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Identification of microalgae in peat swamp waters

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ABSTRACT

Microalgae are a group of autotrophic microorganisms that can live in freshwater and marine ecosystems. Microalgae in peat swamp waters are important to investigate and identify because they are used as aquatic bioindicators and have various potency and applications. This study aims to identify microalgae in peat swamp water in the *Universitas Palangka Raya* area, Central Kalimantan. This research used a purposive sampling method with two stations. The abiotic factors observed were temperature, dissolved oxygen, water transparency, and pH. Microalgae found were 12 genera (Behind the FMIPA UPR Building) and 6 genera (*Jalan B Koetin*). Some of the microalgae included Closterium, Micrasterias, Chlorella, Botryococcus, Cosmarium, Euastrum, Pleurotaenium, Chroococcus, Chlorogonium, Euglena, Selenastrum, Zygnema, Anabaena, Thalassionema, Pinnularia, Staunrastrum, and Gonatozygon. Some of them have morphological characteristics and potential in various fields. The abiotic factors in this research showed the normal temperature (29.5 ^{0}C and 27 ^{0}C), quite low for DO (2.9 mg/L and 4.7 mg/L), low brightness (23.25 and 25 cm), and acidic pH (4). Abiotic factors indicate that the condition of peat swamp water is a reasonably extreme ecosystem but is still suitable for the life of several species of microalgae.

Conflict of interest: The authors declare no conflicts of interest.

Introduction

Peat swamp waters are part of peatlands that accumulate dead and decaying plant material rich in carbon in stagnant water and low oxygen[1]. Peat swamp waters with black or reddish brown color are characterized by high acidity, low turbidity, and high organic matter content[2]. Peat swamp waters are the largest store and provider of carbon in the world[3] and have high biodiversity (gene reservoirs for various organisms)[4]. Assessment of water quality in peat swamps can be observed physically, chemically, and biologically[5]. Biological assessment can be followed based on the condition of the biota within it, such as microalgae. Microalgae in peat swamp waters are important to know and identify because they are used as aquatic bioindicators and have various benefits and applications.

Microalgae are a group of unicellular autotroph microorganisms that can live in freshwater and marine ecosystems and produce organic matter in photosynthesis[6]. Microalgae can be bioindicators of environmental quality because they are primary producers and contribute as oxygen solvents[7]. In addition, microalgae also have a short life cycle and can respond quickly to environmental changes[8]. In addition to being an environmental bioindicator, microalgae also have the potential to be rich in nutrients, such as proteins, polysaccharides, lipids, unsaturated fatty acids, vitamins, pigments, phycobiliproteins, and enzymes[9]. In addition, microalgae also produce bioactive compounds, such as antioxidant, antibacterial, antiviral, antitumor, regenerative, antihypertensive, neuroprotective, and immune stimulant effects[10]. Compounds found in microalgae have been of interest in various fields, such as pharmaceuticals, health, cosmetics, chemical industry, fisheries, energy industry, and agriculture[11]. This makes microalgae have a high potential to be applied in various fields of science and technology[12].

This research aims to identify microalgae from peat waters in *Universitas Palangka Raya*, Central Kalimantan, Indonesia. The peat swamp waters at *Universitas Palangka Raya* have both flowing and stagnant water types. The two types of peat swamp waters are thought to have different contents of living organisms, especially microalgae. Several abiotic factors were also observed to know the living environment of microalgae in each peat swamp water location. This research is preliminary as a primary database regarding the presence of microalgae in peat swamp waters in the *Universitas Palangka Raya* area and as a first step to determine the potential of microalgae and its application in various fields of biotechnology.

Method

Sampling Location

Sampling in this study was conducted from January until Juni 2023. Determination of sampling locations using purposive sampling method with 2 location points, namely peat swamp waters located behind the Faculty of Mathematics and Natural Sciences (FMIPA) Building, *Universitas Palangka Raya* (UPR) (Lat -2.220405° Long 113.887326°) and *Jalan B Koetin*, Jekan Raya, Palangka Raya (Lat -2.215638° Long 113.892468°) (Fig 1). A sampling at two different points considered the condition of the waters, where the peat swamp waters behind the FMIPA UPR building are lentic, while the peat waters on *Jalan B Koetin* are slightly flowing (lotic). In addition, another consideration is that the peat swamp behind the FMIPA UPR building is still natural and is thought to have more microalgae genera. In contrast, the peat swamp on *Jalan B Koetin* is close to residential areas, so it is thought that there are fewer microalgae genera.

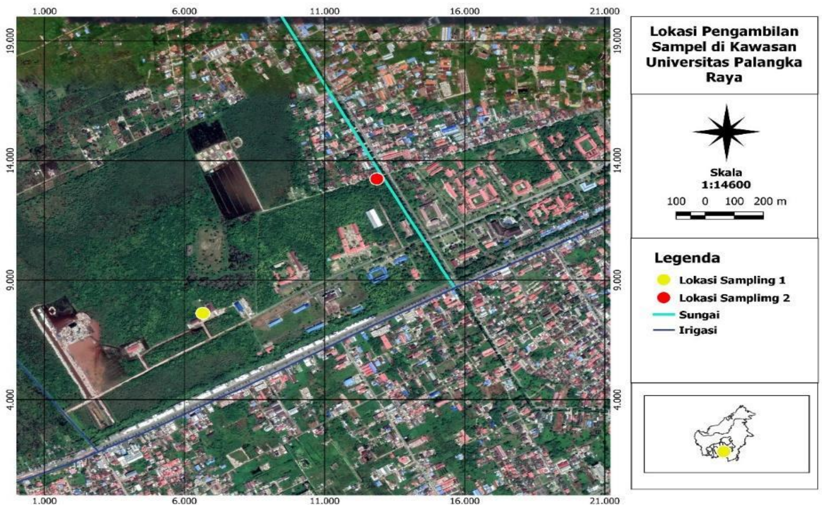

Fig 1. Sampling location map (Yellow: location 1; Red: location 2)

Microalgae identification and observation of aquatic abiotic factors

Sampling was conducted during the day or in the afternoon. Water was taken as much as 50 liters from peat water at each location and filtered using a plankton net with a mesh size of 25 µm. Water was collected at a certain depth from the water surface. Water was collected using a 5-liter bucket so that water was collected 10 times at each sampling point. Water taken with a bucket is filtered using a plankton net. After filtering, the water sample in the collection bottle is put into a 50 ml sample. Then, each sample is dripped with 1% lugol, as much as 2-3 drops as a preservative. Furthermore, the samples were brought to the laboratory and stored in the refrigerator to be observed for several days. Microalgae samples obtained are then identified morphologically using a binocular compound microscope with a magnification of 400x. Microalgae were documented, observed, and identified. Microalgae identification is based on the book Freshwater Algae: Identification, enumeration, and Use as Bioindicators[13], The Fresh-Water Algae[14], and several other references. The abiotic factors measured in this study were temperature, dissolved oxygen (DO), acidity (pH), and brightness to support information about the environmental conditions where microalgae live. Water temperature was observed using a thermometer, water DO was observed using a DO meter, pH was observed using a pH meter, and water brightness was observed using a Secchi disk.

Results and Discussion

Microalgae Identification

The results of this study obtained 12 genera of microalgae in peat swamp waters behind the FMIPA UPR Building consisting of *Closterium*, *Micrasterias*, *Chlorella*, *Botryococcus*, *Cosmarium*, *Euastrum*, *Pleurotaenium*, *Chroococcus*, *Chlorogonium*, *Euglena*, *Selenastrum*, and *Zygnema* (Fig 2) and 6 genera on *Jalan B Koetin*, consisting of *Anabaena*, *Chroococcus*, *Thalassionema*, *Pinnularia*, *Staurastrum*, and *Gonatozygon* (Fig 3). Microalgae in the peat swamp waters behind the FMIPA UPR building were more abundant than those in *Jalan B Koetin*. The waters behind the FMIPA UPR Building are stagnant (lentic) and colorless peat swamp waters, while the peat waters on *Jalan B Koetin* are less flowing and more brownish.

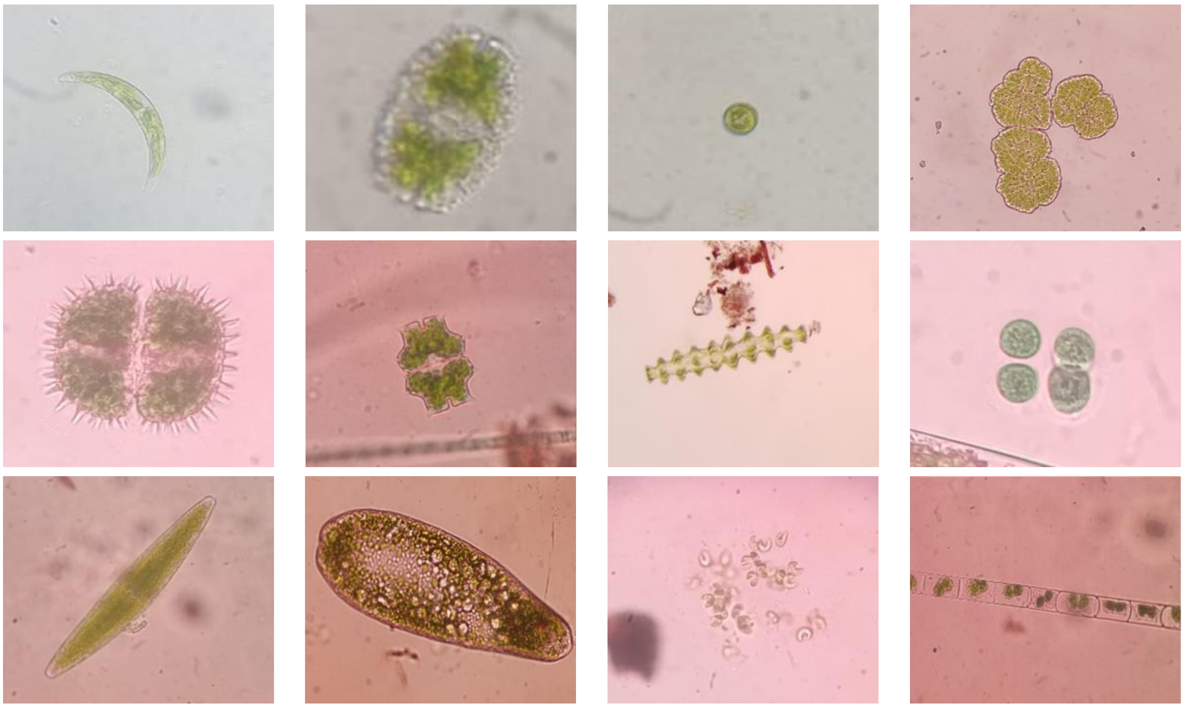

Fig 2. Genera of microalgae found in peat swamp waters behind FMIPA UPR Building (Description left to right: *Closterium*, *Micrasterias*, *Chlorella*, and *Botryococcus* (line one); *Cosmarium*, *Euastrum*, *Pleurotaenium*, and *Chroococcus* (line two); *Chlorogonium*, *Euglena*, *Selenastrum*, and *Zygnema* (line three))

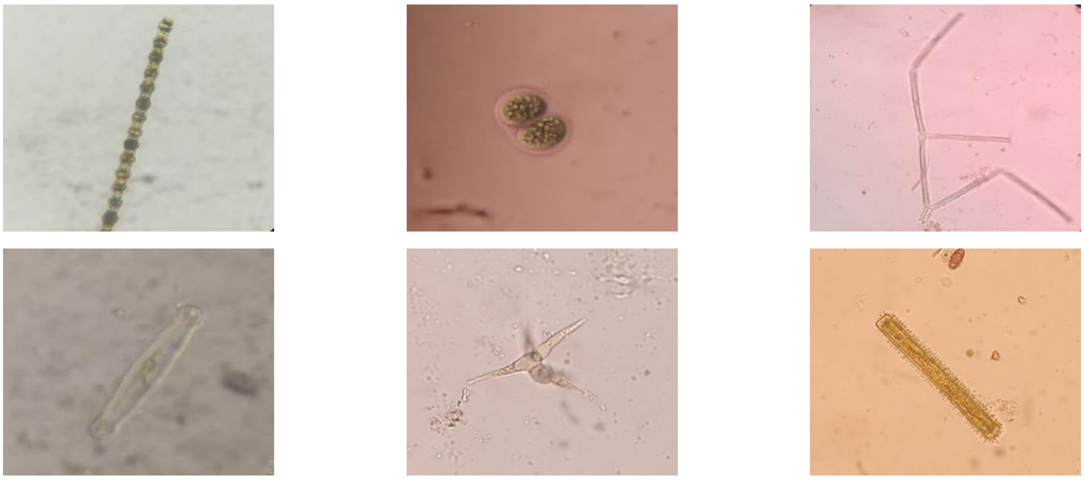

Fig 3. Microalgae genera found in peat swamp waters at *Jalan B Koetin*. (Description left to right: *Anabaena*; *Chroococcus*; *Thalassionema* (line one); *Pinnularia*; *Staunrestrum*; *Gonotozygon* (line two))

Closterium

Closterium is one of the most common microalgae found in peat bogs. This microalgae belongs to the Desmid group, a type of microalgae for several genera, such as *Closterium*, *Cosmarium*, and *Staurastrum*. This group of microalgae is found in swamps ranging from nutrient-poor to nutrient-rich acidic environments. *Closterium* has a slightly or strongly curved cell shape with tapered ends, elongated cells (35-1000 µm), arc or sickle-shaped. This microalga is divided into two parts and contains two chloroplasts, each located on both sides in the center area, and several prominent pyrenoids in each chloroplast[13]. This Desmid group is an environmental bioindicator or an assessment of water quality. Desmid communities have been used as ecological evaluations for aquatic ecosystems in Lake Artabel Natural Park in Turkey. Desmid species such as *Closterium* live mostly in benthic habitats and are mostly acidophilic species with low salinity[15].

Micrasterias

Micrasterias are mostly composed of single cells symmetrically divided in half (Desmid Placoderm). *Micrasterias* commonly found in Indonesia is *Micrasterias foliacea*. This microalgae has cells that bind to each other by forming ribbon-like bonds of 2-100 cells. Cells are shaped like a box with pulls on the holes. Each semi-cell is divided into three curves, two of which are lateral and the third is apical (polar). The habitat of *Micrasterias* consists of lentic waters, including wetlands, bogs, and a wide variety of peat bog waters and ponds, dams, lakes, etc., with varying pHs of 5.8 and 6.4[16]. *Micrasterias* are very sensitive indicators of environmental stresses such as heavy metals, high salinity, and oxidative stress. This desmid grows in clear, nutrient-poor freshwater and can be used to assess and monitor wetland waters[17].

Chlorella

Chlorella is small and round (2-10 µm) with one chloroplast that almost fills the cell. Chlorella belongs to the class Chlorophyta (green algae). Chlorella is an alga that lives in acidic environmental conditions[13]. Chlorella is a microalgae produced commercially for use as food and as a source of chemical compounds. *Chlorella vulgaris* and Chlorella *pyrenoidosa* are used commercially for food supplements. The compounds contained in Chlorella can improve human health and prevent certain diseases. This microalga is currently a green nutraceutical because it contains 55-60% protein, 1-4% chlorophyll, 9-18% dietary fiber, minerals, and vitamins[18].

Botryococcus

Botryococcus is a microalga that is distributed in brackish and fresh waters. Morphologically, this microalga has no flora, and its cells are densely connected by a waxy substance covered with chlorophyll with orange-red carotenoid pigments. There are thick filaments in this microalga. The cell body is 6-10 µm long and 3-6 µm wide. *Botryococcus* usually grows in oligotrophic waters but more in eutrophic environments with slightly acidic pH levels[13]. *Botryococcus* is a unique and special microalgae because it is a source of hydrocarbon and lipid production. Its cells can produce and accumulate oily hydrocarbons called botryococcenes (36% of organic matter) and contain a high lipid content of 30-17% of its dry weight under different growth conditions. Therefore, these microalgae are commonly used as a potential source of renewable fuel[19].

Cosmarium

Cosmarium is morphologically composed of two symmetrically divided semi-cells called an isthmus. The cell wall is filled with pores and pectin spicules. The irregular movement of *Cosmarium* is caused by the flow of gelatinous substances through the pores. The thicker part of the cell wall secretes mucus. Vegetative cells usually have no spines. Each semi-cell has at least one chloroplast in the center. The habitat of this microalgae is in an acidic and oligotrophic environment. It is sometimes found in subaerial or bottom of eutrophic waters[13]. *Cosmarium* microalgae have the potential to produce antioxidant compounds, where extraction using ethanol showed strong antioxidant activity against ABTS radical cations[6]. Based on identifying compounds in the selected fraction, this microalgae contains fatty acids with 9, 12-octadecadienoic acid (linoleic acid) and hexadecanoic acid (palmitic acid) as the dominant compounds[20].

Euastrum

Euastrum has broad and small cells or cells with narrow and deep gaps in the semi-cells. The apex of the *Euastrum* does not have spines. *Euastrum* has indentations on its semi-cells. This microalga has bulges and swellings on the surface of its semi-cells. The chlorophyll part is simple and compressed[21]. Euastrum is also included in Desmid microalgae, which can live in oligotrophic waters[22]. *Euastrum* is known to have pigments containing polyphenolic compounds that can be antioxidant substances. *Euastrum* also has the highest yield for methanolic extract compared to *Nostoc* sp.[23].

Pleurotaenium

Pleurotaenium found in this study is *Pleurotaenium nodosum*. This microalga has a long cylindrical cell shape, slightly narrowed in the middle, and a semi-cellular cross-section that is round and slightly swollen in the middle but does not have a folded structure. In addition, the shape is slightly tapered towards both ends, surrounded by small granules. This species has spiny protrusions and 2-10 band-like chloroplasts in each semicell[24]. This microalga lives in acidic water bodies such as swamps, ponds, old dams, and mountain bogs. Cell length 230-300 µm, width 45-30 µm, and isthmus 23-33 µm[25].

Chroococcus

Chroococcus consists of one or more cells that are usually enveloped by mucus. A single cell is round, but it is often hemispherical in groups of cells because the cell does not divide completely after division. *Chroococcus* has a dense envelope that contains polymers of several sugars, such as glucose and 2-O-methyl deoxygenated, and some other sugars and proteins, but contains few lipids. The cells of these microalgae are gray-green, green-blue, purple-violet, orange, and others. Some are metaphysical species generally found in acidic wetlands, but epiphytes and periphyton are found in freshwater lakes and ponds[26]. *Chroococcus* belongs to a group of cyanobacterial microalgae that have phycobiliprotein pigments. Hidhayati stated that phycobiliprotein pigments extracted from *Chroococcus turgidus* using water solvents are active against antioxidants and are not toxic, so they can be utilized as natural antioxidants[27].

Chlorogonium

Chlorogonium is spindle-shaped or elongated about 20-53 µm with two flagella at the anterior end. It has two contractile vacuoles at each end of the cell. Chloroplasts are on the edge (parietal) and without pyrenoids or 2 pyrenoids[28]. *Chlorogonium* is distributed in fresh waters. This microalgae habitat also exists in eutrophic soil, ponds, and lakes[29]. Starch degradation has been investigated using *Chlorogonium elongatum*. Acidic fermentation has been known to produce formate, acetate, and ethanol with small amounts of H_2 and CO_2[30].

Euglena

Euglena has grass-green chloroplast cells, is flagellated, and has red eye spots. Some *Euglena* are flat and slow-moving. *Euglena* is a facultative heterotroph that can take nutrients heterotrophically when photosynthesis is little or when the concentration of dissolved organic matter around it is high. This microalgae is commonly found in environments with abundant decaying organic matter, such as swamps, wetlands, ponds, mud, etc. *Euglena mutabilis* can live at low pH and an optimum pH of 3.0[13]. Maghfiroh et al. stated that *Euglena* sp., which is given photoperiodism, can increase protein, paramylon, and chlorophyll b, while *Euglena sp.*, which is given full light, can increase carbohydrates, lipids, chlorophyll a, and carotenoid production so that it can improve the quality of food nutrition[31]. In addition, *Euglena* is a highly adaptable protist that can be an autotroph, heterotroph, or mycotroph[32].

Selenastrum

Selenastrum has highly curved, crescent-like cells and is often separated without any mucus layer. The cells usually consist of groups of 4-32 cells and have a convex wall or outer wall. Its habitat is in warm water and temperate waters. One species of the genus *Selenastrum*, namely *Selenastrum capricornutum* is a strain of algae that can be used for biodiesel production[33]. *Selenastrum minutum* grown in wastewater for 6 days under mycotrophic and salt stress conditions is known to have significantly increased saturated fatty acid (SFA) concentration and decreased polyunsaturated fatty acid (PUFA) concentration, which are characteristics of good biodiesel quality[34]. In addition to biodiesel, *Selenastrum capricornutum* can remove Polycyclic Aromatic Hydrocarbons (PAHs) and heavy metals, which shows that *Selenastrum* can also be a microorganism that can treat waste or pollutants[35].

Zygnema

Zygnema has filaments with two chloroplasts in each cell. *Zygnema* cells are cylindrical with two chloroplasts separated by a clear area. Each chloroplast has a pyrenoid. The filaments usually have fine mucilage sheaths that are unbranched and not long. *Zygnema* can adhere to the substrate due to the presence of rhizoids. The chloroplasts are relatively small and shaped like biscuits or stars connected in the center of the cell by cytoplasmic strands close to the nucleus. This microalga belongs to the starch-containing Chlorophyta. *Zygnema* usually lives in acidic shallow waters. *Zygnema heydrichii* has potential as a biodiesel raw material because it contains 14.75% DW fat at 31 days of growth on a nutrient-rich medium[36].

Anabaena

Anabaena has trichomes that are easy to find, have a uniform width throughout their layers, and often have invisible mucus on their surface. Filaments on these microalgae are straight, curved, or coiled, depending on the species. Some species also produce vacuolar gas and can form colonies (blooms); cells are spherical. Heterocysts are spherical and may not be present in nitrogen-rich waters. *Anabaena* are found in lakes, ponds, and ditches and have the potential to produce taste and odor in water used for drinking[14]. *Anabaena* is included in Cyanobacteria, which have the potential to be nitrogen fixers in the air, where there are heterocysts, which are cells that fix the nitrogen. One of them is *Anabaena azollae* used as a natural biofertilizer for rice plants in China and Vietnam[37]. *Anabaena* is included in a genus of microalgae that is toxic and can cause harmful algal blooms (Habs)[38]. *Anabaena* is one of the microalgae that causes Habs in estuarine waters in the Cilacap area, Central Java because it is caused by excess nutrients and causes oxygen reduction[39].

Pinnularia

Pinnularia has linear, lanceolate, or even elliptical cells. There are rib-like striae. The poles are round. Striae (stretch marks) are usually rough, widespread, and common in sediments and other substrates or can also be mixed with lichen clumps. The aquatic habitats of these microalgae range from nutrient-poor and slightly alkaline to slightly acidic environments[13]. One species of *Pinnularia* is *Pinnularia borealis*, known to have high EPA (eicosapentaenoic) and can be projected as a commercial source for dietary supplements. In the study of Świderska-Kołacz et al., it was found that cholesterol and triacylglycerol concentrations in the liver and kidneys were the lowest in mice fed with the highest concentration of *Pinnularia borealis*[40]. In addition, the lowest concentration of *Pinnularia* could also increase antioxidant capacity.

Gonatozygon

Gonatozygon lives in swamp habitats dominated by peat moss (Sphagnum) and slightly acidic oligotrophic lakes. This microalgae has setae (bristles) along the lateral walls. It is cylindrical and is a desmid placoderm[41]. *Gonatozygon* is similar to desmid microalgae and lives solitary. This species has long or short walls that are spiny and not narrowed in the center. Although usually free-floating and mixed with desmids, its cells can also be attached to aquatic plants. There are ribbon-like chloroplasts that are axial rather than parietal[14].

Abiotic Factor Analysis

Some abiotic factors observed were temperature, dissolved oxygen (DO), pH, and brightness. The results of observations of aquatic abiotic factors are listed in Table 1.

Table 1. Abiotic factors of peat swamp waters in the *Universitas Palangka Raya*

Parameters	**Behind FMIPA UPR Building**	***Jalan B Koetin***
Temperature (^{0}C)	29.5	27
DO (mg/L)	2.9	4.7
pH	4	4
Brightness (cm)	23.25	25

Temperature

Based on Table 1, the temperature in both locations was by the waters in general or still in normal condition. Different sunlight intensities cause the temperature difference in the two locations; the location on *Jalan B Koetin* is more overgrown by canopy plants compared to the location behind the FMIPA UPR Building. The intensity of sunlight influences the different temperature patterns in a water body and is also influenced by time. Temperature parameters are taken during the day when the temperature is higher than the temperature in the morning[42]. Solar radiation, geographical location, cloud conditions, evaporation, and wind gusts influence the temperature of a body of water[43]. However, the temperature in both locations is still suitable for living organisms' lives, especially microalgae[43]. The temperature range that is suitable for the life of aquatic organisms is 25-32^{0}C[44]. The optimal water temperature for microalgae growth is 20-30^{0}C; therefore, the temperature of peat waters in both locations is still suitable and supports aquatic microalgae in peat swamps[45].

DO (Dissolved Oxygen)

Dissolved oxygen (DO) levels at both locations were 2.9 mg/L (behind the FMIPA UPR building) and 4.7 mg/L (*Jalan B Koetin*) (Table 1). DO from both locations has a significant difference. DO levels behind the FMIPA UPR building are quite low because the location is included in swamp waters that are always stagnant. However, several species of microalgae were found in that location. This is because some microalgae species, such as *Chroococcus* and Cyanobacteria can adapt to waters with low DO[46]. Some peat waters in the Riau Islands also have low DO, around 2.7 to 4.3[47]. The low DO condition of peat waters can still support microalgae life. However, for normal waters, the occurrence of low dissolved oxygen concentrations in a body of water can reduce the efficiency of oxygen uptake by aquatic biota, reducing the ability of these biota to live[48]. High and low DO is also caused by the photosynthetic activity of plants in a body of water. The location on *Jalan B Koetin* has more plants and several species of aquatic plants than behind the FMIPA UPR Building. This causes the waters on *Jalan B Koetin* to have higher dissolved oxygen content than behind the FMIPA UPR Building.

Brightness

The brightness behind the FMIPA UPR Building is 23.25 cm, and on *Jalan B Koetin*, it is 25 cm (Table 1). The results of brightness observations show that both locations have low brightness. This is because peat swamp waters are shallow waters. However, the brightness of peat swamps is still considered optimal water for the growth of organisms because it is still in optimal conditions if the Secchi pieces still reach 20-40 cm from the surface of the water. Water brightness is also related to photosynthetic activity and primary producers in water[49]. In normal weather, water conditions with low brightness indicate the presence of suspended particles[50]. The brightness value of a body of water is influenced by several parameters, such as weather, collection time, turbidity, and suspended solids.

pH

The degree of acidity (pH) measured at both locations is 4 (Table 1). The acidity in peat swamp water is caused by the composition of peat air formed due to rotting material that contains humic substances, which are components that form humus. Humic substances also cause high organic matter content. Apart from that, humic substances also consist of oxygen, which contains functional groups and fractions, such as humic acid, fulvic acid, and humin[51]. The acidic pH condition in the peat swamp water ecosystem is an extreme environmental condition. The degree of dryness is an inhibiting factor for microalgae life. Only a few species of microalgae can live in peat swamp waters[52].

Conclusion

Based on the research results, it can be concluded that some of the microalgae identified from peat swamp waters consist of 12 genera (Behind the FMIPA UPR Building) and 6 genera (*Jalan B Koetin*). The microalgae consist of *Closterium*, *Micrasterias*, *Chlorella*, *Botryococcus*, *Cosmarium*, *Euastrum*, *Pleurotaenium*, *Chroococcus*, *Chlorogonium*, *Euglena*, *Selenastrum*, *Zygnema*, *Anabaena*, *Pinnularia*, and Gonatozygon. Several genera of microalgae have morphological characteristics and potential in various fields. The observed abiotic factors showed normal temperature (29.5^{0}C and 27^{0}C), quite a low DO (2.9 mg/L and 4.7 mg/L), quite low brightness (23.25 and 25 cm), and pH which tended to be acidic. (pH 4). Abiotic factors indicate that the condition of peat swamp waters is a fairly extreme ecosystem, but is still suitable for the life of several species of microalgae.

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Author contributions

All authors contributed to this article. The conception and design, material preparation, data collection, and analysis were performed by **[Faridah Tsuraya], [M Sadam Rahmansyah]**, **[Fauzan Fikri]** and **[Rizka Hasanah]**. The first draft of the manuscript was written by **[Faridah Tsuraya]**. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.