JJBS

Volume 16, Number 1,March 2023
ISSN 1995-6673
Pages 27 – 34
https://doi.org/10.54319/jjbs/160105

Jordan Journal of Biological Sciences

Species Diversity of Freshwater Microalgae in Dramaga, Bogor Based on Morpho-ecological Identification between Low and High Light Intensity Environment

Miftahul Huda Fendiyanto[1,2], Mentari Putri Pratami[1,*], Rizky Dwi Satrio[1,2] , Isna Arofatun Nikmah[1], Nastiti Intan Permata Sari[1], Mo Awwanah[1], Nadya Farah[1], Nurhadiyanta[1]

[1]Study Program of Biology, Faculty of Military Mathematics and Natural Sciences, Indonesia Defense University. Kampus Universitas Pertahanan Sentul Bogor, Kawasan IPSC, 16810, West Java, Indonesia; [2]Laboratory Plant Physiology and Molecular Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University. Kampus IPB Darmaga Bogor 16680, West Java, Indonesia

Received: April 1, 2022; Revised: June 9, 2022; Accepted: June 24, 2022

Abstract.

Microalgae are prokaryotic or eukaryotic organisms that have photosynthetic anabolic activity. This anabolic photosynthesis is related to the abiotic factor of light. Therefore, this study aimed to identify the morphological diversity level of microalgae based on their environment, especially light intensity. We collected samples based on two types of environment, namely high light community (HL-1D and HL-2D) and low light community (LL-1D and LL-2D). The Data were analyzed using Species Richness Indices (Menhinick and Margalef indices), Species Diversity (Shannon-Wiener and Simpson Indices), and Eveness Index. Based on the results of morphological identification, we found the presence of algae from the divisions Cyanophyta, Chlorophyta, and Euglenophyta in both communities. The most dominant species found in the HL environment were Cyanophyta (*Chroococcus* sp., *Microcystis* sp., *Nostoc* sp., *Oscilatoria* sp.), Chlorophyta (*Actinastrum* sp., *Ankistrodesmus* sp., *Centritractus belanophorus*, *Coelastrum* sp., *Closterium* sp., *Gleocapsa* sp., *Mougeutia* sp., *Pediastrum* sp., *Scenedesmus* sp., *Selenastrum* sp., *Sphaeroplea* sp., *Tetraspora cylindrica*, *Ulothrix* sp., and *Volvox* sp.), Euglenophyta (*Cryptoglena* sp. and *Lepocinclis* sp.). Species found in the LL environment were Cyanophyta (*Bulbochaeta* sp.) and Chlorophyta (*Pleurotaenium* sp., *Uronema elongasi*, and *Zygnema* sp.). Microalgae communities in high light communities have higher diversity than low light communities. This study can be used as a reference for the diversity of microalgae in two different types of environments, especially in the tropics and in freshwater microalgae communities. This diversity data could be a reference for researchers and provide preliminary information of microalgae potency as alternative biofuels in the future.

Keywords: Abiotic Factor, Freshwater Microalgae, Light Intensity, Photosynthesis

1. Introduction

Fossil fuels or mineral fuels are natural resources that contain hydrocarbons such as coal, oil, and natural gas. Along with the increase in the world's population, the availability of fossil-based fuels has been dwindling. This condition will have an impact on various sectors such as transportation, industry, agriculture, and others. With the limited amount of fossil fuels available, humans will switch to using petroleum. If used continuously, there will be an increase in CO_2 emission levels in the atmosphere, which will affect global warming and climate change (Chisti, 2007; Handoko *et al.*, 2008; Paynter and Frölicher, 2015; Williams *et al*., 2017). To overcome this problem, we need a substitute fuel that is renewable and environmentally friendly such as biodiesel (Mahyudin and Kusnandar, 2006; Williams *et al.*, 2017). Biodiesel is an alternative fuel derived from plants or animals through a transesterification process with alcohol (Chisti, 2007; Litinas *et al*., 2020). Indonesia is an archipelagic country with abundant aquatic biological resources, both in type and quantity. One aspect of the richness of biological resources is the abundance of plants that have the potential as a source of biodiesel, one of which is microalgae. Microalgae can produce 150-200 times more lipids than lipid-producing plants, i.e., *Elaeis* sp. and *Jatropha* sp.(Chisti, 2007).

Microalgae are a group of microscopic low-level plants that live in both fresh and marine waters. Microalgae are unicellular organisms that live in colonies or live as solitary and are generally photosynthetic because they live by utilizing light energy. Microalgae are rich in nutrients that can be developed as a source of raw materials for the pharmaceutical, cosmetic, and biofuel industries (Ghufran and Kordi, 2010). In addition, the biomass of many microalgae has a high lipid content of approximately 60% of the dry weight. Such microalgae are also tolerant of changes in extreme environmental conditions such as soil,

* Corresponding author. e-mail: mentariry@gmail.com.

lakes, wastewater, snow, high temperatures of as hot springs, and high salt content of the sea (Agustini, 2006).

Several types of microalgae are being developed in several countries as producers of bio-oil which can be further utilized in biodiesel (Moshood *et al.*, 2020). In addition, the potential of microalgae which is currently receiving special attention is as a producer of biohydrogen that can be used as renewable energy (Richmond and Emeritus, 2013; Moshood *et al*., 2020). The advantages of microalgae for biodiesel raw materials include not only requiring a large area of land, but also fast harvesting time; the biomass has a high lipid content and a fast cell growth rate with cell doubling occurring every 3.5 hours and not causing competition between energy and food needs (Chisti, 2007; Moshood *et al*., 2020).

The growth and production of microalgae are strongly influenced by environmental conditions. The environmental factors that affect the growth and productivity of microalgae lipids include light intensity, temperature, osmotic pressure, pH, and nutrient concentration in the media (Becker, 1994). Nybakken (1982) suggested that the main inorganic nutrients needed by phytoplankton to grow and reproduce are N in the form of NO^{3-} and P in the form of PO_4^{3-}. One of the known cell disruption methods is the osmotic shock method. It performs a sudden decrease in osmotic pressure on a microorganism so that it will cause cell damage. This method can be used to remove cellular components such as oil.

According to Teresa *et al.*, (2010), *Botryococcus braunii* can contain quite a lot of lipids up to 20-75% dry weight of biomass. Long-chain hydrocarbons in the form of oil or unbranched triterpenes of this species are known as botryococcene is very potential as an energy source or biodiesel (Metzger and Largeau, 2005; Rao *et al.*, 2007).

Several groups of microalgae can be used as biodiesel raw materials, namely diatoms (Bacillariophyceae), green microalgae (Chlorophyta), and blue-green microalgae (Cyanophyta) (Griffiths and Harrison, 2009). Microalgae contain several important components including carbohydrates, fatty acids, and proteins so that microalgae can be used as raw materials to produce their derivative products. The content and productivity of microalgae lipids are influenced by nitrogen concentration and light intensity (Gunawan, 2010). Microalgae have been investigated as an alternative as substitute for land plant commodities as a source of oil production because of their ability to grow in a short time with abundant biomass (Prartono *et al.*, 2013). To utilize microalgae as raw material for biodiesel, it is necessary to characterize and identify species first (Moshood *et al.*, 2020). Species identification is a step that can be taken to determine the types and relationships of a group at the taxonomic level and is important to be the basis for applied research from Microalgae (Prartono *et al.*, 2013). Therefore, the purpose of this study is to identify morphologically the diversity level of microalgae based on their environment, especially light intensity to provide a primary data to get a species which used a fossil fuels as renewable energy sources for the next research.

2. Materials And Methods

2.1. Sample collection

Freshwater samples were collected from two sites in Dramaga, Bogor Regency based on high (open, 6.500-10.000 lux) and low light intensity (shaded, 1.000-2.000 lux) environments. We used four sites with 20 replicates, so in total we evaluated 80 samples.. The sites in this study included high light community 1 (HL-1D), high light community 2 (HL-2D), low light community 1 (LL-1D), and low light community 2 (LL-2D). We collected fresh water at these four locations particularly 5 plots with 5 m in distance, respectively and then observed it directly using a compound microscope (Olympus, Japan). Sampling was carried out by filtering 5-10 liters of water with a vacuum pump in which a 0.45-micrometer millipore was installed. Water was taken at a depth of 50 cm to 1 m depending on the intensity of sunlight in the research location so that the filtered microalgae were microalgae that live above the water surface and float in the water. Then, the millipore was taken and put into a falcon bottle that had been filled with Industry Daigo Microalgae (IMK) media (Gunawan, 2010). For the microalgae samples to be identified, 4% formalin was added to keep the chlorophyll from being damaged (Gunawan, 2010).

2.2. Morphological identification

Microalgae from freshwater collections were identified based on cell surface structure, cell shape (globose, filamentous), living type (solitary or colony), species or genus characteristics, and cell motility. These three algae are very abundant in freshwater, according to a report by Gunawan (2010). Identification of microalgae morphology was carried out by observing using a light microscope (Olympus CX41, Japan), and identification was carried out by referring to the identification book entitled "The Freshwater Algae" (Prescot, 1978) and the book “Key to Freshwater Algae: A Web-Based Tool to Enhance Understanding of Microscopic Biodiversity” (Shayler and Siver, 2006).

2.3. Diversity parameter analysis

We tested the species richness (SR) parameter using the R program with the syntax “apply(data[,-1]>0,1, sum)” (Lander, 2014). Species richness was measured based on the number of species at each site specifically in the four communities HL-1D, HL-2D, LL-1D, and LL-2D. In addition to species richness, we also measure richness based on two kinds of indices, namely Menhinick's index (May) and Margalef's index (Mai) with the following formula (Peng *et al.*, 2018) where n was the number of species and N was the total number of individuals.

$$MeI = \frac{n}{\sqrt{N}}$$

$$MaI = \frac{n-1}{lnN}$$

For species abundance, we used the syntax "apply(data[,-1],1,sum)" in the R program. We also tested Rarefaction (Ra) with the formula below, where n was the sub-sample, N was the total number of individuals in the new rarefied taxa, and Ni was the total number of individuals in each of the original taxa (Peng *et al.*, 2018).

$$Ra = \sum 1 - \left[\frac{\left(\frac{N - Ni}{n} \right)}{\left(\frac{N}{n} \right)} \right]$$

For species diversity, we used the Shannon-Wiener Index (H') and Simpson's Index (λ) formulas (Peng *et al.*, 2018) with the syntax "diversity(data[-1], index="Shannon")" and "diversity(data[-1], index= "Simpson")" in the vegan package in the R program where ni is the number of individuals of the amount of each of the i species and N is the total number of individuals for the site. The criteria of species diversity was H' < 1 considered low; 1< H' < 3 is in the middle, and H' > 3 is high.

$$H' = -\sum (\frac{ni}{N} \times \ln \frac{ni}{N})$$

$$\lambda = \sum \frac{ni(ni-1)}{N(N-1)}$$

For the evenness parameter, we used Pilou evenness (J) and Hill's ratios (Ea:b) as below (Peng *et al.*, 2018), where H' is the actual diversity value (the Shannon-Wiener Index) and Hmax is the maximum possible diversity value. In addition, Na is the diversity numbers of order a (community 1) and Nb is the diversity numbers of order b (community 2).

$$J = \frac{H'}{H \max}$$

$$Ea : b = \frac{Na}{Nb}$$

2.4. Statistical test and data analysis

Morphological characteristic data were analyzed descriptively to obtain a complete description of each species. For data analysis, we tested the data statistically (descriptive statistics, analysis of variance, and T-test) by using the program R version 3.5.1 (Lander, 2014).

3. Results and Discussion

3.1. Biodiversity of freshwater microalgae in Dramaga, Bogor in four sites community

Freshwater microalgae in Dramaga showed a relatively abundant level of diversity, especially in high light communities (HL-1D and HL-2D) compared to low light communities (LL-1D and LL-2D). In the high light community (HL), we found many species, i.e. *Actinastrum* sp., *Ankistrodesmus* sp., *Chroococcus* sp., *Coelastrum* sp., *Gleocapsa* sp., *Microcystis* sp., *Oscilatoria* sp., *Pediastrum* sp., *Scenedesmus* sp., *Selenastrum* sp., *Ulothrix* sp., and *Volvox* sp. (Figure 1). In the low light community (LL), we found species *Bulbochaeta* sp., *Closterium* sp., *Closterium* sp2, *Pleurotaenium* sp., *Uronema* sp., and *Zygnema* sp. (Figure 1).

Chroococcus sp. has general characteristics, namely prokaryotic organisms, coccus cell shape, is unicellular, has chlorophyll pigment, and forms a mucous membrane. We found the species *Chroococcus* sp. in abundance in the HL community. *Chroococcus* sp. is classified as division Cyanophyta, Class Cyanophyceae, Order Chroococcales, and Genus *Chroococcus*. In the new classification system, *Chroococcus* sp. is classified closer to bacteria than other eukaryotic algae organisms based on microalgae phylogeny studies (Shayler and Siver, 2006).

Volvox sp. tends to form colonies where it is divided into two types, i.e. main colonies and auto-colonies. Another characteristic that we found in the species *Volvox* sp. In this study, the colonies were spherical, the cells were round in shape, and each cell was covered with mucilage (Figure 1). *Volvox* sp. has a classification system, namely Kingdom Protista, Division Chlorophyta, Class Chlorophyceae, Order Volvocales, Family Volvocaceae, Genus *Volvox*. Different from *Chroococcus* sp., *Volvox* sp. tend to be advanced because they have a nuclear membrane and are classified as eukaryotic organisms. Different from *Volvox* sp., *Coelastrum* sp. Morphologically, no mucilage was found even though they were both Division Chlorophyta.

Other microalgae belonging to the Division Chlorophyta include *Gleocapsa* sp., *Sphaeroplea* sp., *Scenedesmus* sp., and *Actinastrum* sp. *Gleocapsa* sp. is a eukaryotic organism belonging to the division Chlorophyta and Class Chlorophyceae. We identified this species based on the presence of a gelatinous cell envelope. *Sphaerolea* sp. has a characteristic where cells tend to be filamentous and chloroplasts are spiral. *Scenedesmus* sp. in this study has a morphological structure where the spine is relatively long, cells are oval, chloroplasts are found in almost all parts of the cell, and visually it is found the presence of pyrenoid as a place to store food reserves. In this study, *Scenedesmus* sp. also has the number of cells in the colony as many as four, the form of chloroplast laminate, and the colony is coenobitic. *Actinastrum* sp. Morphologically has a cell shape that resembles a star.

Oscilatoria sp. belongs to the Kingdom Monera group, especially the Cyanophyta Division where the cell structure is in the form of filaments.

Based on the identification results, we found that *Pediastrum* sp., has a polygonal cell shape where the colonies are without a conspicuous gelatin sheath, and the cells tend to be green indicated by the presence of high chlorophyll (Figure 1). *Pediastrum* sp. is classified into Kingdom Protista, Division Chlorophyta, Class Chlorophyceae, Order Chlorooccales, Family Hydrodictyaceae, and Genus *Pediastrum*.

Ulothrix sp. has a characteristic filamentous (thread) and cylindrical cell shape (Figure 1). In this study, we found the presence of chlorophyll in the filaments of *Ulothrix* sp. *Ulothrix* sp. classified into Kingdom Protista, Division Chlorophyta, Class Chlorophyceae, Order Ulotrichales, Family Ulotrichaceae, and Genus *Ulothrix*.

Microcystis sp. has a characteristic that is unicellular and round cells (Figure 1). Based on the results of our identification, the chlorophyll in *Microcystis* sp. is located in almost all parts of the cell. *Microcystis* sp. has a classification system, namely Kingdom Monera, Division Cyanophyta, Class Cyanophyceae, Order Nostocales, Family Nostocaceae, and Genus *Microcystis*.

Uronema sp. In this study, it can be categorized as an elongated *Uronema* where the cells are filamentous and there is a partition between the filaments in the microalgae. The elongated *Uronema* in this study is classified as Division Chlorophyta with the cell nucleus surrounded by a nuclear membrane or eukaryotic. *Zygnema* sp. relatively

have the same cell shape as *Uronema* sp. mainly in the form of filaments, but both are distinguished by the location of the bulkhead, and morphologically in the structure of the ends of the filaments.

Based on the identification results, we found the presence of *Bulbochaeta* sp. on the LL site. *Bulbochaeta* sp. grouped into Kingdom Monera, Division Cyanophyta, Class Cyanophyceae, Order Oedogenales, Family Oedogoniaceae, and Genus *Bulbochaeta*. We identified the species *Bulbochaeta* sp. based on the characteristics in the form of branched filaments and chloroplasts are morphologically shaped like a net.

Pleurotaenium sp. and *Closterium* sp. both have cell forms in the form of filaments and belong to the Division Chlorophyta, Kingdom Protista, including eukaryotic organisms. Both differ in cell shape and chloroplasts, where *Pleurotaenium* sp. is wider in shape with rectangular chloroplasts while the cell and chloroplast forms of *Closterium* sp. are slender in shape with relatively tapered ends.

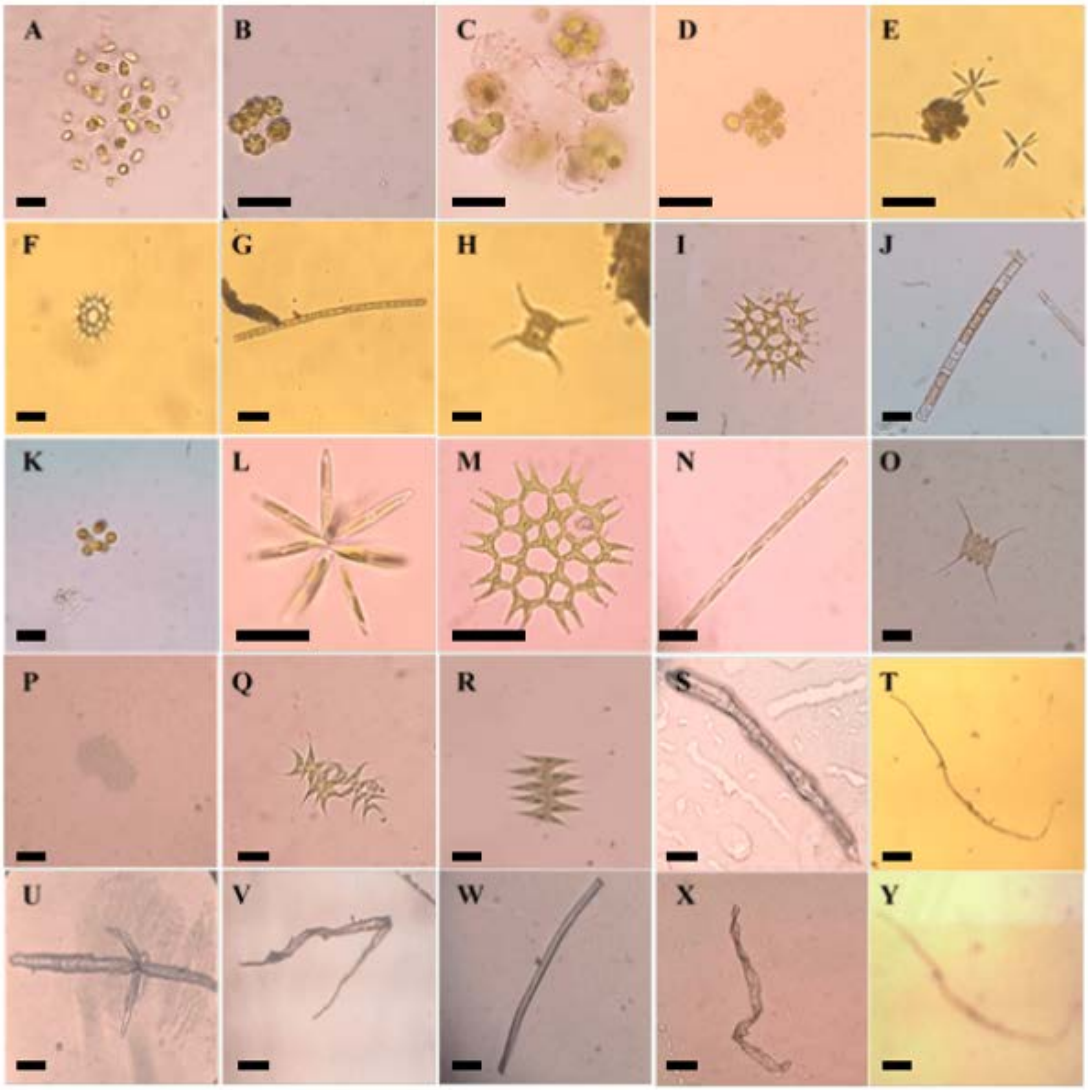

Figure 1. Freshwater-Microalgae Diversity in Dramaga, Bogor particularly in each site. HL-1D: high light community 1: A-H (A: *Chroococcus*, B: *Volvox* sp., C: *Coelastrum*, D: *Gleocapsa*, E: *Actinastrum*, F: *Pediastrum*, G: *Oscilatoria*, H: *Scenedesmus*), HL-2D: high light community 2: I-R (I: *Pediastrum*, J: *Ulothrix* sp1, K: *Microcystis*, L: *Actinastrum*, M: *Pediastrum*, N: *Ulothrix* sp2, O: *Scenedesmus*, P: *Mycrocystis*, Q: *Selenastrum*, R: *Ankistrodesmus*) LL-1D: low light community 1: S-T (S:*Uronema*, T: *Closterium*), LL-2D: low light community 2: U-Y (U: *Bulbochaeta*, V: *Pleurotaenium*, W: *Closterium* sp1., X: *Zygnema*, Y: *Closterium* sp2.). Bar = 10 μm.

Table 1. Species diversity of microalgae in four sites community in Dramaga, Bogor

No.	Sites	Species	Division
1	HL-1D	*Volvox* sp.	Chlorophyta
2		*Chroococcus* sp.	Cyanophyta
3		*Coelastrum* sp.	Chlorophyta
4		*Tetraspora cylindrica*	Chlorophyta
5		*Mougeutia* sp.	Chlorophyta
6		*Lepocinclis* sp.	Euglenophyta
7		*Gleocapsa* sp.	Chlorophyta
8		*Sphaeroplea* sp.	Chlorophyta
9		*Scenedesmus* sp.	Chlorophyta
10		*Cryptoglena* sp.	Euglenophyta
11		*Actinastrum* sp.	Chlorophyta
12		*Pediastrum* sp.	Chlorophyta
13		*Oscilatoria* sp.	Cyanophyta
14		*Nostoc* sp.	Cyanophyta
15	HL-2D	*Volvox* sp.	Chlorophyta
16		*Ulothrix* sp.	Chlorophyta
17		*Pediastrum* sp.	Chlorophyta
18		*Microcystis* sp.	Cyanophyta
19		*Actinastrum* sp.	Chlorophyta
20		*Closterium* sp.	Chlorophyta
21		*Scenedesmus* sp.	Chlorophyta
22		*Gleocapsa* sp.	Chlorophyta
23		*Selenastrum* sp.	Chlorophyta
24		*Centritractus belanophorus*	Chlorophyta
25		*Coelastrum* sp.	Chlorophyta
26		*Ankistrodesmus* sp.	Chlorophyta
27	LL-1D	*Closterium* sp.	Chlorophyta
28		*Uronema elongasi*	Chlorophyta
29	LL-2D	*Bulbochaeta* sp.	Cyanophyta
30		*Zygnema* sp.	Chlorophyta
31		*Closterium* sp.	Chlorophyta
32		*Pleurotaenium* sp.	Chlorophyta

Note: HL-1D: high light community 1, HL-2D: high light community 2, LL-1D: low light community 1, LL-2D: low light community 2

In general, we found differences in species composition or division among the two sites in this research. Specifically, to site HL, we can find all Division Chlorophyta, Cyanophyta, and Euglenophyta. In low light community (LL), we did not find Euglenophyta in our freshwater sample. This is probably related to the behavior of the motile and the presence of eye-spot in Euglenophyta, which tends to like high light intensity. At the species level, we found few species of microalgae at the LL sites but the species at these sites tended to be unique and were not found at the HL sites, i.e., *Uronema elongation*, *Bulbochaeta* sp., *Pleurotaenium* sp., and *Zygnema* sp. Interestingly, at the HL sites, we found *Lepocinclis* sp. and *Cryptoglena* sp. where both are classified as Division Euglenophyta and are motile and have chloroplasts. Species that can be found at both HL and LL sites are *Closterium* sp. Meanwhile, species that could only be found at HL sites were *Volvox* sp., *Chroococcus* sp., *Coelastrum* sp., *Tetraspora cylindrica*, *Mougeutia* sp., *Lepocinclis* sp., *Gleocapsa* sp., *Sphaeroplea* sp., *Scenedesmus* sp., *Cryptoglena* sp., *Actinastrum* sp., *Pediastrum* sp., *Oscilatoria* sp., *Nostoc* sp., *Ulothrix* sp., *Pediastrum* sp., *Microcystis* sp., *Closterium* sp., *Selenastrum* sp., *Centritractus belanophorus*, and *Ankistrodesmus* sp.

3.2. Species richness, abundance, diversity, and evenness indices of freshwater microalgae among high and low light intensity environment

The highest species richness of freshwater microalgae was obtained at the HL-1D site and the lowest was at the LL-1D site. In general, species richness in high light intensity communities (HL-1D and HL-2D) was higher than in low light intensity communities (LL-1D and LL-2D). Richness can also be tested with Menhinick's index (MeI) and Margalef's index (MaI). The values of the MeI and MaI indices are relatively similar to the SR values where the order of communities that have the highest to lowest species richness are HL-1D, HL-2D, LL-2D, and LL-1D (Table 2). Species abundance in this study shows something different from species richness where the highest value is indicated by the HL-2D site, while the LL-1D and LL-2D sites are relatively similar.

On the other hand, the Rarefaction value also shows that the highest Ra value is in the LL-2D community. This means that the species at the site are unique, and the majority can only be found in that community.

In addition to the value of species richness, abundance, and rarefaction, Diversity of Microalgae can also be tested with two types of indices, namely the Shannon-Wiener Index (H') and Simpson's Index (λ). Based on the test of the two types of indices, it shows that the microalgae community as a whole has relatively varied diversity, from low to relatively high. Based on the values of the Shannon-Wiener Index and Simpson's Index, the HL-1D and HL-2D sites have relatively high biodiversity, the LL-2D sites are classified as having moderate biodiversity, and the LL-1D sites are grouped as communities with relatively low biodiversity. In general, the biodiversity of freshwater microalgae in a high light environment is higher than that in a low light environment (Table 2).

Evenness values usually show the inverse value of the diversity index value (both in the Shannon-Wiener Index or Simpson's Index). In this study, we used two kinds of evenness index, namely Pilou evenness (J) and Hill's ratios (Ea:b). Although the two indices have different statistical calculations, they both show the same trend where the LL-1D site has a high Pilou evenness and Hill's ratios value, while the HL-1D site shows a low value (Table 1).

Table 2. Species richness, abundance, diversity, and evenness indices of four community of microalgae

Sites	SR	MeI	MaI	SA	Ra	H'	λ	J	$E_{a:b}$
HL-1D	13±0.03	3.15±0.01	4.24±0.02	17±0.21	3.79±0.01	2.48±0.01	0.91±0.01	0.35±0.00	0.19±0.01
HL-2D	11±0.11	2.40±0.00	3.28±0.01	21±0.32	3.64±0.00	2.31±0.02	0.89±0.01	0.37±0.00	0.22±0.00
LL-1D	2±0.02	1.15±0.01	0.91±0.00	3±0.52	2.00±0.00	0.64±0.01	0.44±0.00	0.64±0.01	0.78±0.02
LL-2D	4±0.01	2.00±0.00	2.16±0.01	4±0.31	4.00±0.01	1.39±0.01	0.75±0.01	0.54±0.01	0.53±0.01

Note: SR: Species richness; MeI: Menhinick's index; MaI: Margalef's index; SA Species abundance Ra Rarefaction; H': Shannon-Wiener Index; λ: Simpson's Index; J: Pilou evenness; $E_{a:b}$: Hill's ratios

3.3. Shannon-Wiener index in each dominant species of microalgae

The Shannon-Wiener Index is a biodiversity parameter that is commonly used in several communities of organisms, both organisms in the blood and in the waters. Specifically, on the calculation of the Shannon-Wiener Index in aquatic environments, we tested the level of H' value in several species of microalgae. Species that have a high level of diversity in freshwater microalgae in this study is *Closterium* sp., *Gleocapsa* sp., *Actinastrum* sp., *Volvox* sp., *Coelastrum* sp., *Scenedesmus* sp., and *Pediastrum* sp. (Table 3).

Table 3. Top seven species diversity of microalgae

Code	Species	H’
Sp17	*Closterium* sp.	1.055±0.02
Sp7	*Gleocapsa* sp.	0.693±0.01
Sp11	*Actinastrum* sp.	0.693±0.01
Sp1	*Volvox* sp.	0.637±0.01
Sp3	*Coelastrum* sp.	0.562±0.01
Sp9	*Scenedesmus* sp.	0.562±0.03
Sp12	*Pediastrum* sp.	0.562±0.01

4. Discussion

Microalgae are microorganisms that have a relatively wide distribution in both freshwater and seawater. Microalgae are divided into two types based on the Whittaker classification system, especially from the presence or absence of a nuclear membrane. The two types are prokaryotic microalgae and eukaryotic microalgae (Branco-Vieira *et al*., 2020). Prokaryotic microalgae are classified into two divisions, i.e. Prochlorophyta and Cyanophyta. Eukaryotic microalgae are divided into nine divisions, i.e. Glaucophyte, Rhodophyta, heterokont, Haptophyta, Cryptophyta, Dinoflagellate, Euglenophyta, Chlorarachniophyta, and Chlorophyta (Barsanti and Gualtieri 2006). In this study, we found Division Cyanophyta, Chlorophyta, and Euglenophyta (Figure 1, Table 1).

Microalgae have a lot of potential in their utilization such as biofuels, bioenergy, cosmetics, pharmaceuticals, and health (Ghufran and Kordi, 2010; Chia *et al*., 2018). Especially for the use of microalgae as biofuels and bioenergy, the important macromolecules produced in microalgae are lipids. High lipid content can determine a high level of biofuel potential in microalgae. The use of markers (morphological, anatomical, metabolite, and molecular markers) can be considered to exploit triacylglyceride-producing metabolic pathways in microalgae such as in higher plants.

Microalgae are photosynthetic microorganisms that have the potential to be used for fine chemicals products (Ghufran and Kordi, 2010; Barbera *et al*., 2018), food additives for humans and animals, immobilization systems for the formation of extracellular compounds, heavy metal biosorption, and CO_2 fixation. With an oil content of 77%, microalgae also have the potential to be used as biodiesel, which is an alternative energy source; and based on calculations, microalgae can produce 200 times more oil than other vegetable sources (Litinas *et al.*, 2020). The advantage of microalgal biodiesel is that it is a renewable source (Moshood *et al.*, 2020). In addition, with its location at the equator, Indonesia has a very sufficient source of sunlight as an energy source for photosynthetic microalgae (Gunawan, 2010).

Microalgae are the only source of biodiesel that has the potential to completely replace fossil fuels (Brennan and Owende, 2010; Hajar *et al*., 2017; Pratami *et al*., 2022). Unlike other plants, microalgae grow very rapidly and contain lots of oil/lipids. Microalgae undergo exponential growth in about 3.5 hours. The lipid content of microalgae can exceed 80% of their dry weight. Each microalga has a different amount of lipid content (Gunawan, 2010; Pratami *et al*., 2022). In other organisms, for example in snake fruit, lipid metabolites are relatively diverse (Fendiyanto *et al*., 2020; Fendiyanto et al., 2021).

Microalgae collection in the laboratory can use one or more types of microalgae which are given special nutrients into a culture such as NO^{3-} or PO_4^{3-} and carried out with lamp irradiation (Bold and Wynne, 1985; Moshood *et al*., 2020). The microalgae growing media used must contain inorganic elements in the form of N, P, Fe, and Si (Chisti, 2007). Microalgae culture requires inorganic nutrients in the form of macronutrients including C, H, O, N, P, K, S, Mg, Si, and Ca, while microelements include Fe, Zn, Cu, Na, Mo, Co, B, Mn, Cl and Ni (Agustini, 2006; Hamim, 2007). The main macronutrients in the form of N in the form of NO3⁻ and P in the form of PO43- are needed for microalgae growth, so that these two elements are limiting factors in microalgae growth. Research conducted by Griffiths and Harrison, (2009) reported that the reduction of nitrogen concentration in microalgae media Greens can increase the lipid content from 41% to two times. Elemental P is needed by microalgae in regulating growth and metabolic processes, which is used to compose cell membranes (phospholipids), as a basic material for ATP and nucleic acid synthesis (Theodorou *et al*., 1991; Barbera et al. 2018). Elemental P in the nutrient solution is usually in the form of PO_4^{3-} which will be absorbed by microalgae under conditions that receive a lot of light and in a pH between 6-7 (Lewin 1962). Macronutrients such as

P are important in the formation of proteins. Restriction of P in green microalgae *Selenastrum minutum* reduces its protein content (Theodorou *et al*., 1991). Gunawan (2010) also reported that microalgae produced high lipid content at 0.2 M concentration of N and 0.6 mM of P concentration in BG 11 medium. K element functions in carbohydrate metabolism, Fe and Na elements play a role in the formation of chlorophyll. The elements Si and Ca are materials in the formation of cell walls.

Micronutrients are given in small amounts and must remain with the function of a catalyst during the biosynthetic process to support the growth of organisms. In microalgae growing media, EDTA or citrate is usually added to stabilize the micronutrient function and also functions as a chelator (Widianingsih *et al*., 2008). In addition, the addition of phosphate salts as a buffer solution or buffer solution will cause the pH of the growing medium to become stable (Sidabutar, 1999). Microalgae culture is usually in the pH range between 7 to 9 and the optimum pH is between 8.2-8.7 which will increase the growth rate of microalgae (Abdulazis, 2010). There are many variations of the growing media used on a laboratory scale or cultivation scale with different mineral compositions according to the needs and types of microalgae used. Microalgae culture under exponential phase conditions is usually carried out within 4 to 7 days of inoculation, during which time microalgae should be given medium conditions with optimal nutrient concentrations for microalgae growth (Sutomo *et al*., 2007). Based on research conducted by Gunawan, (2010), it is known that BG 11 medium is the best culture medium for microalgae. According to Gunawan, (2010), BG 11 is usually used to isolate Cyanophyta and freshwater microalgae. BG 11 medium contains NO^{3-} as a source of N, while HPO_4^{2-} and H_2PO^{4-} as a source of P and as buffering agents (Reine and Trono, 2002).

Molecular identification using DNA is not influenced by morphological characteristics, is easier, and accurate (Pandin, 2010; Fendiyanto *et al*., 2019a; Fendiyanto *et al*., 2019b; Satrio *et al*., 2019; Miftahudin *et al*., 2021; Satrio *et al*., 2022; Pratami *et al*., 2022), i.e. gene expression analysis and metabolomic approaches are used in broad studies especially in many crops (Bendjedid *et al*., 2022; Deabes *et al*., 2022; Kamillah *et al*., 2022). One of the molecular markers that can be used in the identification process of microalgae is the 18S rRNA gene. Wang *et al*., (2014) in their study used the 18S rRNA marker which is a ribosomal recommendation genome used to detect eukaryotic microscopic organisms in ecosystems. In this study, we tried to find ecological markers (especially abiotic factors) to predict the level of microalgae diversity.

5. Conclusion

In general, we found differences in species composition or division among the two sites in this research. Specifically, to site HL, we can find all Division Chlorophyta, Cyanophyta, and Euglenophyta. In low light community (LL), we did not find Euglenophyta in our freshwater sample. The most dominant species found in the HL environment were *Volvox* sp., *Chroococcus* sp., *Coelastrum* sp., *Tetraspora cylindrica*, *Mougeutia* sp., *Lepocinclis* sp., *Gleocapsa* sp., *Sphaeroplea* sp., *Scenedesmus* sp., *Cryptoglena* sp., *Actinastrum* sp., *Pediastrum* sp., *Oscilatoria* sp., *Nostoc* sp., *Ulothrix* sp., *Pediastrum* sp., *Microcystis* sp., *Closterium* sp., *Selenastrum* sp., *Centritractus belanophorus*, and *Ankistrodesmus* sp. Species found in the LL environment were *Uronema elongasi*, *Bulbochaeta* sp., *Pleurotaenium* sp., and *Zygnema* sp. Microalgae communities in high light communities have higher diversity than low light communities. This study can be used as a reference for the diversity of microalgae in two different types of environments, especially in the tropics and in freshwater microalgae communities. This diversity data could be a reference for researchers and provide preliminary information of microalgae potency as alternative biofuels in the future.

Acknowledgements

This research was funded by Ministry of Defense, the Republic of Indonesia through Dr. Mentari Putri Pratami, S.Si., M.Si fiscal year 2021 through LP2M, particularly the Republic of Indonesia Defense University (RIDU) Funding. We thank Laboratory of Plant Physiology and Genetics, Department of Biology, IPB University for supporting facilities to algal culture.

References

Abdulazis SNB. 2010. A Cultivation of *Chlorella vulgaris* under Heterotrophic Condition for Growth and Lipid Production in Various Waste. Universiti Teknologi Malaysia Press, Kuala Lumpur.

Agustini R. 2006. Utilization of thermophilic proteases that live in the hot springs of Batu Cangar Malang. *Indones J Chem.*, **6(2)**: 205-211.

Barbera E, Bertucco A and Kumar S. 2018. Nutrients recovery and recycling in algae pro- cessing for biofuels production. *Renew Sustain Energy Rev.*, **90**: 28-42.

Barsanti L and Gualtieri P. 2006. Microalgae. CRC Press-Taylor & Francis, Paris.

Becker. 1994. Microalge Biotechnology and Microbiology. Cambridge University Press, New York.

Bendjedid S, Bazine I, Tadjine A, Djelloul R, Boukhari A and Bensouici C. 2022. Analysis of phytochemical constituents by using LC-MS, antifungal and allelopathic activities of leaves extracts of aloe vera. *Jordan J Biol Sci.*, **15(1):** 21-28.

Bold HC and Wynne MJ. 1985. Introduction to the Algae Structure and Reproduction Second edition. Prentice-Hall Inc, New Jersey.

Branco-Vieira M, Costa D, Mata TM, Martins AA, Freitas MAV and Caetano NS. 2020. A life cycle inventory of microalgae-based biofuels production in an industrial plant concept. *Energy Rep.*, **6**:397-402.

Brennan L and Owende P. 2010. Biofuels from microalgae- a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew Sustain Energy Rev.*, **14**: 557–577.

Chia SR, Chew KW, Show PL, Yap YJ, Ong HC, Ling TC and Chang J. 2018. Analysis of economic and environmental aspects of microalgae biorefinery for biofuels production: a review. *Biotechnol J*., **13(6):** 1700618.

Chisti Y. 2007. Biodiesel from microalgae. *J Biotech Advances.*, **25**: 294–306.

Deabes MM, Khalil WKB, SKh AEA, Ibrahim SD and Naguib KM. 2022. The Inhibitory Effect of Different Ephedra Plant Extracts on the Aspergillus flavus Growth and Aflatoxin B1 Gene Expression. *Jordan J Biol Sci.*, **15(1):** 59-66.

Fendiyanto MH, Satrio RD, Suharsono, Tjahjoleksono A and Miftahudin. 2019a. Correlation among Snpb11 markers, root growth, and physiological characters of upland rice under aluminum stress. *Biodiversitas.*, **20(5):** 1243-1254.

Fendiyanto MH, Satrio RD, Suharsono, Tjahjoleksono A, Hanarida I and Miftahudin. 2019b. QTL for aluminum tolerance on rice chromosome 3 based on root length characters. *SABRAO J Breed Genet.*, **51(4):** 451-469.

Fendiyanto MH, Satrio RD and Darmadi D. 2020. Metabolic profiling and pathway analysis in red arillus of Salacca sumatrana demonstrate significant pyruvate, sulfur, and fatty acid metabolisms. *Biodiversitas.*, **21(9):** 4361-4368.

Fendiyanto MH, Satrio RD, Widana IDKK, Pratami MP, Nikmah IA and Darmadi D. 2021. Differential hierarchical metabolites expression of red/white Salacca sumatrana arillus and its molecular docking studies. *Biodiversitas.*, 22(2): 1014-1024.

Griffiths MJ and Harrison STL. 2009. Lipid productivity as a key characteristic for choosing algal species for biodesel production. *J Appl Phycol.*, **21**:493- 507.

Ghufran A and Kordi L. 2010. Aquatic Bioata Cultivation for Food, Cosmetics, and Medicine. Andi, Yogyakarta.

Gunawan. 2010. Diversity and characterization of microalgae from hot springs in West Java which are potential sources of biodiesel. Thesis, IPB University, Bogor.

Hajar HAA, Riefler RG and Stuart BJ. 2017. Cultivation of the microalga Neochloris oleoabundans for biofuels production and other industrial applications. *Appl Biochem Microbiol.*, **53(6):** 640-653.

Hamim. 2007. Fisiologi Tumbuhan. Universitas Terbuka Press, Jakarta.

Handoko, Sugiarto Y and Syaukat Y. 2008. The Linkage of Climate Change and Strategic Food Production: An Independent Policy Review in the Sector of Trade and Development. SEAMEO Biotrop, Bogor.

Kamillah WO, Kusumaningrum HP, Ferniah RS, Herida AP and Nasansia GL. 2022. Physiological response and detection of *Inh2* gene in dieng red potato (*Solanum tuberosum* L.) Affected by Frost. *Jordan J Biol Sci.*, **15(1):** 101-106.

Lander JP. 2014. R for Everyone: Advanced Analytics and Graphics. Addison-Wesley, Boston.

Litinas A, Geivanidis S, Faliakis A, Courouclis Y, Samaras Z, Keder A, Krasnoholovets V, Gandzha I, Zabulonov Y, Puhach O and Dmytriyuk M. 2020. Biodiesel production from high FFA feedstocks with a novel chemical multifunctional process intensifier. *Biofuel Res J.*, **26**: 1170-1177.

Mahyudin AR and Koesnandar. 2006. Biohydrogen production: prospects and limitations to practical application. *Akta Kimia Indonesia.*, **1**:73-78.

Metzger P and Largeau C. 2005. *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. *Appl Microbiol Biotech.*, **66(5):** 486–496.

Miftahudin M, Roslim DI, Fendiyanto MH, Satrio RD, Zulkifli A, Umaiyah EI, Chikmawati T, Sulistyaningsih YC, Suharsono S, Hartana A, Nguyen HT and Gustafson JP. 2021. *OsGERLP*: A novel aluminum tolerance rice gene isolated from a local cultivar in Indonesia. *Plant Physiol Biochem.*, **162**: 86-99.

Moshood TD, Nawanir G and Mahmud F. 2020. Microalgae biofuels production: A systematic review on socioeconomic prospects of microalgae biofuels and policy implications. *Environmental Challenges.*, **5**: 1-18.

Nybakken JW. 1982. Marine Biology, An Ecologycal Approach. Harper and Row Publisher, New York.

Pandin DS. 2010. Penanda DNA untuk pemulian tanaman kelapa (*Cocos nucifera* L). *Jurnal Perspektif.*, **9(1):** 21-35.

Paynter D and Frölicher TL. 2015. Sensitivity of radiative forcing, ocean heat uptake, and climate feedback to changes in anthropogenic greenhouse gases and aerosols. *J Geophys Res Atmos.*, **120**: 9837-9854.

Peng Y, Fan M, Song J, Cui T, and Li R. 2018. Assessment of plant species diversity based on hyperspectral indices at a fine scale. *Sci Rep*., **8**: 4776.

Pratami MP, Fendiyanto MH, Satrio RD, Nikmah IA, Awwanah M, Farah N, Sari NIP, Nurhadiyanta N. 2022. In-silico Genome Editing Identification and Functional Protein Change of Chlamydomonas reinhardtii Acetyl-CoA Carboxylase (CrACCase). *Jordan J Biol Sci.*, **15(5):** 431-440.

Prartono T, Kawaroe M and Katili V. 2013. Fatty acid composition of three diatom species *Skeletonema costatum*, *Thalassiosira* sp. and *Chaetoceros gracilis*. *Int J Environ Bioenergy.*, **6(1):** 28-43.

Prescott GW. 1978. How to Know the Freshwater Algae, Third edition. Wm C Brown Company Publishers, New York.

Rao AR, Dayananda C, Sarada R, Shamala TR and Ravishankar GA. 2007. Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. *Biores Technol*., **1(98):** 560–564.

Reine WFP and Trono JGC. 2002. Cryptograms: Algae. Prosea Foundation, Bogor.

Richmond A and Emeritus C. 2013. Handbook of Microalgal Culture Applied Phycology and Biotechnology. Wiley-Blackwell, New York.

Satrio RD, Fendiyanto MH, Suharsono, Supena EDJ and Miftahudin. 2019. Identification of drought-responsive regulatory genes by hierarchical selection of expressed sequence tags and their expression under drought stress in rice. *Intl J Agric Biol.*, **22(6):** 1524-1532.

Satrio RD, Fendiyanto MH, Suharsono, Supena EDJ and Miftahudin. 2021. Genome-wide SNP discovery, linkage mapping, and analysis of QTL for morpho-physiological traits in rice during vegetative stage under drought stress. *Physiol Mol Biol Plants*., **27(11):** 2635-2650.

Shayler HA and Siver PA. 2006. Key to freshwater algae: a web-based tool to enhance understanding of microscopic biodiversity. *J Sci Educ Technol*., **15**: 298–303.

Sidabutar EA. 1999. The Effect of the Type of Microalgae Growth Medium *Chlorella* sp. to the Growth-Promoting Compounds Produced. IPB University Pr, Bogor.

Sutomo A, Ani G and Sutomo G. 2007. Kultur tiga jenis mikroalga (*Tetraselmis* sp., *Chlorella* sp.dan *Chaetoceros gracilis*) dan pengaruh kepadatan awal terhadap pertumbuhan *C. Gracilis* di laboratorium. *Oseano Limnol*., **37**: 43-58.

Teresa M, Anto´nio A, Martins and Nidia SC. 2010. Microalgae for biodiesel production and other application. *Biotech.*, **14**:217–232.

Theodorou ME, Elrifi IR, Turpin DH and Plaxton WC. 1991. Effects of phosphorus limitation on respiratory metabolism in the green alga *Selenastrum minutum*. *Plant Physiol.*, 95: 1089-1095.

Wang Y, Tian RM, Gao ZM, Bougoffa S and Qian PY. 2014. Optimal eukaryotic 18S and universal 16S/18S ribosomal RNA primers and their application in a study of symbiosis. *PloS One*., **9(3):**e90053.

Widianingsih, Ridho A, Hartati R and Harmoko. 2008. Kandungan nutrisi Spirulina platensis yang dikultur pada media yang berbeda. Ilmu Kelautan., **3:** 167-170.

Williams RG, Roussenov V, Goodwin P, Resplandy L and Bopp L. 2017. Sensitivity of global warming to carbon emissions: effects of heat and carbon uptake in a suite of earth system models. *J Climate.*, **12:**9343–9363.