7896)
Sep-17	3	6.8633	0.610	(6.7670, 6.9596)
Oct-17	3	6.5583	0.550	(6.4620, 6.6546)
Nov-17	3	7.0583	0.500	(6.9620, 7.1546)
Dec-17	3	6.2333	0.330	(6.1370, 6.3296)
Jan-18	3	6.0833	0.440	(5.9870, 6.1796)
Feb-18	3	6.1733	0.550	(6.0770, 6.2696)
Mar-18	3	6.2283	0.503	(6.1320, 6.3246)
Apr-18	3	6.0683	0.710	(5.9720, 6.1646)
May-18	3	6.2533	0.601	(6.1570, 6.3496)
Jun-18	3	6.4333	0.230	(6.3370, 6.5296)

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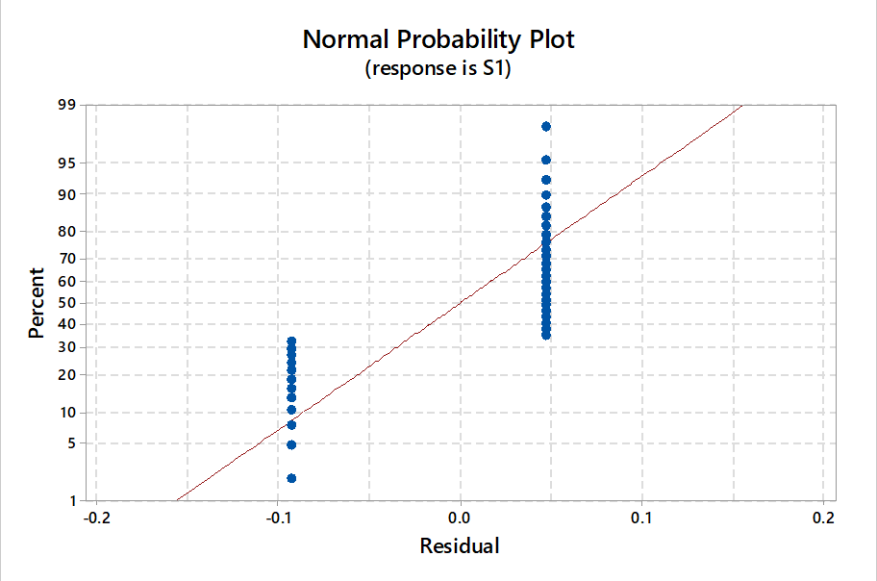
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Jul-18	3	5.9333	0.107 (5.8370, 6.0296)

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping					
Nov-17	3	7.0583	A					
Sep-17	3	6.8633	A	B				
Aug-17	3	6.6933		B	C			
Oct-17	3	6.5583			C	D		
Jun-18	3	6.4333				D	E	
May-18	3	6.2533					E	F
Dec-17	3	6.2333					E	F
Mar-18	3	6.2283					E	F
Feb-18	3	6.1733						F
Jan-18	3	6.0833						F G
Apr-18	3	6.0683						F G
Jul-18	3	5.9333						G

Means that do not share a letter are significantly different.



Normplot of Residuals for S1

One-way ANOVA Post Hoc: S2 versus TIME
Method

Null hypothesis	All means are equal

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Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	1.8688	0.169886	30.16	0.000
Error	24	0.1352	0.005633		
Total	35	2.0040			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0750555	93.25%	90.16%	84.82%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	6.6867	0.440	(6.5972, 6.7761)
Sep-17	3	6.6617	0.550	(6.5722, 6.7511)
Oct-17	3	6.3817	0.500	(6.2922, 6.4711)
Nov-17	3	6.6517	0.700	(6.5622, 6.7411)
Dec-17	3	6.2867	0.600	(6.1972, 6.3761)
Jan-18	3	6.0517	0.440	(5.9622, 6.1411)
Feb-18	3	6.1867	0.550	(6.0972, 6.2761)
Mar-18	3	6.2517	0.500	(6.1622, 6.3411)
Apr-18	3	6.1817	0.700	(6.0922, 6.2711)
May-18	3	6.4567	0.600	(6.3672, 6.5461)
Jun-18	3	6.4167	0.230	(6.3272, 6.5061)
Jul-18	3	5.9667	0.100	(5.8772, 6.0561)

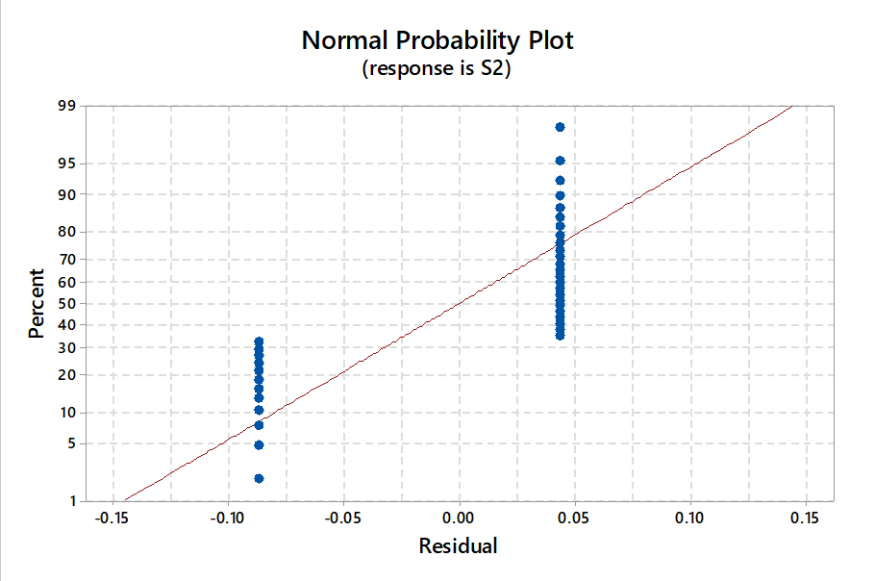
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Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping				
Aug-17	3	6.6867	A				
Sep-17	3	6.6617	A	B			
Nov-17	3	6.6517	A	B			
May-18	3	6.4567		B	C		
Jun-18	3	6.4167			C		
Oct-17	3	6.3817			C	D	
Dec-17	3	6.2867			C	D	
Mar-18	3	6.2517			C	D	E
Feb-18	3	6.1867				D	E F
Apr-18	3	6.1817				D	E F
Jan-18	3	6.0517					E F
Jul-18	3	5.9667					F

Means that do not share a letter are significantly different.



Normplot of Residuals for S2
One-way ANOVA Post Hoc: S3 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

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Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	11.8949	1.08136	46.95	0.000
Error	24	0.5528	0.02303		
Total	35	12.4477			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.151767	95.56%	93.52%	90.01%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	7.4633	0.100	(7.2825, 7.6442)
Sep-17	3	7.6433	0.255	(7.4625, 7.8242)
Oct-17	3	7.0783	0.500	(6.8975, 7.2592)
Nov-17	3	7.5483	0.270	(7.3675, 7.7292)
Dec-17	3	6.2133	0.330	(6.0325, 6.3942)
Jan-18	3	6.1483	0.440	(5.9675, 6.3292)
Feb-18	3	6.2383	0.255	(6.0575, 6.4192)
Mar-18	3	6.3483	0.500	(6.1675, 6.5292)
Apr-18	3	6.1983	0.270	(6.0175, 6.3792)
May-18	3	6.5733	0.160	(6.3925, 6.7542)
Jun-18	3	6.4333	0.230	(6.2525, 6.6142)
Jul-18	3	6.0433	0.110	(5.8625, 6.2242)

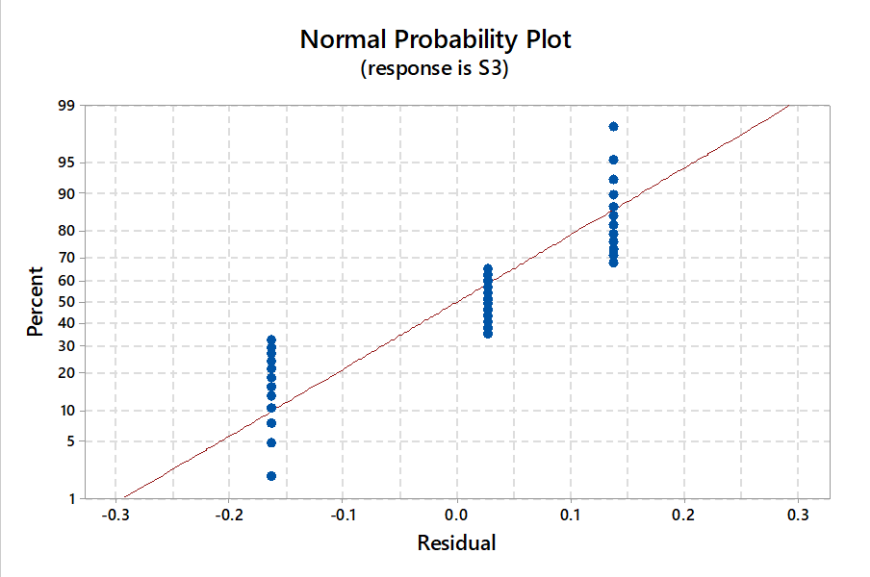
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Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping			
Sep-17	3	7.6433	A			
Nov-17	3	7.5483	A			
Aug-17	3	7.4633	A	B		
Oct-17	3	7.0783		B		
May-18	3	6.5733			C	
Jun-18	3	6.4333			C	D
Mar-18	3	6.3483			C	D
Feb-18	3	6.2383			C	D
Dec-17	3	6.2133			C	D
Apr-18	3	6.1983			C	D
Jan-18	3	6.1483			C	D
Jul-18	3	6.0433				D

Means that do not share a letter are significantly different.



Normplot of Residuals for S3
One-way ANOVA Post Hoc: S4 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

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Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	11.8352	1.07593	44.58	0.000
Error	24	0.5792	0.02413		
Total	35	12.4144			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.155349	95.33%	93.20%	89.50%

Means

TIME	N	Mean	Std Error	95% CI
May-18	3	7.1733	0.940	(6.9882, 7.3584)
Jun-18	3	7.4533	0.550	(7.2682, 7.6384)
Jul-18	3	7.5733	0.500	(7.3882, 7.7584)
Aug-18	3	7.5283	1.600	(7.3432, 7.7134)
Sep-18	3	7.1683	0.600	(6.9832, 7.3534)
Oct-18	3	6.9483	0.440	(6.7632, 7.1334)
Nov-18	3	7.7683	0.550	(7.5832, 7.9534)
Dec-18	3	7.0533	0.500	(6.8682, 7.2384)
Jan-19	3	6.4383	0.700	(6.2532, 6.6234)
Feb-19	3	6.0683	0.600	(5.8832, 6.2534)
Mar-19	3	6.2133	0.230	(6.0282, 6.3984)
Apr-19	3	6.1683	0.100	(5.9832, 6.3534)

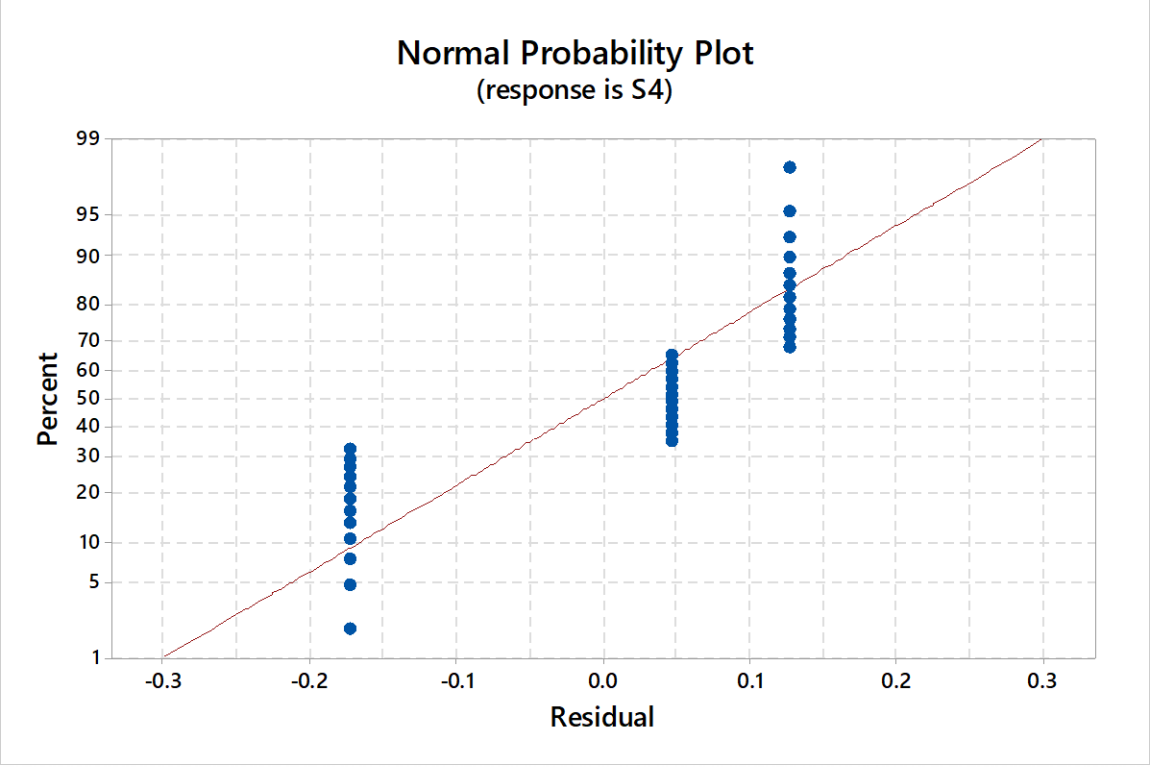
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Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping				
Nov-18	3	7.7683	A				
Jul-18	3	7.5733	A	B			
Aug-18	3	7.5283	A	B			
Jun-18	3	7.4533	A	B	C		
May-18	3	7.1733		B	C	D	
Sep-18	3	7.1683		B	C	D	
Dec-18	3	7.0533			C	D	
Oct-18	3	6.9483				D	
Jan-19	3	6.4383					E
Mar-19	3	6.2133					E
Apr-19	3	6.1683					E
Feb-19	3	6.0683					E

Means that do not share a letter are significantly different.



Normplot of Residuals for S4
One-way ANOVA Post Hoc: S5 versus TIME
Method

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Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	16.6921	1.51746	66.56	0.000
Error	24	0.5472	0.02280		
Total	35	17.2393			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.150997	96.83%	95.37%	92.86%

Means

TIME	N	Mean	Std Error	95% CI
May-18	3	7.4600	0.610	(7.2801, 7.6399)
Jun-18	3	7.8000	1.550	(7.6201, 7.9799)
Jul-18	3	7.5500	0.500	(7.3701, 7.7299)
Aug-18	3	7.5800	0.700	(7.4001, 7.7599)
Sep-18	3	7.4700	0.330	(7.2901, 7.6499)
Oct-18	3	6.9250	1.440	(6.7451, 7.1049)
Nov-18	3	8.0100	0.550	(7.8301, 8.1899)
Dec-18	3	7.2450	0.500	(7.0651, 7.4249)
Jan-19	3	6.5250	0.700	(6.3451, 6.7049)
Feb-19	3	5.9900	0.600	(5.8101, 6.1699)
Mar-19	3	6.1700	0.230	(5.9901, 6.3499)
Apr-19	3	6.0600	1.100	(5.8801, 6.2399)

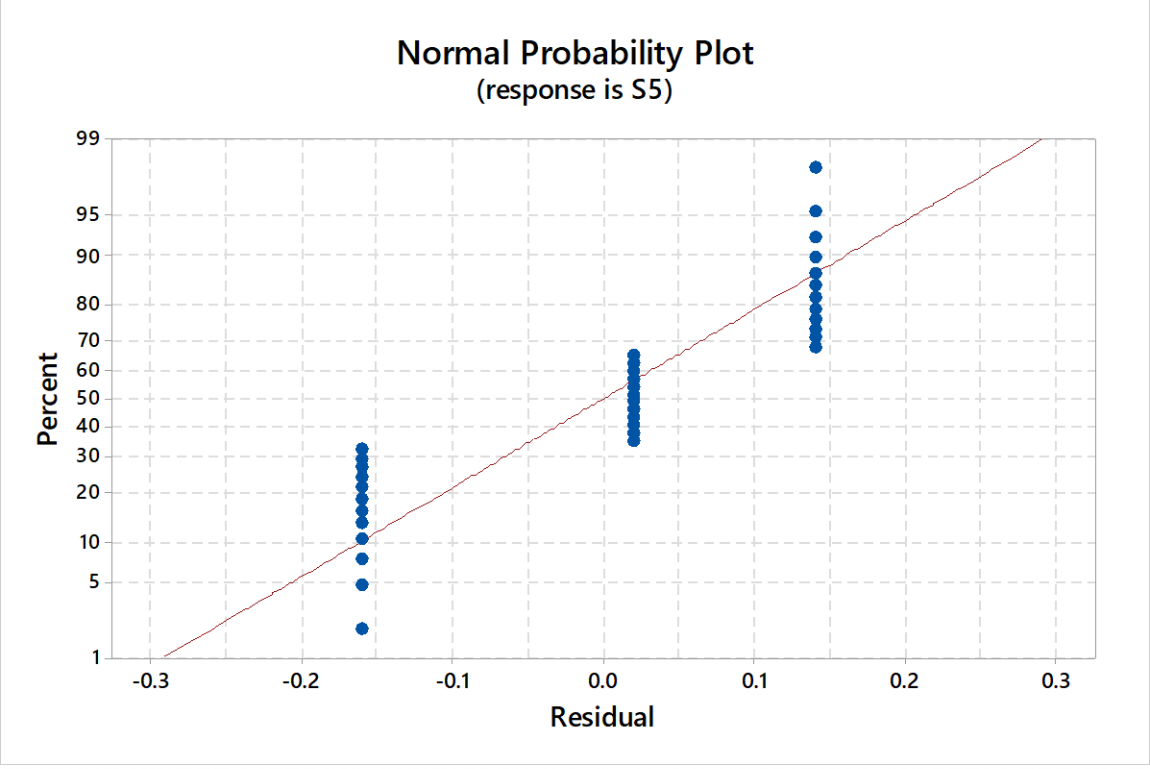
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Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping					
Nov-18	3	8.0100	A					
Jun-18	3	7.8000	A	B				
Aug-18	3	7.5800	A	B	C			
Jul-18	3	7.5500		B	C			
Sep-18	3	7.4700		B	C			
May-18	3	7.4600		B	C			
Dec-18	3	7.2450			C	D		
Oct-18	3	6.9250				D	E	
Jan-19	3	6.5250					E	F
Mar-19	3	6.1700						F G
Apr-19	3	6.0600						G
Feb-19	3	5.9900						G

Means that do not share a letter are significantly different.



Normplot of Residuals for S5

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APPENDIX (D)

Total nitrogen

One-way ANOVA Post Hoc: S1 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	24.891	2.26285	46.24	0.000
Error	24	1.174	0.04893		
Total	35	26.066			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.221209	95.49%	93.43%	89.86%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	1.5000	0.035	(1.2364, 1.7636)
Sep-17	3	2.0000	0.061	(1.7364, 2.2636)
Oct-17	3	3.267	0.055	(3.003, 3.530)
Nov-17	3	1.0200	0.150	(0.7564, 1.2836)
Dec-17	3	2.133	0.133	(1.870, 2.397)
Jan-18	3	3.0000	0.144	(2.7364, 3.2636)
Feb-18	3	3.5000	0.055	(3.2364, 3.7636)
Mar-18	3	3.100	0.150	(2.836, 3.364)
Apr-18	3	3.5000	0.070	(3.2364, 3.7636)
May-18	3	3.5333	0.060	(3.2697, 3.7969)

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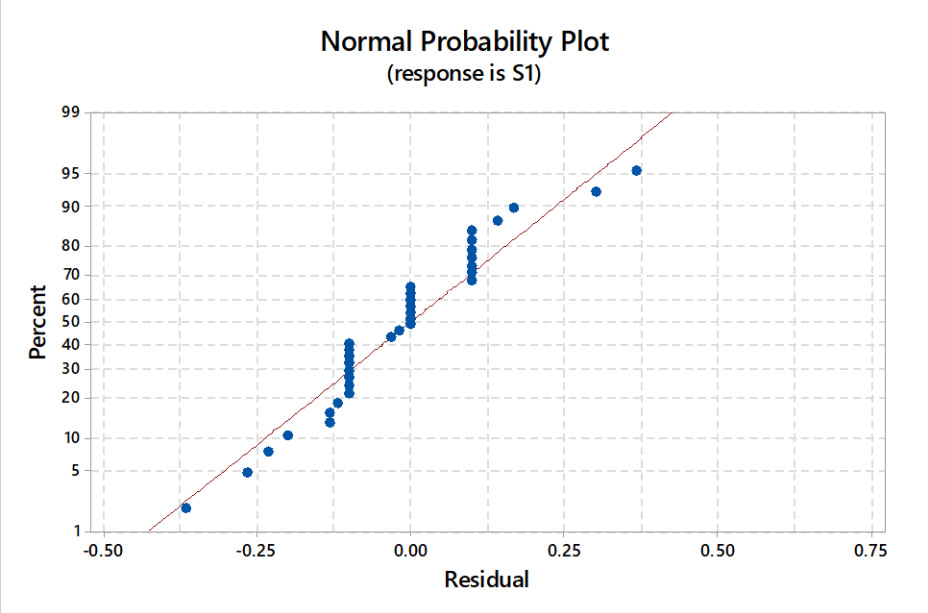
Jun-18	3	2.5000	0.123	(2.2364, 2.7636)
Jul-18	3	3.5000	0.100	(3.2364, 3.7636)

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping			
May-18	3	3.5333	A			
Jul-18	3	3.5000	A			
Apr-18	3	3.5000	A			
Feb-18	3	3.5000	A			
Oct-17	3	3.267	A			
Mar-18	3	3.100	A	B		
Jan-18	3	3.0000	A	B		
Jun-18	3	2.5000		B	C	
Dec-17	3	2.133			C	D
Sep-17	3	2.0000			C	D
Aug-17	3	1.5000				D E
Nov-17	3	1.0200				E

Means that do not share a letter are significantly different.

Normplot of Residuals for S1



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One-way ANOVA Post Hoc: S2 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	23.4704	2.13368	51.86	0.000
Error	24	0.9875	0.04114		
Total	35	24.4579			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.202841	95.96%	94.11%	90.92%

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Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	2.5033	0.144	(2.2616, 2.7450)
Sep-17	3	0.9967	0.055	(0.7550, 1.2384)
Oct-17	3	2.0300	0.050	(1.7883, 2.2717)
Nov-17	3	1.763	0.070	(1.522, 2.005)
Dec-17	3	1.4967	0.060	(1.2550, 1.7384)
Jan-18	3	3.0033	0.001	(2.7616, 3.2450)
Feb-18	3	2.9967	0.055	(2.7550, 3.2384)
Mar-18	3	3.063	0.050	(2.822, 3.305)
Apr-18	3	3.9967	0.0170	(3.7550, 4.2384)
May-18	3	3.0033	0.060	(2.7616, 3.2450)
Jun-18	3	2.0200	0.023	(1.7783, 2.2617)
Jul-18	3	3.0033	0.100	(2.7616, 3.2450)

Tukey Pairwise Comparisons

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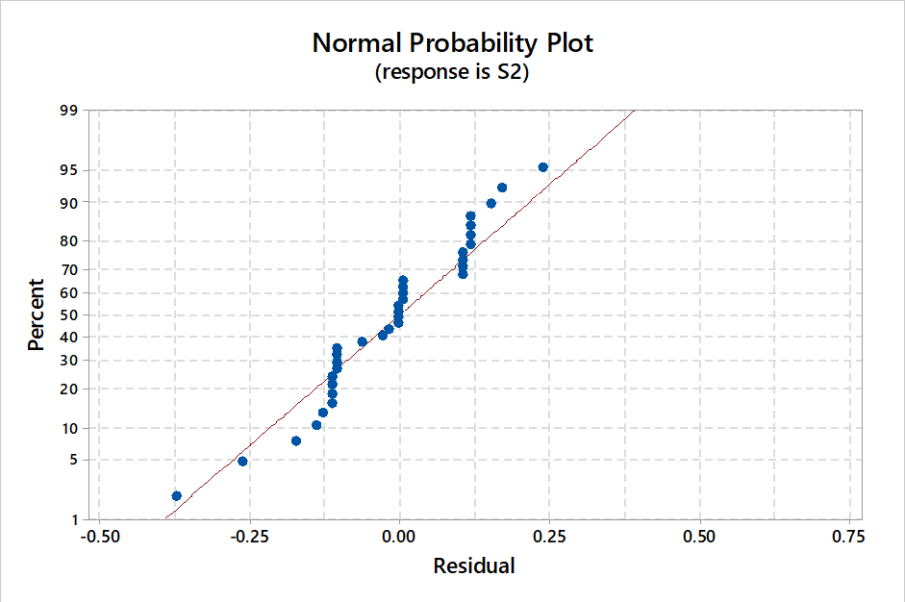
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Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping			
Apr-18	3	3.9967	A			
Mar-18	3	3.063		B		
Jul-18	3	3.0033		B		
May-18	3	3.0033		B		
Jan-18	3	3.0033		B		
Feb-18	3	2.9967		B		
Aug-17	3	2.5033		B	C	
Oct-17	3	2.0300			C	D
Jun-18	3	2.0200			C	D
Nov-17	3	1.763				D
Dec-17	3	1.4967				D	E
Sep-17	3	0.9967					E

Means that do not share a letter are significantly different.

Normplot of Residuals for S2



One-way ANOVA: S3 versus TIME

*** NOTE * Cannot draw the interval plot for the Tukey procedure. Interval plots for comparisons are illegible with more than 45 intervals.**

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Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	20.79	1.8903	3.95	0.002
Error	24	11.49	0.4788		
Total	35	32.28			

Multivariate Analysis Summary

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S	R-sq	R-sq(adj)	R-sq(pred)
0.691976	64.40%	48.09%	19.91%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	2.253	0.0610	(1.429, 3.078)
Sep-17	3	2.740	0.0550	(1.915, 3.565)
Oct-17	3	3.583	0.0500	(2.759, 4.408)
Nov-17	3	1.230	0.0700	(0.405, 2.055)
Dec-17	3	2.753	0.0330	(1.929, 3.578)
Jan-18	3	3.633	0.0440	(2.809, 4.458)
Feb-18	3	3.217	0.0550	(2.392, 4.041)
Mar-18	3	4.313	0.0500	(3.489, 5.138)
Apr-18	3	3.753	0.0700	(2.929, 4.578)
May-18	3	3.020	0.0600	(2.195, 3.845)
Jun-18	3	3.143	0.0230	(2.319, 3.968)
Jul-18	3	2.830	0.0100	(2.005, 3.655)

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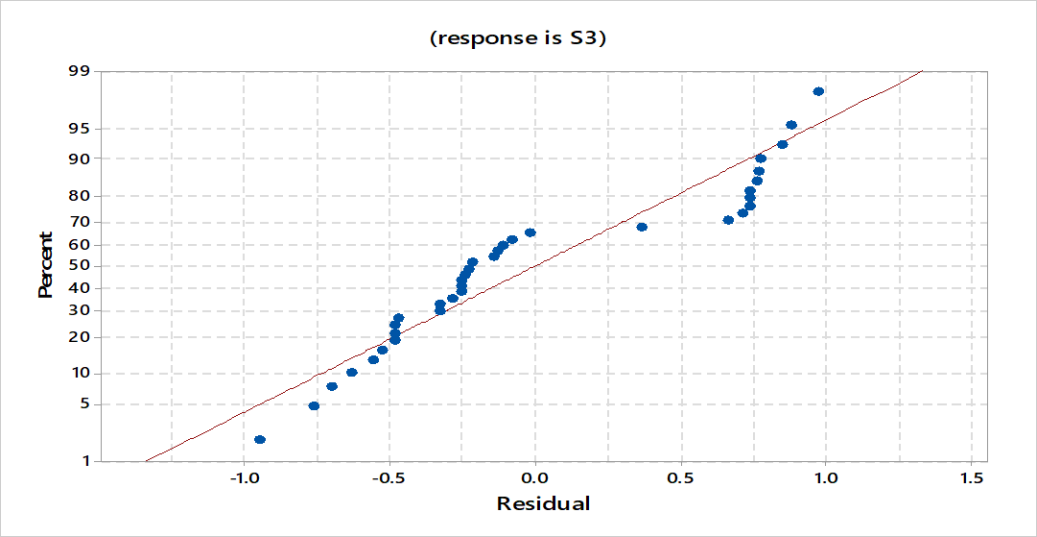
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Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping		
Mar-18	3	4.313	A		
Apr-18	3	3.753	A	B	
Jan-18	3	3.633	A	B	
Oct-17	3	3.583	A	B	
Feb-18	3	3.217	A	B	C
Jun-18	3	3.143	A	B	C
May-18	3	3.020	A	B	C
Jul-18	3	2.830	A	B	C
Dec-17	3	2.753	A	B	C
Sep-17	3	2.740	A	B	C
Aug-17	3	2.253		B	C
Nov-17	3	1.230			C

Means that do not share a letter are significantly different.

Normplot of Residuals for S3



One-way ANOVA: S4 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal

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Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	1.25885	0.114441	1412.85	0.000
Error	24	0.00194	0.000081		
Total	35	1.26079			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.009	99.85%	99.78%	99.65%

Means

TIME	N	Mean	Std Error	95% CI
May-18	3	1.12000	0.035	(1.10928, 1.13072)
Jun-18	3	1.21500	0.061	(1.20428, 1.22572)
Jul-18	3	1.27500	0.055	(1.26428, 1.28572)
Aug-18	3	1.33000	0.0150	(1.31928, 1.34072)
Sep-18	3	1.66500	0.0133	(1.65428, 1.67572)
Oct-18	3	1.77000	0.0144	(1.75928, 1.78072)
Nov-18	3	1.57500	0.055	(1.56428, 1.58572)
Dec-18	3	1.30000	0.0150	(1.28928, 1.31072)
Jan-19	3	1.44000	0.070	(1.42928, 1.45072)
Feb-19	3	1.34000	0.0060	(1.32928, 1.35072)
Mar-19	3	1.54500	0.0123	(1.53428, 1.55572)
Apr-19	3	1.54500	0.0100	(1.53428, 1.55572)

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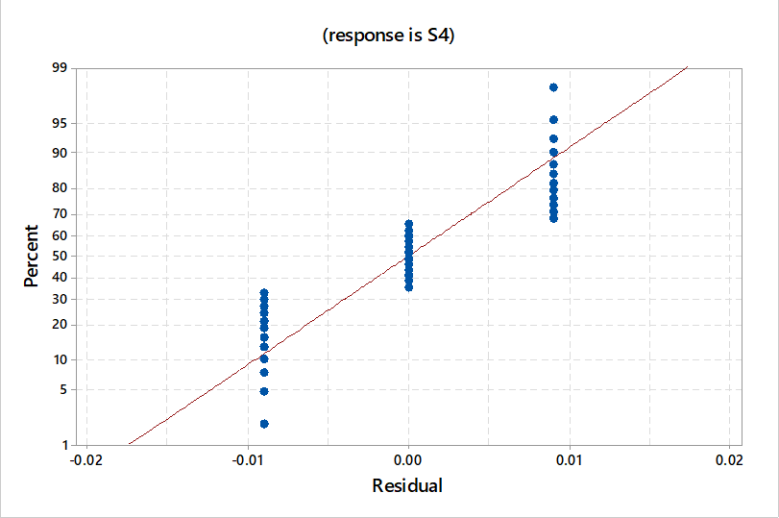
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Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping
Oct-18	3	1.77000	A
Sep-18	3	1.66500	B
Nov-18	3	1.57500	C
Apr-19	3	1.54500	D
Mar-19	3	1.54500	D
Jan-19	3	1.44000	E
Feb-19	3	1.34000	F
Aug-18	3	1.33000	F
Dec-18	3	1.30000	G
Jul-18	3	1.27500	G
Jun-18	3	1.21500	H
May-18	3	1.12000	I

Means that do not share a letter are significantly different.

Normplot of Residuals for S4



One-way ANOVA: S5 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

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Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	3.2968	0.29971	10.75	0.000
Error	24	0.6692	0.02789		
Total	35	3.9660			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.166988	83.13%	75.39%	62.03%

Means

TIME	N	Mean	Std Error	95% CI
May-18	3	1.11480	0.0610	(0.91582, 1.31378)
Jun-18	3	1.95480	0.0550	(1.75582, 2.15378)
Jul-18	3	1.47480	0.0500	(1.27582, 1.67378)
Aug-18	3	1.29980	0.0700	(1.10082, 1.49878)
Sep-18	3	1.75980	0.0330	(1.56082, 1.95878)
Oct-18	3	2.27480	0.0440	(2.07582, 2.47378)
Nov-18	3	1.53480	0.0550	(1.33582, 1.73378)
Dec-18	3	1.58980	0.0500	(1.39082, 1.78878)
Jan-19	3	1.63980	0.0700	(1.44082, 1.83878)
Feb-19	3	1.45980	0.0600	(1.26082, 1.65878)
Mar-19	3	1.63980	0.0230	(1.44082, 1.83878)
Apr-19	3	1.243	0.0100	(1.044, 1.442)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping		
Oct-18	3	2.27480	A		
Jun-18	3	1.95480	A	B	
Sep-18	3	1.75980		B	C
Mar-19	3	1.63980		B	C D
Jan-19	3	1.63980		B	C D

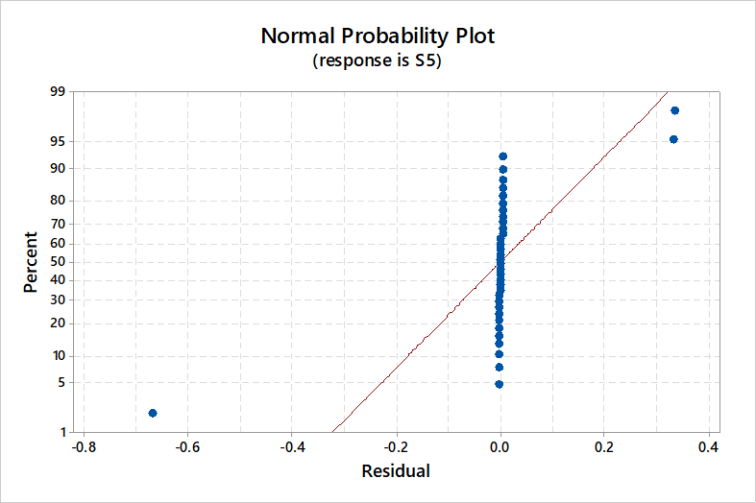
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Dec-18	3	1.58980	B	C	D	E
Nov-18	3	1.53480	B	C	D	E
Jul-18	3	1.47480	B	C	D	E
Feb-19	3	1.45980		C	D	E
Aug-18	3	1.29980		C	D	E
Apr-19	3	1.243			D	E
May-18	3	1.11480				E

Means that do not share a letter are significantly different.

Normplot of Residuals for S5



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APPENDIX (E)

Total phosphorus

One-way ANOVA Post Hoc: S1 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	4.3188	0.39262	20.83	0.000
Error	24	0.4523	0.01884		
Total	35	4.7711			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.137275	90.52%	86.18%	78.67%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	1.3333	0.050	(1.1698, 1.4969)
Sep-17	3	1.0400	0.061	(0.8764, 1.2036)
Oct-17	3	0.8400	0.055	(0.6764, 1.0036)
Nov-17	3	1.1900	0.050	(1.0264, 1.3536)
Dec-17	3	0.4400	0.033	(0.2764, 0.6036)
Jan-18	3	0.3400	0.044	(0.1764, 0.5036)
Feb-18	3	0.5600	0.055	(0.3964, 0.7236)
Mar-18	3	1.0400	0.050	(0.8764, 1.2036)
Apr-18	3	0.5900	0.070	(0.4264, 0.7536)
May-18	3	0.2900	0.060	(0.1264, 0.4536)

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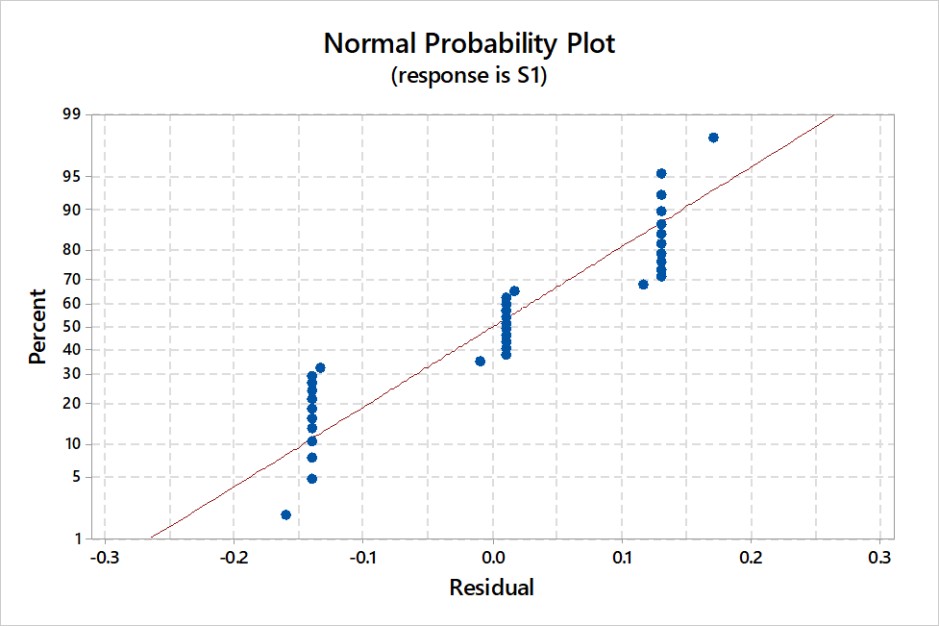
Jun-18	3	1.1900	0.023	(1.0264, 1.3536)
Jul-18	3	0.9900	0.010	(0.8264, 1.1536)

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping				
Aug-17	3	1.3333	A				
Nov-17	3	1.1900	A	B			
Jun-18	3	1.1900	A	B			
Mar-18	3	1.0400	A	B			
Sep-17	3	1.0400	A	B			
Jul-18	3	0.9900	A	B	C		
Oct-17	3	0.8400		B	C	D	
Apr-18	3	0.5900			C	D	E
Feb-18	3	0.5600				D	E
Dec-17	3	0.4400				D	E
Jan-18	3	0.3400					E
May-18	3	0.2900					E

Means that do not share a letter are significantly different.

Normplot of Residuals for S1



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One-way ANOVA Post Hoc: S2 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	8.1813	0.74375	18.21	0.000
Error	24	0.9800	0.04083		
Total	35	9.1613			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.202073	89.30%	84.40%	75.93%

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Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	1.783	0.050	(1.543, 2.024)
Sep-17	3	1.183	0.033	(0.943, 1.424)
Oct-17	3	1.083	0.044	(0.843, 1.324)
Nov-17	3	1.133	0.055	(0.893, 1.374)
Dec-17	3	0.333	0.050	(0.093, 0.574)
Jan-18	3	0.433	0.050	(0.193, 0.674)
Feb-18	3	0.483	0.061	(0.243, 0.724)
Mar-18	3	1.683	0.055	(1.443, 1.924)
Apr-18	3	1.233	0.050	(0.993, 1.474)
May-18	3	0.333	0.033	(0.093, 0.574)
Jun-18	3	0.823	0.044	(0.583, 1.064)
Jul-18	3	1.183	0.033	(0.943, 1.424)

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

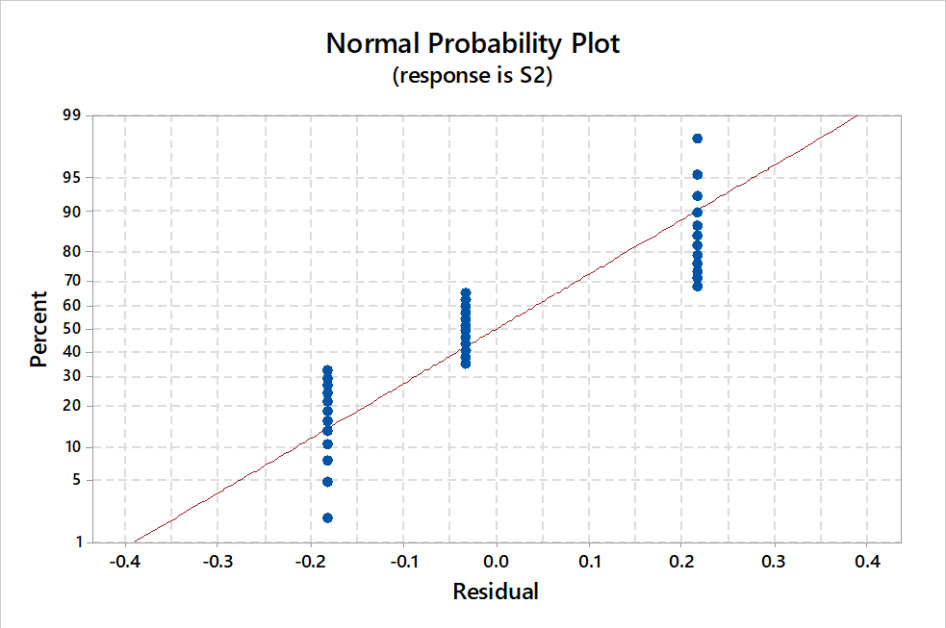
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TIME	N	Mean	Grouping			
Aug-17	3	1.783	A			
Mar-18	3	1.683	A	B		
Apr-18	3	1.233	A	B	C	
Jul-18	3	1.183		B	C	
Sep-17	3	1.183		B	C	
Nov-17	3	1.133		B	C	
Oct-17	3	1.083			C	
Jun-18	3	0.823			C	D
Feb-18	3	0.483				D
Jan-18	3	0.433				D
Dec-17	3	0.333				D
May-18	3	0.333				D

Means that do not share a letter are significantly different.

Normplot of Residuals for S2



One-way ANOVA Post Hoc: S3 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

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Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	5.9300	0.53909	13.76	0.000
Error	24	0.9400	0.03917		
Total	35	6.8700			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.197906	86.32%	80.05%	69.21%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	0.783	0.055	(0.548, 1.019)
Sep-17	3	0.933	0.050	(0.698, 1.169)
Oct-17	3	1.383	0.070	(1.148, 1.619)
Nov-17	3	1.333	0.060	(1.098, 1.569)
Dec-17	3	0.233	0.023	(-0.002, 0.469)
Jan-18	3	0.833	0.010	(0.598, 1.069)
Feb-18	3	0.483	0.050	(0.248, 0.719)
Mar-18	3	1.633	0.050	(1.398, 1.869)
Apr-18	3	0.683	0.061	(0.448, 0.919)
May-18	3	0.433	0.055	(0.198, 0.669)
Jun-18	3	1.233	0.050	(0.998, 1.469)
Jul-18	3	0.8333	0.033	(0.5975, 1.0692)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping
Mar-18	3	1.633	A
Oct-17	3	1.383	A B
Nov-17	3	1.333	A B C
Jun-18	3	1.233	A B C D

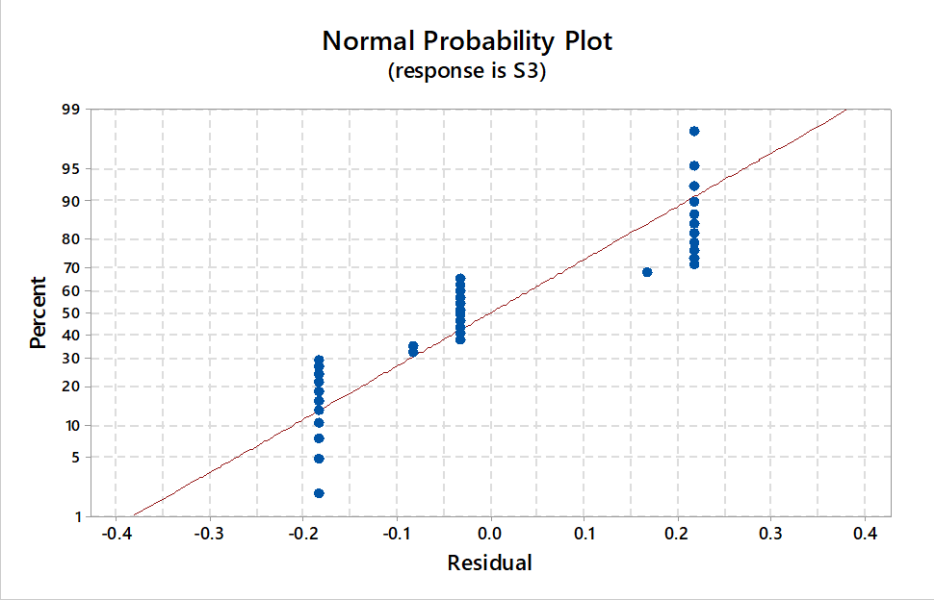
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Sep-17	3	0.933	B	C	D	E	
Jul-18	3	0.8333	B	C	D	E	
Jan-18	3	0.833	B	C	D	E	
Aug-17	3	0.783		C	D	E	F
Apr-18	3	0.683			D	E	F
Feb-18	3	0.483				E	F
May-18	3	0.433				E	F
Dec-17	3	0.233					F

Means that do not share a letter are significantly different.

Normplot of Residuals for S3



One-way ANOVA Post Hoc: S4 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

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Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	2.6809	0.24372	15.59	0.000
Error	24	0.3752	0.01563		
Total	35	3.0561			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.125033	87.72%	82.10%	72.38%

Means

TIME	N	Mean	Std Error	95% CI
May-18	3	1.1267	0.050	(0.9777, 1.2757)
Jun-18	3	1.7817	0.073	(1.6327, 1.9307)
Jul-18	3	1.6067	0.044	(1.4577, 1.7557)
Aug-18	3	1.1467	0.055	(0.9977, 1.2957)
Sep-18	3	1.5367	0.009	(1.3877, 1.6857)
Oct-18	3	1.9567	0.050	(1.8077, 2.1057)
Nov-18	3	1.4967	0.061	(1.3477, 1.6457)
Dec-18	3	1.1167	0.055	(0.9677, 1.2657)
Jan-19	3	1.1067	0.050	(0.9577, 1.2557)
Feb-19	3	1.7067	0.033	(1.5577, 1.8557)
Mar-19	3	1.4967	0.044	(1.3477, 1.6457)
Apr-19	3	1.4867	0.033	(1.3377, 1.6357)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping		
Oct-18	3	1.9567	A		
Jun-18	3	1.7817	A	B	
Feb-19	3	1.7067	A	B	
Jul-18	3	1.6067	A	B	
Sep-18	3	1.5367		B	
Mar-19	3	1.4967		B	C
Nov-18	3	1.4967		B	C
Apr-19	3	1.4867		B	C D

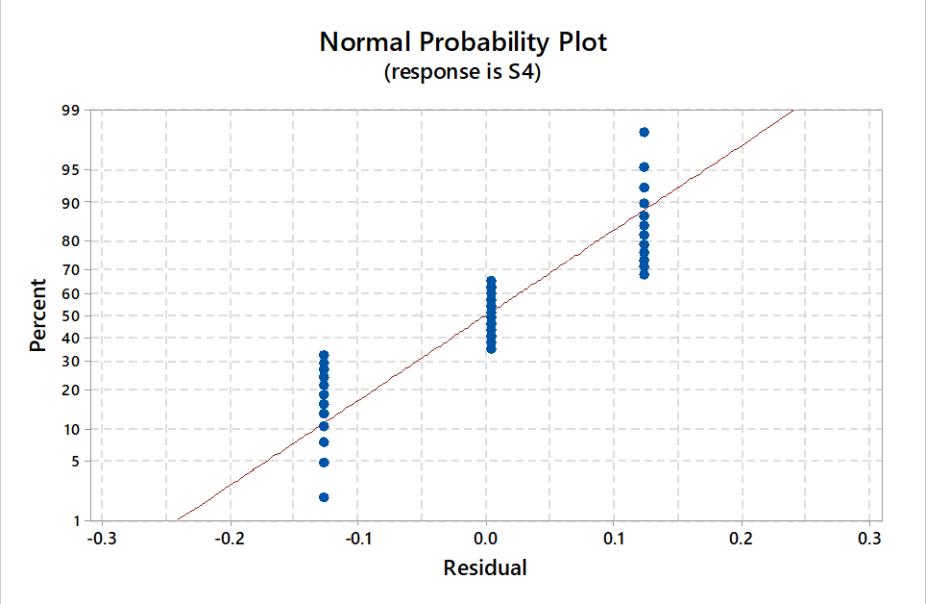
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Aug-18	3	1.1467	C	D	E
May-18	3	1.1267		D	E
Dec-18	3	1.1167			E
Jan-19	3	1.1067			E

Means that do not share a letter are significantly different.

Normplot of Residuals for S4



One-way ANOVA Post Hoc: S5 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	4.1982	0.38165	34.38	0.000
Error	24	0.2664	0.01110		

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Total	35	4.4646

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.105357	94.03%	91.30%	86.57%

Means

TIME	N	Mean	Std Error	95% CI
May-18	3	0.7900	0.050	(0.6645, 0.9155)
Jun-18	3	1.5700	0.061	(1.4445, 1.6955)
Jul-18	3	1.2100	0.055	(1.0845, 1.3355)
Aug-18	3	1.7500	0.050	(1.6245, 1.8755)
Sep-18	3	1.7600	0.033	(1.6345, 1.8855)
Oct-18	3	1.8300	0.044	(1.7045, 1.9555)
Nov-18	3	1.1700	0.055	(1.0445, 1.2955)
Dec-18	3	1.7500	0.050	(1.6245, 1.8755)
Jan-19	3	1.7500	0.070	(1.6245, 1.8755)
Feb-19	3	1.7200	0.060	(1.5945, 1.8455)
Mar-19	3	1.1100	0.023	(0.9845, 1.2355)
Apr-19	3	1.1100	0.010	(0.9845, 1.2355)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

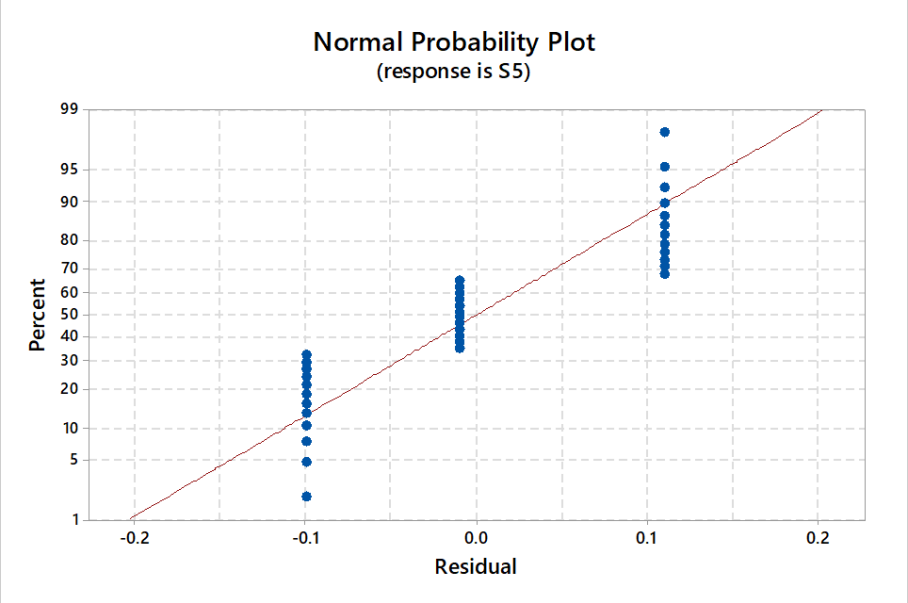
TIME	N	Mean	Grouping		
Oct-18	3	1.8300	A		
Sep-18	3	1.7600	A		
Jan-19	3	1.7500	A		
Dec-18	3	1.7500	A		
Aug-18	3	1.7500	A		
Feb-19	3	1.7200	A		
Jun-18	3	1.5700	A		
Jul-18	3	1.2100		B	
Nov-18	3	1.1700		B	
Apr-19	3	1.1100		B	
Mar-19	3	1.1100		B	
May-18	3	0.7900			C

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Means that do not share a letter are significantly different.

Normplot of Residuals for S5



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APPENDIX (F)

Chemical oxygen demand

One-way ANOVA Post Hoc: S1 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	14338.5	1303.50	1707.00	0.000
Error	24	18.3	0.76		
Total	35	14356.8			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.873855	99.87%	99.81%	99.71%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	52.067	1.350	(51.025, 53.108)
Sep-17	3	23.010	0.610	(21.969, 24.051)
Oct-17	3	39.097	0.550	(38.055, 40.138)
Nov-17	3	22.070	0.500	(21.029, 23.111)
Dec-17	3	13.067	0.330	(12.025, 14.108)
Jan-18	3	51.097	0.440	(50.055, 52.138)
Feb-18	3	82.080	0.550	(81.039, 83.121)
Mar-18	3	43.253	0.500	(42.212, 44.295)
Apr-18	3	61.597	0.700	(60.555, 62.638)
May-18	3	16.597	0.600	(15.555, 17.638)
Jun-18	3	28.030	0.230	(26.989, 29.071)

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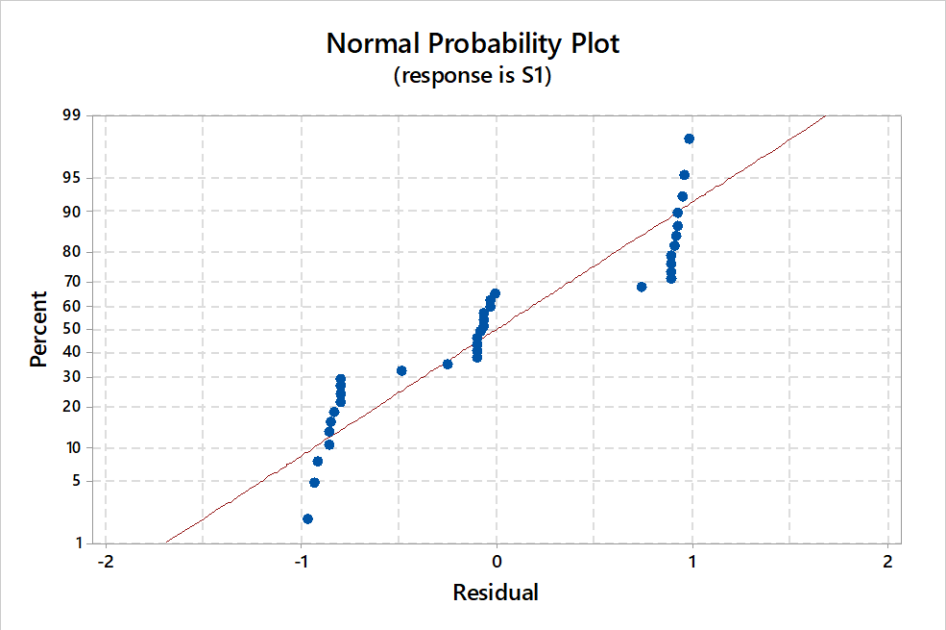
Jul-18	3	23.037	1.100 (21.995, 24.078)

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping
Feb-18	3	82.080	A
Apr-18	3	61.597	B
Aug-17	3	52.067	C
Jan-18	3	51.097	C
Mar-18	3	43.253	D
Oct-17	3	39.097	E
Jun-18	3	28.030	F
Jul-18	3	23.037	G
Sep-17	3	23.010	G
Nov-17	3	22.070	G
May-18	3	16.597	H
Dec-17	3	13.067	I

Means that do not share a letter are significantly different.

Normplot of Residuals for S1



One-way ANOVA Post Hoc: S2 versus TIME
Method

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Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	14948.7	1358.98	3081.83	0.000
Error	24	10.6	0.44		
Total	35	14959.3			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.664051	99.93%	99.90%	99.84%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	20.933	0.440	(20.142, 21.725)
Sep-17	3	15.947	0.550	(15.155, 16.738)
Oct-17	3	17.933	0.500	(17.142, 18.725)
Nov-17	3	30.913	0.700	(30.122, 31.705)
Dec-17	3	22.933	0.600	(22.142, 23.725)
Jan-18	3	21.903	0.440	(21.112, 22.695)
Feb-18	3	75.007	0.550	(74.215, 75.798)
Mar-18	3	54.433	0.500	(53.642, 55.225)
Apr-18	3	58.843	0.700	(58.052, 59.635)
May-18	3	27.990	0.600	(27.199, 28.781)
Jun-18	3	60.413	0.230	(59.622, 61.205)
Jul-18	3	11.433	0.100	(10.642, 12.225)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping
Feb-18	3	75.007	A

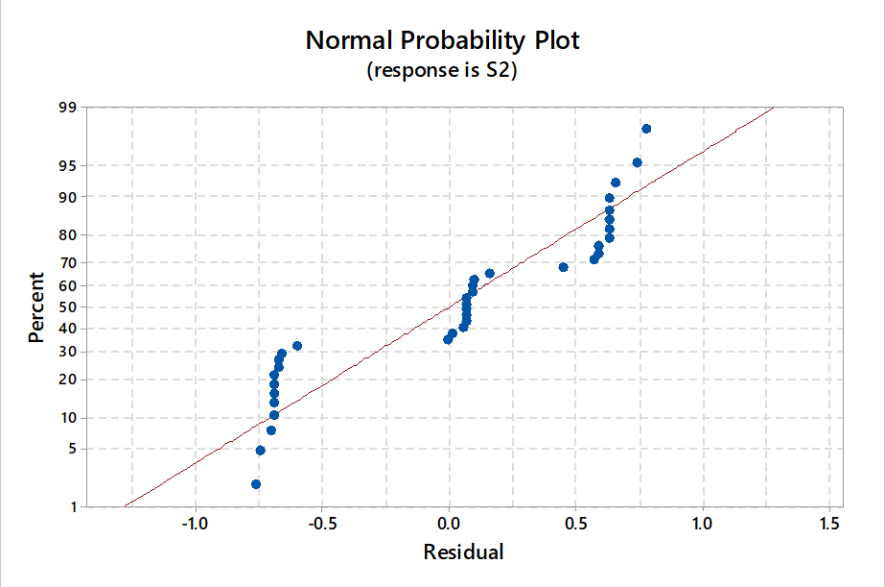
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Jun-18	3	60.413		B					
Apr-18	3	58.843		B					
Mar-18	3	54.433			C				
Nov-17	3	30.913				D			
May-18	3	27.990					E		
Dec-17	3	22.933						F	
Jan-18	3	21.903						F	G
Aug-17	3	20.933							G
Oct-17	3	17.933							
Sep-17	3	15.947							
Jul-18	3	11.433							

Means that do not share a letter are significantly different.

Normplot of Residuals for S2



One-way ANOVA Post Hoc: S3 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values

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TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	14423.2	1311.20	1780.85	0.000
Error	24	17.7	0.74		
Total	35	14440.9			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.858066	99.88%	99.82%	99.72%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	43.067	0.610	(42.044, 44.089)
Sep-17	3	59.067	0.550	(58.044, 60.089)
Oct-17	3	78.067	0.500	(77.044, 79.089)
Nov-17	3	48.567	0.700	(47.544, 49.589)
Dec-17	3	23.067	0.330	(22.044, 24.089)
Jan-18	3	66.067	0.440	(65.044, 67.089)
Feb-18	3	78.067	0.550	(77.044, 79.089)
Mar-18	3	48.567	0.500	(47.544, 49.589)
Apr-18	3	59.537	0.700	(58.514, 60.559)
May-18	3	32.270	0.600	(31.248, 33.292)
Jun-18	3	9.567	0.230	(8.544, 10.589)
Jul-18	3	40.540	0.100	(39.518, 41.562)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping
Feb-18	3	78.067	A
Oct-17	3	78.067	A
Jan-18	3	66.067	B
Apr-18	3	59.537	C
Sep-17	3	59.067	C
Mar-18	3	48.567	D

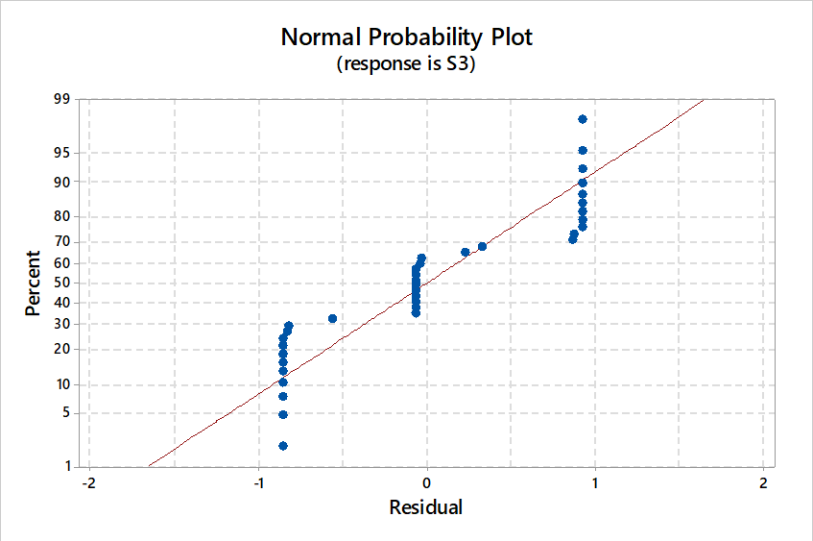
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Nov-17	3	48.567		D				
Aug-17	3	43.067			E			
Jul-18	3	40.540				F		
May-18	3	32.270					G	
Dec-17	3	23.067						H
Jun-18	3	9.567						I

Means that do not share a letter are significantly different.

Normplot of Residuals for S3



One-way ANOVA Post Hoc: S4 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	14293.6	1299.41	6306.99	0.000
Error	24	4.9	0.21		
Total	35	14298.5			

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Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.453903	99.97%	99.95%	99.92%

Means

TIME	N	Mean	Std Error	95% CI
May-18	3	33.560	0.940	(33.019, 34.101)
Jun-18	3	9.567	0.550	(9.026, 10.108)
Jul-18	3	40.560	0.500	(40.019, 41.101)
Aug-18	3	43.067	1.600	(42.526, 43.608)
Sep-18	3	59.093	0.600	(58.552, 59.634)
Oct-18	3	78.037	0.440	(77.496, 78.578)
Nov-18	3	48.537	0.550	(47.996, 49.078)
Dec-18	3	23.023	0.500	(22.482, 23.564)
Jan-19	3	66.053	0.700	(65.512, 66.594)
Feb-19	3	78.033	0.600	(77.492, 78.574)
Mar-19	3	48.533	0.230	(47.992, 49.074)
Apr-19	3	59.523	0.100	(58.982, 60.064)

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Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

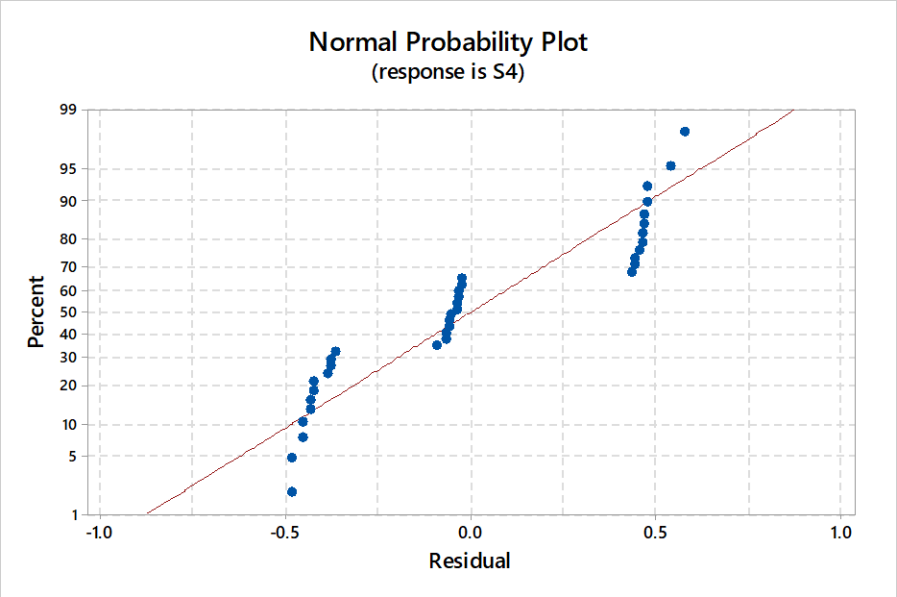
TIME	N	Mean	Grouping
Oct-18	3	78.037	A
Feb-19	3	78.033	A
Jan-19	3	66.053	B
Apr-19	3	59.523	C
Sep-18	3	59.093	C
Nov-18	3	48.537	D
Mar-19	3	48.533	D
Aug-18	3	43.067	E
Jul-18	3	40.560	F
May-18	3	33.560	G
Dec-18	3	23.023	H
Jun-18	3	9.567	I

Means that do not share a letter are significantly different.

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Normplot of Residuals for S4



One-way ANOVA Post Hoc: S5 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	14183.6	1289.42	6396.46	0.000
Error	24	4.8	0.20		
Total	35	14188.5			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.448980	99.97%	99.95%	99.92%

Means

TIME	N	Mean	Std Error	95% CI

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May-18	3	17.630	0.610	(17.095, 18.165)
Jun-18	3	28.130	1.550	(27.595, 28.665)
Jul-18	3	23.130	0.500	(22.595, 23.665)
Aug-18	3	52.197	0.700	(51.662, 52.732)
Sep-18	3	23.080	0.330	(22.545, 23.615)
Oct-18	3	39.053	1.440	(38.518, 39.588)
Nov-18	3	22.007	0.550	(21.472, 22.542)
Dec-18	3	13.180	0.500	(12.645, 13.715)
Jan-19	3	51.120	0.700	(50.585, 51.655)
Feb-19	3	82.0133	0.600	(81.4783, 82.5483)
Mar-19	3	43.193	0.230	(42.658, 43.728)
Apr-19	3	61.687	1.100	(61.152, 62.222)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

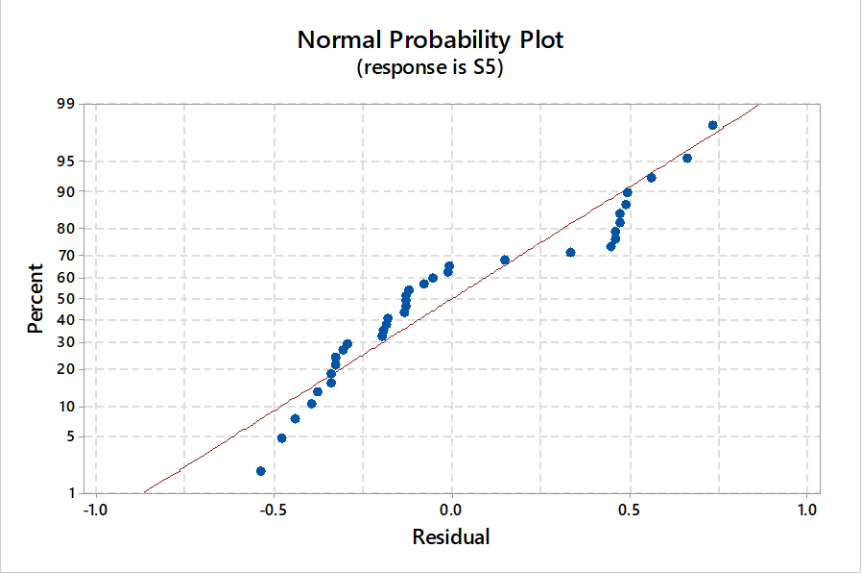
TIME	N	Mean	Grouping
Feb-19	3	82.0133	A
Apr-19	3	61.687	B
Aug-18	3	52.197	C
Jan-19	3	51.120	C
Mar-19	3	43.193	D
Oct-18	3	39.053	E
Jun-18	3	28.130	F
Jul-18	3	23.130	G
Sep-18	3	23.080	G
Nov-18	3	22.007	G
May-18	3	17.630	H
Dec-18	3	13.180	I

Means that do not share a letter are significantly different.

Normplot of Residuals for S5

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APPENDIX (G)

Dissolved oxygen

One-way ANOVA Post Hoc: S1 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	50.3043	4.57312	435.19	0.000
Error	24	0.2522	0.01051		
Total	35	50.5565			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.102510	99.50%	99.27%	98.88%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	7.4033	0.350	(7.2812, 7.5255)
Sep-17	3	6.8483	0.610	(6.7262, 6.9705)
Oct-17	3	7.5233	0.550	(7.4012, 7.6455)
Nov-17	3	7.0783	0.500	(6.9562, 7.2005)
Dec-17	3	6.1733	0.330	(6.0512, 6.2955)
Jan-18	3	6.0233	0.440	(5.9012, 6.1455)
Feb-18	3	9.2283	0.550	(9.1062, 9.3505)
Mar-18	3	7.3783	0.500	(7.2562, 7.5005)
Apr-18	3	6.2283	0.700	(6.1062, 6.3505)
May-18	3	5.9283	0.600	(5.8062, 6.0505)
Jun-18	3	9.4383	0.230	(9.3162, 9.5605)

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Jul-18	3	8.7983	0.610 (8.6762, 8.9205)

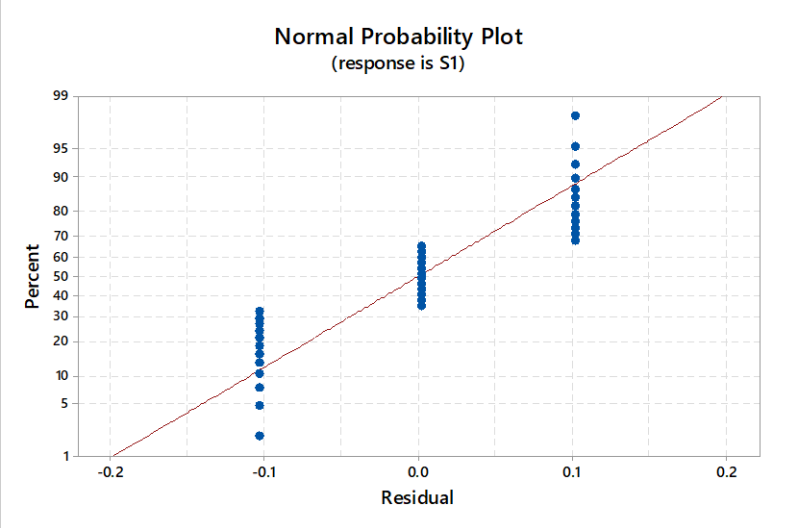
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping				
Jun-18	3	9.4383	A				
Feb-18	3	9.2283	A				
Jul-18	3	8.7983		B			
Oct-17	3	7.5233			C		
Aug-17	3	7.4033			C		
Mar-18	3	7.3783			C	D	
Nov-17	3	7.0783				D	E
Sep-17	3	6.8483					E
Apr-18	3	6.2283					F
Dec-17	3	6.1733					F
Jan-18	3	6.0233					F
May-18	3	5.9283					F

Means that do not share a letter are significantly different.

Normplot of Residuals for S1



One-way ANOVA Post Hoc: S2 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal

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Significance level α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	44.2770	4.02518	171.22	0.000
Error	24	0.5642	0.02351		
Total	35	44.8412			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.153324	98.74%	98.17%	97.17%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	7.2433	0.440	(7.0606, 7.4260)
Sep-17	3	6.8383	0.550	(6.6556, 7.0210)
Oct-17	3	7.4083	0.500	(7.2256, 7.5910)
Nov-17	3	7.2233	0.700	(7.0406, 7.4060)
Dec-17	3	6.4783	0.600	(6.2956, 6.6610)
Jan-18	3	6.3233	0.440	(6.1406, 6.5060)
Feb-18	3	9.1483	0.550	(8.9656, 9.3310)
Mar-18	3	7.3633	0.500	(7.1806, 7.5460)
Apr-18	3	6.5933	0.700	(6.4106, 6.7760)
May-18	3	6.5733	0.600	(6.3906, 6.7560)
Jun-18	3	9.8833	0.230	(9.7006, 10.0660)
Jul-18	3	8.7833	0.100	(8.6006, 8.9660)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping
Jun-18	3	9.8833	A
Feb-18	3	9.1483	B
Jul-18	3	8.7833	B

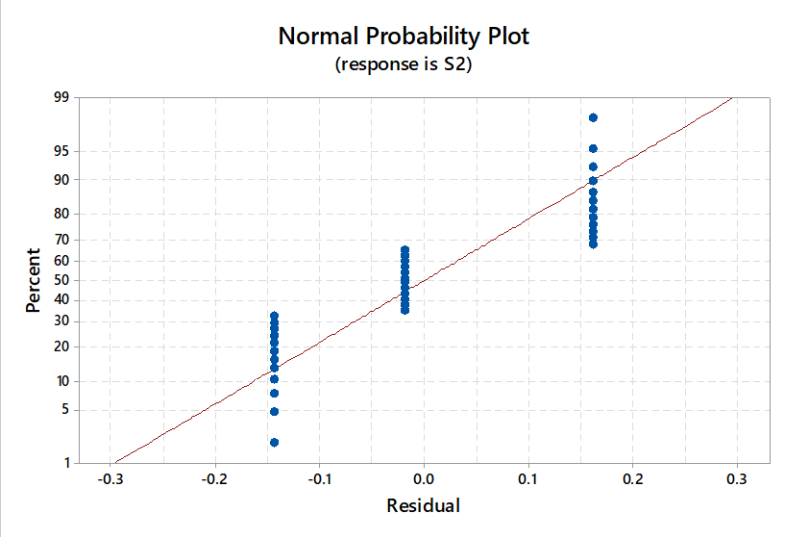
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Oct-17	3	7.4083	C			
Mar-18	3	7.3633	C			
Aug-17	3	7.2433	C	D		
Nov-17	3	7.2233	C	D		
Sep-17	3	6.8383		D	E	
Apr-18	3	6.5933			E	F
May-18	3	6.5733			E	F
Dec-17	3	6.4783			E	F
Jan-18	3	6.3233				F

Means that do not share a letter are significantly different.

Normplot of Residuals for S2



One-way ANOVA Post Hoc: S3 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value

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TIME	11	67.0904	6.09913	1010.91	0.000
Error	24	0.1448	0.00603		
Total	35	67.2352			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0776745	99.78%	99.69%	99.52%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	9.1633	0.610	(9.0708, 9.2559)
Sep-17	3	8.5833	0.550	(8.4908, 8.6759)
Oct-17	3	6.7933	0.500	(6.7008, 6.8859)
Nov-17	3	7.2383	0.700	(7.1458, 7.3309)
Dec-17	3	5.3933	0.330	(5.3008, 5.4859)
Jan-18	3	6.5883	0.440	(6.4958, 6.6809)
Feb-18	3	9.1683	0.550	(9.0758, 9.2609)
Mar-18	3	7.2433	0.500	(7.1508, 7.3359)
Apr-18	3	6.3633	0.700	(6.2708, 6.4559)
May-18	3	6.4533	0.600	(6.3608, 6.5459)
Jun-18	3	9.6883	0.230	(9.5958, 9.7809)
Jul-18	3	9.1883	0.100	(9.0958, 9.2809)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping				
Jun-18	3	9.6883	A				
Jul-18	3	9.1883		B			
Feb-18	3	9.1683		B			
Aug-17	3	9.1633		B			
Sep-17	3	8.5833			C		
Mar-18	3	7.2433				D	
Nov-17	3	7.2383				D	
Oct-17	3	6.7933					E
Jan-18	3	6.5883					E F
May-18	3	6.4533					F

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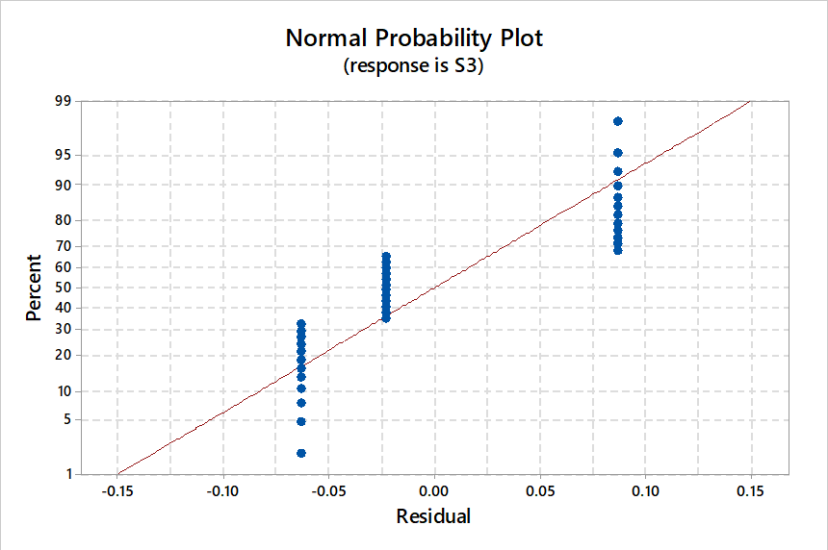
05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

Apr-18	3	6.3633	F
Dec-17	3	5.3933	G

Means that do not share a letter are significantly different.

Interval Plot of S3 vs TIME

Normplot of Residuals for S3



One-way ANOVA Post Hoc: S4 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	54.157	4.92334	107.89	0.000
Error	24	1.095	0.04563		
Total	35	55.252			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.213620	98.02%	97.11%	95.54%

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Means

TIME	N	Mean	Std Error	95% CI
May-18	3	5.852	0.340	(5.597, 6.106)
Jun-18	3	7.782	0.550	(7.527, 8.036)
Jul-18	3	7.247	0.500	(6.992, 7.501)
Aug-18	3	4.712	0.600	(4.457, 4.966)
Sep-18	3	4.237	0.600	(3.982, 4.491)
Oct-18	3	4.192	0.440	(3.937, 4.446)
Nov-18	3	4.567	0.550	(4.312, 4.821)
Dec-18	3	5.112	0.500	(4.857, 5.366)
Jan-19	3	4.602	0.270	(4.347, 4.856)
Feb-19	3	3.597	0.600	(3.342, 3.851)
Mar-19	3	5.772	0.230	(5.517, 6.026)
Apr-19	3	4.227	0.100	(3.972, 4.481)

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

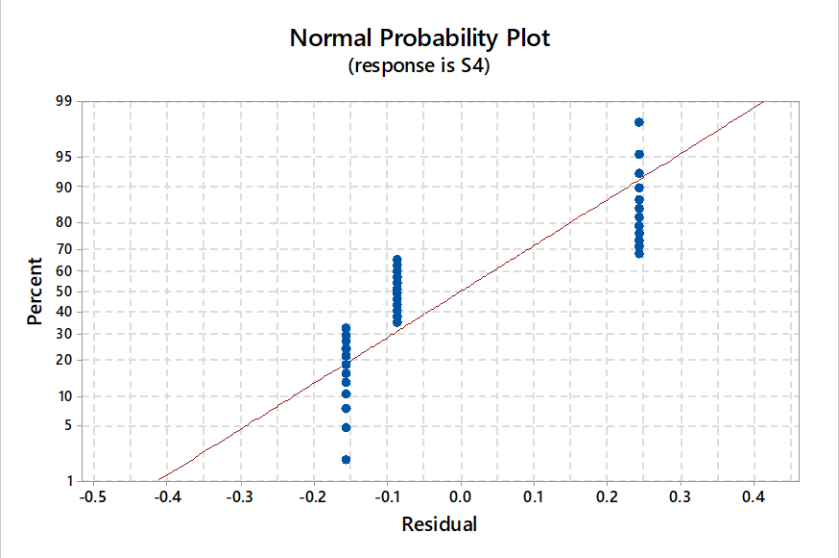
TIME	N	Mean	Grouping			
Jun-18	3	7.782	A			
Jul-18	3	7.247	A			
May-18	3	5.852		B		
Mar-19	3	5.772		B		
Dec-18	3	5.112			C	
Aug-18	3	4.712			C	D
Jan-19	3	4.602			C	D
Nov-18	3	4.567			C	D
Sep-18	3	4.237				D
Apr-19	3	4.227				D
Oct-18	3	4.192				D	E
Feb-19	3	3.597					E

Means that do not share a letter are significantly different.

Interval Plot of S4 vs TIME
Normplot of Residuals for S4

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One-way ANOVA Post Hoc: S5 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	80.8203	7.34730	725.06	0.000
Error	24	0.2432	0.01013		
Total	35	81.0635			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.100664	99.70%	99.56%	99.32%

Means

TIME	N	Mean	Std Error	95% CI
May-18	3	5.6017	0.610	(5.4817, 5.7216)
Jun-18	3	7.3567	0.550	(7.2367, 7.4766)

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Jul-18	3	7.6567	0.500	(7.5367, 7.7766)
Aug-18	3	4.2267	0.700	(4.1067, 4.3466)
Sep-18	3	4.0117	0.330	(3.8917, 4.1316)
Oct-18	3	4.4767	0.440	(4.3567, 4.5966)
Nov-18	3	4.1167	0.550	(3.9967, 4.2366)
Dec-18	3	4.0367	0.500	(3.9167, 4.1566)
Jan-19	3	2.7617	0.700	(2.6417, 2.8816)
Feb-19	3	3.0967	0.600	(2.9767, 3.2166)
Mar-19	3	5.0817	0.230	(4.9617, 5.2016)
Apr-19	3	3.1567	0.100	(3.0367, 3.2766)

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

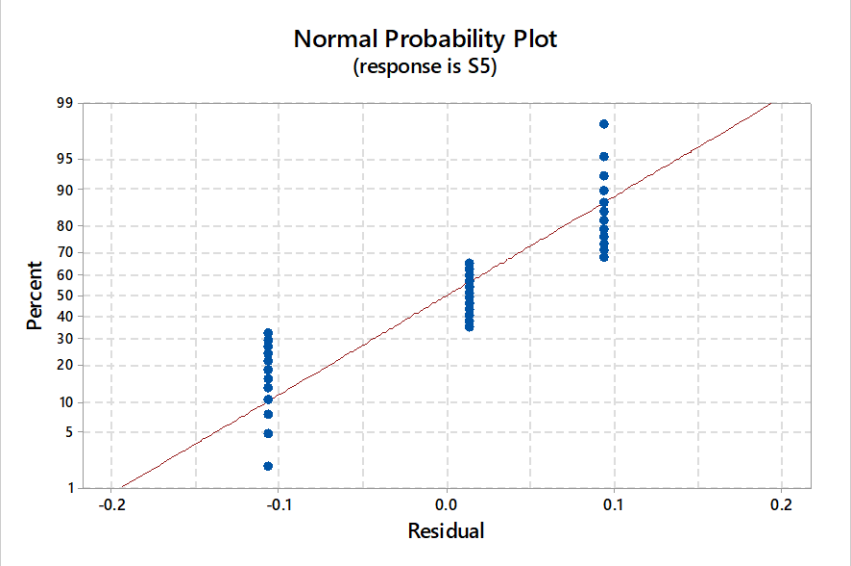
TIME	N	Mean	Grouping					
Jul-18	3	7.6567	A					
Jun-18	3	7.3567		B				
May-18	3	5.6017			C			
Mar-19	3	5.0817				D		
Oct-18	3	4.4767					E	
Aug-18	3	4.2267					E	F
Nov-18	3	4.1167						F
Dec-18	3	4.0367						F
Sep-18	3	4.0117						F
Apr-19	3	3.1567						G
Feb-19	3	3.0967						G
Jan-19	3	2.7617						H

Means that do not share a letter are significantly different.

Interval Plot of S5 vs TIME
Normplot of Residuals for S5

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APPENDIX (H)

Chlorophyll-*a*

One-way ANOVA Post Hoc: S1 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	4231.03	384.639	751.39	0.000
Error	24	12.29	0.512		
Total	35	4243.31			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.715472	99.71%	99.58%	99.35%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	20.570	0.350	(19.717, 21.423)
Sep-17	3	6.485	0.610	(5.632, 7.338)
Oct-17	3	5.335	0.550	(4.482, 6.188)
Nov-17	3	4.410	0.500	(3.557, 5.263)
Dec-17	3	2.105	0.330	(1.252, 2.958)
Jan-18	3	3.255	0.440	(2.402, 4.108)
Feb-18	3	40.810	0.550	(39.957, 41.663)
Mar-18	3	4.255	0.500	(3.402, 5.108)
Apr-18	3	2.950	0.700	(2.097, 3.803)
May-18	3	9.955	0.600	(9.102, 10.808)

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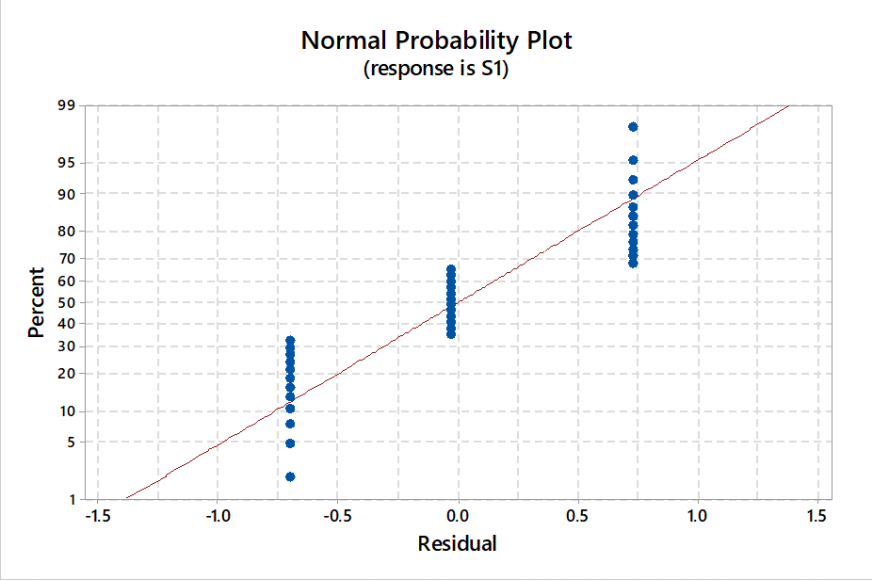
Jun-18	3	2.720	0.230	(1.867, 3.573)
Jul-18	3	2.335	0.100	(1.482, 3.188)

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping					
Feb-18	3	40.810	A					
Aug-17	3	20.570		B				
May-18	3	9.955			C			
Sep-17	3	6.485				D		
Oct-17	3	5.335				D	E	
Nov-17	3	4.410				D	E	F
Mar-18	3	4.255					E	F
Jan-18	3	3.255					E	F G
Apr-18	3	2.950						F G
Jun-18	3	2.720						F G
Jul-18	3	2.335						F G
Dec-17	3	2.105						G

Means that do not share a letter are significantly different.

Normplot of Residuals for S1



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One-way ANOVA Post Hoc: S2 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	9089.60	826.327	4366.71	0.000
Error	24	4.54	0.189		
Total	35	9094.14			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.435010	99.95%	99.93%	99.89%

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Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	56.882	0.440	(56.363, 57.400)
Sep-17	3	24.767	0.550	(24.248, 25.285)
Oct-17	3	3.917	0.500	(3.398, 4.435)
Nov-17	3	3.452	0.700	(2.933, 3.970)
Dec-17	3	3.827	0.600	(3.308, 4.345)
Jan-18	3	2.302	0.440	(1.783, 2.820)
Feb-18	3	26.147	0.550	(25.628, 26.665)
Mar-18	3	2.842	0.500	(2.323, 3.360)
Apr-18	3	2.377	0.700	(1.858, 2.895)
May-18	3	12.227	0.600	(11.708, 12.745)
Jun-18	3	2.377	0.230	(1.858, 2.895)
Jul-18	3	2.837	0.100	(2.318, 3.355)

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

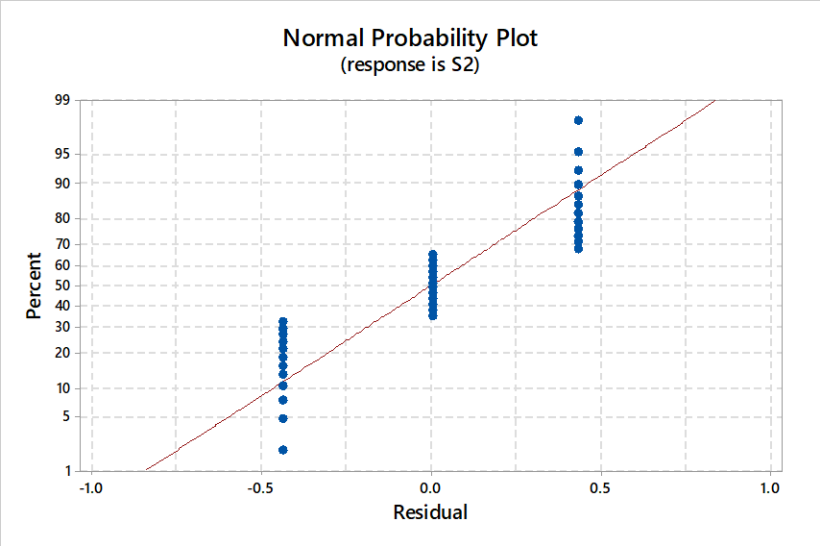
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TIME	N	Mean	Grouping				
Aug-17	3	56.882	A				
Feb-18	3	26.147		B			
Sep-17	3	24.767			C		
May-18	3	12.227				D	
Oct-17	3	3.917					E
Dec-17	3	3.827					E
Nov-17	3	3.452					E F
Mar-18	3	2.842					E F
Jul-18	3	2.837					E F
Jun-18	3	2.377					F
Apr-18	3	2.377					F
Jan-18	3	2.302					F

Means that do not share a letter are significantly different.

Normplot of Residuals for S2



One-way ANOVA Post Hoc: S3 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

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Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	4093.18	372.107	7943.47	0.000
Error	24	1.12	0.047		
Total	35	4094.30			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.216436	99.97%	99.96%	99.94%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	14.000	0.610	(13.742, 14.258)
Sep-17	3	7.800	0.550	(7.542, 8.058)
Oct-17	3	6.650	0.500	(6.392, 6.908)
Nov-17	3	41.315	0.700	(41.057, 41.573)
Dec-17	3	2.765	0.330	(2.507, 3.023)
Jan-18	3	3.460	0.440	(3.202, 3.718)
Feb-18	3	9.920	0.550	(9.662, 10.178)
Mar-18	3	2.150	0.500	(1.892, 2.408)
Apr-18	3	2.9533	0.700	(2.6954, 3.2112)
May-18	3	16.145	0.600	(15.887, 16.403)
Jun-18	3	2.380	0.230	(2.122, 2.638)
Jul-18	3	2.610	0.100	(2.352, 2.868)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping
Nov-17	3	41.315	A
May-18	3	16.145	B
Aug-17	3	14.000	C
Feb-18	3	9.920	D

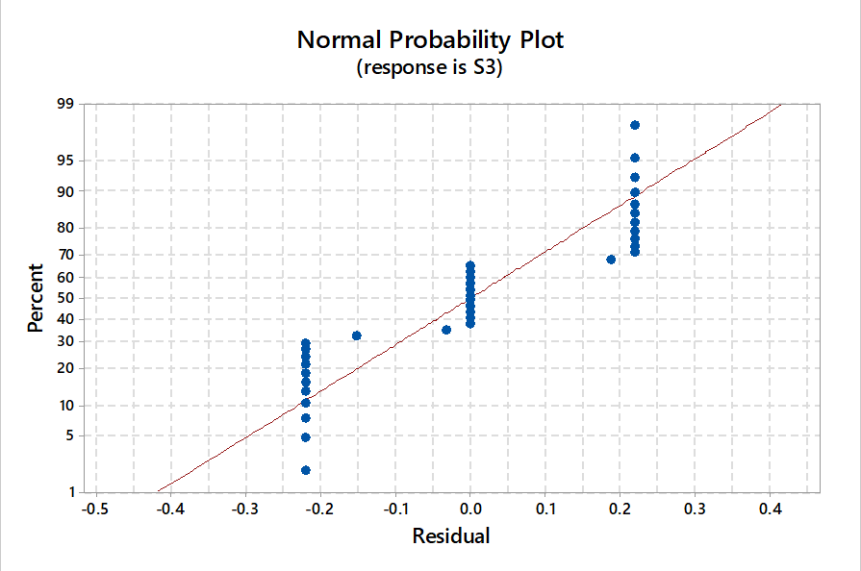
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Sep-17	3	7.800		E			
Oct-17	3	6.650			F		
Jan-18	3	3.460				G	
Apr-18	3	2.9533				G	H
Dec-17	3	2.765					H I
Jul-18	3	2.610					H I
Jun-18	3	2.380					H I
Mar-18	3	2.150					I

Means that do not share a letter are significantly different.

Normplot of Residuals for S3



One-way ANOVA Post Hoc: S4 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

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Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	48907.9	4446.18	4975.02	0.000
Error	24	21.4	0.89		
Total	35	48929.4			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.945357	99.96%	99.94%	99.90%

Means

TIME	N	Mean	Std Error	95% CI
May-18	3	102.880	1.350	(101.754, 104.006)
Jun-18	3	38.685	0.610	(37.559, 39.811)
Jul-18	3	28.855	2.550	(27.729, 29.981)
Aug-18	3	62.840	0.500	(61.714, 63.966)
Sep-18	3	105.335	1.330	(104.209, 106.461)
Oct-18	3	107.465	0.440	(106.339, 108.591)
Nov-18	3	99.975	1.550	(98.849, 101.101)
Dec-18	3	33.715	0.500	(32.589, 34.841)
Jan-19	3	60.760	0.700	(59.634, 61.886)
Feb-19	3	4.915	0.600	(3.789, 6.041)
Mar-19	3	17.735	0.230	(16.609, 18.861)
Apr-19	3	18.325	2.100	(17.199, 19.451)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping					
Oct-18	3	107.465	A					
Sep-18	3	105.335	A	B				
May-18	3	102.880		B				
Nov-18	3	99.975			C			
Aug-18	3	62.840				D		
Jan-19	3	60.760				D		
Jun-18	3	38.685					E	
Dec-18	3	33.715						F
Jul-18	3	28.855						G

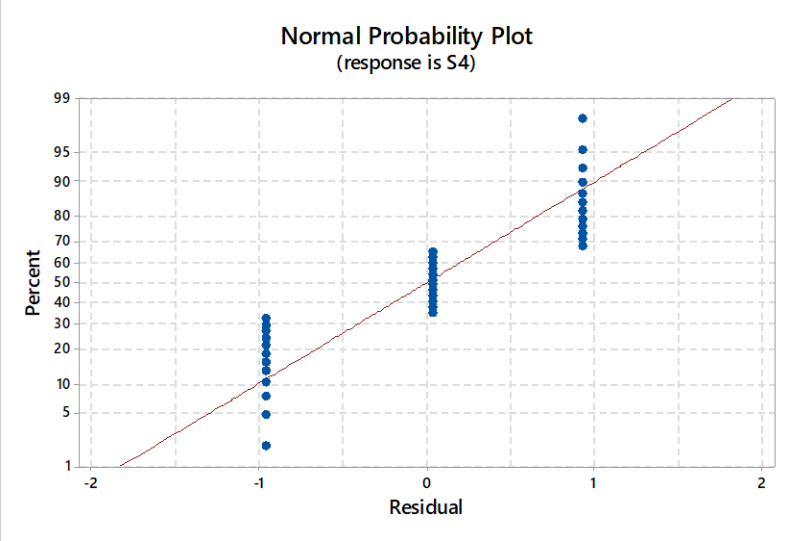
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Apr-19	3	18.325		H
Mar-19	3	17.735		H
Feb-19	3	4.915		I

Means that do not share a letter are significantly different.

Normplot of Residuals for S4



One-way ANOVA Post Hoc: S5 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	55447.0	5040.64	5195.29	0.000
Error	24	23.3	0.97		
Total	35	55470.3			

Multivariate Analysis Summary

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S	R-sq	R-sq(adj)	R-sq(pred)
0.985004	99.96%	99.94%	99.91%

Means

TIME	N	Mean	Std Error	95% CI
May-18	3	132.053	2.440	(130.880, 133.227)
Jun-18	3	106.228	0.550	(105.055, 107.402)
Jul-18	3	99.523	2.500	(98.350, 100.697)
Aug-18	3	106.878	0.700	(105.705, 108.052)
Sep-18	3	82.548	0.600	(81.375, 83.722)
Oct-18	3	55.828	0.440	(54.655, 57.002)
Nov-18	3	69.183	0.550	(68.010, 70.357)
Dec-18	3	18.693	0.500	(17.520, 19.867)
Jan-19	3	23.548	0.700	(22.375, 24.722)
Feb-19	3	23.243	0.600	(22.070, 24.417)
Mar-19	3	18.653	0.230	(17.480, 19.827)
Apr-19	3	30.483	0.100	(29.310, 31.657)

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Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

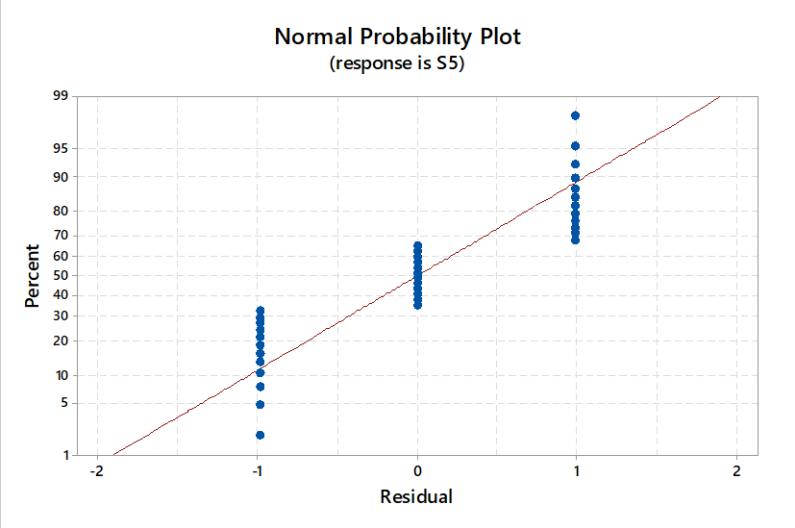
TIME	N	Mean	Grouping
May-18	3	132.053	A
Aug-18	3	106.878	B
Jun-18	3	106.228	B
Jul-18	3	99.523	C
Sep-18	3	82.548	D
Nov-18	3	69.183	E
Oct-18	3	55.828	F
Apr-19	3	30.483	G
Jan-19	3	23.548	H
Feb-19	3	23.243	H
Dec-18	3	18.693	I
Mar-19	3	18.653	I

Means that do not share a letter are significantly different.

Normplot of Residuals for S5

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APPENDIX (I)

Biovolumes of *Microcystis* spp

One-way ANOVA Post Hoc: S1 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	3.11463E+16	2.83148E+15	2.74098E+11	0.000
Error	24	247925	10330		
Total	35	3.11463E+16			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
101.638	100.00%	100.00%	100.00%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	143519395	1003,509	(143519274, 143519516)
Sep-17	3	145503534	209,9961	(145503413, 145503655)
Oct-17	3	141535256	200,9855	(141535135, 141535377)
Nov-17	3	136905598	31	(136905477, 136905719)
Dec-17	3	103175233	436,2476	(103175112, 103175354)
Jan-18	3	114418688	464,6245	(114418567, 114418809)
Feb-18	3	164683545	263,457	(164683424, 164683666)
Mar-18	3	99868335	437,572	(99868214, 99868456)
Apr-18	3	72090387	457,9876	(72090266, 72090508)
May-18	3	99868335	578,4	(99868214, 99868456)
Jun-18	3	70106248	547,3486	(70106127, 70106369)

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Jul-18	3	91931778	567,8365	(91931657, 91931899)

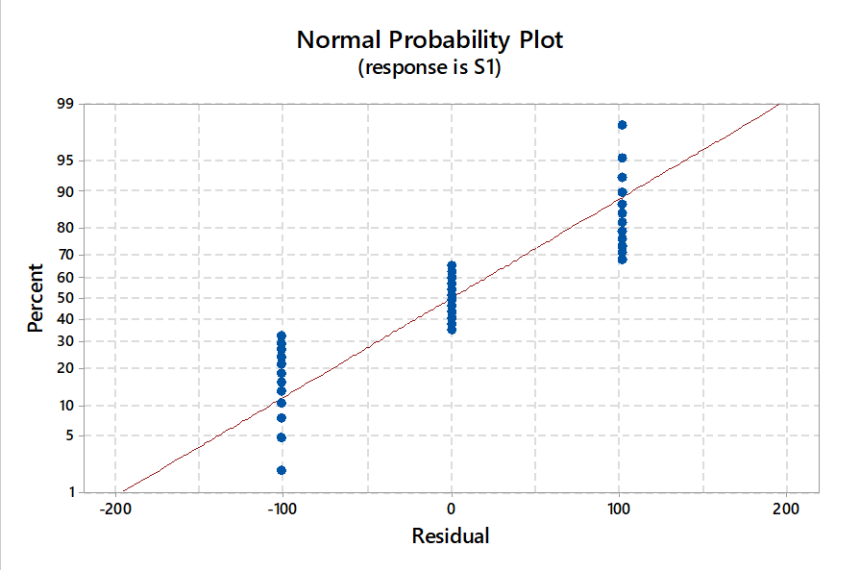
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping
Feb-18	3	164683545	A
Sep-17	3	145503534	B
Aug-17	3	143519395	C
Oct-17	3	141535256	D
Nov-17	3	136905598	E
Jan-18	3	114418688	F
Dec-17	3	103175233	G
May-18	3	99868335	H
Mar-18	3	99868335	H
Jul-18	3	91931778	I
Apr-18	3	72090387	J
Jun-18	3	70106248	K

Means that do not share a letter are significantly different.

Normplot of Residuals for S1



One-way ANOVA Post Hoc: S2 versus TIME

Method

Null hypothesis	All means are equal

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Alternative hypothesis Not all means are equal

Significance level $\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	1.76010E+16	1.60009E+15	1.11581E+11	0.000
Error	24	344166	14340		
Total	35	1.76010E+16			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
119.751	100.00%	100.00%	100.00%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	142196635	1003,509	(142196492, 142196778)
Sep-17	3	143519394	209,9961	(143519252, 143519537)
Oct-17	3	114418688	200,9855	(114418545, 114418830)
Nov-17	3	132937319	302	(132937177, 132937462)
Dec-17	3	89947639	436,2476	(89947496, 89947781)
Jan-18	3	81349703	464,6245	(81349560, 81349845)
Feb-18	3	132275939	263,457	(132275797, 132276082)
Mar-18	3	130953180	1003,509	(130953037, 130953323)
Apr-18	3	80688323	1003,50	(80688180, 80688466)
May-18	3	125000763	209,9961	(125000620, 125000905)
Jun-18	3	97884195	200,9855	(97884052, 97884338)
Jul-18	3	104497992	100,09	(104497849, 104498135)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping
Sep-17	3	143519394	A
Aug-17	3	142196635	B

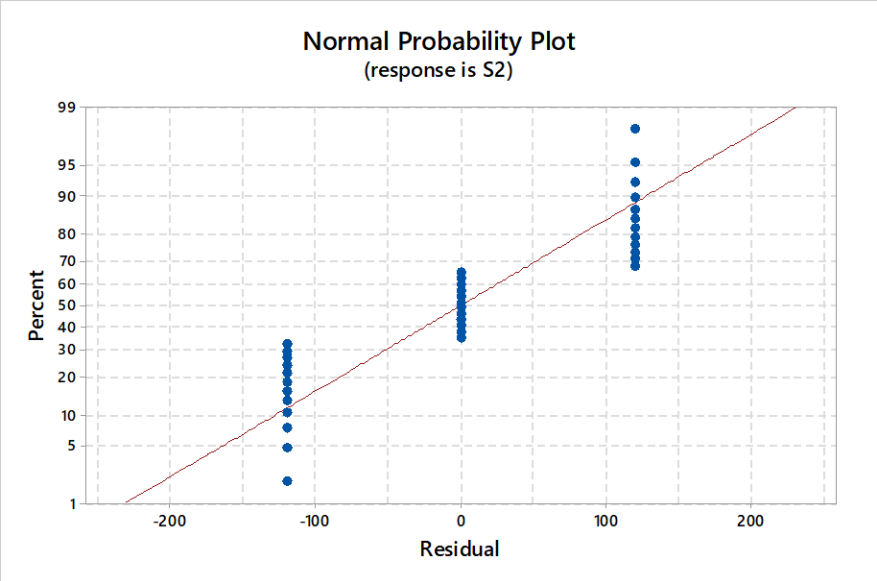
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Nov-17	3	132937319	C
Feb-18	3	132275939	D
Mar-18	3	130953180	E
May-18	3	125000763	F
Oct-17	3	114418688	G
Jul-18	3	104497992	H
Jun-18	3	97884195	I
Dec-17	3	89947639	J
Jan-18	3	81349703	K
Apr-18	3	80688323	L

Means that do not share a letter are significantly different.

Normplot of Residuals for S2



One-way ANOVA Post Hoc: S3 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	Aug-17, Sep-17, Oct-17, Nov-17, Dec-17, Jan-18, Feb-18, Mar-18, Apr-18, May-18, Jun-18, Jul-18

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Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	2.08200E+16	1.89273E+15	1.90796E+11	0.000
Error	24	238084	9920		
Total	35	2.08200E+16			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
99.6000	100.00%	100.00%	100.00%

Means

TIME	N	Mean	Std Error	95% CI
Aug-17	3	87302120	150.35	(87302002, 87302239)
Sep-17	3	132937320	740.61	(132937201, 132937438)
Oct-17	3	121032485	210.55	(121032366, 121032604)
Nov-17	3	163360786	210.5	(163360667, 163360905)
Dec-17	3	132937320	90.33	(132937201, 132937438)
Jan-18	3	97884196	120.44	(97884077, 97884314)
Feb-18	3	119048346	310.55	(119048227, 119048465)
Mar-18	3	93915917	460.5	(93915799, 93916036)
Apr-18	3	80688323	670.7	(80688205, 80688442)
May-18	3	130291801	650.6	(130291682, 130291920)
Jun-18	3	93915917	130.23	(93915799, 93916036)
Jul-18	3	89286260	90.1	(89286141, 89286378)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TIME	N	Mean	Grouping
Nov-17	3	163360786	A
Dec-17	3	132937320	B
Sep-17	3	132937320	B
May-18	3	130291801	C
Oct-17	3	121032485	D
Feb-18	3	119048346	E
Jan-18	3	97884196	F
Jun-18	3	93915917	G

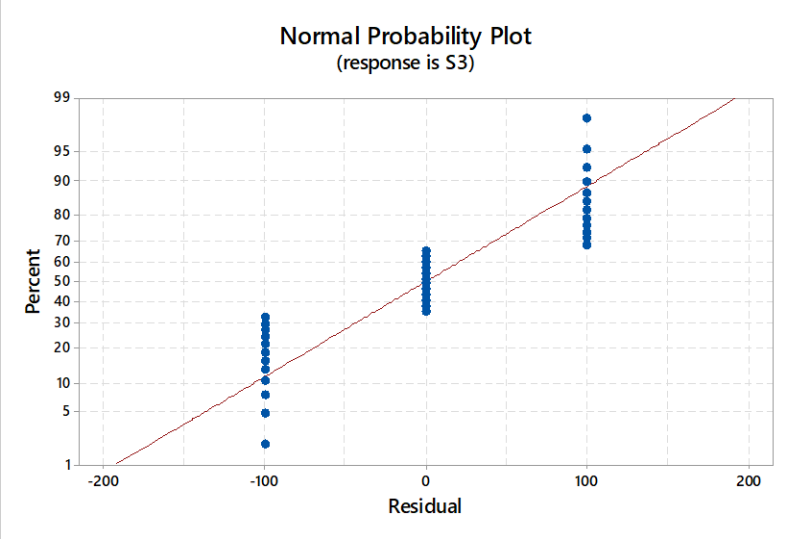
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Mar-18	3	93915917		G		
Jul-18	3	89286260			H	
Aug-17	3	87302120				I
Apr-18	3	80688323				J

Means that do not share a letter are significantly different.

Normplot of Residuals for S3



One-way ANOVA Post Hoc: S4 versus TIME
Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	5.61594E+15	5.10540E+14	*	*
Error	24	0	0		
Total	35	5.61594E+15			

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Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0	100.00%	100.00%	100.00%

Means

TIME	N	Mean	Std Error	95% CI
May-18	3	6338544	35	(6338544, 6338544)
Jun-18	3	40684342	450.61	(40684342, 40684342)
Jul-18	3	22205891	230.55	(22205891, 22205891)
Aug-18	3	21702166	520.5	(21702166, 21702166)
Sep-18	3	50246601	820.33	(50246601, 50246601)
Oct-18	3	6128658	40.44	(6128658, 6128658)
Nov-18	3	22457754	510.55	(22457754, 22457754)
Dec-18	3	8689262	440.5	(8689262, 8689262)
Jan-19	3	26025808	580.7	(26025808, 26025808)
Feb-19	3	16538981	220.6	(16538981, 16538981)
Mar-19	3	24094861	23.23	(24094861, 24094861)
Apr-19	3	21366349	650.1	(21366349, 21366349)

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Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

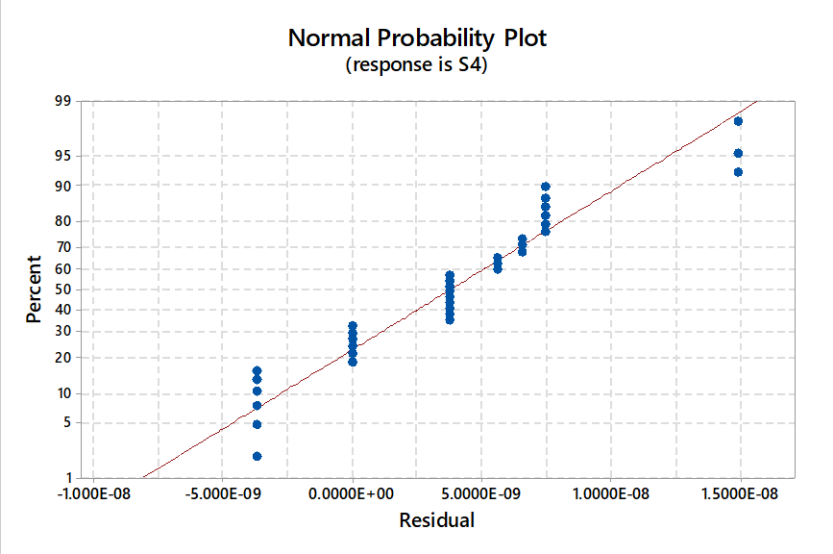
TIME	N	Mean	Grouping
Sep-18	3	50246601	A
Jun-18	3	40684342	B
Jan-19	3	26025808	C
Mar-19	3	24094861	D
Nov-18	3	22457754	E
Jul-18	3	22205891	F
Aug-18	3	21702166	G
Apr-19	3	21366349	H
Feb-19	3	16538981	I
Dec-18	3	8689262	J
May-18	3	6338544	K
Oct-18	3	6128658	L

Means that do not share a letter are significantly different.

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Normplot of Residuals for S4



One-way ANOVA Post Hoc: S5 versus TIME

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TIME	12	May-18, Jun-18, Jul-18, Aug-18, Sep-18, Oct-18, Nov-18, Dec-18, Jan-19, Feb-19, Mar-19, Apr-19

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TIME	11	3.78501E+15	3.44092E+14	*	*
Error	24	0	0		
Total	35	3.78501E+15			

Multivariate Analysis Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0	100.00%	100.00%	100.00%

Means

TIME	N	Mean	Std Error	95% CI

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May-18	3	14733966	563	(14733966, 14733966)
Jun-18	3	22814559	340.61	(22814559, 22814559)
Jul-18	3	26487556	870.55	(26487556, 26487556)
Aug-18	3	41809202	340.5	(41809202, 41809202)
Sep-18	3	42396881	780.33	(42396881, 42396881)
Oct-18	3	35302750	240.44	(35302750, 35302750)
Nov-18	3	37653468	170.55	(37653468, 37653468)
Dec-18	3	9780667	730.5	(9780667, 9780667)
Jan-19	3	26739419	980.7	(26739419, 26739419)
Feb-19	3	15153737	60.6	(15153737, 15153737)
Mar-19	3	24136838	890.23	(24136838, 24136838)
Apr-19	3	30223519	180.1	(30223519, 30223519)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

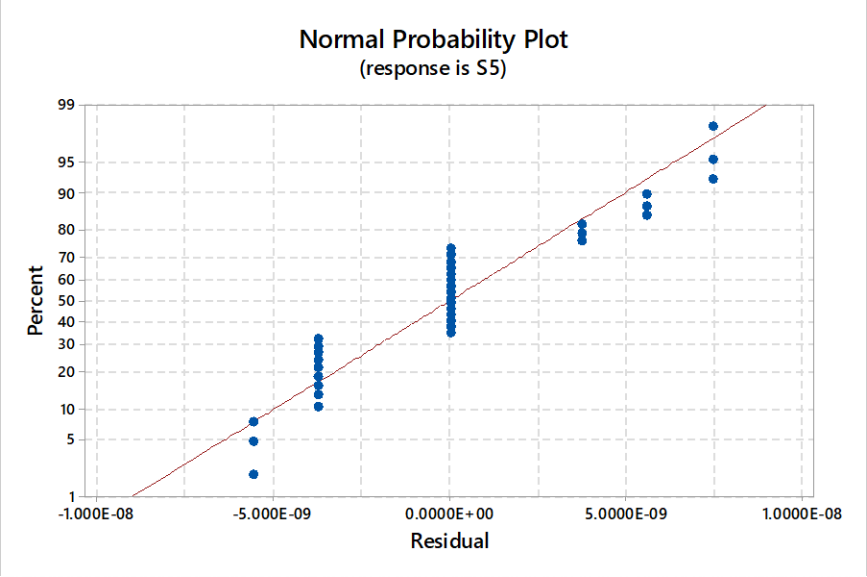
TIME	N	Mean	Grouping
Sep-18	3	42396881	A
Aug-18	3	41809202	B
Nov-18	3	37653468	C
Oct-18	3	35302750	D
Apr-19	3	30223519	E
Jan-19	3	26739419	F
Jul-18	3	26487556	G
Mar-19	3	24136838	H
Jun-18	3	22814559	I
Feb-19	3	15153737	J
May-18	3	14733966	K
Dec-18	3	9780667	L

Means that do not share a letter are significantly different.

Normplot of Residuals for S5

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APPENDIX(J)

Published Articles

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INFLUENCE OF SEASONAL RAINFALL TO THE WATER QUALITY OF SLIM RIVER LAKE IN PERAK, MALAYSIA

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Abstract

Rapid pace of development surrounding many lake catchments substantially affects the waterbody quality, thus causing public health concerns, threats to endangered aquatic species, aesthetic issues and algal blooms. This study assessed physicochemical and biological profiles of water quality in Slim River Lake (Perak, Malaysia), and relate with rainfall. On-site and laboratory analyses performed include turbidity, dissolved oxygen (DO), total nitrogen, total phosphorus, water temperature, chlorophyll-*a* and cyanobacteria biovolume. The effects of rainfall on the lake's water quality were analysed based on the Pearson correlation analysis. Results showed a significant correlation between rainfall and water temperature ($r = 0.480$ $p < 0.01$), total phosphorus ($r = 0.478$, $p < 0.01$) and DO ($r = -0.406$, $p < 0.05$). In addition, the results also showed a positive correlation between the rainfall and biovolume of *Phormidium* spp. test ($r = 0.372$, $p < 0.05$). This study provides significant contribution on the impact of seasonal rainfall to the variability of physical, chemical and biological profiles of the lake ecosystem, and reflected its health status and pollution.

Key words: Eutrophication, Lakes, Water quality, Slim River Lake, Rainfall

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Introduction

Lake has performed a variety of functions including water source, drainage, food supply, flood control, hydroelectricity generation, transportation, recreational and eco-tourism (Kertesz *et al.*, 2019). Due to the detrimental impacts on environmental, economic and health aspects, lake degradation has been a major concern recently and was addressed as serious issues both locally and globally.

Lakes receive a various source of pollutants, primarily from changes in land use, domestic activities and farming (Kertesz *et al.*, 2019). Even from visual assessment alone, it can be deduced that there is something wrong with the current water quality status of the lake. However, from the scientific standpoint, determination of lake water properties is needed to identify its trophic level and water quality classification (Zaki, 2010). Tremendous efforts have been made to investigate the factors that affecting lake's water quality. These efforts were taken to prevent lake degradation from happening and to ensure adequate management and remediation efforts are made to restore its stability (Zaki *et al.*, 2014; Vogt *et al.*, 2018). Nevertheless, complex and inter-connected impacts of natural factors such as local weather and rainfall, industrialisation and urbanisation have become the key challenges and obstacles in maintaining the lake's water quality and ecosystems (Muzzammil-Shahabudin & Musa, 2018).

Eutrophication is one of the major concerns of lake pollution. Eutrophication is a natural process by which organic productivity increases within a water body to the point where the ground surface has built up, DO is reduced and microorganism concentrations of the water are altered (Ansari *et al.*, 2011). This phenomenon can be classified into two categories; natural and cultural. The process of natural eutrophication happens gradually and very slowly in geological time, but it can be dramatically accelerated by anthropogenic actions

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Evaluation of Eutrophication Indicators in Slim River Lake, Perak – Malaysia

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ARTICLE DETAILS

Article History
Published Online: 14 November 2020

Keywords
Eutrophication, NLWQS, Slim River Lake, Cyanobacteria, Nutrients, Local Weather

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ABSTRACT

This study investigated the trophic state of Slim River Lake, and evaluated its water quality according to National Lake Water Quality Criteria and Standards. (NLWQS) Water samples were collected twice per month from August 2017 to July 2018 in three sampling stations around the Slim River Lake. The tested water quality parameters include water temperature, total nitrogen, total phosphorus, chl-a, chemical oxygen demand and cyanobacterial cells number. The rainfall and air temperature data from August 2017 to July 2018 were obtained from the Malaysian Meteorological Department and the mean values were recorded as 252.93 ± 36.84 mm and26.96 ± 0.46 °C, respectively. The mean and standard error of water temperature, total nitrogen, total phosphorus, chemical oxygen demand, chl-a, cells number of cyanobacterial genera were29.52 ± 0.30°C, 5.32± 0.95 mg/L, 0.82± 0.12 mg/L, 31.89 ± 3.93 mg/L, 7.26±1.96 mg/L, 18.3 × 10⁴ cells /mL, respectively. High levels of total nitrogen and total phosphorus were quantified during high rainfall period, thus imply the significant load from the storm water runoff. High cyanobacterial cell numbers were also detected, which indicated the occurrence of cyanobacterial bloom. The comparison of water quality parameters with NLWQS suggests a hypereutrophic condition in the study lake. In conclusion, the state of eutrophication in Slim River Lake poses potential health risks, and future changes in climatic factors will enhance this risk. Therefore, more attention and control by local authorities are needed to reduce the severity of eutrophication progression in this lake.

1. Introduction

Eutrophication is the process of increasing nutrients represented by nitrogen and phosphorus in water bodies such as lakes, often accompanied by algae bloom(1). Eutrophication is a natural process by which organic productivity increases within a water body can degrade water quality and changes the microorganism concentrations in water ecosystems(2). In eutropic lakes, phytoplankton is often dominated by cyanobacteria. Cyanobacterial bloom is a term used to describe a lake ecosystem condition where its phytoplankton community is mainly dominated by cyanobacteria(3). Cyanobacteria can produce toxins such as microcystins, poses potential health risks in water ecosystems(4). Local weather, hydrologic conditions, and land-use patterns are examples of factors that influence the nutrient loads and eutrophication in a water body (5). When the dissolved oxygen percentage in the water is less than 3mg /L, the condition is called hypoxia, and it is considered a significant problem in the lake (6).

In Malaysia, there are approximately 80 major lakes, of which 60 suffer from eutrophication. This was confirmed by a study conducted by the National Institute for Hydraulic Research in Malaysia(7).The assessment of lakes' eutrophication status was made based on NLWQS. This standard utilized water quality parameters including phosphorus, nitrogen, and chlorophyll-a. Phosphorus and nitrogen are the critical determinant of algae level, while chlorophyll is a good indicator for assessing and determining algae biomass(8).

This study was conducted to evaluate the state of the eutrophication problem in a local urban shallow lake using the NLWQS standard. This study also investigates the influence of local weather patterns on the water quality and succession of cyanobacteria in Slim River Lake.

2. Methodology

Lake Description and Water Sampling

Slim River Lake (3° 49' 35.688" N; 101° 24' 30.6216" E) is a human-made shallow lake located in the Muallim district of Perak state, Malaysia. Slim River Lake is an abandoned ore mine and currently used for recreation, fishing, and entertainment among local communities. Slim River Lake was chosen as a study site due to its high algae and floating macrophyte growth. Three locations around the lake were selected as sampling stations. The area of the Slim River Lake and sampling stations are illustrated in Figure 1. The first sampling station was located at the upstream of the lake. Meanwhile, the second station was on the western side of the lake, exactly beside the highway. The third sampling station was situated at the opposite side of sampling station 1, parallel to the Bernama River.

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