

Biodiversity and distribution of freshwater microalgae from the black-water ecosystem of Rungan River, Central Kalimantan, Indonesia

LISWARA NENENG¹, KIKI LOUISA², YULIANA^{3,✉}, ZAHROTUN NAFISAH³, DIAH RADINI NOERDJITO⁴

¹Departemen of Biology, Faculty of Mathematic and Natural Science, Universitas Palangka Raya. Jl. Hendrik Timang, Palangka, Jekan Raya, Palangka Raya 74874, Central Kalimantan, Indonesia

²Departemen of Biology Education, Postgraduate Programme, Universitas Palangka Raya. Jl. Hendrik Timang, Palangka, Jekan Raya, Palangka Raya 74874, Central Kalimantan, Indonesia

³Departemen of Chemistry, Faculty of Mathematic and Natural Science, Universitas Palangka Raya. Jl. Hendrik Timang, Palangka, Jekan Raya, Palangka Raya 74874, Central Kalimantan, Indonesia. Tel./fax.: +62-857-4820842, ✉email: yuliana@mipa.upr.ac.id

⁴Research Center for Biosystematics and Evolution National Research and Innovation Agency of Republic Indonesia (BRIN). Jl. Raya Jakarta-Bogor Km. 46, Cibinong, Bogor 16911, West Java, Indonesia

Manuscript received: 14 November 2024. Revision accepted: 9 April 2025.

Abstract. *Neneng L, Louisa K, Yuliana, Nafisah Z, Noerdjito DR. 2025. Biodiversity and distribution of freshwater microalgae from the black-water ecosystem of Rungan River, Central Kalimantan, Indonesia. Biodiversitas 26: 1789-1798.* The black-water ecosystem is strongly influenced by the physical appearance of peatlands, which include low pH, low Dissolved Oxygen (DO), high Dissolved Organic Carbon (DOC), Biological Oxygen Demand (BOD), and Chemical Oxygen Demand (COD), high tannins, and low nutrients. Identifying microalgae from this extreme environment enables us to isolate species that are naturally tolerant and exhibit unique features. This research aimed to explore, identify, and find information on the distribution of freshwater microalgae species from the black-water ecosystem in Rungan River of Central Kalimantan, Indonesia. This research also investigated the physicochemical parameters of the river to observe their impacts on microalgae distribution. There were 15 study sites in this research, including five main rivers (Rungan Rivers), five small rivers (Anjung, Tabatik, Panya, Karahang and Sahang), and five lakes (Humbang, Kuning, Petuk Uwah, Tahai Hulu and Rasau). Water samples were collected, and physicochemical parameters were measured and analyzed using standard protocols. The results showed that 59 species of freshwater microalgae were found in the study sites. These species belong to the classes Bacillariophyceae, Mediophyceae, Chlorophyceae, Zygnematophyceae, Charophyceae, Xantophyceae, Cyanophyceae, and Euglenoidea. *Chlorella* sp. and *Clostridium* sp. were discovered in all of study sites, while *Botryococcus brownii* and *Spirulina* sp. were only discovered at one site. The biodiversity index (H': 2.81) was included in the medium category. The highest abundance of microalgae species was found in lakes, and the lowest number was in the main rivers. The distribution of many microalgae (61%) in the black-water ecosystem was found in the pH range of 4.4-6.0. Besides, 71% of microalgae in the black-water ecosystem prefer to live at a temperature range of 28-32°C. Only *Anabaena* sp. was discovered at a temperature of 26-34°C. Principal Component Analysis (PCA) results showed that environmental parameters, including pH and nitrate, were the dominant factors that influenced the distribution and abundance of microalgae in this ecosystem.

Keywords: Biodiversity, black-water ecosystem, microalgae, PCA analysis, peatland

INTRODUCTION

Black-water ecosystem is a unique aquatic environment which strongly influenced by the characteristics of peatlands, which include low pH, low Dissolved Oxygen (DO), high Dissolved Organic Carbon (DOC), Biological Oxygen Demand (BOD), and Chemical Oxygen Demand (COD), high tannins, and low nutrients (Swaleh et al. 2022). Microalgae represent an extraordinarily diverse but highly specialized group of microorganisms that adapt to various ecological habitats. Their primary habitats are freshwater, brackish, and marine ecosystems. Microalgae are eukaryotic microorganisms that can be found in various aquatic habitats with sufficient sunlight (Sari et al. 2019; Liao et al. 2020). Microalgae are incredibly diverse, and their species are estimated to be about 200,000-800,000 (Undan et al. 2021). Indonesian microalgae have a biodiversity of up to 2060 species (Andriani et al. 2024). Microalgae play an important role in aquatic ecosystems as

the primary producers and the main link in the food chain (Nava and Leoni 2021). The ability to do photosynthesis makes microalgae the primary supplier of dissolved oxygen in the water. Furthermore, they can be used as a bioindicator to assess the environmental health status (O'Neill and Rowan 2022). They also have benefits for the sustainability of human needs in various fields, such as nutritional sources (Koyande et al. 2019; Noerdjito 2019), biorefinery (Bayu et al. 2020), bioremediation agents (Neneng et al. 2020), cosmetics (Bayu et al. 2023), and biological indicators of environmental quality (Poot-Delgado et al. 2015; Prabowo et al. 2023). In China, people have long utilized microalgae as food sources, including *Arthrospira*, *Nostoc*, and *Aphanizomenon* (Prihanto et al. 2022).

Identifying microalgae from this extreme environment enables us to isolate naturally tolerant species and exhibit unique characteristics. In particular, the correlation of ecological parameters with the physicochemical

characteristics of the sampling site greatly influences the diversity of microalgal morphophytes in water bodies (Severes et al. 2018; Dasgupta et al. 2024). Besides, the elevating values of physicochemical parameters (temperature, pH, Total Dissolved Solids (TDS), alkalinity, nitrate, phosphate, DO, etc.) supported by phytoplankton in nutrient-rich waters provide an idea of the trend of water bodies toward eutrophication. In any aquatic ecosystem, the growth, density, and abundance of phytoplankton are primarily governed by interactions between environmental factors and biotic entities (Naik et al. 2010). The influx of freshwater and tidal activity are abiotic interactions that can play crucial roles in phytoplankton growth and their abundance in the estuary (Dasgupta et al. 2024). The constant nutrient supply always supports the rich phytoplankton production. Still generally, nitrogen (N) and phosphorus (P) have been considered as the potentially limiting nutrients for phytoplankton growth in aquatic ecosystems (O'Neill and Rowan 2022).

The biodiversity of microalgae in the world has been estimated to include millions of species. Still, many of them have not yet been identified. Studies related to the distribution of microalgae in black-water ecosystems have also not yet been widely reported. Meanwhile, environmental factors that play a role in the distribution and abundance of microalgae are critical to understanding to increase the potential for optimal microalgae utilization. Accordingly, this research aimed to explore, identify, and find information on the distribution of freshwater microalgae species from the black-water ecosystem of Rungan River in Central Kalimantan, Indonesia. The physicochemical parameters of the river were also investigated to observe their impacts on microalgae distribution. Further, this research was done to explore the presence and occurrence of native microalgae as promising strains for many purposes. Finally, the results hopefully can provide a scientific contribution regarding the ecology of microalgae in the black-water ecosystems, including in lakes, rivers, or swamps.

MATERIALS AND METHODS

Study area

This study area was in the Rungan River located in Central Kalimantan, Indonesia (Figure 1), especially flowing along the districts of Palangka Raya, Katingan, and Gunung Mas. There were 15 sites along the river for the study. They included five main rivers, five small rivers, and five lakes, as shown in Figure 2 and Table 1. The selected of study sites based on the different characteristics they possess, such as current strength, water pH, and the availability of nutrient. Three sampling points were selected from each location for the study.

Procedures

Sample collection and analysis

The microalgae sampling was conducted by taking water along the Rungan River, at a depth of around 20-30 cm using 1 L of polyethylene bottle. Clean labeled polyethylene bottles were used to sample 50 mL of concentrate sampled using 5-20 micron phytoplankton net to obtain microalgae cells. Filtered microalgae were put in a 50 mL bottle, preserved with 4% Lugol, and stored in a cooler box for further identification. The pH, temperature, TDS, and brightness of water were measured onsite via Advantec® pH test paper, water thermometer, TDS meter, and lux meter, respectively. Meanwhile, the parameters such as phosphate, nitrate, BOD, COD, and DO were analyzed in the Laboratory of Regional Health Center (Labkesda) of Central Kalimantan. Cell density (cells mL) of microalgae was determined using the drop count method (Bartram and Rees 2000). The microalgae were identified based on their morphological characteristics and enumerated using Olympus light microscope BX5 and Camera Olympus DP26. The identification referred to the Handbook of Identification, Enumeration, and Use as Bioindicators (Edward and David 2015).

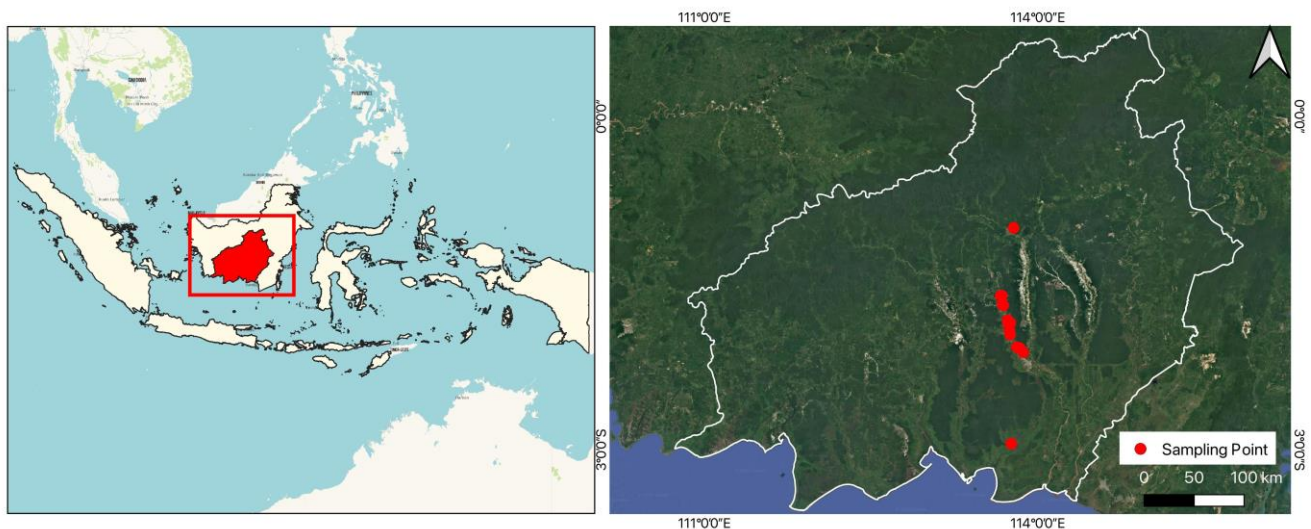


Figure 1. Study sites in Rungan River, Central Kalimantan, Indonesia

Table 1. Sampling sites and coordinates

Sampling locations	Coordinate sites	Describe of site
Rungan River 01	1°00'11.358"S, 113°47'1.263"E	Width of rivers up to 250 meters, and a depth of 10 m or more; pH between 4.12-5.09
Rungan River 02	2°04'34.0"S, 113°48'46.1"E	
Rungan River 03	2°07'20.0"S, 113°52'15.2"E	
Rungan River 04	2°56'39.503"S, 113°45'45.149"E	
Rungan River 05	2°05'06.2"S, 113°50'23.9"E	
Small River Anjung	1°49'56.377"S, 113°43'52.453"E	Width of rivers between 5 and 7 meters, and a depth ranging from 5 to 7 m; pH between 4.12-6.22
Small River Tabatik	1°41'25.679"S, 113°41'0.725"E	
Small River Panya	1°42'0.772"S, 113°41'23.284"E	
Small River Karahang	1°51'45.817"S, 113°44'8.279"E	
Small River Sahang	1°51'24.781"S, 113°45'16.995"E	
Lake Humbang	1°36'44.265"S, 113°40'25.472"E	Depth of lakes ranging from 5 to 7 meters, with a width of between 10 and 15 meters; pH between 4.80-6.54
Lake Kuning	1°36'51.859"S, 113°40'0.692"E	
Lake Petuk Uwah	1°36'51.536"S, 113°40'38.604"E	
Lake Tahai Hulu	1°55'56"S, 113°44'47"E	
Lake Rasau	1°57'45.921"S, 113°45'6.376"E	

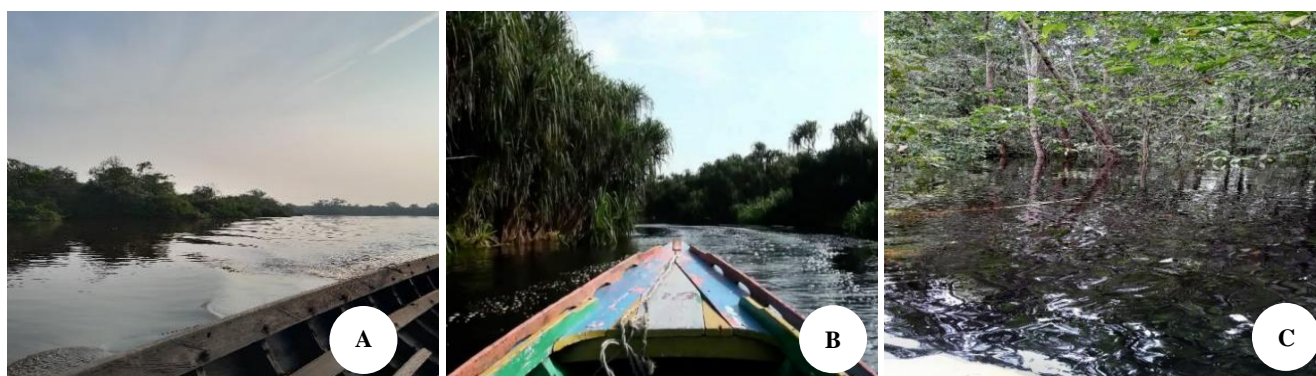


Figure 2. Sampling sites in Rungan River, Central Kalimantan, Indonesia. A. Main rivers; B. Small rivers; C. Lakes

Data analysis

Principal Component Analysis (PCA) was applied to determine the parameters that are strongly associated with each other. PCA was chosen to reduce the dimension of complex data sets, which are correlated with each other, so that new variables (called principal components) are smaller. The biodiversity index was estimated using Shannon-Weaver assuming individual species were sampled randomly from a large population (APHA 2005) using Equation:

$$H' = - \sum_{n=1}^s P_i \ln P_i$$

Where:

- H' : Shannon-Weaver diversity index
- S : Total species
- Pi : Relative abundance of each species (ni/N)

The criteria for this diversity index are: H' < 1 considered low; 1 < H' < 3 is in the middle; H' > 3 is high.

RESULTS AND DISCUSSION

Environmental parameters

The study sites were divided into three zones in the black-water ecosystem. First are the main river zones (large rivers) characterized by a flowing river, with a width ranging from 10 to 15 meters, and a depth of 10 m. The second zones are small rivers or tributaries that flows into a large river. The current conditions in these zones are categorized as the low to medium category, with a tributary width between 5 and 7 meters, and a depth ranging from 5 to 7 m. The third zone is the lakes that remain in the Rungan River basin. The lakes were formed from a relative deep basin, ranging from 5 to 7 meters, with a width of between 10 and 15 meters. The lakes were lentic water bodies, because the water flow was very slow or even non-existent. Of the total 15 study sites, they included five main rivers (Rungan River 01, Rungan River 02, Rungan River 03, Rungan River 04, and Rungan River 05), five small rivers (Anjung, Tabatik, Panya, Karahang, and Sahang), and five lakes (Humbang, Kuning, Petuk Uwah, Tahai Hulu, and Rasau). The environmental parameters are shown in the Table 2.

According to Table 2, the environmental temperatures of the study sites were not significantly different, and they have close values. However, in terms of acidity, the pH values of all sites were acidic, ranging from 4.12 to 6.5. Nevertheless, the main rivers had relatively lower pH compared to small rivers and lakes. TDS values in all sites still comply with water quality standards (Omer 2019).

pH is one of the most critical environmental conditions in microalgal cultivation since it determines the solubility and availability of CO₂ and nutrients, and it has a significant influence on microalgal metabolism. Each microalgal species has an optimal pH range for biomass and lipid production, which is narrow and strain-specific. The pH of microalgal cultures rises gradually during the day due to the uptake of inorganic carbon by microalgae. Higher pH limits the availability of CO₂, thus inhibiting cell growth. On the other hand, algal cultivation at high pH can suppress undesired biological contaminants (Qiu et al. 2017).

In an aquatic ecosystem, the amount of DO is chiefly influenced by temperature, photosynthetic activity, respiration, and richness of organic matter. DO concentrations in the lakes and small rivers were sufficient to support microalgae growth, except in the main rivers, where the value was below the standard of 6.5 mg mg L⁻¹ (Figure 3). DO concentration was found to have a negative effect on microalgal growth. CO₂ concentrations of more 30 g/m³ were known to induce a loss of biomass productivity of 30% in *Chlorella vulgaris* (Kazbar et al. 2019). However, since 2003 algal populations in the Tualatin River have tended to be smaller, and algal blooms have terminated earlier compared to conditions in the 1990s. This resulted in a further decrease in DO concentrations. Consequently, the DO levels did not meet the standard in countries worldwide (Kazbar et al. 2019). The low levels (6.2 mg L⁻¹) of DO at the main Rungan River suggests a higher density of aerobic microorganisms and micro-fauna. Further, it is also a sign that the water is moderately polluted, so it is slightly turbid, and anoxic conditions occur in the water body (Purbani et al. 2021).

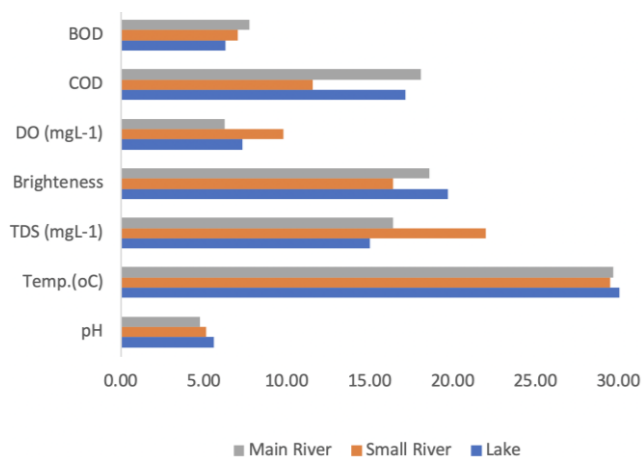


Figure 3. Physicochemical parameters at lakes, small rivers, and main rivers

The concentration of nitrates and phosphates was relatively low in all sites. However, the nitrate concentrations in the lake sites were relatively higher compared to small rivers and main rivers (Figure 4). It is reported that a nitrate level of 0.3 mg L⁻¹ is sufficient to stimulate algae growth (Vazirzadeh et al. 2022). The acceptable natural nitrate-nitrogen concentration in surface waters is less than 1 mg L⁻¹, and the maximum permissible level of nitrate-nitrogen in drinking water is 10 mg L⁻¹. The permissible nitrite nitrogen level in public water supply is 1 mg L⁻¹. Meanwhile, the minimum phosphorus level for stimulating algal growth in quiescent lakes is around 0.015 mg L⁻¹ (Metcalf and Eddy 1991), equivalent to a phosphate (PO₄³⁻) concentration of 0.046 mg L⁻¹. Similarly, nitrogen discharges must be minimized. Mainisa et al. (2023) stated that microalgae species in these habitat are influenced by phosphate nutrition rather than nitrate.

Microalgae biodiversity indices

A total of 59 species of freshwater microalgae were discovered in the black-water ecosystem along the Rungan River. These species belonging to classes Bacillariophyceae, Mediophyceae, Chlorophyceae, Zygnematophyceae, Charophyceae, Xantophyceae, Cyanophyceae, and Euglenoidea, as shown in Table 3. According to Table 3, 36 genera of microalgae were found in the black-water ecosystem of Rungan River, and most of them belong to the classes Chlorophyta (15 genera), Bacillariophyta (9 genera), Charophyta (4 genera), Chrysophyta (4 genera), Cyanophyta (3 genera) and Euglenophyta (1 genera). The Chlorophyta group had the highest composition and abundance of microalgae, as presented in Figure 5. Among the 36 genera found in this research, only about 30% were discovered in all study sites, while about 70% existed in different sites. This means the composition and distribution of all microalgae found only had 30% similar in all six sites in the black-water ecosystem in the Rungan River. Most of the genera existing in all sites mainly belong to Chlorophyceae (Figure 5).

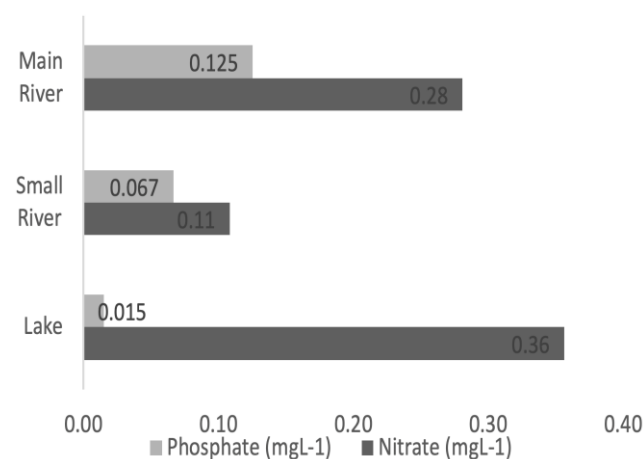


Figure 4. Physicochemical parameters of main rivers, small rivers, and lakes in the black-water ecosystem

Table 2. The environmental parameters of study sites in Rungan River, Central Kalimantan, Indonesia

Location	Number of microalgae species	Physicochemical parameters								
		pH	Temperature (°C)	TDS (mg L ⁻¹)	Brightness (cm)	DO (mg L ⁻¹)	Nitrate (mg L ⁻¹)	Phosphate (mg L ⁻¹)	COD	BOD
Lake Humbang	19	6±0.71	30 ±1.88	12±3.08	25.5±5.11	7.11±0.30	0.282±0.17	0.024±0.008	12.2±7.65	6.28±0.83
Lake Kuning	8	6.54±0.71	31.5±1.88	15±3.08	16±5.11	7.02±0.30	0.497±0.17	0.005±0.008	11.7±7.65	6.83±0.83
Lake Petuk Uwah	8	5.75±0.71	32.4±1.88	19±3.08	17±5.11	7.68±0.30	0.194±0.17	0.016±0.008	10.9±7.65	4.93±0.83
Lake Tahai Hulu	10	4.8±0.71	28.2±1.88	12±3.08	15±5.11	7.32±0.30	0.584±0.17	<0,005±0.008	26.3±7.65	7.03±0.83
Lake Rasau	14	5.03±0.71	28.3±1.88	17±3.08	25±5.11	7.64±0.30	0.224±0.17	0.016±0.008	24.7±7.65	6.58±0.83
Small River Anjung	14	4.35±0.93	29±0.48	22±6.44	27±7.02	4.88±7.12	<0,003±0.073	0.149±0.07	18.4±5.87	6.68±0.56
Small River Tabatik	8	6.22±0.93	29±0.48	18±6.44	10±7.02	21.9±7.12	0.159±0.073	0.013±0.07	6.91±5.87	7.65±0.56
Small River Panya	6	5.91±0.93	30±0.48	17±6.44	13±7.02	6.67±7.12	0.004±0.073	0.142±0.07	17.6±5.87	7.68±0.56
Small River Karahang	8	5.2±0.93	29.5±0.48	20±6.44	12±7.02	10.5±7.12	0.156±0.073	0.014±0.07	7.02±5.87	6.67±0.56
Small River Sahang	3	4.12±0.93	29.9±0.48	33±6.44	20±7.02	5.1±7.12	0.115±0.073	0.015±0.07	8.03±5.87	6.58±0.56
Rungan River 01	2	5.09±0.38	28.9±1.16	13±5.08	18±4.67	8.32±1.31	0.442±0.13	0.196±0.07	28.8±6.22	7.38±0.43
Rungan River 02	2	4.92±0.38	29.5±1.16	17±5.08	20±4.67	6.71±1.31	0.402±0.13	0.129±0.07	17±6.22	7.48±0.43
Rungan River 03	3	4.8±0.38	31.7±1.16	25±5.08	25±4.67	4.93±1.31	0.183±0.13	0.052±0.07	13.7±6.22	8.23±0.43
Rungan River 04	2	4.97±0.38	28.9±1.16	13±5.08	18±4.67	5.82±1.31	0.183±0.13	0.053±0.07	17.1±6.22	8.22±0.43
Rungan River 05	2	4.12±0.38	29.4±1.16	14±5.08	12±4.67	5.58±1.31	0.194±0.13	0.197±0.07	13.8±6.22	7.49±0.43

Table 3. Microalgae biodiversity in the black-water ecosystem of Rungan River, Central Kalimantan, Indonesia

Divisio/Classes	Ordo	Family	Genera	Spesies
Bacillariophyta				
Bacillariophyceae	Naviculales	Pinnulariaceae	<i>Pinnularia</i>	<i>Pinnularia</i> sp. <i>Pinnularia viridis</i> <i>Pinnularia gibba</i>
		Stauroneidaceae	<i>Stauroneis</i>	<i>Stauroneis anceps</i> <i>Stauroneis smithii</i>
	Eunotiales	Brachysiraceae	<i>Brachysira</i>	<i>Brachysira</i> sp.
		Eunotiaceae	<i>Eunotia</i>	<i>Eunotia valida</i> <i>Eunotia</i> sp.
	Striatellales	Striatellaceae	<i>Grammatophora</i>	<i>Grammatophora</i> sp.
	Bacillalales	Bacillariaceae	<i>Nitzschia</i>	<i>Nitzschia</i> sp.
	Cymbellales	Cymbellaceae	<i>Cymbella</i>	<i>Cymbella</i> sp.
	Pennales	Diatomaceae	<i>Synedra</i>	<i>Synedra</i> sp. <i>Synedra ulna</i>
Mediophyceae	Stephanodiscales	Stephanodiscaceae	<i>Cyclotella</i>	<i>Cyclotella</i> sp.
Chlorophyta				
Chlorophyceae	Chlorococcales	Oocystaceae	<i>Chlorella</i>	<i>Chlorella</i> sp.
		Scenedesmaceae	<i>Scenedesmus</i>	<i>Scenedesmus</i> sp. <i>Scenedesmus longispina</i>
		Desmidiaceae	<i>Cosmarium</i> <i>Staurastrum</i>	<i>Cosmarium</i> sp. <i>Staurastrum</i> sp. <i>Staurastrum margaritaceum</i>
	Zygnematales	Zygnemataceae	<i>Closterium</i> <i>Spirogyra</i>	<i>Closterium</i> sp. <i>Spirogyra</i> sp.
	Zygnematophyceae	Desmidia	<i>Euastrum</i> <i>Xanthidium</i>	<i>Euastrum</i> sp. <i>Xanthidium</i> sp.
	Volvocales	Chlamydomonadaceae	<i>Chlamydomonas</i>	<i>Chlamydomonas</i> sp.
	Speropheaales	Selenastraceae	<i>Ankistrodesmus</i>	<i>Ankistrodesmus</i> sp.
	Trebouxiophyceae	Botryococcaceae	<i>Botryococcus</i>	<i>Botryococcus</i> sp.
	Zygnematales	Mesotaeniaceae	<i>Mesotaenium</i>	<i>Mesotaenium</i> sp.
		Platymonaceae	<i>Platymonas</i>	<i>Platymonas</i> sp.
Zygnematophyceae	Zygnemataceae	Zygnematales	<i>Zygnema</i>	<i>Zygnema</i> sp.
Charophyta				
Charophyceae	Zygnematales	Zygnemataceae	<i>Mougeotia</i>	<i>Mougeotia</i> sp.
	Desmidiales	Desmidiaceae	<i>Bambusina</i>	<i>Bambusina borleri</i>
Chrysophyta				
	Pennales	Naviculaceae	<i>Naviculla</i>	<i>Naviculla</i> sp. <i>Navicula protracoides</i>
	Fragilariales	Fragilariaceae	<i>Fragilaria</i>	<i>Fragilaria</i> sp.
	Coscinodiscophycidae	Melosirales	<i>Melosira</i>	<i>Melosira</i> sp.
Xantophyceae	Vancheriales	Tribonemaceae	<i>Tribonema</i>	<i>Tribonema</i> sp.
Cyanophyta				
Cyanophyceae	Oscillatoriales	Nostoceceae	<i>Anabaena</i>	<i>Anabaena</i> sp.
	Chroococcales	Chroococcaceae	<i>Choroococcus</i>	<i>Choroococcus</i> sp.
Oscillatoriophycidae	Oscillatoriales	Oscillatoriaceae	<i>Oscillatoria</i>	<i>Oscillatoria</i> sp.
Euglenophyta				
Euglenoidea	Euglenida	Phacaceae	<i>Phacus</i>	<i>Phacus</i> sp.

The microalgae biodiversity index in the black-water ecosystem of Rungan River was at a moderate level (H' : 2.805499981). It was higher than that in the waters of Lake Dendam, South Sumatra, which is below 1 (classified as low level) (Wijnana et al. 2023). Still, they are both influenced by peat. One factor causing the diversity and abundance of microalgae in the black-water ecosystem of

Central Kalimantan is the value of water pH. The average pH in Lake Dendam is below 6. In contrast, at several sampling sites, the pH values were above 6, even though the nitrate content in the Rungan River was, on average, lower than in Lake Dendam. Based on these results, it is suspected that in aquatic environments, water pH plays a more critical role in the distribution of microalgae.

Distribution of species microalgae in all location

Microalgae with the species Chlorophyta (*Chlorella* sp., *Closterium* sp., *Scenedesmus* sp., *Chlamydomonas* sp.), Cyanophyta (*Anabaena* sp., *Oscillatoria* sp.), Bacillariophyta (*Pinnularia* sp.), and Chrisophyta (*Navicula* sp.), were often discovered in almost all sampling sites. Among them, *Chlorella* sp. and *Closterium* sp. were the most dominant species. The representation of microalgae species from the black-water ecosystem is shown in Figure 5. This study showed that *Chlorella* sp. was a type of microalgae found in almost all study sites (Figure 5). The abundance of *Chlorella* sp. and *Chlamydomonas* sp. (Figure 6) is one of the indicators that influence environmental quality. *Chlorella* species mostly live in fresh water and are especially common in very nutrient-rich waters. They are also often found growing in soil. More than 100 *Chlorella* species have been described from freshwater, marine, and soil habitats (Krienitz 2009). *Chlorella* sp. is a microalga that has potential for food supplements, pharmaceuticals, animal feed, aquaculture, and cosmetics. It also commonly grows in seawater (Widayat et al. 2018). It is a green unicellular alga commercially produced and distributed worldwide as a dietary supplement. Its products contain numerous nutrients and vitamins, including D and B₁₂, that are absent in plant-derived food sources. *Chlorella* contains larger amounts of folate and iron than other plant-derived foods. *Chlorella* supplementation in mammals, including humans, has been reported to exhibit various pharmacological activities, including immunomodulatory, antioxidant, antidiabetic, antihypertensive, and antihyperlipidemic activities (Bito et al. 2020).

Microalgae distribution in three water zone

Figure 7 demonstrates that the highest abundance of microalgae species in the black-water ecosystem was found in the lakes. Meanwhile, the lowest number was discovered in the main rivers. According to Krustok et al. (2015), the inoculation of the reactor with lake water had significant benefits on the overall growth of microalgae biomass. The most abundant algal phyla in these reactors were Chlorophyta with *Scenedesmus*.

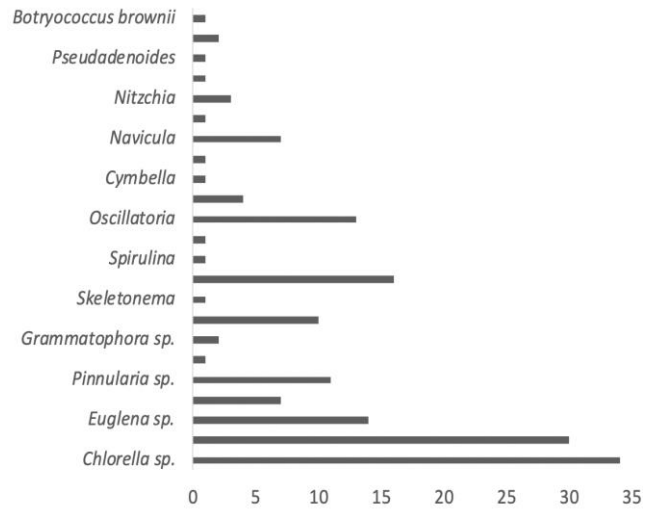


Figure 5. Distribution of microalgae species in all sampling sites

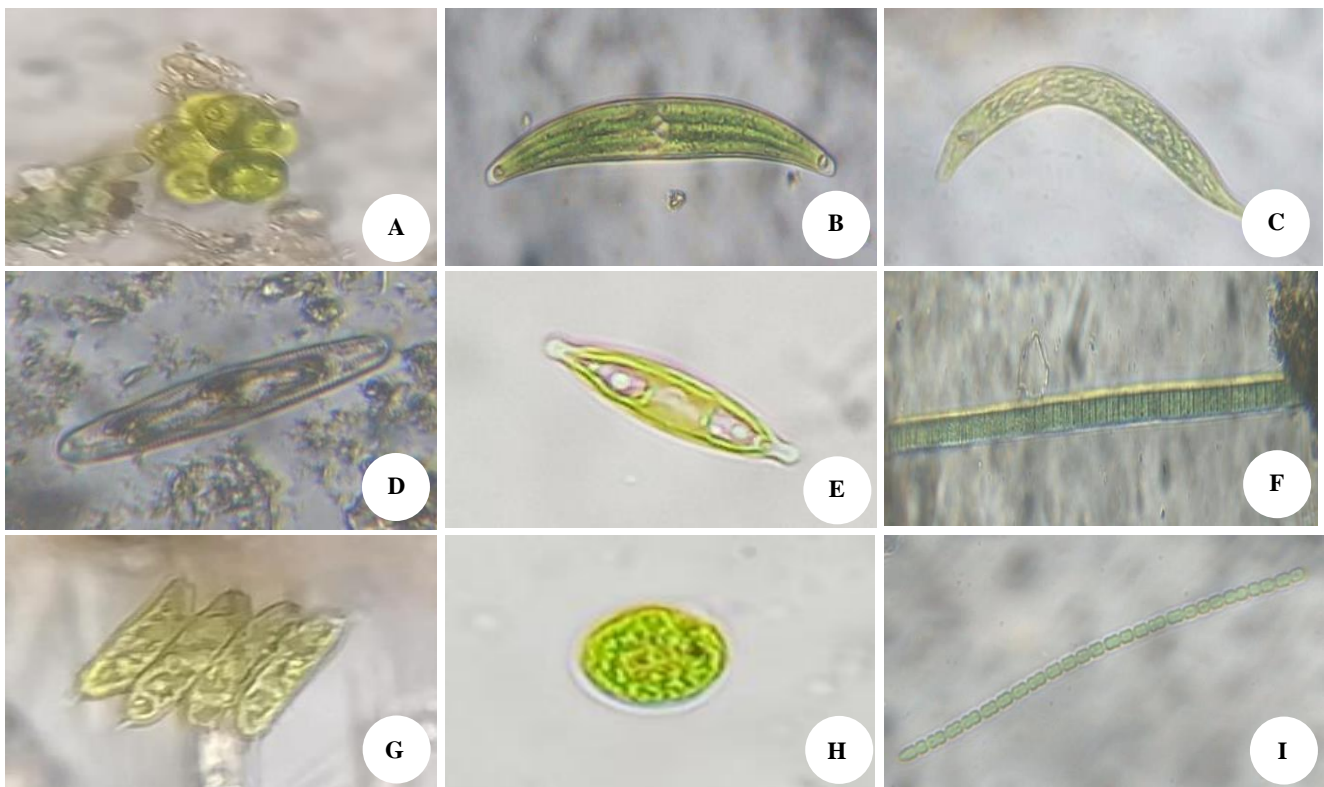


Figure 6. Representatives of microalgae from the black-water ecosystem (magnification 4x10). A. *Chlorella* sp.; B. *Closterium* sp.; C. *Euglena* sp.; D. *Pinnularia* sp.; E. *Navicula* sp.; F. *Oscillatoria* sp.; G. *Scenedesmus* sp.; H. *Chlamydomonas* sp.; I. *Anabaena* sp.

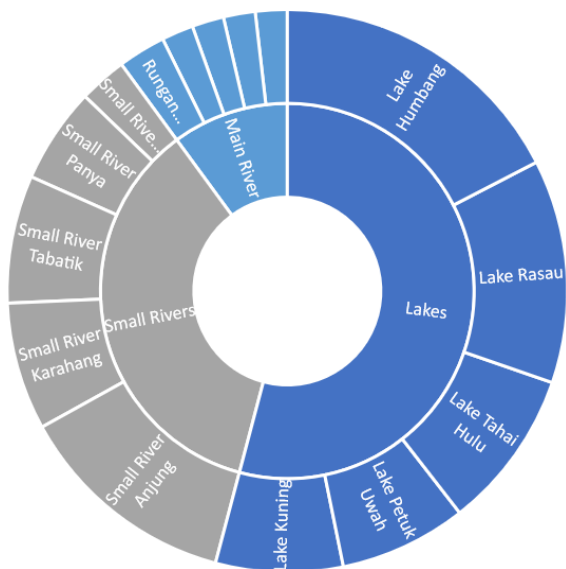


Figure 7. Microalgae distribution in different water zones (main rivers, small rivers, and lakes)

Microalgae distribution based on the differences in pH value and temperature

This study revealed that the largest distribution of microalgae (61%) was found in the black-water ecosystem with a pH value of 4.4-6 (Figure 8.A). There were differences in the average pH level among the main rivers (4.78), small rivers (5.16), and lakes (5.62). According to (Sakarika and Kornaros 2016), in most cases, microalgae prefer to live in waters with a pH value close to 7. Still, there are staggering exceptions, such as *C. vulgaris*, which can grow heterotrophically in waters with a wide pH range of 5.0-8.0. In this study, *Oscillatoria* sp. and *Anabaena* sp. were both discovered in waters with a wide pH range of 3.7 to 6.5. Other microalgae commonly found in the black-water ecosystem, including *Chlorella* sp. and *Closterium* sp., were only discovered in the pH range of 3.7-6.1. As

71% of microalgae species in the black water ecosystem prefer to live in waters with a temperature range of 28-32°C. Only the species *Anabaena* sp. was found at a temperature of 26-34°C (Figure 8.B). Most microalgae species are capable of conducting photosynthesis and cellular division over a wide range of temperatures, generally 15-30°C. Still the optimal conditions can be achieved at 20-25°C (Ras et al. 2013).

Principal Component Analysis (PCA)

PCA results showed that environmental parameters in the black-water ecosystem have an effect of 48.67% on the distribution of microalgae species throughout the study sites (Figure 9). Besides, pH and nutrients (nitrate) were the dominant factors influencing the distribution and abundance of microalgae in black-water ecosystem. Further, the yields revealed that pH and DO influence the Little Panya River and Lake Kuning sites. Meanwhile, nitrate and phosphate concentrations affected microalgae in the Rungan Rivers 02, 04, and 05. TDS impacted microalgae in the Sahang Little River and Rungan River 03 because, at these sites, the TDS concentrations were higher than at other sites.

The presence of microalgae at the Lake Petuk Uwah site is influenced by high temperatures (32.4°C). In the Anjung small river, the higher brightness value induces a greater effect on microalgae distribution. The abundance and distribution of microalgae are highly affected by environmental conditions, including the biotic and abiotic components of the waters (Mahmudi et al. 2023). Variations in the physicochemical parameters in each sampling site had an impact on the microalgae population during each season. The physicochemical parameters influenced the dominant taxa of microalgae Chlorellaceae, Scenedesmaceae, and Chlorococcaceae in the Noyyal River. Besides, they give base data for the seasonal and dynamic relationship between environmental parameters and microalgae populations (Kumar and Thomas 2019).

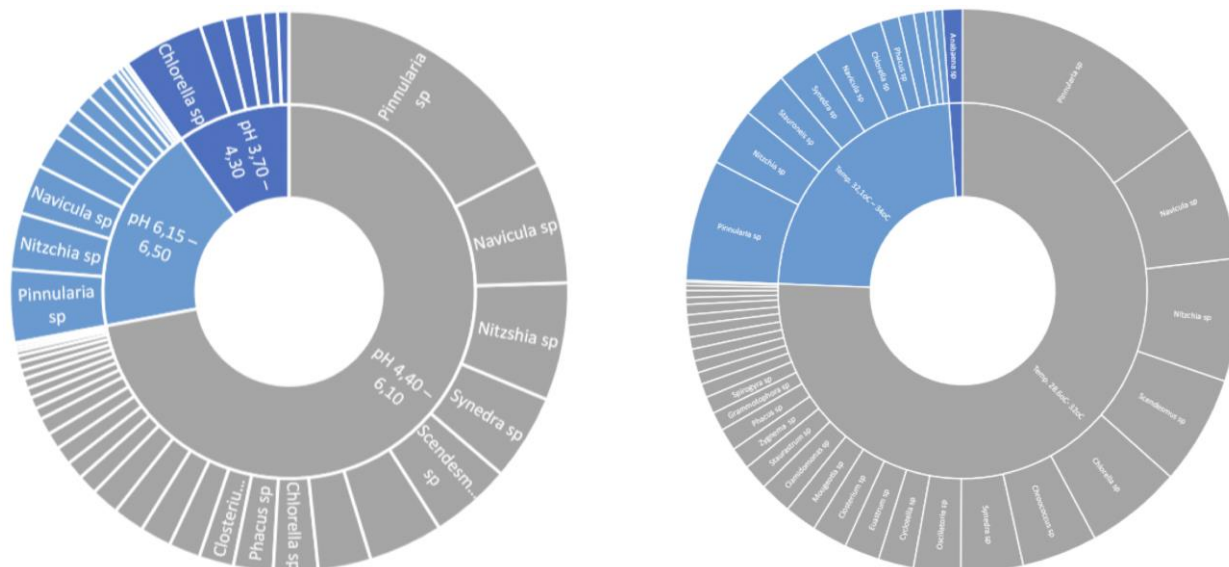


Figure 8. Microalgae distribution in different pH values and temperatures

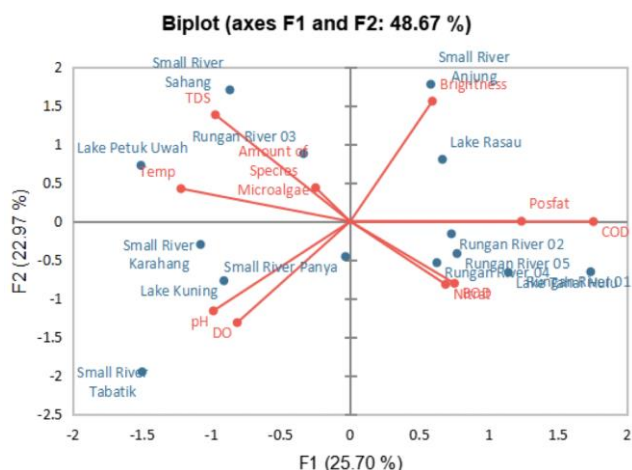


Figure 9. Principal Component Analysis (PCA)

In conclusion, the results showed that 59 species of freshwater microalgae were found in the black-water ecosystem in the Rungan River, Central Kalimantan. These species belong to the classes Bacillariophyceae, Mediophyceae, Chlorophyceae, Zygnematophyceae, Charophyceae, Xantophyceae, Cyanophyceae, and Euglenoidea. The species *Chlorella* sp. and *Clostridium* sp. were discovered in all study sites, while *Botryococcus brownii* and *Spirulina* sp. were only found at one site. The biodiversity index was included in the medium category. The highest abundance of microalgae species was found in lakes, and the lowest number in the main rivers. The distribution of many microalgae was found in the pH range of 4.4–6.0. Many microalgae species found at a temperature range of 28–32°C. PCA results showed that environmental parameters such as pH and nutrients (nitrate), were the dominant factors that influenced the distribution and abundance of microalgae in the black-water ecosystem. *Chlorella* sp., *Closterium* sp., and *Chlamydomonas* sp. were the dominant microalgae in study locations. These microalgae have the potential to be developed in the field of bioremediation, antioxidants, feed ingredients, bioethanol production, to improve water quality, and to be used in research and education.

ACKNOWLEDGEMENTS

The author would like to thank the National Research and Innovation Agency (BRIN), Jakarta, Indonesia, through the RIIM Expedition Batch I grant No. 11/IV/KS/01/2024 and the Education Endowment Fund Institute (LPDP) for financial support for this research.

REFERENCES

Andriani F, Hendrayanti D, Yasman Y. 2024. Exploring microalgae diversity in Indonesia: Harnessing potential for lead bioremediation. *Bioeduscience* 8 (1): 26–42. DOI: 10.22236/jbes/13225.

- APHA. 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.
- Bayu A, Noerdjito DR, Rahmawati SI, Putra MY, Karnjanakom S. 2023. Biological and technical aspects on valorization of red microalgae genera *Porphyridium*. *Biomass Convers Biorefin* 13 (14): 12395–12411. DOI: 10.1007/s13399-021-02167-5.
- Bayu A, Rachman A, Noerdjito DR, Putra MY, Widayatno WB. 2020. High-value chemicals from marine diatoms: A biorefinery approach. *IOP Conf Ser Earth Environ Sci* 460 (1): 012012. DOI: 10.1088/17551315/460/1/012012.
- Bitto T, Okumura E, Fujishima M, Watanabe F. 2020. Potential of *chlorella* as a dietary supplement to promote human health. *Nutrients* 12 (9): 1–21. DOI: 10.3390/nu12092524.
- Dasgupta CN, Nayaka S, Singh AK. 2024. Effects of water quality on diversity of freshwater green microalgae in semiurban areas. Preprints 1–16. DOI: 10.20944/preprints202404.1623.v1.
- Edward GB, David CS. 2015. *Freshwater Algae Identification: Identification and Use as Bioindicators*. Wiley-Blackwell, United States.
- Kazbar A, Cogne G, Urbain B, Marec H, Le-Gouic B, Tallec J, Takache H, Ismail A, Pruvost J. 2019. Effect of dissolved oxygen concentration on microalgal culture in photobioreactors. *Algal Res* 39: 101432. DOI: 10.1016/j.algal.2019.101432.
- Koyande AK, Chew KW, Rambabu K, Tao Y, Chu DT, Show PL. 2019. Microalgae: A potential alternative to health supplementation for humans. *Food Sci Hum Well* 8 (1): 16–24. DOI: 10.1016/j.fshw.2019.03.001.
- Krienitz L. 2009. Algae. In: Likens GE (eds). *Earth Systems and Environmental Sciences Likens. Encyclopedia of Inland Waters*. Academic Press, Cambridge. DOI: 10.1016/B978-012370626-3.00132-0.
- Krustok I, Truu J, Odlare M, Truu M, Ligi T, Tiirik K, Nehrenheim E. 2015. Effect of lake water on algal biomass and microbial community structure in municipal wastewater-based lab-scale photobioreactors. *Appl Microbiol Biotechnol* 99 (15): 6537–6549. DOI: 10.1007/s00253-015-6580-7.
- Kumar PS, Thomas J. 2019. Seasonal distribution and population dynamics of limnic microalgae and their association with physicochemical parameters of river Noyyal through multivariate statistical analysis. *Sci Rep* 9 (1): 15021. DOI: 10.1038/s41598-019-51542-w.
- Liao Y, Jiang X, Xiao Y, Li M. 2020. Exposure of microalgae *Euglena gracilis* to polystyrene microbeads and cadmium: Perspective from the physiological and transcriptional responses. *Aquat Toxicol* 228: 105650. DOI: 10.1016/j.aquatox.2020.105650.
- Mahmudi M, Arsad S, Lusiana ED, Musa M, Fitrianesia F, Ramadhan SF, Arif AR, Savitri FR, Dewinta AA, Ongkosongo AD. 2023. Microalgae diversity in varying habitat characteristics in Pasuruan and Sidoarjo coastal areas, East Java, Indonesia. *Biodiversitas* 24 (8): 4418–4426. DOI: 10.13057/biodiv/d240823.
- Mainisa, Adhar S, Muliani, Saputra F, Hsb S. 2023. Analysis of microalgae growth based on availability of nutrients in freshwater. *MICoMS* 3: 00023. DOI: 10.29103/micoms.v3i.182.
- Metcalf, Eddy Inc. 1991. *Rekayasa Air Limbah: Pengolahan, Pembuangan, dan Penggunaan Kembali*. Edisi ke-3, McGraw-Hill, Inc., Singapura.
- Naik UG, Nayak VV, Kusuma N. 2010. Relationship between Abundance of Microalgae and Ecosystem of Sunkeri Backwaters, Karwar. *Lake 2010: Wetlands, Biodiversity and Climate Change*. Bangalore, India, 22–24 December 2010.
- Nava V, Leoni B. 2021. A critical review of interactions between microplastics, microalgae and aquatic ecosystem function. *Water Res* 188: 116476. DOI: 10.1016/j.watres.2020.116476.
- Neneng L, Ardianoor A, Usup HLD, Adam C, Zakaria Z, Ghazella A, Perangin-angin SB, Alvianita V. 2020. Potensi *Chlorella* sp. dan *Pseudomonas* sp. dari areal tambang emas sebagai mikroorganisme potensial pereduksi merkuri. *Jurnal Ilmu Lingkungan* 18 (3): 617–625. DOI: 10.14710/jil.18.3.617-625. [Indonesian]
- Noerdjito DR. 2019. Perkembangan, produksi, dan peran kultur mikroalga laut dalam industri. *Oseana* 42 (1): 18–27. DOI: 10.14203/oseana.2017.vol.42no.1.35. [Indonesian]
- O'Neill EA, Rowan NJ. 2022. Microalgae as a natural ecological bioindicator for the simple real-time monitoring of aquaculture wastewater quality including provision for assessing impact of

- extremes in climate variance: A comparative case study from the Republic of Ireland. *Sci Total Environ* 802: 149800. DOI: 10.1016/j.scitotenv.2021.149800.
- Omer NH. 2019. Water Quality Parameters-Science, Assessments and Policy. *Water Quality: Science, Assessments and Policy*. IntechOpen, United Kingdom.
- Poot-Delgado CA, Okolodkov YB, Aké-Castillo JA, Rendón-von OJ. 2015. Ciclo anual del fitoplancton con énfasis en las especies potencialmente nocivas en bancos ostrícolas de la laguna de Términos, sureste del Golfo de México. *Rev Biol Mar Oceanog* 50 (3): 465477. DOI: 10.4067/S0718-19572015000400006.
- Prabowo DA, Noerdjito DR, Purbani DC, Ragamustari SK. 2023. Unwanted hitchhikers: The potential dispersal of harmful marine microalgae via plastic garbage and debris in Indonesian waters. *AIP Conf Proc* 2972 (1): 70001. DOI: 10.1063/5.0182790.
- Prihanto AA, Jatmiko YD, Nurdiani R, Miftachurrochmah A, Wakayama M. 2022. Freshwater microalgae as promising food sources: Nutritional and functional properties. *Open Microbiol J* 16 (1): e2206200. DOI: 10.2174/18742858-v16-e2206200.
- Prabowo DA, Noerdjito DR, Purnaningsih I, Yuliani Y, Prabowo DA. 2021. Analisis morfologi dan filogenetik molekuler alga hijau coccoid yang diisolasi dari Pulau Enggano. *Jurnal Ilmu Hayati* 20 (3): 1-12. DOI: 10.14203/beritabiologi.v20i1.3991. [Indonesian]
- Qiu R, Gao S, Lopez PA, Ogden KL. 2017. Effects of pH on cell growth, lipid production and CO₂ addition of microalgae *Chlorella sorokiniana*. *Algal Res* 28: 192-199. DOI: 10.1016/j.algal.2017.11.004.
- Ras M, Steyer JP, Bernard O. 2013. Temperature effect on microalgae: A crucial factor for outdoor production. *Rev Environ Sci Biotechnol* 12 (2): 153-164. DOI: 10.1007/s11157-013-9310-6.
- Sakarika M, Kornaros M. 2016. Effect of pH on growth and lipid accumulation kinetics of the microalga *Chlorella vulgaris* grown heterotrophically under sulfur limitation. *Bioresour Technol* 219: 694-701. DOI: 10.1016/j.biortech.2016.08.033.
- Sari LA, Pursetyo KT, Arsad S, Masithah ED, Setiawan E, Affandi M. 2019. The effect of nutrient abundance on distribution of *Cyanobacteria* and *Chlorophyll-A* in Sedati Water, Sidoarjo Luthfiana. *Poll Res* 38: S38-S43.
- Severes A, Nivas S, Souza LD, Hegde S. 2018. Diversity study of freshwater microalgae of some unexplored water bodies of a rapidly developing industrial region in India. *J Algal Biomass Util* 9 (2): 31-40.
- Swaleh M, Abubakar L, Mwanguni S, Munga D, Okuku E, Dzoga M, Fulanda A. 2022. Effect of selected environmental factors on microalgae diversity and abundance in Gazi Bay, South Coast Kenya. *J Sea Res* 184: 102217. DOI: 10.1016/j.seares.2022.102217.
- Undan JR, Martin LO, De Leon AM. 2021. The first report on the molecular identification of fresh water microalgae from waterfalls of Paracelis, Mountain Province, Philippines. *Intl J Sci Basic Appl Res* 56 (2): 117-129.
- Vazirzadeh A, Jafarifar K, Ajdari A, Chisti Y. 2022. Removal of nitrate and phosphate from simulated agricultural runoff water by *Chlorella vulgaris*. *Sci Total Environ* 802: 149988. DOI: 10.1016/j.scitotenv.2021.149988.
- Widayat PJ, Wibisono J. 2018. Cultivation of microalgae *Chlorella* sp. on fresh water and waste water of tofu industry. *E3S Web Conf* 31 (4): 04009. DOI: 10.1051/e3sconf/20183104009.
- Wijnana APA, Martono A, Martono D, Susatya A, Yansen Y. 2023. Kualitas perairan dan keanekaragaman mikroalga di Danau Dendam untuk menyusun strategi pengelolaan kawasan TWA Danau Dendam. *Naturalis* 12 (1): 1-7. DOI: 10.31186/naturalis.12.1.24223. [Indonesian]