UNIVERSITI PUTRA MALAYSIA

PHYTOPLANKTON COMMUNITY STRUCTURE IN TURBID RIVERS AND EFFECT OF TURBIDITY ON THE GROWTH OF A MICROALGA, Chlorella vulgaris BEYERINCK [BEIJERINCK] 1890

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Phytoplankton is one of the primary producers of aquatic ecosystems and shapes the base of the food pyramid that supports the rest of the trophic levels such as zooplankton and fishes in aquatic food webs. Turbidity is one of the main factors that control phytoplankton growth since it is very effective in attenuating light levels below the water surface. Any perturbation that would disrupt the aquatic food chain would change the phytoplankton community structure. Escalations of turbidity in the water body would deteriorate water quality, which would, in turn, affect the phytoplankton distribution that role as the main source of a producer. This study aimed to assess the phytoplankton community structure in turbid rivers and coastal waters in Sarawak and to determine the effects of turbidity on *Chlorella vulgaris* growth under laboratory conditions. The study was carried out from August 2016 to June 2017 along the coastal waters in Sarawak and three major rivers; Batang Lupar and its associated coastal waters, Batang Saribas, Batang Lassa, and Mukah (coastal area). Phytoplankton samples were collected from 16 stations in three rivers and coastal waters in Sarawak and preserved using Lugol's iodine solution and were identified and enumerated under an inverted microscope. Physico-chemical parameters including water temperature, turbidity, dissolved oxygen, salinity, and pH were measured *in-situ* at all sampling stations by using HydroLab multiparameter. Phytoplankton Shannon-Weiner diversity index was calculated for all stations, and multivariate analyses were used to determine the important factors controlling the phytoplankton distribution using PRIMER software version 7. Phytoplankton species composition for all sampling stations comprised mainly of diatoms, dinoflagellates, and blue-green algae, whereas a few species of green algae were also found in the rivers. A total of 135 species of phytoplankton were recorded from all stations throughout the season, with diatoms dominating all stations, both in terms of number and species. Phytoplankton composition and distribution in the estuaries were more similar to marine phytoplankton due to the tidal influences. Mukah coastal area (Mu_Coast15 and Mu_Coast16) showed the highest total phytoplankton density ($50.22 \pm 3.30 \times 10^4$ cells L^{-1}) compared to the other stations.

For Batang Lupar, the estuarine station (Lu_Est1) showed the highest total phytoplankton density (4.81±0.20 x 10^4 cells L^{-1}) and the lowest total phytoplankton density was recorded at Lu_Mid5 (2.30±0.03 x 10^4 cells L^{-1}) where salinity was less than 10 ppt for every month except for August 2016. Batang Saribas waters were mostly saline with salinity >20 ppt even in the upstream. In this river, the highest total phytoplankton density was at the estuary (Sa_Est6) with (5.04±0.22 x 10^4 cells L^{-1}) and the lowest in the upstream Sa_Mid8 (2.08±0.05 x 104 cells L^{-1}). In Batang Lassa, the highest total phytoplankton density was at the mid-stream at La_Ups12 (5.32±0.22 x 10^4 cells L^{-1}) and the lowest at La_Ups13 (1.39±0.03 x 10^4 cells L^{-1}) where the salinity at both stations was low (< 2 ppt). The marine stations had the highest ($p < 0.05$) species density throughout the sampling period compared to the other stations in the estuaries and the rivers. Multidimensional scaling analysis based on the phytoplankton densities revealed two distinct groups. The first group consisted of the phytoplankton with high density in coastal waters area with moderate turbidity, whereas the other group consisted of the phytoplankton with low density in the rivers with high turbidity levels. A turbidity simulation study was conducted in the laboratory to understand the results obtained from the field sampling. A chlorophyte, *Chlorella vulgaris* was examined with five different turbidity concentrations in triplicates including 0 NTU as the control, 100 NTU, 200 NTU, 400 NTU, and 600 NTU. Microalgae pigment was measured using Shimadzu Prominence-i high-performance liquid chromatography (LC 2030). The highest number of cell densities (5.7 x 10^7 cell ml^{-1}) was produced in 0 NTU on day 21. The cell density of the culture increased over time in all concentrations, except for 200 NTU, 400 NTU, and 600 NTU where cell density decreased at the early stage of the experiment. Similarly, the highest specific growth rate (0.38±0.022 µ) was achieved in 0 NTU ($p < 0.05$) followed by 100 NTU, 200 NTU, 400 NTU and 600 NTU. The results of the present study illustrated that although the growth performance of *C. vulgaris* decreased in turbidity treatments, this species showed excellent self-adaptation capabilities to cope with stress condition and capable to produce of pigments including astaxanthin, beta-carotene and lutein.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

STRUKTUR KOMUNITI FITOPLANKTON DI SUNGAI YANG KERUH DAN KESAN KEKERUHAN TERHADAP PERTUMBUHAN MIKROALGA, *Chlorella vulgaris* BEYERINCK [BEIJERINCK] 1890

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Fitoplankton adalah salah satu pengeluar utama ekosistem akuatik dan membentuk dasar piramid makanan yang menyokong sisa tahap trofik seperti zooplankton dan ikan dalam jaring makanan akuatik. Kekeruhan adalah salah satu faktor utama yang mengawal pertumbuhan fitoplankton kerana sangat penting dalam mengurangkan tahap cahaya di bawah permukaan air. Segala gangguan yang mengganggu rangkaian makanan akuatik akan mengubah struktur komuniti fitoplankton. Peningkatan kekeruhan di dalam air akan mengakibatkan kemerosotan kualiti air, yang pada gilirannya akan mempengaruhi taburan fitoplankton yang berperanan sebagai sumber utama pengeluar. Kajian ini bertujuan untuk menilai struktur komuniti fitoplankton di sungai yang keruh dan pesisir pantai perairan di Sarawak dan untuk mengetahui kesan kekeruhan terhadap pertumbuhan fitoplankton yang diuji di dalam makmal. Kajian ini telah dijalankan dari bulan Ogos 2016 hingga Jun 2017 di sepanjang perairan pantai di Sarawak dan tiga sungai utama; Batang Lupar dan perairan pantai yang berkaitan dengannya, Batang Saribas, Batang Lassa, dan Mukah (kawasan pantai). Sampel fitoplankton dikumpulkan dari 16 stesen di tiga sungai dan pesisir pantai perairan Sarawak dan diawetkan menggunakan larutan Lugol iodin, kemudian dikenal pasti dan dihitung di bawah mikroskop terbalik. Parameter fisiko-kimia termasuk suhu air, kekeruhan, oksigen terlarut, kemasinan, dan pH diukur secara *in-situ* di semua stesen persampelan dengan menggunakan multiparameter HydroLab. Indeks kepelbagaian fitoplankton Shannon-Weiner dihitung untuk semua stesen, dan analisis kepelbagaian digunakan untuk menentukan faktor-faktor penting yang mengawal taburan fitoplankton menggunakan perisian PRIMER versi 7. Komposisi spesies fitoplankton untuk semua stesen persampelan terdiri terutamanya dari diatom, dinoflagellate, dan alga biru-hijau, sedangkan beberapa spesies alga hijau juga ditemukan di sungai. Sebanyak 135 spesies fitoplankton direkodkan dari semua stesen sepanjang musim, dengan diatom mendominasi semua stesen, baik dari segi jumlah dan spesies. Komposisi dan penyebaran fitoplankton di muara lebih serupa dengan fitoplankton laut kerana pengaruh pasang surut. Kawasan pesisir Mukah (Mu_Coast15 dan

Mu_Coast16) menunjukkan jumlah ketumpatan fitoplankton tertinggi ($50.22 \pm 3.30 \times 10^4$ sel L^{-1}) berbanding dengan stesen lain. Bagi Batang Lupar, stesen muara (Lu_Est1) menunjukkan ketumpatan fitoplankton tertinggi ($4.81 \pm 0.20 \times 10^4$ sel L^{-1}) dan jumlah ketumpatan fitoplankton terendah dicatat pada Lu_Mid5 ($2.30 \pm 0.03 \times 10^4$ sel L^{-1}) di mana kemasinan kurang daripada 10 ppt untuk setiap bulan kecuali Ogos 2016. Perairan Batang Saribas kebanyakannya masin dengan kemasinan > 20 ppt termasuk di hulu sungai. Di sungai ini, jumlah ketumpatan fitoplankton tertinggi adalah di muara (Sa_Es6) dengan ($5.04 \pm 0.22 \times 10^4$ sel L^{-1}) dan terendah di hulu Sa_Est8 ($2.08 \pm 0.05 \times 10^4$ sel L^{-1}). Di Batang Lassa, jumlah ketumpatan fitoplankton tertinggi berada di aliran tengah di La_Ups12 ($5.32 \pm 0.22 \times 10^4$ sel L^{-1}) dan terendah di La_Ups13 ($1.39 \pm 0.03 \times 10^4$ sel L^{-1}) di mana kemasinan di kedua-dua stesen rendah (<2 ppt). Stesen laut mempunyai kepadatan spesies tertinggi ($p<0.05$) sepanjang tempoh pengambilan sampel berbanding stesen lain di muara dan sungai. Analisis penskalaan multidimensi berdasarkan kepadatan fitoplankton menunjukkan dua kumpulan yang berbeza. Kumpulan pertama terdiri dari fitoplankton dengan kepadatan tinggi di kawasan pesisir pantai perairan dengan kekeruhan sederhana, sedangkan kumpulan yang lain terdiri dari fitoplankton dengan kepadatan rendah di sungai-sungai dengan kadar kekeruhan yang tinggi. Kajian simulasi kekeruhan dilakukan di makmal untuk memahami hasil yang diperoleh dari persampelan lapangan. Klorofil *Chlorella vulgaris* diperiksa dengan lima kepekatan kekeruhan yang berbeza dalam tiga replika termasuk 0 NTU sebagai kawalan, 100 NTU, 200 NTU, 400 NTU, dan 600 NTU. Pigmen mikroalga diukur menggunakan kromatografi cecair berprestasi tinggi Shimadzu Prominence-i (LC 2030). Ketumpatan sel tertinggi (5.7×10^7 sel ml^{-1}) dihasilkan dalam 0 NTU pada hari ke 21. Ketumpatan sel kultur meningkat dari masa ke masa dalam semua kepekatan, kecuali 200 NTU, 400 NTU, dan 600 NTU di mana sel ketumpatan menurun pada peringkat awal eksperimen. Begitu juga, kadar pertumbuhan spesifik tertinggi (0.38 ± 0.022 µ) dicapai dalam 0 NTU ($p <0.05$) diikuti oleh 100 NTU, 200 NTU, 400 NTU dan 600 NTU. Keputusan kajian ini menggambarkan bahawa walaupun prestasi pertumbuhan *C. vulgaris* menurun dalam sampel kepekatan kekeruhan, spesies ini menunjukkan keupayaan penyesuaian diri yang sangat baik untuk menghadapi keadaan tekanan dan mampu menghasilkan pigmen termasuk astaxanthin, beta-karotena dan lutein.

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%	Percentage
ºC	Degree Celsius
µg	Microgram
mg	Milligram
µs	Micro Siemens
Chl a	Chlorophyll *a*
Cm	Centimetre
DO	Dissolved Oxygen
g	Gram
H’	Diversity Index
J’	Evenness Index
Ind	Individual
L	Litre
ml	Millilitre
m^3	Cubic meter
OD	Optical Density
NTU	Nephelometric Turbidity Unit

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Water resources and aquatic foods undergo pressure when the global population increases as climate changes have modified the natural supplies (Trenberth et al., 2003). It is very important to manage and sustain aquatic resources in terms of quantity and quality. The presence of suspended particulate matter (SPM), from very small size particles to colloids sand-sized sediments become one of the reasons for the deteriorating of water quality worldwide (Billota et. al., 2012; Gray, 2008; Restrepo et al., 2018; Richter et al., 2005). A large amount of SPM water leads to suspended sediments that have significant destructive impacts. For example, severe biological degradation, a decrease of fisheries resources, aesthetic issues, and higher costs of water quality management (Bilotta and Brazier, 2008; Grove et al., 2015; Owens et al., 2005; Wood and Armitage, 1997).

An increase in sedimentation and siltation in the aquatic ecosystems results in harmful impacts on fish and other aquatic life (Taylor and Owens, 2009; Zauwiyah and Alias, 1997). Reduced light penetration with high concentrations of suspended sediments also gives unfavourable effects to primary production (Mitchell et al., 2017; Obrador and Pretus, 2008; Pedersen et al., 2012). Turbidity in water increases when concentrations of organic matter, suspended solids, effluents and surface run-off increase (Yisa and Jimoh 2010).

Turbidity can be defined as a measurement of suspended matters to the degree where the water loses its transparency. Turbidity also can be expressed from the amount of scattering light by materials in the water bodies. Turbidity inhibits the process of photosynthesis by blocking sunlight to penetrate, making the light less available to phytoplankton (Dejen et al., 2004; Domingues et al., 2014; Hart, 1992; Jones et al., 2015). In aquatic food chains where fish are the main predator, phytoplankton essentially plays an important part (Saifullah et al., 2016). Phytoplankton is the vital organism that acts as a primary producer in aquatic ecosystems by forming the base that supports zooplankton and fish of aquatic food webs.

Energy is transferred by primary biological components to higher organisms through the food chain (Ananthan et al., 2004; Tiwara and Chauhan 2006). Phytoplankton also plays many ecological roles in aquatic ecosystems and affects human affairs in numerous ways (Rochelle-Newall et al., 2011). In addition, the almost universal presence of phytoplankton in all water bodies and the most extreme environment indicates their important role in the ecological complex (Graham and Wilcox, 2000). The diversity of organisms at higher trophic levels are regulated by biomass and production of phytoplankton, also dependent on time and seasonal disparities (Saifullah et al., 2016). It may be difficult or even impossible to restore to original condition after deterioration has occurred on the ecosystem (Duarte et al., 2009; Landis et al., 2000; Lovett et al., 2007; Scheffer et al., 2001).

1.2 Problem Statement

Plenty supplies of water resources from the previous time have driven to negligible alarm about their conservation and feasible misuse (Yusoff et al., 2006). Negative consequences due to many factors such as anthropogenic activities and environmental changes include negative alterations in aquatic ecosystems and a decrease in biodiversity (Seifert et al., 2015; Stocker et al., 2014). Sarawak rivers have been facing an increase of environmental stress and severe water pollution due to rapid population growth, land development along the river basin, urbanization and industrialization. All these factors led to more serious problems and eventually affected the organisms that live inside the water body.

The knowledge of phytoplankton distribution concerning spatial and temporal patterns is important in determining the status and functioning of the ecosystem community structure (Effendi et al., 2016). Most studies explored Sarawak rivers focused only on certain rivers and touched physicochemical factors of water quality without considering other micro aquatic organisms that are also affected by the changes (Saifullah et al., 2014; Saifullah et al., 2016). The study of phytoplankton community responses to these environmental variables is considered beneficial to understanding biological events and predicting the future trend (Troccoli et al., 2004). The combination study of physicochemical parameters and biological aspects would provide more profound data to understand conditions in Sarawak water ecosystems that already effected the food chain as well as spawning ground and nursery ground of the aquatic organisms.

1.3 Objectives

Thus, the objectives of this study were:

1. To analyze the changes of phytoplankton community structures in Sarawak turbid rivers and coastal waters associated with environmental variables.

2. To determine the effects of turbidity on *Chlorella vulgaris* growth performance under laboratory conditions.

1.4 Hypothesis

The hypotheses of the study were:

1. Null hypothesis (H_o): Increase in turbidity level of Sarawak rivers and coastal waters do not cause changes on phytoplankton community structures.

2. Alternative hypothesis (H_A): Increase in turbidity level of Sarawak rivers and coastal waters do cause changes on phytoplankton community structures.

3. Null hypothesis (H_o): Increase in turbidity concentrations does not affect the growth performance of *Chlorella vulgaris*

4. Alternative hypothesis (H_A): Increase in turbidity concentrations does affect the growth performance of *Chlorella vulgaris*

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