



UNIVERSITI PUTRA MALAYSIA

***DIVERSITY OF PHYTOPLANKTON
ASSOCIATED WITH WATER QUALITY IN
SELECTED AREA IN BINTULU, SARAWAK***

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**DIVERSITY OF PHYTOPLANKTON ASSOCIATED WITH WATER QUALITY IN
SELECTED AREA IN BINTULU, SARAWAK**

By

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**A Project Report Submitted in Partial Fulfillment of the Requirements for the Degree
of Bachelor of Science (Honors) Aquaculture in the
Faculty of Agricultural Science and Forestry
Universiti Putra Malaysia Bintulu Sarawak Campus**

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DECLARATION

I declare that this project report DIVERSITY OF PHYTOPLANKTON ASSOCIATED WITH WATER QUALITY IN SELECTED AREA IN BINTULU, SARAWAK is fully based on my own under the supervision of Ts. Dr. Suhaili Mustafa and co-supervised Dr. Tan Toh Hii. This report has not been submitted for another degree requirement of this or any others higher institution.

I declare the statements that I have made and the conclusion of this report is based on my research outcome. I also certify that;

- I. The content of the report is originally done by me under the supervision of my supervisor.
- II. The work has not been submitted to any others institution or university in Malaysia or abroad.
- III. I have followed the guideline given by the university in writing the report.
- IV. All the material, data, text and theoretical analysis from the other author or sources, I have given the credit to them in the text of the report and giving the details on my references.

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ABSTRACT

Bintulu is one of the largest industrial area where it located in the middle parts of Sarawak with a total area of 7,220.40 square kilometers including 2 others district which is Sebauh and Tatau. Unfortunately, there are lack of information regarding the phytoplankton species especially on freshwater environments has been measured in the current study area. The early hypothesis stated that the high diversity of phytoplankton can be used as an indicator for water quality conditions. *In-situ* and *ex-situ* method which include 12 water quality parameters has been measured, which is consisting of temperature, dissolve oxygen, salinity, turbidity, pH, total dissolved solids, conductivity, total suspended solids, nitrite, nitrate, ammonia, and phosphate. Physico-chemical of water not showing a significant result. While, correlation coefficient shows both positive and negative result on several phytoplankton species and physico-chemical of water. Physico-chemical of water is affecting the phytoplankton availability in each sampling station, as for the phytoplankton species recorded in each sampling station is different.

ABSTRAK

Bintulu merupakan salah satu bandar yang mempunyai Kawasan industri yang terbesar, dimana ia nya mempunyai keluasan kira-kira 7,220.40km persegi terletak ditengah-tengah negeri Sarawak. Bandar Bintulu merangkumi dua daerah, iaitu daerah Sebauh dan Tatau. Namun begitu, infomasi tentang spesies fitoplankton, terutamanya yang terdapat di persekitaran air tawar masih kurang di Kawasan penyelidikan. Hipotesis awal menyatakan bahawa kadar fitoplankton yang tinggi mampu menjadi reflektor kepada tahap kualiti air. Kaedah analisis *in-situ* dan *ex-situ* telah digunapakai dalam melaksanakan kajian ini, yang merangkumi suhu, oksigen terlarut, saliniti, turbiditi, pH, kadar pepejal telarut, konduktiviti air, kadar pepejal tersejat, nitrit, nitrat, ammonia dan fosfat, dimana kesemuanya tidak menunjukkan nilai signifikan. Sementara itu, analisis korelasi menunjukkan nilai positif dan negatif pada sesetengah spesies yang dijumpai dan fisiko-kimia air. Fisiko-kimia air mempengaruhi keberadaan fitoplankton di setiap lokasi persampelan, dimana spesies fitoplankton yang dijumpai di setiap lokasi persampelan adalah berlainan.

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APPROVAL SHEET

I certify that this research project report entitled **Diversity of Phytoplankton Associated with Water Quality in Selected Area in Bintulu, Sarawak** has been examined and approved as the partial fulfillment of the requirement for the degree of Bachelor of Science (Honors) Aquaculture in the Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus.

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LIST OF ABBREVIATIONS

pH	Potential of Hydrogen
TSS	Total Suspended Solid
TDS	Total Dissolved Solids
DO	Dissolved Solid
NH ₃ N	Ammonia Nitrogen
NO ₂ ⁻	Nitrite
NO ₃ ⁻	Nitrate
°C	Degree Celsius
µS/cm	MicroSiemens Per Centimeter
NTU	Nephelometric Turbidity Units
mg/L	Milligrams Per Liter
ppt	Part Per Thousand
Temp.	Temperature
Cond.	Conductivity
GPS	Global Positioning System
HU	Hatchery Pond 1 UPMKB
KJ	Kampung Jepak
MJ	Martek Pangkalan Polis Marin Wilayah 5 Jetty
ML	Muhibbah Lake
UW	UPMKB Waterfall
BV	Boulevard Valve

CHAPTER I INTRODUCTION

1.1 Background of The Study

In Malaysia, Bintulu is one of the largest industrial area where it located in the middle parts of Sarawak with a total area of 7,220.40 square kilometers including 2 others district which is Sebauh and Tatau. Bintulu is also known as a coastal city and the nearest beach known as *Pantai Temasya Tanjung Batu* which is located about 10KM from the city itself. The current main activities in this area, including fisheries, food and beverages and recreational activities. Along the way to develop as a well-known industrial city, the environmental effects towards the presence of the giant industrial sector around Bintulu should be monitor, especially on the water quality. The terminology of the water quality itself is referred to the characteristic of waters which includes chemical, biological, and physical to determine the sustainability of the waters for it used and process (Meybeck et al., 2006, Hastuti, 2011).

The definition of water pollutions itself is referred to the excessive materials in the waters and bring harm to the humans, animals and aquatic life that also caused by the daily activities of several communities inside or near to the body of the water (Trivedi and Raj, 1992). It occurs when the water bodies contain excessive amounts of materials and bring adverse effects to the water. Chemical characteristic encompasses the analysis of water whether it is filtered or unfiltered, particles matter, tissues of biota and whole organism for organic components and inorganic components such as metals, radionuclides and elemental isotope. For physical characteristic, it is including the measurements that relate to the habitat, time and the variability of the space of aquatics life while biological characteristics is including all organisms that live in the water that have a selected environmental condition in which they grow (Meybeck et al., 2006). It is important to monitor water quality to get the latest data of the water bodies that will determine the health condition of the monitored area. Due to the essential needs for water in

our daily life, it proves that water is one of the main elements that play an important role in life on Earth.

The Biological indicators usually used to indicate the pollution in freshwater or even marine. For example, phytoplankton. It can be categorized as a plant of water and contribute to the primary production. Phytoplankton also plays an important role to build the base of food chain in the ecosystem. It is very sensitive to the environmental condition especially on physio-chemical and biological factors. From that, the sensitivity and the huge varieties in the species composition are usually used as a reflector towards the water condition in the ecosystem (Malik et al., 2012). Based on this study, it was hypothesis that the high diversity of phytoplankton can be used as an indicator for water quality conditions.

1.2 Problem Statement

In Sarawak, especially in Bintulu Sarawak there are lack of information regarding the phytoplankton species. Some of the papers about phytoplankton in Sarawak has been published by Saifullah et al. (2014), but this paper is more focusing on estuaries environment. This study aims to contribute a data on phytoplankton in Bintulu, Sarawak, due to the human population is higher in the coastal region, the coastline and estuaries experienced rapid development which at the same time affected the household discharged into the body of the water. This continuous economic rising and industrialization will bring negative impacts to the coastal area and estuaries due to increase in the concentration of the household discharged nutrients into the river caused by the increment in population percentage in the region (Kamaruzzaman and Zawawi, 2010).

1.3 Objective of The Study

1. To identify the diversity of phytoplankton in different types of water in Bintulu area.
2. To observe the effects of the water quality towards the diversity of phytoplankton.

CHAPTER 2 LITERATURE REVIEW

2.1 Characteristic of Phytoplankton

Half of the global primary production is contributed by the marine microalgae due to the ability to provide organic feed to most marine aquatic life and at the same time contribute to the worldwide carbon cycle (Lewitus et al., 1998). It can be found mostly on the top water due to the easy access to light for the photosynthesis process (Muhammad Adlan et al., 2012b). It is usually sensitive to organic, inorganic matter and the content of heavy metals. Phytoplankton is one of the fastest changes in nutrient contents compare to the others organisms and this is as an indicator of the water quality condition itself (Hötzel and Croome, 1999).

2.1.1 Phytoplankton Composition

Cynobacteria and Chlorophyceae is some of the phylogenetic classes in phytoplankton that have wide range of traits compare to the others species (Kruk et al., 2011). This both groups are show the broad variety of growth forms, wide range of environmental conditions and with specialized cells, it is varying from small single cell (unicell) to filaments. While, for Chrysophytes, it is more restricted to environmental distribution and variety of morphological variation(Sandgreen and phytoplankton, 1988).

2.1.2 Phytoplankton Diversity in Malaysia

In Malaysia, the common phylum was found are Bacillariophyta, Chlorophyta, Cyanophyta, and Dinophyta (Muhammad Adlan et al., 2012b). According to Shamsudin et al. (1999), the distributions and the abundance of the phytoplankton are affected by the physico-chemical factors. While another report by Boonyapiwat (1999) mentions that the highest diversity of phytoplankton is associated with the chlorophyll maximum layer. Due to little data on freshwater phytoplankton, this could be a sub-indicator for phytoplankton diversity in freshwater.

The main factor that affects the distribution of phytoplankton is the salinity of the water itself as in Langat and Perak estuary is dominated by the diatoms (Lassen et al., 2004). Other than salinity, phytoplankton diversity also depending on the weather, where in the current region is affected by the monsoon season and result in freshwater diatom is more dominant compared to marine diatom as in Perak estuary. According to Khan Chowdhury et al. (2011) reports, the dominant phytoplankton found in the Pahang estuary was *Leptocylindrus sp*, which is 19.05% and the least was *Gymnodinium sp*. 0.04%

2.1.2 Seasonality and Succession of Phytoplankton

According to Wei et al. (2020), during the summer and winter in 2017, the Juehe Wetland and Xiaoxihu Lake were dominated by Cyanobacteria and slowly evolved to Bacillariophyta. Wei et al. (2020) also reported that there was a positive correlation between Cyanobacteria biomass and the temperature, it is more resistant to high temperature and able to growth in range of 25°C to 30°C.

Another study was perform by Yamaguchi et al. (2000) reported that, when the temperature dropped from 18°C to 14°C, the dominant phylum was Bacillariophyta. However, when the temperature increase during summer, Bacillariophyta is still the dominant phylum recorded. This is a prove that Bacillariophyta can optimize the growth and being reproductive in high temperature (Beliveau and Hickman, 1982). Therefore, even though Bacillariophyta is more prefer to low temperature during winter, they also able to survive productively during summer.

2.3 Environmental Factor and Phytoplankton Distribution.

According to Goldman and Horn (1983), the growth of phytoplankton is complicated and affected by physical and chemical characteristics, for example, DO, temperature, light visibility, nutrient availability and the presence of nitrogen and phosphorus. Another research

by Ghosh et al. (2012) mention that the diversities of phytoplankton is depending on the availability of the supplement, temperature, light visibility, and other limnology elemental.

2.3.1 Vertical and Horizontal Distribution

According to De Queiroz et al. (2015), in tropical and subtropical ocean region, the structure of water is a vertical and horizontal. This structure will cause a small physical change between the 60 meters to 120 meters depths when the thermocline occurs. In this study also mention that, the richest water is found in the photic layer. Photic layer is the first layer in the ocean where the energy from heat is absorbed by the water itself and the light energy from the sunlight is absorbed by the inhibits phytoplankton.

2.4 National Water Quality Index (Malaysia)

Table 1: National Water Quality Index (Malaysia)

Parameter	Unit	Class					
		I	IIA	IIB	III	IV	V
Ammonia Nitrogen	mg/l	0.1	0.3	0.3	0.9	2.7	>2.7
Biochemical Oxygen Demand	mg/l	1	3	3	6	12	>12
Chemical Oxygen Demand	mg/l	10	25	25	50	100	>100
Dissolved Oxygen	mg/l	7	5-7	5-7	3-5	< 3	<1
pH	-	6.5-8.5	6-9	6-9	5-9	5-9	-
Color	TCU	15	150	150	-	-	-
Electrical Conductivity*	µS/cm	1000	1000	-	-	6000	-
Floatables	-	N	N	N	-	-	-
Odour	-	N	N	N	-	-	-
Salinity	%	0.5	1		-	2	-
Taste	-	N	N	N	-	-	-
Total Dissolved Solid	mg/l	500	1000		-	4000	
Total Suspended Solid	mg/l	25	50	50	150	300	300
Temperature	°C	-	Normal + 2°C	-	Normal + 2°C	-	-
Turbidity	NTU	5	50	50		-	-

Faecal Coliform**	count/100ml	10	100	400	5000 (20000) a	5001 (20000) a	-
Total Coliform	count/100ml	100	5000	5000	50000	50001	>5000

Notes:

N: No visible floatable materials or debris, no objectional odor or no objectional taste

*: Related parameters, only one recommended for use

** : Geometric mean

a: Maximum not to be exceeded

Table 2: Water Class and Uses

Class	Uses
Class I	Conservation of natural environment. Water Supply I – Practically no treatment necessary. Fishery I – Very sensitive aquatic species.
Class IIA	Water Supply II – Conventional treatment required. Fishery II – Sensitive aquatic species.
Class IIB	Recreational use with body contact.
Class III	Water Supply III – Extensive treatment required. Fishery III – Common, of economic value and tolerant species; livestock drinking.
Class IV	Irrigation
Class V	None of the above

This national water quality index (Malaysia) shows the 5 classes of water. Where Class I is identified as a clean water and class V is polluted water(Zainudin, 2010).

CHAPTER 3 Materials and Method

3.1 Sampling Sites

Six station were selected to perform this study which is, Kampung Jepak (KJ), Boulevard Valve (BV), Hatchery Pond 1 UPMKB (HU), UPMKB Waterfall (UW), Muhibbah Lake (ML), and Martek Pangkalan Polis Marin Wilayah 5 Jetty (MJ). The field work was carried out twice which is on 1st April 2021 (0800 a.m. -1200 p.m.) for Kampung Jepak, Boulevard Valve, Martek Pangkalan Polis Marin Wilayah 5 Jetty, and 6th April 2021 (0800 a.m. -1200 p.m.) for Hatchery Pond 1 UPMKB, UPMKB Waterfall and Muhibbah Lake. Each sample from the station were taken for three replicates.

Table 3: GPS reading of sampling station

Station	GPS
KJ	3°09'39.2"N 113°02'04.9"E
BV	3°11'59.8"N 113°02'40.4"E
HU	3°12'35.4"N 113°04'48.2"E
UW	3°12'28.3"N 113°04'30.0"E
ML	3°12'42.5"N 113°03'19.5"E
MJ	3°12'42.5"N 113°03'19.5"E

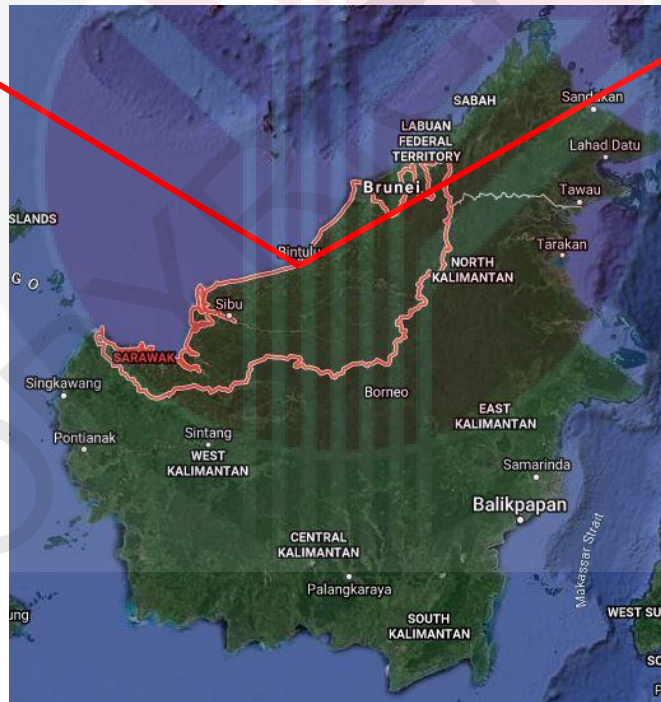


Figure 1: Map showing sampling station in Bintulu, Sarawak, Malaysia (Source: Google Earth)

3.2 Phytoplankton Collection Method

Plankton net with 20 μ m size was used to collect the water sample by towed it on the surface of the water on each station(Hötzel and Croome, 1999). The collected sample was preserved using acidic Lugol's solution before doing the identification process. The 500ml of water sample was divided into 5 bottles (100ml each bottle) and analyzed under the compound microscope (Brand: Leica, Country: Germany, Magnification: 10x).

3.3 Water Sample Collection

The water was collected using a water sampler and transferred into a sample bottle (1 Liter) for each station (3 replicates). During the water collection period, *in-situ* water quality data was taken using TOA-DKK WQ-24 water quality multiparameter which is consists of temperature, pH, salinity, dissolved oxygen (DO), conductivity, total dissolved solids (TDS), and water turbidity data. For *ex-situ* water quality data analysis, five analysis has been selected, which is ammonia, nitrite, nitrate, phosphate and total suspended solids (TSS)

3.4 Phosphorus, Reactive (Orthophosphate) Analysis

To observe the phosphorus, 10ml water sample from each station was used and added with PhosVer 3 Phosphate reagent powder pillow by using method 8048(Ray et al., 2010). Each station is consisting of 3 replications. The sample was shaken between 20 and 30 seconds and proceed with 20 minutes reaction time were taken. The phosphorus was measured by using a spectrophotometer (Brand: Hach, Model: DR2800, Country: United States).

3.5 Total Suspended Solids (TSS)

The total suspended solids was measured by using the APHA method(Association et al., 1912). In this method, one liter of water sample was used from each station (3 replicates). The water sample was filtered using chlorophyll filter paper together with an electric vacuum pump. The

residue in the filter paper was dried for 60 minutes within a 103°C to 105°C in an oven and placed into a desiccator for 20 minutes.

After 20 minutes, the weight of chlorophyll filter paper was taken using an electronic balancer (Sartorius BS 224 S) and the TSS is calculated using the specific formula;

$$TSS \left(\frac{mg}{l} \right) = \frac{\text{Final Weight (g)} - \text{Initial Weight (g)}}{\text{Sample Volume (ml)}}$$

3.6 Nitrite (NO₂⁻) and Nitrate (NO₃⁻)

For nitrate, 10ml of water sample from each station were mixed with NitraVer 5 Nitrate Reagent Powder Pillow (3 replicates) exclude the blank sample. The sample was shaken for 1 minute and followed by 5 minutes reaction times. The samples were measured by using a spectrophotometer (Brand: Hach, Model: DR2800, Country: United States). For nitrite analysis, the 10ml blank sample are mixed with NitriVer 3 Nitrite Reagent Powder Pillow. 20 minutes reaction time and measured using a spectrophotometer (Brand: Hach, Model: DR2800, Country: United States).

3.7 Ammonia

Salicylate method was used to perform this analysis. 30ml of water sample from each station and deionized water as a blank sample and mixed with Ammonia Salicylate Powder Pillow were prepared. The reaction time for this process is 3 minutes and immediately add the Ammonia Cyanurate Reagent Powder Pillow into each sample including the blank sample and shaken to ensure all the reagents were well mixed. This process took 15 minutes for the sample to start reacting and after the 15 minutes timer beep, all of the samples were measured using a spectrophotometer.

3.8 Phytoplankton Identification

Microalgae were identified until the lowest taxonomic rank possible. All the characteristics such as the colors, size, shape, and the presence of flagella are important in the process of

identification. 1ml of water sample was taken by using a dropper from the bottle sample and put onto the microscopic slides. The slides were observed under 10x magnification using compound microscope (Brand: Leica, Country: Germany, Magnification: 10x), and the picture were taken using mobile phone camera (Brand: Redmi Note 9s, Resolution: 4000x3000 pixels) if possible phytoplankton appears and identified using the phytoplankton and diatoms identification key by Tan et al. (2016), Pandey et al. (2017) and Samanta and Bhadury (2018). The ImageJ software was used to add the scale bar onto the micrograph.

3.9 Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) was used to perform the Pearson correlation coefficient of different physical-chemical parameters and phytoplankton and One Way Anova analysis for physico-chemical parameters. For the cluster analysis, PAST version 4.03 was used to divide the species into its group.

CHAPTER 4

Results

4.1 Water Quality Parameter

There are 12 water quality parameters has been measured using *in-situ* and *ex-situ* measurement, which is Temperature, Dissolve Oxygen, Salinity, Turbidity, pH, Total Dissolved solids, Conductivity, Total Suspended Solids, Nitrite, Nitrate, Ammonia, and Phosphate as shown on the Table 4. The DO value recorded at BV and ML are in the same classes which is class III, MJ and UW class IV, while for HU is in class V. For pH, sampling station KJ, BV and ML are categorized in the same classes, which is class I. While for both UW and HU is categorized in class V and MJ at class IIA. Salinity shows all the sampling station are in the same class I except for sampling station BV in class V. While for TDS and TSS shows that the all sampling station are categorized in class I.

4.1.2 Physico-Chemical Water Parameter by Location

The total of water quality parameter was recorded by six selected area in Bintulu, Sarawak. Mean value of physico-chemical parameters by location at selected sampling station in Bintulu as shown in Table 4. The temperature ranged from $26.35 \pm 0.04^{\circ}\text{C}$ to $31.76 \pm 0.14^{\circ}\text{C}$, dissolved oxygen ranged from 0.006 ± 0.01 mg/L to 3.41 ± 0.15 mg/L, salinity ranged from 0.00 ± 0.00 ppt to 21.91 ± 0.01 ppt, turbidity ranged from 0.003 ± 0.005 NTU to 77.80 ± 0.005 NTU, pH ranged from 5.58 ± 0.005 to 10.10 ± 0.15 , total dissolved solids ranged from 0.00 ± 0.00 mg/L to 34.13 ± 0.03 mg/L, conductivity ranged from 0.57 ± 0.02 ms/s to 4.91 ± 0.01 ms/s, total suspended solids ranged from 0.19 ± 0.005 mg/L to 0.59 ± 0.005 mg/L, nitrite ranged from 0.005 ± 0.000 mg/L NO_2^- N to 0.543 ± 0.011 mg/L NO_2^- N, nitrate ranged from 0.63 ± 0.11 NO_3^- N to 5.03 ± 0.77 NO_3^- N, ammonia ranged from 0.250 ± 0.00 mg/L to 2.833 ± 0.023 mg/L and phosphate ranged from 0.23 ± 0.01 mg/L to 1.12 ± 0.005 mg/L

Table 4: Mean value of physico-chemical parameters by location at selected sampling station in Bintulu

Water Parameter	Station					
	Mean \pm SD					
	MJ	KJ	BV	ML	UW	HU
Temp(°C)	27.16 \pm 0.20	26.48 \pm 0.01	30.05 \pm 0.05	30.33 \pm 0.40	26.35 \pm 0.04	31.76 \pm 0.14
DO (mg/L)	1.85 \pm 0.005	0.006 \pm 0.01	3.35 \pm 0.005	3.41 \pm 0.15	1.30 \pm 0.005	0.72 \pm 0.005
Salt (ppt)	0.00 \pm 0.00	0.00 \pm 0.00	21.91 \pm 0.01	0.00 \pm 0.00	0.20 \pm 0.005	0.00 \pm 0.00
Turb (NTU)	34.80 \pm 0.08	44.88 \pm 0.01	77.80 \pm 0.005	55.41 \pm 0.01	0.003 \pm 0.005	19.60 \pm 0.005
pH	6.01 \pm 0.01	6.72 \pm 0.01	7.34 \pm 0.005	6.48 \pm 0.005	10.10 \pm 0.15	5.58 \pm 0.005
TDS (mg/L)	0.09 \pm 0.005	0.00 \pm 0.00	34.13 \pm 0.03	0.00 \pm 0.00	0.50 \pm 0.005	0.00 \pm 0.00
Cond (ms/s)	3.05 \pm 0.01	3.33 \pm 0.40	4.91 \pm 0.01	4.20 \pm 0.005	4.30 \pm 0.09	0.57 \pm 0.02
TSS (mg/L)	0.20 \pm 0.005	0.20 \pm 0.00	0.19 \pm 0.005	0.40 \pm 0.01	0.40 \pm 0.00	0.59 \pm 0.005
Nitrite (mg/L NO ₂ ⁻ N)	0.543 \pm 0.011	0.020 \pm 0.000	0.313 \pm 0.519	0.015 \pm 0.00	0.005 \pm 0.000	0.028 \pm 0.004
Nitrate (mg/L NO ₃ ⁻ N)	5.03 \pm 0.77	1.23 \pm 0.11	4.66 \pm 0.55	2.16 \pm 0.57	0.63 \pm 0.11	1.73 \pm 0.11
Ammonia (mg/L)	0.380 \pm 0.52	0.283 \pm 0.023	0.686 \pm 0.085	1.792 \pm 0.002	0.250 \pm 0.00	1.721 \pm 0.001
Phosphate (mg/L)	0.32 \pm 0.01	0.46 \pm 0.005	1.12 \pm 0.005	0.47 \pm 0.005	0.23 \pm 0.01	0.23 \pm 0.01

MJ= Martek Pangkalan Polis Marin Wilayah 5 Jetty, **KJ**= Kampung Jepak, **BV**= Boulevard Valve, **ML**= Muhibbah Lake, **UW**= UPMKB Waterfall, **HU**= Hatchery Pond 1 UPMKB,

4.2 Phytoplankton Availability

A total of 20 species, 17 families, six classes and 12 orders were observed and identified at all the sampling station; MJ, KJ, BV, ML, UW and HU as shown on the Table 5. The six classes were consisting of Bacillariophyceae, Chlorophyceae, Conjugatophyceae, Cyanophyceae, Dinophyceae and Euglenoidea. Bacillariophyceae class recorded the most species, with a total of 11 species found; *Bacillaria sp*, *Cocconeis sp*, *Coscinodiscus sp*, *Cylindrotheca sp*, *Gyrosigma sp*, *Navicula sp*, *Nitzschia sp*, *Sellaphora sp*, *Skeletonema sp*, *Stauroneis sp*, and *Thalassionema sp*. Second most species found followed by Dinophyceae class, with a total of five species, which is *Alexandrium sp*, *Ceratium furca*, *Coolia sp*, *Gonyaulax* and *Protoperidinium sp*. While for Chlorophyceae, Conjugatophyceae, Cyanophyceae and Euglenoidea classes recorded only one species found, similar to what has been stated by Muhammad Adlan et al. (2012a).

4.3 Cluster Analysis

The cluster analysis between species and water quality are shown in figure 2. There has been divided into 3 cluster. Cluster A content 3 species which is *Chroococcus sp*, *Coconeis sp* and *Thalassionema sp*. Cluster B consist of 10 species *Closterium sp*, *Coolia sp*, *Euglena*, *Stauroneis sp*, *Coscinodiscus sp*, *Navicula sp*, *Gonyaulax sp*, *Bacillaria sp*, *Gyrosigma sp*, and *Alexandrium sp*. While cluster C, consist of *Ceratium furca*, *Pandorina sp*, *Protoperidinium sp*, *Sellaphora sp*, *Skeletonema sp*, *Cylindrotheca sp*, and *Nitzschia sp*.

As for figure 3, the figure shows the cluster analysis between phytoplankton species with sampling station. There are two big cluster was recorded and categories as group A and B. Group A consist of 2 sampling station, which is HU and KJ. Both of this sampling station is freshwater region. For group B, it consists of 4 sampling station, which is BV, ML, MJ, and UW.

4.4 Correlation Coefficient

Table 7 show the correlation coefficients for comparison for physiochemical parameter and phytoplankton species. The result will be highlighting the significant correlation between species and water quality parameter.



Table 5: A checklist of phytoplankton found in every selected station in Bintulu area.

Class	Order	Family	Species
Bacillariophyceae	Bacillariales	Bacillariaceae	<i>Bacillaria sp</i>
	Achnanthes	Cocconeidaceae	<i>Cocconeis sp</i>
	coscinodiscales	Coscinodiscaceae	<i>Coscinodiscus sp</i>
	Bacillariales	Bacillariaceae	<i>Cylindrotheca sp</i>
	Naviculales	Pleurosigmaaceae	<i>Gyrosigma sp</i>
		Naviculaceae	<i>Navicula sp</i>
	Bacillariales	Bacillariaceae	<i>Nitzschia sp</i>
	Naviculales	Sellaphoraceae	<i>Sellaphora sp</i>
	Thalassiosirales	Skeletonemataceae	<i>Skeletonema sp</i>
	Naviculales	Stauroneidaceae	<i>Stauroneis sp</i>
	Thalassionematales	Thalassionemataceae	<i>Thalassionema sp</i>
Chlorophyceae	Chlamydomonadales	Volvocaceae	<i>Pandorina sp</i>
Conjugatophyceae	Desmidiales	Closteriaceae	<i>Closterium sp</i>
Cyanophyceae	Chroococcales	Chroococcaceae	<i>Chroococcus sp</i>
Dinophyceae	Gonyaulacales	Ostreopsidaceae	<i>Alexandrium sp</i>
		Ceratiaceae	<i>Ceratium furca</i>
		Ostreopsidaceae	<i>Coolia sp</i>
		Gonyaulacaceae	<i>Gonyaulax</i>
	Peridiales	Protopteridiniaceae	<i>Protopteridium sp</i>
Euglenozoa	Euglenida	Euglenaceae	<i>Euglena</i>

Table 6: Species availability based on sampling location.

Species	Sampling Location					
	BV	HU	KJ	MJ	ML	UW
<i>Alexandrium sp</i>	+	-	-	+	+	+
<i>Bacillaria sp</i>	-	+	-	+	+	-
<i>Ceratium furca</i>	+	-	-	-	-	-
<i>Chroococcus sp</i>	-	-	+	-	-	+
<i>Closterium sp</i>	-	+	-	-	-	-
<i>Cocconeis sp</i>	-	+	-	-	-	-
<i>Coolia sp</i>	-	-	+	-	-	-
<i>Coscinodiscus sp</i>	-	-	+	+	-	-
<i>Cylindrotheca sp</i>	+	-	-	-	-	-
<i>Euglena</i>	-	-	+	-	-	-
<i>Gonyaulax</i>	-	-	-	+	-	-
<i>Gyrosigma sp</i>	-	-	-	+	+	+
<i>Navicula sp</i>	-	-	+	+	-	-
<i>Nitzschia sp</i>	+	-	-	-	-	-
<i>Pandorina sp</i>	+	-	-	-	-	-
<i>Protoperidinium sp</i>	+	-	-	-	-	-
<i>Sellaphora sp</i>	+	-	-	-	-	-
<i>Skeletonema sp</i>	+	-	-	-	-	-
<i>Stauroneis sp</i>	-	-	+	-	-	-
<i>Thalassionema sp</i>	-	+	-	-	-	-

MJ= Martek Pangkalan Polis Marin Wilayah 5 Jetty, **KJ**= Kampung Jepang, **BV**= Boulevard Valve, **ML**= Muhibbah Lake, **UW**= UPMKB Waterfall, **HU**= Hatchery Pond 1 UPMKB, **+**: Present, **-**: Absent

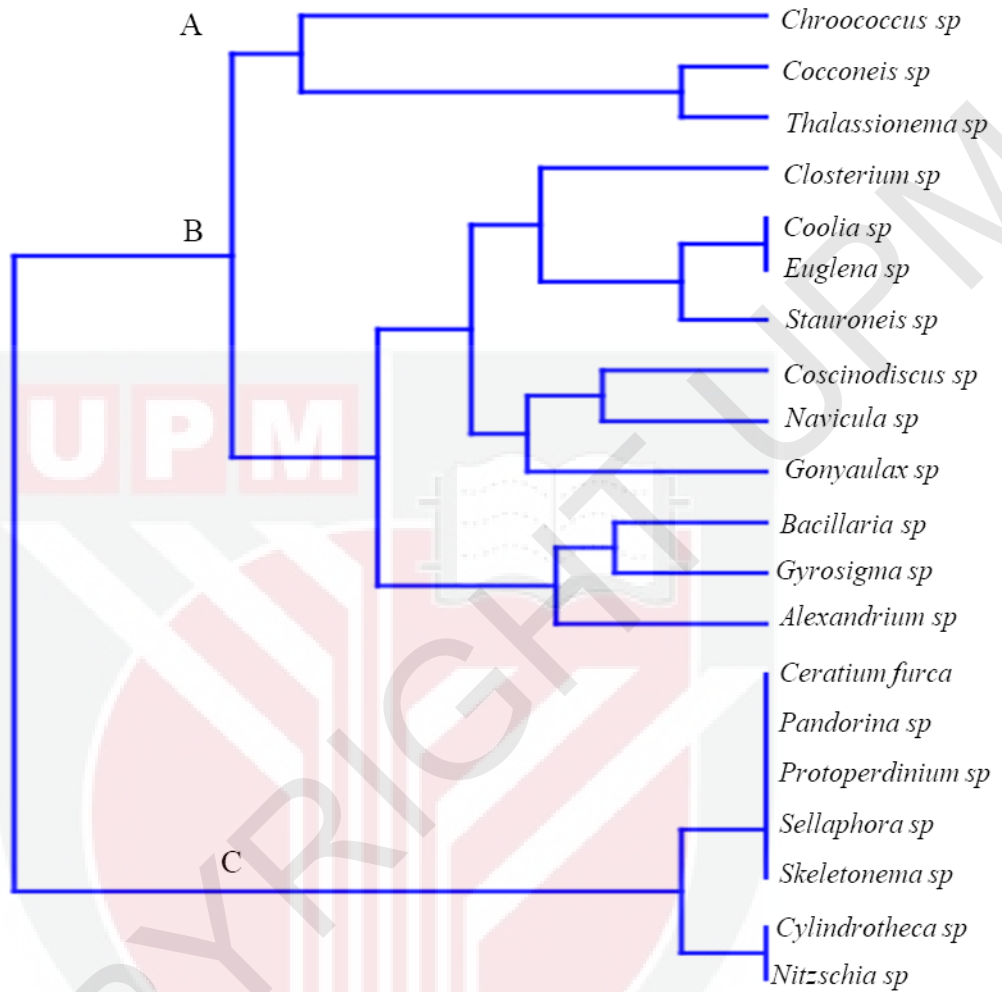
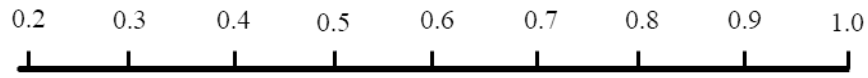


Figure 2: Cluster Analysis using Bray-Curtis similarity between phytoplankton species and water quality parameter

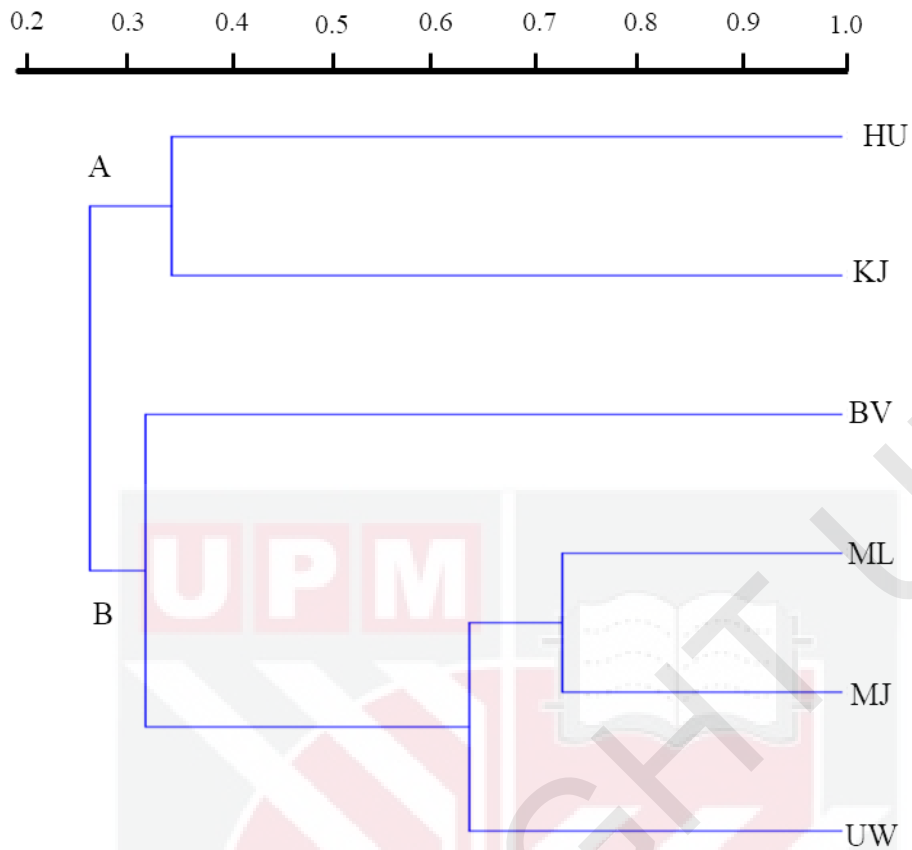


Figure 3: Cluster Analysis using Bray-Curtis similarity between phytoplankton species and sampling station. **MJ**= Martek Pangkalan Polis Marin Wilayah 5 Jetty, **KJ**= Kampung Jepak, **BV**= Boulevard Valve, **ML**= Muhibbah Lake, **UW**= UPMKB Waterfall, **HU**= Hatchery Pond 1 UPMKB.

Table 7: Pearson correlation coefficient of different physical-chemical parameters and phytoplankton in Bintulu area.

SPECIES	NH ₃	NO ₂ ⁻	NO ₃ ⁻	PO ₄	Temp	Cond	Turb	TDS	pH	Sal	DO	TSS
<i>Alexandrium sp</i>	ns	ns	ns	ns	ns	.722**	ns	ns	ns	ns	.787**	ns
<i>Bacillaria sp</i>	ns	ns	ns	ns	ns	-.793**	ns	ns	-.597**	ns	ns	ns
<i>Ceratium sp</i>	ns	ns	.547*	.946**	ns	.480*	.701**	1.000**	ns	1.000**	.556*	ns
<i>Chroococcus sp</i>	ns	ns	-.509*	ns	-.495*	ns	-.696**	ns	.927**	ns	ns	ns
<i>Closterium sp</i>	ns	ns	ns	ns	ns	-.722**	ns	ns	ns	ns	-.787**	ns
<i>Cocconeis sp</i>	.592**	ns	ns	ns	.649**	-.893**	ns	ns	ns	ns	ns	.796**
<i>Coolia sp</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-.624**	ns
<i>Coscinodiscus sp</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-.624**	ns
<i>Cylindrotheca sp</i>	ns	ns	.547*	.946**	ns	.480*	.701**	1.000**	ns	1.000**	.556*	ns
<i>Euglena sp</i>	ns	.648**	.643**	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Gonyaulax sp</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-.624**	ns
<i>Gyrosigma sp</i>	ns	ns	ns	ns	-.741**	ns	ns	-.629**	ns	-.631**	ns	ns
<i>Navicula sp</i>	-.561*	ns	ns	ns	-.625**	ns	ns	ns	ns	ns	-.471*	-.626**
<i>Nitzschia sp</i>	ns	ns	.547*	.946**	ns	.480*	.701**	1.000**	ns	1.000**	.556*	ns
<i>Pandorina sp</i>	ns	ns	.547*	.946**	ns	.480*	.701**	1.000**	ns	1.000**	.556*	ns
<i>Protoperidinium sp</i>	ns	ns	.547*	.946**	ns	.480*	.701**	1.000**	ns	1.000**	.556*	ns
<i>Sellaphora sp</i>	ns	ns	.547*	.946**	ns	.480*	.701**	1.000**	ns	1.000**	.556*	ns
<i>Skeletonema sp</i>	ns	ns	.547*	.946**	ns	.480*	.701**	1.000**	ns	1.000**	.556*	ns
<i>Stauroneis sp</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-.624**	ns
<i>Thalassionema sp</i>	.592**	ns	ns	ns	.649**	-.893**	ns	ns	ns	ns	-.371	.796**

Temp= Temperature, **DO**= Dissolved Oxygen, **Sal**= Salinity, **Turb**= Turbidity, **TDS**= Total Dissolved Solid, **Cond**= Conductivity, **NO₂⁻**= Nitrite, **NO₃⁻**= Nitrate, **TSS**= Total Suspended Solid, **NH₃**= Ammonia, **PO₄**= Phosphate, **pH**= Potential of Hydrogen, **ns**= Not Significant

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

CHAPTER 5 DISCUSSION

5.1 Physical-chemical parameter

Generally, all the six-sampling station has its own environmental factors that contribute to the physical-chemical characteristic of the water. For BV, it is located at the connecting valve between freshwater and sea water as located in the Figure 1. Due to its location is located at the Bintulu city and residential area, the physical-chemical characteristic of water is affected by house waste water release from the residential and industrial waste from the city(Zhang et al., 2017). While, MJ is located at Kemena riverbank, where the area is opposite with Bintulu old town. This area is the second largest sampling station behind BV. The physical-chemical of water in this area is affected by the tide itself due it is the open water and obviously active waterflow. While, KJ is located in the small water stream which is the water drainage for residential in Kampung Jepak. Besides, ML is the park pond located near to Bintulu's Stadium. This sampling station is recreational park area. For UW and HU, both of this station is located in Universiti Putra Malaysia Bintulu Campus (UPMKB). UW is waterfall area in UPMKB and this sampling location is actively waterflow where it located at the side of the main road in the campus from Li Hua gate. Besides, HU is one of the fish culture ponds in UPMKB, where the physical-chemical characteristic of the water in this station is affected by the fish culture activities, include active nitrification process by the cultured fish in this pond.

Based on Table 4, HU recorded the highest mean value $31.76 \pm 0.14^{\circ}\text{C}$. This due to this station is directly exposed to sunlight where it affects the temperature of this station. Dissolved oxygen recorded 0.006 ± 0.01 mg/L at KJ, little amount compares to the others, MJ, BV, ML, UW and HU. This is due to the dissolved oxygen is consumed during decomposition process by macroalgae, and this result in low amount of dissolved oxygen(Gao et al., 2013). For turbidity, the highest clarity was recorded at BV with 77.80 ± 0.005 NTU due to this station is a brackish area where the water clarity is slightly murky and the clear water was recorded at UW with

0.003 ± 0.005 NTU. This station also recorded the highest total dissolved solids, which is 34.1 mg/L. This shows the amount of organic matter in BV is higher compared to the other stations (Zhang et al., 2017). As mentioned before, this is affected by the nearby city and residential in the area itself. This sampling station also recorded 21.91 ± 0.01 ppt salinity due to the brackish water region, while the others is 0.

From the study, UW recorded the highest pH compared to the other sampling stations which is 10.10 ± 0.15. This happens due to high photosynthesis reaction among the aquatic plants in the area that decrease the amount of carbon dioxide, this phenomenon is contributing to the rise of the pH (Picot et al., 1993). The mean for total suspended solids was 0.33, where it shows a small difference between sampling stations, where the highest was recorded at HU with 0.59 ± 0.005 mg/L. This is due to the water depth of HU is the shallowest compared to the other stations and it is exposed to the sediment from the bottom water. In addition, the highest concentration of nitrites was recorded at BV with 0.313 ± 0.519 mg/L. While the highest concentration of nitrate was recorded at MJ. This result is affected by the nitrogen cycles in the water itself (Hastuti, 2011). For ammonia, the highest concentration level of ammonia found at ML with 1.792 ± 0.002 mg/L. This is due to this station is considered as a park pond, high diversity of aquatic plants and fish inhabit in this pond will contribute to nitrogen cycles, similar to what has been stated by Hastuti (2011). While for KJ recorded the lowest concentration of ammonia with 0.283 ± 0.023 mg/L, due to its environmental factors and lack of aquatic life inhabits this station. Last but not least, the highest level of phosphate was recorded at BV, with 1.12 ± 0.005 mg/L. This is proving that the diversity of phytoplankton is related to the phosphate level due to the highest species found in BV (Goldman and Horn, 1983).

5.2 Phytoplankton Availability

The most species found in every location was from class Bacillariophyceae, with a total number of 11 order and 11 families, followed by genus from class Dinophyceae with 5 order and families. A checklist of the identified phytoplankton of this research are as shown on the table 5. The table shows Bacillariophyceae class have the highest species found at all the sampling station which is consist of 11 species as listed; *Bacillaria sp*, *Cylindrotheca sp*, *Nitzschia sp*, *Cocconeis sp*, *Coscinodiscus sp*, *Navicula sp*, *Gyrosigma sp*, *Sellaphora sp*, *Skeletonema sp*, *Stauroneis sp*, and *Thalassionema sp*.

This due the diatom abilities to immigrate is related to their abilities for colonizing in the stream substrates. This immigration abilities might affect the fitness of benthic diatom. Besides, monoraphid diatom had a lower ability to immigrate compared to araphid diatom (Stevenson and Peterson, 1989). Another study was performed by Ghosh et al. (2012) state that phytoplankton species diversity is related to the availability of the supplement, temperature, light visibility, and other limnology elemental.

5.3 Cluster analysis

The cluster analysis using Bray-Curtis similarity between phytoplankton species and water quality parameter are shown in figure 2. There has been divided into 3 cluster. Cluster A content three species which is *Chroococcus sp*, *Coconeis sp* and *Thalassionema sp*. These three species are found in UPMKB area which is HU and UW where the water clarity is clear and high availability of light. Cluster B consist of 10 species *Closterium sp*, *Coolia sp*, *Euglena*, *Stauroneis sp*, *Coscinodiscus sp*, *Navicula sp*, *Gonyaulax sp*, *Bacillaria sp*, *Gyrosigma sp*, and *Alexandrium sp*. Where all 6 identified species in KJ are listed in this cluster. The rest 4 more species are recorded at BV, HU and MJ. This results in all the recorded species in cluster B is able to adapt with brackish water ecosystem. While cluster C, consist of *Ceratium furca*, *Pandorina sp*, *Protoperidinium sp*, *Sellaphora sp*, *Skeletonema sp*, *Cylindrotheca sp*, and

Nitzschia sp. These all species were recorded at BV, with a brackish water condition, 21.9 ppt. As stated by Ghosh et al. (2012), different species found on different area is well effected by the physico-chemical parameters in the water itself such as the availability of supplement, As for figure 3, cluster analysis using Bray-Curtis similarity between phytoplankton species and sampling station. There are two big cluster was recorded and categories as group A and B. Group A consist of 2 sampling station, which is HU and KJ. The mean for the nitrate for this both stations is 1.483 mg/L, where it proves the recorded species from both stations is related to the physical-chemical characteristic Besides, both of this sampling station is freshwater region. For group B, it consists of 4 sampling station, which is BV, ML, MJ, and UW. All these sampling station recorded the availability of *Alexandrium sp.* This species has its ability to adapt to change the amount of nitrogen level in the environment(Usup et al., 2002).

5.4 Correlation Coefficients for Comparison for Physiochemical Parameter and Phytoplankton Species

Based on correlation obtained, *Alexandrium sp* and *Closterium sp* shows the negative correlation with a value of -0.722 with conductivity and -0.787 with DO. *Bacillaria sp* shows also negative correlation with a value of -0.793 with conductivity and -0.597 with pH. *Chroococcus sp* obtain a negative correlation value -0.509 with NO_2^- , -0.495 with temperature, -0.696 with turbidity and positive correlation value 0.927 with pH. For *Cocconeis sp*, it shows a positive correlation with a value of 0.592 with NH_3 , 0.649 with temperature, -0.893 with conductivity, and 0.796 with TSS. While for *Ceratium sp*, *Cylindrotheca sp*, *Nitzschia sp*, *Pandorina sp*, *Protoperdinium sp*, *Sellaphora sp* and *Skeletonema sp* shows a positive correlation with a value of 0.547 with NO_3^- , 0.946 with PO_4 , 0.480 with conductivity, 0.701 with turbidity, 1.000 with TDS and 0.556 with DO. PO_4 shows the significant correlation between the correlated species, this indicate that the recycle of the nutrients in the water is high, different from what has been stated by (Palleyi et al., 2011). *Coolia sp*, *Coscinodiscus sp*,

Gonyaulax sp and *Stauroneis sp* shows a negative correlation with a value of -0.624 with DO. *Euglena sp* shows a positive correlation with a value of 0.648 with NO_2^- and 0.643 with NO_3^- . For *Gyrosigma sp*, it shows a negative correlation with a value of -0.742 with temperature, -0.629 with turbidity and -0.631 with salinity. Salinity is measured because it can influence the distribution and the diversity of phytoplankton (Palleyi et al., 2011). *Navicula sp* also obtained a negative correlation with a value -0.561 with NH_3 , -0.625 with temperature, -0.471 with DO and -0.626 with TSS. While for *Thalassionema sp* shows a positive value correlation with 0.592 with NH_3 , 0.649 with temperature and 0.796 with TSS, but negative correlation with a value of -0.893 with conductivity and -0.371 with DO. Based on this study, I suggest that the quantitative and qualitative data on phytoplankton diversity is important to take. To summaries this, all the six-sampling station has its own characteristic that contribute to the physical-chemical characteristic of the water itself and affected the availability of phytoplankton.

CHAPTER 6 CONCLUSION

A study of Diversity of Phytoplankton Associated with Water Quality in Selected Area in Bintulu, Sarawak at 6 sampling station which is Kampung Jepak (KJ), Boulevard Valve (BV), UPMKB Hatchery Pond 1 (HU), UPMKB Waterfall (UW), Martek Pangkalan Polis Marin Wilayah 5 Jetty (MJ) and Muhibbah Lake, Bintulu (ML). All the sampling station is in the range of class I to class IV. A total of 20 species, from 17 families, 6 classes and 12 order were recorded and identified at all the sampling station. The most species found were recorded at Boulevard Valve, with a total of 8 species and 7 families. Bacillariophyceae was the most diverse class found, due to it able to adapt with wide range of environmental factors.

Physico-chemical of water is affecting the phytoplankton availability in each sampling station, as for the phytoplankton species recorded in each sampling station is different. This result is supporting the objective of this study. Therefore, as for the Bacillariophyceae was the most high in species found, it can be an indicators to reflect water quality condition (Malik et al., 2012).

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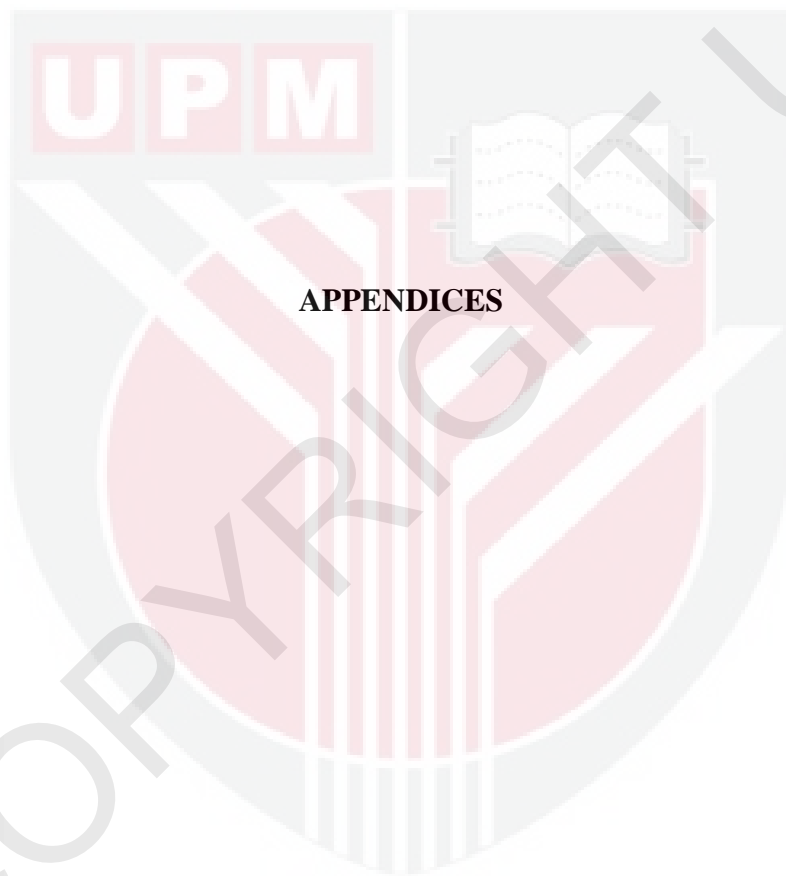
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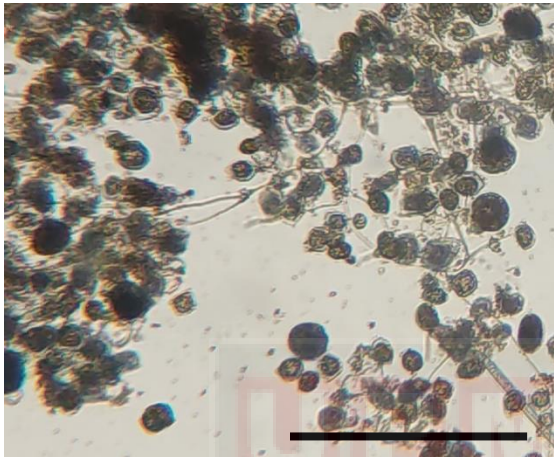
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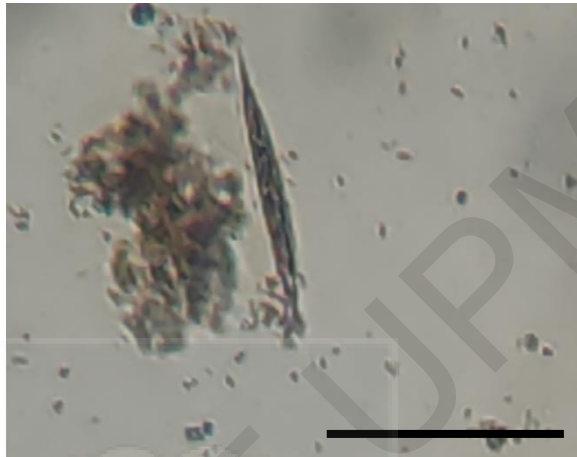
APPENDICES

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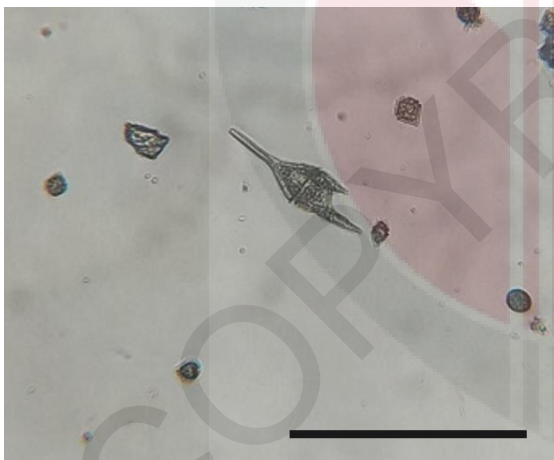
APPENDIX A



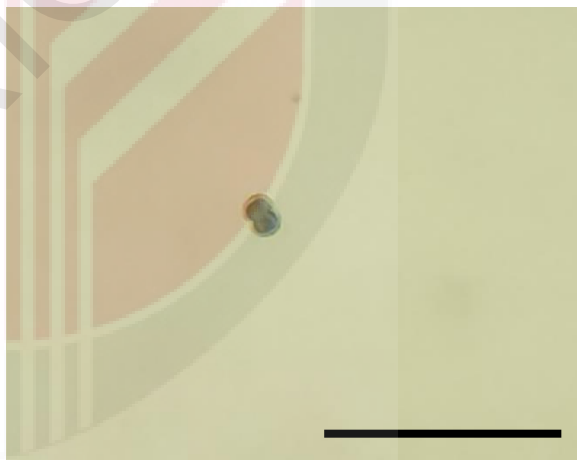
Class : Dinophyceae
 Family : Ostreopsidaceae
 Order : Gonyaulacales
 Genus : *Alexandrium*
 Species : *Alexandrium sp*



Class : Bacillariophyceae
 Family : Bacillariaceae
 Order : Bacillariales
 Genus : *Bacillaria*
 Species : *Bacillaria sp*

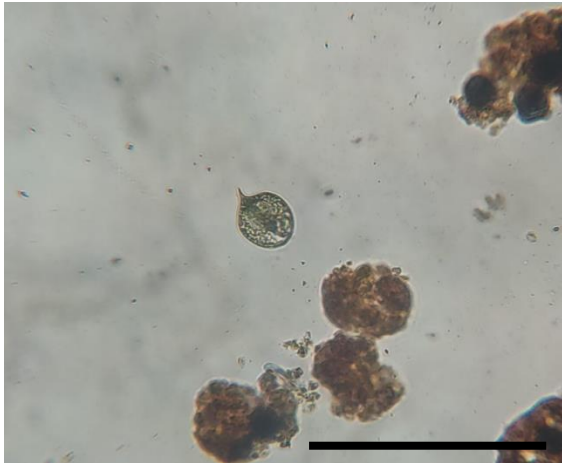


Class : Dinophyceae
 Family : Ceratiaceae
 Order : Gonyaulacales
 Genus : *Ceratium*
 Species : *Ceratium furca*



Class : Cyanophyceae
 Family : Chroococcaceae
 Order : Chroococcales
 Genus : *Chroococcus*
 Species : *Chroococcus sp*

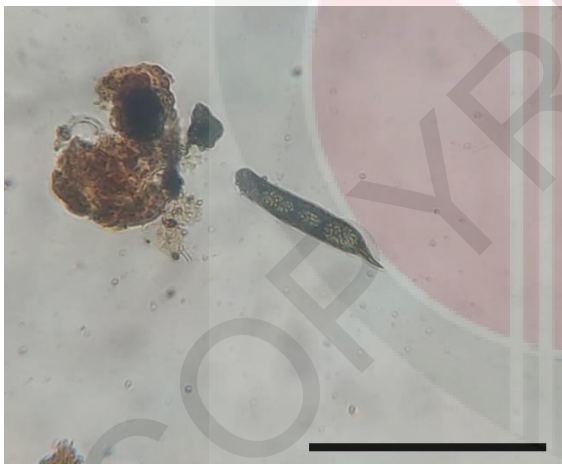
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Class : Bacillariophyceae
 Family : Coscinodiscaceae
 Order : Coscinodiscales
 Genus : Coscinodiscus
 Species : *Coscinodiscus sp*



Class : Bacillariophyceae
 Family : Coscinodiscaceae
 Order : Coscinodiscales
 Genus : Coscinodiscus
 Species : *Coscinodiscus sp*



Class : Euglenoidea
 Family : Euglenaceae
 Order : Euglenida
 Genus : Euglena
 Species : *Euglena sp*



Class : Dinophyceae
 Family : Gonyaulacaceae
 Order : Gonyaulacales
 Genus : Gonyaulax
 Species : *Gonyaulax sp*

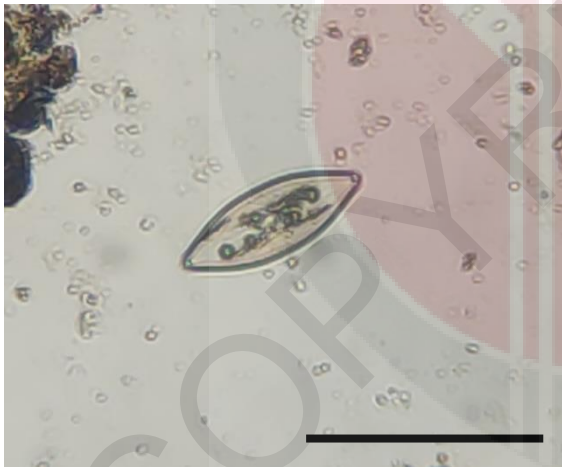
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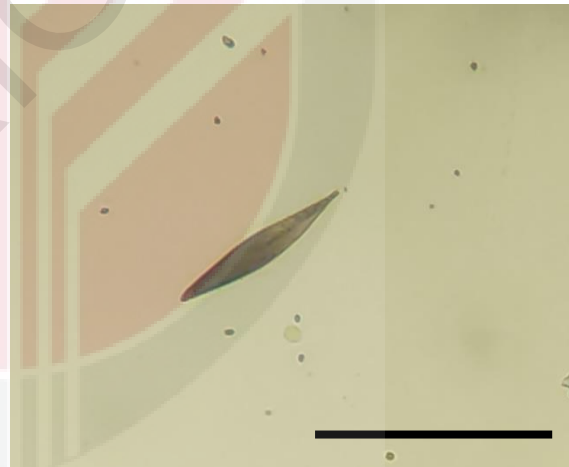
Class : Bacillariophyceae
 Family : Pleurosigmataceae
 Order : Navicules
 Genus : Gyrosigma
 Species : *Gyrosigma sp*



Class : Bacillariophyceae
 Family : Pleurosigmataceae
 Order : Navicules
 Genus : Gyrosigma
 Species : *Gyrosigma sp*

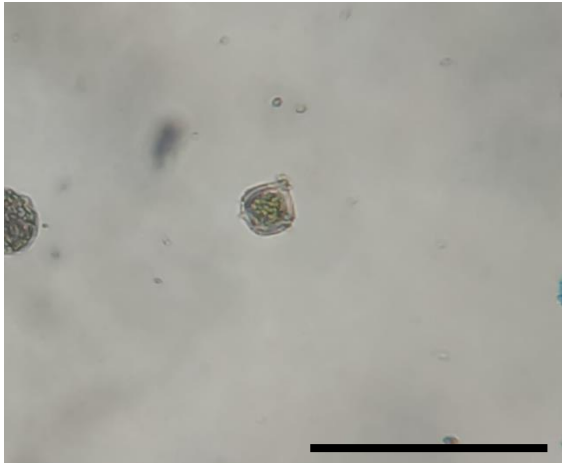


Class : Bacillariophyceae
 Family : Naviculaceae
 Order : Navicules
 Genus : Navicula
 Species : *Navicula sp*

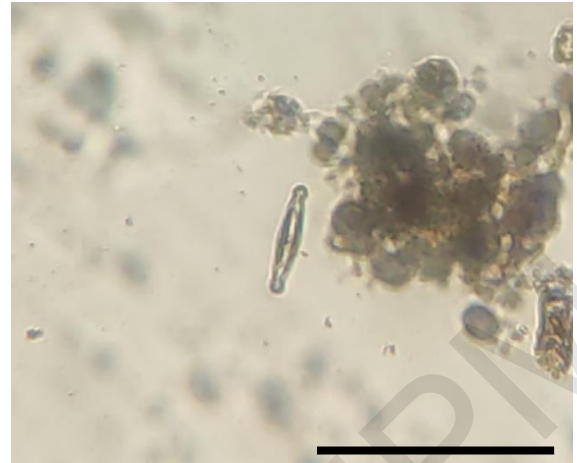


Class : Bacillariophyceae
 Family : Naviculaceae
 Order : Navicules
 Genus : Navicula
 Species : *Navicula sp*

Scale bar = 20µm



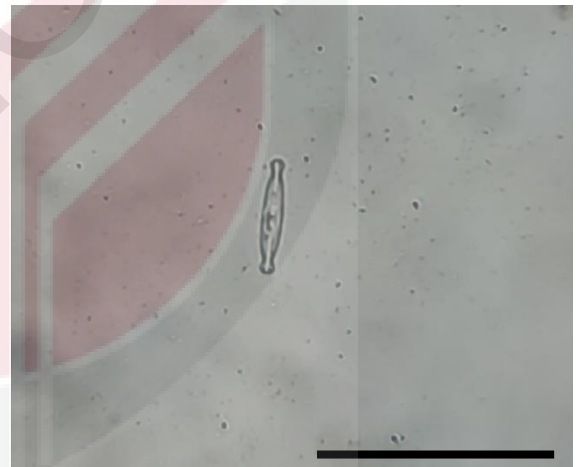
Class : Dinophyceae
 Family : Protoperidiniaceae
 Order : Peridiales
 Genus : Protoperidinium
 Species : *Protoperidinium sp*



Class : Bacillariophyceae
 Family : Sellaphoraceae
 Order : Navicules
 Genus : Sellaphora
 Species : *Sellaphora sp*



Class : Bacillariophyceae
 Family : Skeletonemataceae
 Order : Thalassiosirales
 Genus : Skeletonema
 Species : *Skeletonema sp*



Class : Bacillariophyceae
 Family : Stauroneidaceae
 Order : Navicules
 Genus : Stauroneis
 Species : *Stauroneis sp*

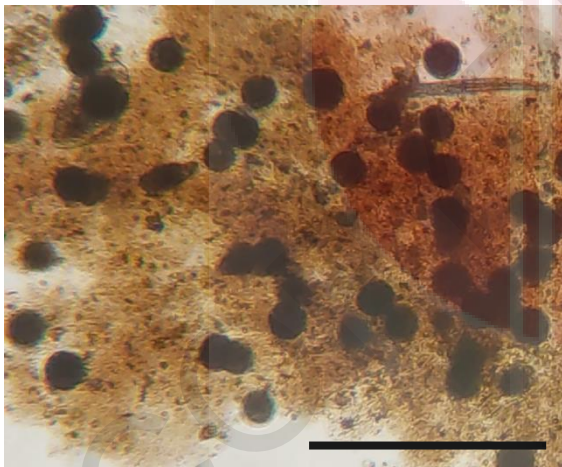
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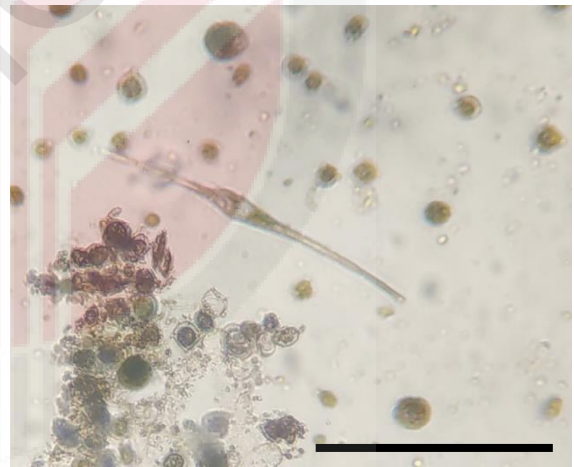
Class : Conjugatophyceae
 Family : Closteriaceae
 Order : Desmidiiales
 Genus : Closterium
 Species : *Closterium sp*



Class : Bacillariophyceae
 Family : Cocconeidaceae
 Order : Achnanthes
 Genus : Cocconeis
 Species : *Cocconeis sp*



Class : Dinophyceae
 Family : Ostreopsidaceae
 Order : Gonyaulacales
 Genus : Coolia
 Species : *Coolia sp*



Class : Bacillariales
 Family : Bacillariaceae
 Order : Gonyaulacales
 Genus : Cylindrotheca
 Species : *Cylindrotheca sp*

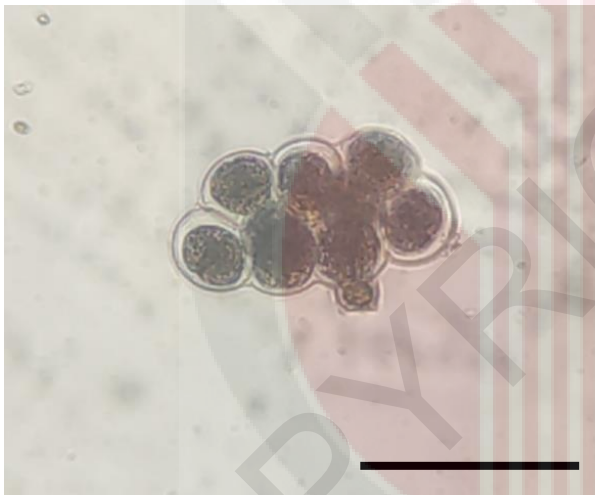
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Class : Bacillariophyceae
 Family : Thalassionemataceae
 Order : Thalassionematales
 Genus : Thalassionema
 Species : *Thalassionema sp*

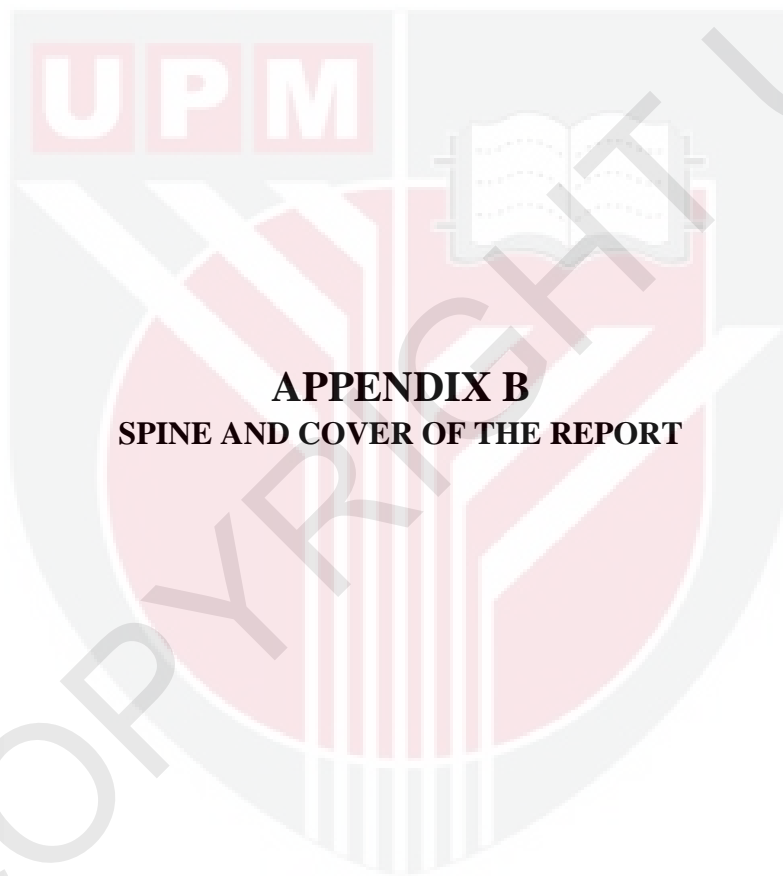


Class : Bacillariophyceae
 Family : Bacillariaceae
 Order : Bacillariales
 Genus : Nitzschia
 Species : *Nitzschia sp*



Class : Chlorophyceae
 Family : Volvocaceae
 Order : Chlamydomonadales
 Genus : Pandorina
 Species : *Pandorina sp*

Scale bar = 20um



APPENDIX B
SPINE AND COVER OF THE REPORT

**DIVERSITY OF PHYTOPLANKTON ASSOCIATED
WITH WATER QUALITY IN SELECTED AREA IN
BINTULU, SARAWAK**



PETRUSON ASAN ANAK BAJING

**FACULTY OF AGRICULTURAL SCIENCE AND
FORESTRY UNIVERSITI PUTRA MALAYSIA
BINTULU SARAWAK CAMPUS**

2021

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled “Diversity of Phytoplankton Associated with Water Quality in Selected Area in Bintulu, Sarawak” by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus and published in the form approved by the Faculty.



Petruson Asan Anak Bajing

Date: 4th August 2021