Kronologi pengajuan naskah "Checklist of Freshwater Periphytic Diatoms in the Midstream of Brantas River, East Java, Indonesia" to Biodiversitas Journal of Biological Diversity"			
No.	Tanggal	Agenda	Halaman Bukti
1.	14 Mei 2023	**Pengajuan naskah:** Penulis mengajukan naskah artikel yang berjudul *"Checklist of Freshwater Periphytic Diatoms in the Midstream of Brantas River, East Java, Indonesia"* pertama kali pada *Biodiversitas Journal of Biological Diversity*	1-20
2.	23 Mei 2023	**Revisi I:** Reviewer menyatakan bahwa naskah perlu adanya revisi	21-41
3.	13 Juni 2023	**Revisi II:** Reviewer menyatakan bahwa naskah perlu adanya sedikit revisi	42-59
4.	16 Juni 2023	**Proofread:** Pihak *Biodiversitas Journal of Biological Diversity* mengirimkan uncorrected proof dengan judul akhir " *Checklist of Freshwater Periphytic Diatoms in the Midstream of Brantas River, East Java, Indonesia* "	60-75
5.	26 Juni 2023	**Pembayaran :** Pihak *Biodiversitas Journal of Biological Diversity* menerima pembayaran	76

1. Halaman bukti pengajuan naskah awal
14 Mei 2023

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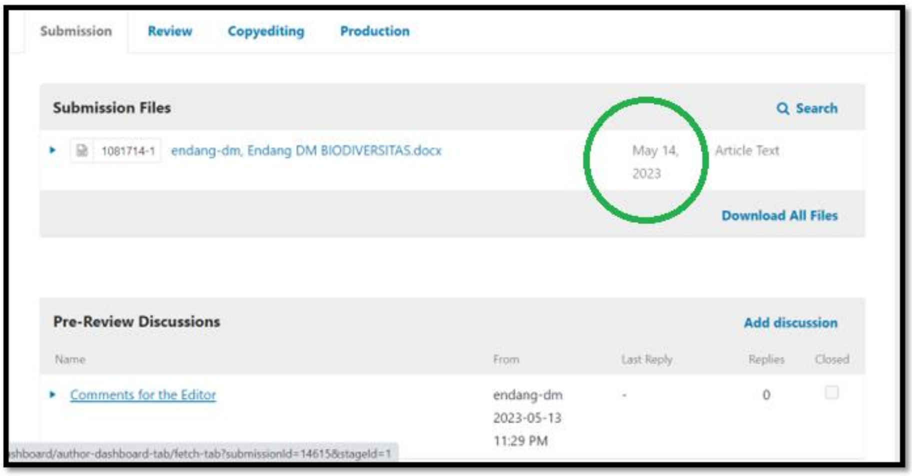
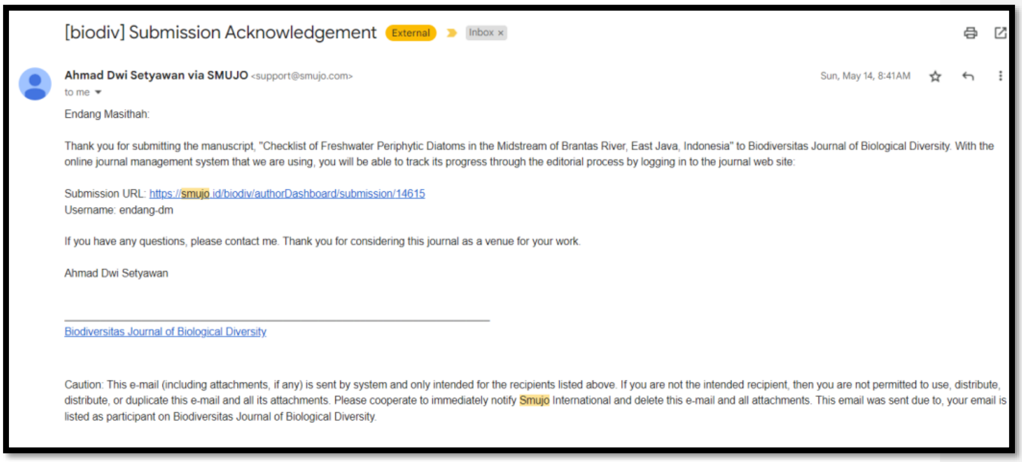


B. Editor mengkonfirmasi bahwa naskah telah diterima untuk proses review 1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Checklist of Freshwater Periphytic Diatoms in the Midstream of Brantas River, East Java, Indonesia

Author(s) name:

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This study provides detailed information on the types of periphytic diatoms found in the area, including species not previously recorded in the area. In addition, this study also provides a better understanding of the structure and composition of the periphytic diatom community which can be used as an indicator of river water quality.

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Endang Dewi Masithah

Checklist of Freshwater Periphytic Diatoms in the Midstream of Brantas River, East Java, Indonesia

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Abstract. Periphytic diatoms are a group of microalgae that live attached to the surface of the substrate such as rocks, aquatic plants, or other objects in the water. This group has potential as an indicator of environmental quality of water because its existence is influenced by water quality, such as nutrient levels and pollution levels. This study provides a checklist of freshwater periphytic diatoms in the midstream of Brantas River, East Java, Indonesia. The sampling and identification according to published methods were carried out at the Laboratory of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya. This study was undertaken once a week in a month (May 2022) between 9 a.m. and 1 p.m (GMT + 7). Determination of sampling sites based on differences in land use. The total abundance of freshwater periphytic diatom ranges from 938,905 Ind / mm2 – 1,597,758 Ind / mm2. The lowest total abundance is at station 1 with a value of 938,905 Ind / mm2 and consists of 17 genera. The highest total abundance is at station 4 with a value of 1,597,758 Ind / mm^2 and consists of 17 genera. In this study, 27 genera were found spread across 3 phylum.

Keywords: Epilithic, microalgae, freshwater periphytic diatom, water quality.

Running title: Biodiversity of Freshwater periphytic diatom and Environmental Condition

INTRODUCTION

Diatoms are an important group of microalgae and are the largest group of periphytic algae in fresh waters. The Diatom group is characterized by a cell body covered with a silica shell, which can take various shapes and have distinctive patterns on its surface (Danielson et al. 2012; Mayzel et al. 2021). The abundance of diatoms in fresh waters is very high and often becomes dominant in fresh waters because of their good ability to utilize nutrients in the waters and their high tolerance for various environmental conditions in fresh waters (De Nicola et al. 2014; Camas-Anzueto et al. 2015; Soraya and Islamy 2022). Therefore, Diatoms are an important group of algae as bioindicators of fresh water environmental conditions (Costache et al. 2013; Charles et al. 2019; Kazbar et al. 2019).

Freshwater periphytic diatoms have an important role as environmental bioindicators (Park et al. 2016; Rivera et al. 2019; Schmidt et al. 2019). The presence or absence of periphytic diatom species can provide very useful information to identify disturbances or changes in fresh waters. Some examples of periphytic diatom and their role as bioindicators are Nitzschia which is used to indicate organic contamination (Ahn et al. 2013; Rovira et al. 2015; Sugie et al. 2020), Fragilaria to indicate changes in water quality such as eutrophication, A study conducted that the presence of metal contamination in waters proved to be a strong driver of the formation of diatom community structure, and allowed the identification of tolerant species such as *Cocconeis placentula* var. euglypta, *Eolimna minima*, *Fragilaria gracilis*, *Nitzschia sociabilis*, *Pinnularia parvulissima*, and *Surirella angusta* (Fernández et al. 2012; Brown et al. 2017; Mori et al. 2017). The use of periphytic diatoms as environmental bioindicators can provide more detailed and accurate information about environmental conditions in fresh waters, so it is important to conduct research on periphytic diatoms as environmental bioindicators.

Drafting a checklist of aquatic organisms is very important, especially those whose role is as bioindicators of aquatic environmental conditions (Bilanovic et al. 2016; Islamy and Hasan 2020; Isroni et al. 2023). The checklist can help researchers and environmentalists to obtain data on the diversity of periphytic diatom species in certain fresh waters so that it can be a reference for determining which species are present or absent in fresh waters (Fortes et al. 2010). By obtaining accurate and detailed information about the condition of the freshwater environment, the periphytic diatom checklist can assist in environmental conservation efforts and improve the sustainability of freshwater management (Wang et al. 2012; Pattanayak et al. 2020).

One location that can be used as a case study to determine water quality is the midstream of Brantas River. This river is one of the rivers that has an important role in supporting the lives of the surrounding community, both as a source of irrigation water, a means of transportation, and as a tourist spot. However, the environmental conditions around the river are getting worse due to domestic and industrial waste pollution, as well as increasing human activities. Therefore, research on periphytic sampling of freshwater diatoms in midstream of Brantas River is important to do. This study aims to provide a checklist of the diversity of freshwater periphytic diatom species and an overview of the environmental conditions of the midstream of Brantas River. The research results can be used as a reference in making policies to maintain the quality of the water environment in the midtsream of River. In addition, this research can also provide a better understanding of the use of periphytic diatoms as an indicator of the environmental quality of water in the tropics (Hariyadi et al. 1992; Mattson 1999; Mason 2010; Martin et al. 2012; Schulte 2015; Sudrajat et al. 2016).

MATERIALS AND METHODS

Study area

This research was conducted at the midstream of Brantas River, Kedungkandang District, Malang City, East Java, Indonesia. Determination of periphytic freshwater diatom sampling locations based on different land uses (Soraya and Islamy 2022). First, various types of land use in the study area, such as agricultural land, residential areas, industrial areas, or natural areas were mapped and then a representative sampling location was determined for each type of land use to provide a comprehensive assessment of the diatom community in the study area.

Figure 1. Location of sampling sites at midstream of Brantas River, Kedungkandang District, Malang City, East Java, Indonesia

Sampling site 1: The sampling site 1 is located Rolak Kedungkandang DAM (7°59'21.9"S 112°39'11.4"E). Ecological and substrate conditions in this area are affected by the presence of dams, which provide a unique habitat for a variety of freshwater organisms. The water inside the dam is relatively clear and shallow, with a depth of about 1-3 meters. The substrate in this area is dominated by rock and gravel, which provide suitable attachment sites for periphytic diatoms. On the other side of the dam is plantation land, with plants such as vegetables and fruits being cultivated in the surrounding area. This can allow the entry of water streams containing pesticides and agricultural fertilizers which can affect water

quality and aquatic ecology.
Sampling site 2: This station is located in Coban Amprong which is a low waterfall tourist spot located in Kedungkandang District, Malang City, East Java, Indonesia. The ecology and substrate in this area are influenced by waterfall flows, vegetation, and other environmental factors. The water in Coban Amprong tends to be cold and fresh with a swift waterfall flow and the natural rocks around it form very different substrate conditions compared to the conditions at the Rolak Kedungkandang Dam. The substrate in this area is dominated by granite and other rocks, which are the attachment sites for periphytic diatoms and other microorganisms. The vegetation around the waterfall consists of plants such as pine trees, teak trees, bamboo, and other shrubs. Around Coban Amprong there are several plantation areas that are used as agricultural land for the cultivation of plants such as vegetables, fruits, and flowers.
Sampling site 3: This station is located in a river branching area near the Kedungkandang bridge, Malang, East Java, Indonesia, which is an area that has a unique ecology and substrate. This spot has a calm and slow water flow, as well as differences in elevation which can affect the condition of the substrate. Due to the calm water conditions, the substrate in these areas tends to be softer and easier to settle. The substrate around the river branching area is dominated by silt and sand. In addition, around this area there is also quite abundant vegetation, such as shrubs and trees. These plants provide shelter for many types of animals, such as fish and insects.
Sampling site 4: This sampling site is located in a residential area near Kedungkandang Sub-District, Malang, East Java, Indonesia. It is an area that has a different environment and substrate than the natural river area. In these areas, the dominant substrate is soil that has been altered by human activities to build houses, roads, and other infrastructure. The river which flows around this residential area has more polluted water conditions compared to the natural area of the river, due to domestic and industrial waste being dumped into the river. Even so, around this residential area, there are still a number of green plants such as trees and shrubs that can provide shelter for various types of animals and insects. This area can also still be a habitat for periphytic diatoms and other microorganisms that can live and thrive on substrates that have been altered by human activities.

Sample collecting

Water quality, Freshwater periphytic diatom sampling, and identification were carried out at the Laboratory of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya. This study was undertaken once a week in a month (May 2020) between 9 and 13 h (GMT + 7). Identification and Freshwater periphytic diatom samples were collected by scraping the substrate (stones/rocks/aquatic plant) in and around the respective water sampling stations.

Freshwater periphytic diatom sampling

The procedure of Freshwater periphytic diatom sampling is based on Freshwater periphytic diatom Collection Protocols (West Virginia Department of Environmental Protection 2018). Sample collected during stable flow conditions and streams are not turbid (i.e., the substrate is visible). Label the sample container with Stream Name, AN-Code, date, and collector. Sample only be collected from rocks (epilithic habitat) from riffle/run areas of the streams. Collect five separate cobble-sized rocks that are exposed to varying light conditions and contain varying Freshwater periphytic diatom communities (brown vs. green). Rinse the PVC ring, toothbrush, micro-spatula, and squirt bottle thoroughly with stream water at the site before each sampling event to avoid contamination from a prior sampling of subsequent collections. Using the PVC ring to delimit the sample area (12.56 cm^2), use the micro-spatula to scrape all algae from the upper surface of rocks into the sample jar. Use the toothbrush to loosen any remaining Freshwater periphytic diatom. Remove the sampler and rinse loosened algae into the sample jar using clear stream water collected from that sampling site in the squirt bottle. Repeat Step 5 until all the Freshwater periphytic diatom from the five rocks (representing 62.8 cm^2 of the sampled area) is composited into one sample jar.
Rinse the micro-spatula, toothbrush, and PVC ring into the sample, removing as much of the lingering Freshwater periphytic diatom as possible. Snap the labeled lid onto the container. Rinse the PVC ring, toothbrush, micro spatula, and squirt bottle thoroughly with stream water at the site after each sampling event to avoid contamination of subsequent collections. For preservation, we assume that the sample jar is about 33 ml full, preserve with an adequate amount of 1 drop Lugol's solution (West Virginia Department of Environmental Protection 2018).

Identification

Freshwater periphytic diatom identification was performed using an Olympus Light Microscope (model CX 40) to determine the types of Freshwater periphytic diatom that had been collected. Identification sampling based on Freshwater periphytic diatom Collection Protocols (West Virginia Department of Environmental Protection 2018). A drop of the sample was placed on glass slides. These were examined at different magnifications using an Olympus light microscope (model CX 40) and illustrate the Freshwater periphytic diatom that is seen in the microscope. Most taxa within samples were identified to species level by reference to standard works, such as identification books and numerous journals (Alika and Akoma 2012).

Freshwater periphytic diatom algae abundance

The calculation of Freshwater periphytic diatom abundance is carried out according to the published procedure (APHA 1985), with the formula:

$$N = \frac{n\ xA_t\ xV_t}{A_c\ x\ V_s\ x\ A_s}$$

Where N = density of Freshwater periphytic diatom algae (ind / mm^2); n = Number of organisms found; At = area of the cover glass (mm^2); Vt = volume of sample accommodated in sample bottle (ml), Ac = area of the field of view multiplied by the number of fields of view observed (mm^2); Vs = volume of water drops used in the observation (ml).

Freshwater periphytic diatom Diversity Index

Diversity assessment was carried out for analysis of the richness of elements, taxonomic diversity (by the number of a taxons in hydrobiological groups), expressed by the number of taxons, and 2) for the relative representation of populations in communities (by abundance or biomass). The indices are as follows (Protasov et al. 2019):

$$\mathrm{H} = -?\frac{\mathrm{ni}}{\mathrm{N}}\ln\frac{\mathrm{ni}}{\mathrm{N}}$$

Where: N = common organism abundance; s = species number; ni = species number of each species; H, Shannon diversity index.

Dominance Index

The dominance index was assessed by formula/equation as the Shannon Index. Simpson index is calculated as follows Simpson Dominance Index (D):

$$D = \ ?\left(\frac{ni}{n}\right)^2$$

Where D = Dominance index; ni = number of individuals of species i; n = total number of individuals.

RESULTS AND DISCUSSION

Composition and abundance of Freshwater periphytic diatom

Data from the composition determination and the results of the average Freshwater periphytic diatom abundance during the study at each sampling site are presented in Table 1. The total abundance of Freshwater periphytic diatom ranges from 938,905 Ind / mm^2 – 1,597,758 Ind / mm^2. The lowest total abundance is at station 1 with a value of 938,905 Ind / mm^2 and consists of 17 genera. The highest total abundance is at station 4 with a value of 1,597,758 Ind / mm^2 and consists of 17 genera. In this study, 27 genera were found spread across 3 phylum. Freshwater periphytic diatom composition and abundance data can be seen in Table 1.

Based on Table 1, at station 1 to station 3, the dominant Freshwater periphytic diatom division is Chrysophyta with relative abundance values of 96%, 91%, and 75%, respectively. Besides, their high abundance, at each station, species from this Division are also more numerous and varied than those from the Chlorophyta, Chyanophyta, and Euglenophyta Divisions that are found in the waters of the Bango River. The Chrysophyta is a group of algae that are qualitatively and quantitatively found in various river type waters (Adjie et al. 2003; Kristiansen and Škaloud 2017). Golden algae are one of the most critical functional components in freshwater microalgae. They are indicators of changes in environmental parameters such as pH, salinity, and climate (Korneva and Solovyeva 2017; Kristiansen and Škaloud 2017). The Chrysophyta, a group of protists containing single-celled individuals as well as quite complex colonial forms, can briefly be defined by the following biochemical and structural criteria: chloroplasts with chlorophylls a and c but lacking b, fucoxanthin as the most critical accessory pigment, β-1, 3-glucan as a storage product, swarmers with heterokont flagella (i.e., one long hairy and one shorter smooth, the latter in many cases only to be detected by EM). Endogenous silicate cysts (stomatocysts) are present throughout the class (Kristiansen and Škaloud 2017).

Meanwhile, at sampling site 4, the dominant Freshwater periphytic diatom was from the Chlorophyta division with a relative abundance value of 63%. Chlorophyta is usually found in stagnant waters and is planktonic. However, Chlorophyta of the Genus Oedogonium and Ulothrix are not. Oedogonium and Ulothrix more often live attached either to plants, rocks, or other surfaces (García et al. 2012). In the Chlorophyta division, at stations 1, 2, and 4, Ulotrhix is more able to grow than other Chlorophyta genera, which was found probably due to the ability of Ulotrhix to be more tolerant of environmental conditions. At station 3, the abundance of Ulothrix is slight due to the condition of station three, which has lower levels of phosphate and nitrate than other stations.

Cyanophyta and Euglenophyta divisions were found at each station. The Euglenophyta are a heterogeneous group of freshwaters. Some of them are naked, covered only by the periplasm, and others have an external lorica (envelope), which encloses the cell (Poniewozik 2017). Euglenophyta has a flagellum that functions as a means of movement in the water. Hence the little Euglenophyta Division is found as Freshwater periphytic diatom attached to the substrate. Meanwhile, benthic cyanophytes or those that become Freshwater periphytic diatoms are less likely to bloom or be abundant. Species that are not planktonic are generally species that rarely result in population explosions (blooming) due to eutrophication (nutrient enrichment) (Prihantini et al. 2010).

Table 1. Data on Composition and Abundance of Freshwater periphytic diatom in the river during the Study

Freshwater Periphytic Diatom		SAMPLING SITES							
		1		2		3		4	
		(Ind/mm²)	**(%)**	**(Ind/mm²)**	**(%)**	**(Ind/mm²)**	**(%)**	**(Ind/mm²)**	**(%)**
No	**Chlorophyta**	**33295**	**100%**	**17757**	**1**	**68440**	**1**	**999198**	**1**
1	*Cosmarium*					370	0,5%		
2	*Entransia*					54751	80,0%		
3	*Oedogonium*					9249	13,5%	69548	7,0%
4	*Ulotrhix*	33295	100,0%	14798	83,3%	2590	3,8%	929650	93,0%
5	*Cenedesmus*			2959	16,7%	1480	2,2%		
	Cyanophyta	**3700**	**100%**	**85826**	**100%**	**186818**	**100%**	**62890**	**100%**
6	*Spirulina*	370	10,0%			370	0,2%		
7	*Oscillatoria*	3330	90,0%	75098	87,5%	179049	95,8%	61410	97,6%
8	*Chroococcus*			10728	12,5%	7399	4,0%	1480	2,4%
	Chrysophyta	**901910**	**100%**	**1042113**	**100%**	**763182**	**100%**	**535670**	**100%**
9	*Navicula*	106912	11,9%	207904	20,0%	169061	22,2%	127628	23,8%
10	*Pinnularia*	41063	4,6%	85825	8,2%	32924	4,3%	45132	8,4%
11	*Terpsinoe*					370	0,05%	370	0,07%
12	*Nitzschia*	44763	5,0%	105432	10,1%	133547	17,5%	89155	16,6%
13	*Stauroneis*			7769	0,7%	4070	0,5%		
14	*Cymbella*	27746	3,1%	58080	5,6%	92854	12,2%	41433	7,7%
15	*Placoneis*	1110	0,1%	2220	0,2%	8509	1,1%	1110	0,2%
16	*Cocconeis*	215673	23,9%	81756	7,8%	73988	9,7%	43653	8,1%
17	*Gomphomena*	270424	30,0%	355509	34,1%	130218	17,1%	117270	21,9%
18	*Aulacoseira*			7029	0,7%	740	0,1%	740	0,1%

19	*Diploneis*	1850	0,2%	1110	0,1%	740	0,1%	4809	0,9%
20	*Achnanthes*	153524	17,0%	100993	9,7%	79166	10,4%	11838	2,2%
21	*Gyrosigma*	370	0,0%	7029	0,7%	7769	1,0%	1110	0,2%
22	*Surirella*				0,0%	8139	1,1%	1480	0,3%
23	*Rhopalodia*	370	0,0%	370	0,04%				
24	*Synedra*	27746	3,1%	14798	1,4%	18497	2,4%	12578	2,3%
25	*Chlorobotrys*	9989	1,1%			370	0,0%	37364	7,0%
26	*Frustulia*	370	0,0%	6289	0,6%	1480	0,2%		
27	*Cyclotella*					740	0,1%		
	TOTAL	**938905**		**1145696**		**1018440**		**1597758**	

In the Cyanophyta Division, the genus Oscillatoria is more dominant at each station than the other genera. Cyanobacteria are a common component of the Freshwater periphytic diatom (the ensemble of microorganisms attached to submerged surfaces), forming crusts and films over rocks (epilithon), plants (epiphytes), sand (epipsam-mon), sediments (epipelon), and other substrates. In many environments, these biofilms accumulate from millimeters to centimeters in thickness as vertically structured, microbial mats that form a benthic layer at the bottom of the water column, or that detach and float at the surface (Vincent 2009).

Sampling site 1

During the study at sampling site 1, the total abundance of freshwater periphytic diatom is 938,905 Ind/mm^2, and 17 genera were found, namely Spirulina, Ulotrhix, Navicula, Pinnularia, Nitzschia, Cymbella, Placoneis, Cocconeis, Gomphomena, Diploneis, Achnanthes, Oscillatoria, Gyrosigma, Rhopalodia, Synedra, Chlorobotrys, and Frustulia. In this sampling site 1, of all genera found at the time of observation, the Genus with the highest abundance was Gomphonema (270,424 Ind/mm^2), Cocconeis (215,673 Ind/mm^2), and Achnanthes (153,524 Ind/mm^2).

Studies show that diatoms such as Cocconeis are sensitive to changes in pH, temperature, salinity, water quality, nutrient availability, and even bathymetry (Martín and Fernandez 2012; Minelgaite et al. 2020). Another study discovered that Achnanthes and Gomphonema are types of periphytic microalgae that live in non-polluted waters (Novais et al. 2015; Noga et al. 2018). Based on the high abundance of Cocconeis, Achnanthes, and Gomphonema, we assume that the waters at sampling station 1 are waters that are still not polluted. Besides, this Genus that only lives in clean waters and has a low tolerance for changes in environmental conditions can be used as an indicator of non-polluted waters.

Sampling site 2

The total average abundance of Freshwater periphytic diatom at sampling station 2 was 1,146,436 Ind / mm^2. At this sampling site, we found 21 genera were found, namely Ulotrhix, Scenedesmus, Chroococcus, Navicula, Pinnularia, Nitzschia, Stauroneis, Cymbella, Placoneis, Cocconeis, Gomphomena, Aulacoseira, Achnanthes, Oscillatoria, Gyrosigma, Rhopalodia, Synedra, and Frustulia. The Chrysophyta Division also dominates this sampling site with the Genus with the highest abundance are Gomphonema (355,509 Ind / mm^2), and Navicula (207,904 Ind / mm^2).

Similar to sampling site 1, Gomphonema at sampling site 2 still dominates. However, the abundance of unpolluted water Freshwater periphytic diatom (Cocconeis and Achnanthes) has decreased. Besides, the abundance of polluted water Freshwater periphytic diatom such as Navicula and Nitzschia at this station is starting to increase. Navicula and Nitzchia are known as microalgae whose existence can indicate that the waters where they live are subject to anthropogenic pollution (sources of unnatural pollution arise due to human influence or intervention or human activities) (Sawaiker and Rodrigues, 2017). We assume that sampling site 2 shows symptoms of increased pollution.

Sampling site 3

Data on the average abundance of Freshwater periphytic diatom at point 3 total sampling was 1,018,440 Ind / mm^2, and 22 genera were found, namely Spirulina, Cosmarium, Entransia, Oedogonium, Ulotrhix, enedesmus, Chroococcus, Navicula, Pinnularia, Terpsinoe, Nitzschia, Stauroneis, Cymbella, Placoneis, Cocconeis, Gomphomena, Aulacoseira, Diploneis, Achnanthes, Oscillatoria, Gyrosigma, Surirella, Synedra, Chlorobotrys, Frustulia, and Cyclotella. The Chrysophyta Division also dominates this station with the Genus with the highest abundance Nitzschia (133.547

Ind/mm^2) dan Navicula (169.061 Ind/mm^2), Oscillatoria (179.049 Ind/mm^2).
The total average abundance of Freshwater periphytic diatom at sampling site 3, it is dominated by the Genus Oscillatoria, Nitzchia, and Navicula. The Genus Oscillatoria is known as a type of microalgae that is very tolerant of organic matter contamination (Salem et al. 2017). However, the Freshwater periphytic diatom of other eutrophic water types such as Navicula and Nitzschia have increased and started to dominate. We assume that the waters at sampling site 3 are currently happening eutrophication or enrichment of organic polluting materials. Besides, Gomphonema, which is an indicator of unpolluted waters, has begun to decline. Likewise, for the types of Cocconeis and Achnanthes.

Sampling site 4

The total average abundance of Freshwater periphytic diatom at sampling site 4 was 1,597,758 Ind/mm^{2,} and 20 genera were found, namely Oedogonium, Ulotrhix, Oscillatoria, Chroococcus, Navicula, Pinnularia, Terpsinoe, Nitzschia, Cymbella, Placoneis, Cocconeis, Gomphomena, Aulacoseira, Diploneis, Achnanthes, Gyrosigma, Surirella, Synedra, and Chlorobotrys. At sampling site 4 here it is dominated by the Chlorophyta Division with the highest abundance of Genus is Ulotrhix (130,218 Ind / mm^2).
The Genus Ulothrix dominated in sampling site 4. It is suspected that in such water conditions, only Ulothrix can tolerate high water environments at sampling site 4. Genus Ulothrix is tolerant of organic pollution and is sometimes used as an indicator of heavily polluted water (Yusuf 2020). Hydrobiota that have high tolerance will be able to survive in polluted ecosystems, while those with low tolerance have a low abundance and eventually disappear (Ramakrishnan et al. 2010). Based on the data above, it can be concluded that at station 4, there has been pollution, which is thought to be due to the high input of household waste and domestic waste because it is in the residential area.

Diversity and Dominance Index

The value of Freshwater periphytic diatom diversity in the Bango river ranged from 0.91 to 2.44 (table 3). The lowest diversity occurred at sampling site 4 with a value of 0.91, and the highest diversity occurred at station 3 with a value of 2.44. This classification of the Shannon-Wiener diversity index value can be used to determine the distribution of each species and the stability of the community: H> 3 = high diversity, high distribution of individual numbers of each species, and high community stability; 1 <H <3 = Moderate diversity, moderate distribution of the number of individuals per species and moderate community stability; H <1 = low diversity, low distribution of the number of individuals per species, and low community stability. According to the data, Bango River is categorized into a small diversity category.

Table 2. Diversity and dominance index during the study

Sampling site	Dominance index (D)	Diversity Index (H)
1	0.18275	1.94
2	0.15468	2.18
3	0.10909	2.44
4	0.66237	0.91

Based on the data, we assume that the Bango River has a low to moderate Freshwater periphytic diatom diversity, individual distribution of each species, and moderate community stability. Sampling site 1 to Sampling site 3 can still be categorized into moderate diversity, moderate distribution of the number of individuals of each species, and moderate community stability. Whereas sampling site 4 has low diversity, the distribution of the number of individuals of each species is low, and the stability of the community is low.
In terms of the dominance index, a study states that the dominance value ranges between 0 and 1 (Hossain et al. 2017). If the dominance value is close to 0, it means that almost no individuals dominate. In contrast, if the dominance is close to 1, it means that there are individuals who dominate the population. According to the dominance index value in sampling site 1 to sampling site 3, the index value is close to the value of 0, so we assume that there is no species dominance. While at station 4, the dominance index value is close to the value 1, so we assume that there is species dominance at station 4.

The Checklist

The periphytic diatom checklist (figure 2) is a list of diatom species found on periphytic substrates, namely on the surface of solid objects such as rocks, wood, or leaves in the aquatic environment of the river. This periphytic diatom checklist is usually used to study the biological and ecological diversity of diatoms in a body of water. This checklist includes the identification of diatom species that can be found in various types of periphytic substrates in the waters. In addition, this checklist can also be used as a basis for further research on the ecology and dynamics of diatoms in waters, as well as an important source of information for biologists and managers of the aquatic environment.

Cosmarium
Cosmarium is a genus of blue-green algae belonging to the division Chlorophyta. These algae can be found in a variety of freshwater habitats such as rivers, lakes and ponds. Cosmarium has a distinctive and unique cell shape, which is shaped like a half ball or semi-lunar with a hollow in the middle. Each Cosmarium cell consists of a tough cell wall and two semivacuoles, which are internal structures that look like the bubbles in an algal cell. This algae has chloroplasts which play a role in the process of photosynthesis. Cosmarium usually grows attached to substrates such as rocks, leaves or aquatic plants. Cosmarium is an important type of algae in aquatic ecosystems. This algae can be used as an indicator of water quality, because tolerance to different environmental conditions can distinguish one species from another. Apart from that, Cosmarium also plays a role in the food chain in the waters, because it is a food source for the higher organisms in it.

Entransia
Entransia is a genus of green algae belonging to the Selenastraceae family. These algae can be found in a variety of freshwater habitats such as rivers, lakes, and ponds. Entransia have a distinctive and unique cell shape, which is round or oval in shape and looks like a small bottle or tube. Each Entransia cell consists of a tough cell wall and one or two chloroplasts located near the base of the cell. This algae also has additional pigments such as carotenoids and xanthophylls which play a role in photosynthesis. Usually, Entransia grows attached to substrates such as rocks or aquatic plants. Entransia is an important type of algae in aquatic ecosystems, because it is a source of food for the higher organisms in it and also plays a role in the water nutrient cycle. In addition, Entransia can also be used as an indicator of water quality, because tolerance to different environmental conditions can differentiate one species from another.

Oedogonium
Oedogonium is a genus of filamentous green algae found in freshwater environments such as rivers, lakes, and ponds. It typically grows in dense mats on rocks or other submerged surfaces. Oedogonium can serve as a bioindicator of water quality because it is sensitive to changes in environmental conditions such as water temperature, nutrient levels, and water flow. High levels of nutrients like nitrogen and phosphorus can cause excessive growth of Oedogonium and other algae, leading to eutrophication and a decrease in dissolved oxygen levels, which can negatively impact aquatic life. Therefore, the presence of Oedogonium in a water body may indicate high nutrient levels, poor water quality, and a potential risk to aquatic life. Its abundance and distribution can be used as a tool for monitoring and assessing the ecological health of freshwater environments.

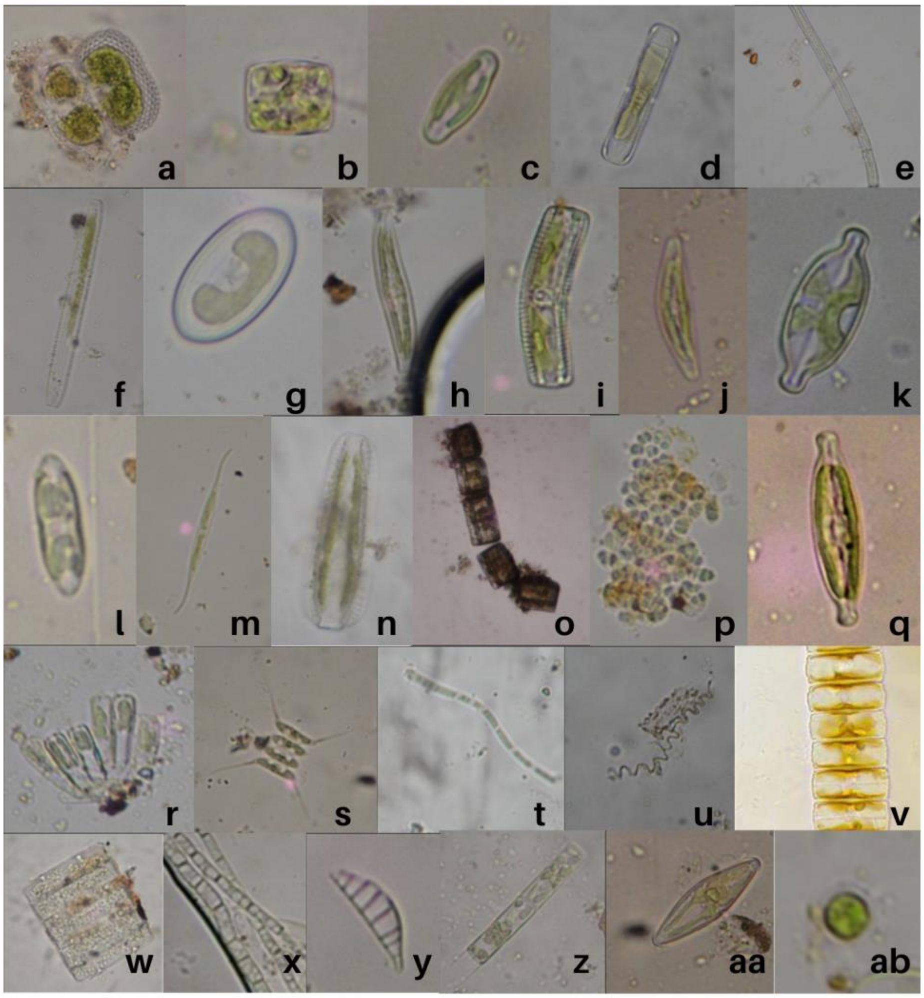

Figure 2. The checklist of freshwater periphytic diatom in stream of the brantas river during the research. (a) Cosmarium; (b) Cyclotella; (c) Navicula; (d) Pinnularia; (e) Oedogonium; (f) Synedra; (g) Cocconeis; (h) Gyrosigma; (i) Achnanthes; (j) Cymbella; (k) Placoneis; (l) Diploneis; (m) Nitzschia; (n) Surirella; (o) Melosira; (p) Chroococcus; (q) Stauroneis; (r) Gomphonema; (s) Scenedesmus; (t) Oscillatoria; (u) Spirulina; (v) Fragilaria; (w) Terpsinoe; (x) Entransia; (y) Rhopalodia; (z) Aulacoseira; (aa) Frustulia; and (ab) Chlorobotrys.

Ulotrhix

Ulothrix is a genus of filamentous green algae that are commonly found in freshwater habitats such as rivers, streams, ponds, and lakes. They are free-floating and can form mats or colonies on submerged surfaces. Ulothrix is known for its ability to fix carbon dioxide and produce oxygen through photosynthesis. As a bioindicator, Ulothrix is useful for monitoring water quality in freshwater systems. High levels of Ulothrix in a water body may indicate nutrient pollution, such as excess nitrogen and phosphorus, which can lead to harmful algal blooms and degraded water quality. In addition, changes in the morphology of Ulothrix colonies, such as a decrease in size or branching, can indicate changes in water chemistry or environmental stressors, making it a valuable tool in assessing the health of aquatic ecosystems.

Scenedesmus

Scenedesmus is a genus of green algae that has a characteristic cell shape, where its cells are arranged in groups called colonies or coenobia. Each Scenedesmus colony consists of several cells that are well-connected and are triangular or circular in shape, with the outermost cell having small protrusions called lobes. Each cell has chloroplasts that function in photosynthesis. Scenedesmus can reproduce asexually by cell division or sexually through the formation of zoospores. In aquatic environments, Scenedesmus is an important primary producer and serves as a food source for many aquatic organisms. Additionally, its abundance and diversity can be used as an indicator of water quality and environmental health.

Spirulina

Spirulina is a type of blue-green algae that belongs to the phylum Cyanobacteria. It is characterized by its spiral shape and blue-green color. Spirulina can be found in both freshwater and marine environments, and it is known for its ability to perform photosynthesis and fix atmospheric nitrogen. As a bioindicator, Spirulina can be used to monitor water quality. It is particularly sensitive to changes in nutrient levels and can indicate the presence of pollutants, such as heavy metals and organic compounds. Spirulina is also used in aquaculture as a source of food for fish and other aquatic animals due to its high protein content.

Oscillatoria

Oscillatoria is a type of cyanobacteria (blue-green algae) which has the form of single-celled filaments or many cells arranged in an unbranched manner. The main feature of the Oscillatoria is that it forms long, thin filaments with tapered ends. The cells usually have photosynthetic pigments such as chlorophyll a, phycocyanin, and phycoerythrin which give the filaments a greenish or bluish color. Oscillatoria can be found in fresh water, marine and wet land with high humidity. Oscillatoria's natural habitats include stagnant fresh water, brackish water, sea water, and wetlands on the banks of rivers or lakes. Oscillatoria usually lives attached to substrates such as rocks, gravel, or aquatic plants. Oscillatoria plays an important role in the carbon and nitrogen cycles in aquatic ecosystems (Morales et al. 2017). As a primary producer, Oscillatoria can convert sunlight into energy through the process of photosynthesis, so that it becomes a food source for other organisms in the aquatic food chain. In addition, Oscillatoria can also act as a bioindicator for water quality, because its presence can show the level of pollution and nutrient content in the waters.

However, some Oscillatoria species can also be a problem in the environment. Some species can produce toxins that are harmful to human and animal health. In addition, Oscillatoria can also thrive in eutrophic conditions (too many nutrients), causing the growth of other invasive algae and damaging the balance of aquatic ecosystems.

Chroococcus

Chroococcus is a genus of non-chlorophyll photosynthetic bacteria belonging to the Cyanobacteria group. These bacteria are usually spherical or nearly spherical, about 1-10 micrometers in diameter and usually live together in dense colonies. The cells are protected by a thick, bluish-green cell wall. Chroococcus habitat is very diverse, it can be found in fresh water, sea water or wet soil environments. These bacteria can live in highly acidic or alkaline water and are able to survive in extreme environmental conditions. Chroococcus has an important role as an oxygen producer in biogeochemical cycles, and is able to fix atmospheric nitrogen into ammonia which is used by plants. In addition, Chroococcus can also be used as a water quality bioindicator, because its presence is sensitive to environmental changes such as the level of water pollution or water acidity. If the Chroococcus population decreases drastically, this can be a bad indication for the health of the aquatic ecosystem.

Navicula

Navicula is a type of diatom that belongs to the Bacillariophyceae family. The characteristics of Navicula are its flat and sharp cell shape, and it has a special structure called a raphe which functions to control cell movement. Navicula generally live as periphyton on rock, sand, or aquatic plant substrates in fresh or seawater. In addition, Navicula can also be found in polluted waters. Navicula has an important role as a water quality bioindicator. Its presence can indicate the ecological condition of a waters. Navicula is often used as a tool to monitor water quality and heavy metal pollution, because of its ability to absorb these metals. In addition, Navicula is also used as a bioindicator of water temperature and acidity, as well as changes in water flow patterns in the waters that affect the condition of the substrate.

Pinnularia

Pinnularia is a genus of diatoms that generally live in fresh water. The characteristics of Pinnularia are the shape of the cells which are long and flat like ribbons, and have a special structure called a raphe which functions to control cell movement. Pinnularia can be found in a variety of freshwater habitats such as rivers, lakes, ponds, and swamps. Pinnularia has an important role as a water quality bioindicator. Its presence can indicate the ecological condition of waters and can be used to monitor organic and inorganic pollutants in waters. Pinnularia can also be used to indicate water quality in waters affected by industrial waste, such as waste from agriculture, livestock, and sewage treatment. In addition, Pinnularia can also show good water quality and stable ecosystem conditions, which are needed by other organisms in it.

Terpsinoe

Terpsinoe is a genus of diatoms that generally live in fresh water, especially in shallow waters such as lakes, ponds and rivers. The defining characteristic of Terpsinoe is the shape of the cells which are flat, round, and convex in the center, giving it a hat- or umbrella-like appearance when viewed from the side. Terpsinoe also have a raphe which functions to control cell movement. Terpsinoe can have an important role as a water quality bioindicator, especially in monitoring changes in the ecological conditions of waters and the impact of pollution on waters. Its presence can indicate the ecological conditions of water, including the level of acidity, nutrient levels, and the level of water pollution. Terpsinoe can also show good water quality and stable ecosystem conditions, which are needed by other organisms in it.

Nitzschia

Nitzschia is a genus of diatoms that have a long, cylindrical shape. These diatoms belong to the pennate group which means they have two valves and generally have bilateral symmetry. Nitzschia can be found in both fresh and salt water. Several Nitzschia species can be used as bioindicators of water conditions because their presence is highly dependent on water quality. For example, Nitzschia palea is an indicator of a state of eutrophication or excessive increase in nutrients in the waters. The presence of excessive Nitzschia in water can be a sign that the water is experiencing eutrophication. In addition, several Nitzschia species can also be used in the cosmetic and pharmaceutical industries due to their natural fatty acid and pigment content.

Stauroneis

Stauroneis is a genus of diatoms that have a shape similar to an ax or knife. These diatoms belong to the pennate group which means they have two valves and generally have bilateral symmetry. Stauroneis can be found in fresh waters, both cold and warm waters. Several Stauroneis species can also be used as bioindicators of water conditions because their presence is highly dependent on water quality. For example, Stauroneis anceps is often used as a bioindicator of clean and good quality natural water conditions. The presence of other Stauroneis species may indicate water conditions that are more acidic or alkaline. In addition, several Stauroneis species can also be used in biogeochemical studies due to their ability to absorb heavy metals from the aquatic environment.

Cymbella

Cymbella is a genus of diatoms that have a cylindrical or ellipsoidal shape and are generally small to medium in size. These diatoms belong to the pennate group which means they have two valves and generally have bilateral symmetry. Cymbella can be found in freshwater, in both cold and warm water environments. They are often found on the surface of moist substrates such as rocks, wood, or leaves. Several Cymbella species can be used as bio-indicators of water conditions because their presence is highly dependent on water quality. For example, Cymbella cistula is often used as a bioindicator of clean and good quality water conditions. The presence of other Cymbella species may indicate water conditions that are more acidic or alkaline. In addition, several Cymbella species can also be used in biogeochemical studies because of their ability to absorb heavy metals from the aquatic environment.

Placoneis

Placoneis is a genus of diatoms belonging to the family Naviculaceae. The distinctive feature of this genus is the shape of the cells which are flat and tight, and have transverse raphe bands. In addition, in the cell there are chloroplasts which are flat and numerous. The habitat of Placoneis is in clean fresh water, such as rivers, lakes and swamps. This genus can be found on the surface of waterlogged substrates such as rocks, sand, and other organic matter. As a bioindicator, the presence of Placoneis can be an indicator of the condition of a clean freshwater ecosystem. Its dominant presence in an environment that is kept clean indicates good water quality and is not polluted by chemicals that are harmful to life in it. Apart from that, Placoneis can also be used to measure the level of water pollution by heavy metals, because its cells are able to absorb heavy metals and store them in the cells.

Cocconeis

Cocconeis is a genus of diatoms that have a vessel-like cell shape with two valves or valves, usually symmetrical. These cells are usually small, about 20-200 micrometers. Consistent shape and size make this genus an easy species to identify and measure, so it is often used as a bioindicator in fresh and marine waters. Cocconeis usually live in relatively calm waters, such as lakes, ponds and rivers that are not too fast. They can attach to different substrates, such as rocks, wood, and aquatic plants. As a bioindicator, Cocconeis can provide information about water conditions and pollution levels. Different species of the Cocconeis genus have different tolerances to changing environmental conditions, such as temperature, pH, and nutrient content. Therefore, by studying the presence and abundance of Cocconeis species in a body of water, it can provide an overview of the water quality and environmental conditions that exist there.

Gomphomena

Gomphonema is a genus of diatoms that are commonly found in freshwater environments. They are characterized by their elongated shape and their ability to form chains of cells. Gomphonema cells are usually attached to substrates such as rocks, sediments, or aquatic plants by a mucilaginous stalk that is secreted by the cell. The cells are usually rectangular or trapezoidal in shape and have a raphe, a slit-like opening that runs the length of the cell. The raphe is used for movement and attachment to substrates. Gomphonema species are known to be indicators of environmental conditions, such as nutrient levels and water flow rates. They are commonly used as bioindicators for water quality assessments and are sensitive to changes in water chemistry and pollution levels. Some species of Gomphonema are also known to produce bioactive compounds with potential uses in the pharmaceutical and biotechnology industries.

Aulacoseira

Aulacoseira is a genus of freshwater diatoms that are commonly found in lakes and rivers. They are characterized by their distinctive circular or elliptical shape and their radial symmetry. Aulacoseira cells are enclosed in a siliceous frustule, which consists of two overlapping halves. Aulacoseira species are important indicators of past and present environmental conditions, especially in lake sediments. Their abundance and composition can provide information about past nutrient levels, climate change, and other environmental factors. Aulacoseira is also important primary producers in freshwater ecosystems, contributing to the food web and supporting aquatic life. Some species of Aulacoseira are known to produce toxins, which can have harmful effects on aquatic organisms and human health.

Diploneis

Diploneis is a genus of freshwater and marine diatoms that are commonly found in a variety of aquatic habitats, such as lakes, rivers, and estuaries. They are characterized by their distinctive boat-shaped or elliptical frustules, which are composed of overlapping silica valves. Diploneis species play an important role in aquatic ecosystems as primary producers, contributing to the food web and supporting the growth of other organisms. They are also important indicators of environmental conditions, as their abundance and species composition can provide information about water quality and nutrient levels. Some species of Diploneis have been found to produce toxins that can have harmful effects on aquatic organisms and human health. Additionally, they are often used in environmental monitoring and assessment programs to evaluate the health of aquatic ecosystems and the effectiveness of management strategies.

Achnanthes

Achnanthes is a genus of diatoms with a distinctive feature of this genus is the shape of the cells which are lancet or elliptical with tight lines located in the middle of the cell. These cells have many microscopic pits called areolae which form a characteristic pattern and are often used for identification of diatoms. Achnanthes habitat is fresh water, whether in rivers, lakes or swamps. Some species can be found in nutrient-rich substrates, such as in water polluted by organic wastes or in eutrophic waters. However, most Achnanthes species are considered indicators of a relatively clean environment. Achnanthes has an important role as a water quality bioindicator. Some species are sensitive to environmental changes, such as increased nutrients, increased temperature, or decreased pH, so their presence can indicate environmental problems in these waters.

Gyrosigma
Gyrosigma is a genus of freshwater diatoms that are commonly found in streams, rivers, and lakes. They have a unique sigmoid shape that resembles a human ear or a boomerang, and are often used as bioindicators of water quality due to their sensitivity to environmental changes. In terms of ecology, Gyrosigma is an important part of the food chain in freshwater ecosystems, as they are a primary producer and provide a source of nutrition for other organisms such as zooplankton and small fish. They also play a role in nutrient cycling, helping to regulate levels of nitrogen and phosphorus in the water. Some species of Gyrosigma have been found to produce toxins that can have harmful effects on aquatic organisms and even humans. As such, monitoring populations of Gyrosigma can provide valuable information about the health of freshwater systems and potential risks to human health.

Surirella
Surirella is a type of diatom that can be found in both freshwater and marine environments. This diatom has a curved shape and consists of two cells tightly packed together so that it looks like a small cylindrical box. The cells have fine fibers or regular stripes, and there are many different species. Surirella's natural habitat includes clean and sufficiently flowing fresh water, and can occasionally be found in brackish or seawater environments that enter estuaries. Surirella can act as a water quality bioindicator because its existence is very sensitive to environmental changes caused by pollution, eutrophication, and other factors.

Rhopalodia
Rhopalodia is a genus of a group of diatoms that can be found in fresh waters, including rivers, lakes, and swamps. Diatoms of the genus Rhopalodia have general characteristics such as a flat or elliptical shape, and sizes that vary from microscopic to several millimeters. Rhopalodia is often found in waters with eutrophic or nutrient-abundant conditions, where high levels of phosphorus and nitrogen lead to algal overgrowth. As a bioindicator, Rhopalodia can be used to indicate the eutrophication level of water. If Rhopalodia is found in large numbers, it indicates a eutrophication problem. On the other hand, Rhopalodia can provide ecological benefits, including as a food source for aquatic organisms such as plankton, fish, and other aquatic animals.

Synedra
Synedra is a genus of diatoms that are commonly found in fresh and marine water. The distinguishing feature of this genus is its long, cylindrical shape and the transverse central slit (raphe). The natural habitats of Synedra vary from fresh water, such as lakes and rivers, to marine environments, such as estuaries and estuaries. Some Synedra species can also be found in muddy substrates or in nutrient-rich waters. Synedra can act as a bioindicator in assessing water quality because of its sensitivity to various environmental parameters, such as temperature, brightness, pH, and nutrients. The presence or abundance of Synedra species in an aquatic ecosystem can provide information about water quality and environmental conditions that may affect the life of other organisms in it.

Chlorobotrys
Chlorobotrys is a genus of green algae that is commonly found in freshwater environments. It is a unicellular organism that has a distinctive shape, with two long flagella that are used for movement. Chlorobotrys is often found in stagnant or slow-moving water bodies such as ponds, swamps, and wetlands. As a bioindicator, Chlorobotrys is particularly useful for monitoring changes in water quality and nutrient levels. This is because it is highly sensitive to changes in the environment, and can quickly respond to changes in nutrient levels, temperature, and other environmental factors. In polluted water bodies, Chlorobotrys can be used to detect the presence of excess nutrients such as nitrogen and phosphorus, which can lead to eutrophication and harmful algal blooms. By monitoring the presence and abundance of Chlorobotrys, researchers can gain insights into the overall health of freshwater ecosystems, and take steps to mitigate the effects of pollution and other environmental stressors.

Frustulia

Frustulia is a genus of diatoms that are commonly found in freshwater environments. They are characterized by having a high valve density and an elongated cell shape. Several species of Frustulia can be found in clean waters with good water quality. Frustulia can be used as a water quality bioindicator because of its sensitivity to environmental changes. Several species of Frustulia can indicate the presence of pollutant substances in water such as heavy metals and pesticides, so that they can be used to monitor pollution levels in waters. In addition, a decrease in the number of species and density of Frustulia can also be an indicator of a decrease in water quality and disruption of aquatic ecosystems. Therefore, Frustulia can be used as a tool for monitoring water quality and environmental sustainability.

Cyclotella

Cyclotella is a genus of diatoms belonging to the centric group of diatoms. A distinctive feature of Cyclotella is the shape of the flattened round cells, with many apexes surrounding the cells. In nature, Cyclotella can be found in a variety of freshwater environments, such as lakes, rivers, and marshes. Cyclotella is used as a bioindicator because of its sensitivity to changes in the aquatic environment. Nutrient concentration, temperature, pH, and water clarity can affect the presence and abundance of Cyclotella in a body of water. Therefore, changes in Cyclotella populations can provide clues to the current state of the aquatic environment. In addition, several Cyclotella species are also used as indicators to monitor heavy metal pollutants in waters, because they can absorb these metals and concentrate in their cells.

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2. Halaman bukti revisi I
23 Mei 2023

A. Email dari editor berisi detail revisi yang disampaikan oleh reviewer **(instruksi revisi 1)**

Revisions External Inbox ×

Aditya Triyanto <biodiv33@gmail.com>
to me

Tue, May 23, 2:03 PM

Endang Masithah:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Checklist of Freshwater Periphytic Diatoms in the Midstream of Brantas River, East Java, Indonesia".

Our decision is: Revisions Required

Reviewer A:

Dear Author(s),

Thank you very much for your submission. Here is my review.

1. The abstract is too brief to be published in the journal. Abstract is about 200-300 words

2. This manuscript has lacks references. At least, you need to compose a minimum of 20 references which 80% of scientific journals published in the last 10 years (2013-2023), and a maximum of 10% of references in local references.

B. Author menjawab semua saran reviewer **(perbaikan revisi 1)**

Dear Editor,

All the corrections suggested by the reviewer A were included in the new version of the manuscript:

Reviewer A:

1. The abstract is too brief to be published in the journal. Abstract is about 200-300 words

2. This manuscript has lacks references. At least, you need to compose a minimum of 20 references which 80% of scientific journals published in the last 10 years (2013-2023), and a maximum of 10% of references in local references.

Answer:

1. The abstract has as suggested around 200-300 words

2. References have been added according to reviewer suggestions, more than 80% are articles above 2013

All the best,

Mashitah and Islamy

COVERING LETTER

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

- [x] The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
- [x] The submission file is in OpenOffice, Microsoft Word (DOC, not DOCX), or RTF document file format.
- [ ] The text is single-spaced; uses a 10-point font; employs italics, rather than underlining (except with URL addresses); and all illustrations, figures, and tables are placed within the text at the appropriate points, rather than at the end.
- [x] The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines.
- [x] Most of the references come from current scientific journals (c. 80% published in the last 10 years), except for taxonomic papers.
- [x] Where available, DOIs for the references have been provided.
- [x] When available, a certificate for proofreading is included.

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Ensure that the following items are present:

The first corresponding author must be accompanied with contact details:

- [x] E-mail address
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All necessary files have been uploaded, and contain:

- [x] Keywords
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- [x] Charts (graphs and diagrams) are drawn in black and white images; use shading to differentiate

Title:

Checklist of Freshwater Periphytic Diatoms in the Midstream of Brantas River, East Java, Indonesia

Author(s) name:

Endang Dewi Masithah[1], R Adharyan Islamy[2]

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- [x] Biodiversitas Journal of Biological Diversity
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Novelty:

(state your claimed novelty of the findings versus current knowledge)

This study provides detailed information on the types of periphytic diatoms found in the area, including species not previously recorded in the area. In addition, this study also provides a better understanding of the structure and composition of the periphytic diatom community which can be used as an indicator of river water quality.

Statements:

This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.
Author(s) has been read and agree to the Ethical Guidelines.

List of five potential reviewers

(Fill in names of five potential reviewers **that agree to review your manuscpt** and their **email** addresses. He/she should have Scopus ID and come from different institution with the authors; and from at least three different countries)

Ren Fitriadi, Universitas Jenderal Soedirman, renfitriadi@unsoed.ac.id
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Place and date:

Surabaya, 8 May 2023

Sincerely yours,

(fill in your name, no need scanned autograph)

Endang Dewi Masithah

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Checklist of Freshwater Periphytic Diatoms in the Midstream of Brantas River, East Java, Indonesia

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Abstract. Periphytic diatoms are a group of microalgae that live attached to the surface of the substrate such as rocks, aquatic plants, or other objects in the water. This group has potential as an indicator of environmental quality of water because its existence is influenced by water quality, such as nutrient levels and pollution levels. They are also useful in describing the ecological state, performances, and sustainability of ecosystems because of their ability to measure various environmental parameters and correlate them with diversity, evenness, and richness. This study provides a checklist of freshwater periphytic diatoms in the midstream of Brantas River, East Java, Indonesia. The sampling and identification according to published methods were carried out at the Laboratory of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya. This study was undertaken once a week in a month (May 2022) between 9 a.m. and 1 p.m (GMT + 7). Determination of sampling sites based on differences in land use. Sampling site 1 is located at the Rolak Kedungkandang DAM, sampling site 2 is located at the Waterfall Coban Amprong tourist area, sampling site 3 is located at the river branch below the Kedungkandang bridge, and sampling site 4 is located in a residential and agricultural area. The total abundance of freshwater periphytic diatom ranges from 938,905 Ind / mm2 – 1,597,758 Ind / mm2. The lowest total abundance is at station 1 with a value of 938,905 Ind / mm2 and consists of 17 genera. The highest total abundance is at station 4 with a value of 1,597,758 Ind / mm^2 and consists of 17 genera. In this study, 27 genera were found spread across 3 phylum.

Keywords: Epilithic, microalgae, freshwater periphytic diatom, water quality.

Running title: Biodiversity of Freshwater periphytic diatom and Environmental Condition

INTRODUCTION

Diatoms are an important group of microalgae and are the largest group of periphytic algae in fresh waters. The diatom group is characterized by a cell body covered with a silica shell, which can take various shapes and have distinctive patterns on its surface (Danielson et al. 2012; Mayzel et al. 2021). The abundance of diatoms in fresh waters is very high and often becomes dominant in fresh waters because of their good ability to utilize nutrients in the waters and their high tolerance for various environmental conditions in fresh waters (De Nicola et al. 2014; Camas-Anzueto et al. 2015; Soraya and Islamy 2022). Therefore, Diatoms are an important group of algae as bioindicators of fresh water environmental conditions (Costache et al. 2013; Charles et al. 2019; Kazbar et al. 2019).

Freshwater periphytic diatoms have an important role as environmental bioindicators (Park et al. 2016; Rivera et al. 2019; Schmidt et al. 2019). The presence or absence of periphytic diatom species can provide very useful information to identify disturbances or changes in fresh waters. Some examples of periphytic diatom and their role as bioindicators are Nitzschia which is used to indicate organic contamination (Ahn et al. 2013; Rovira et al. 2015; Sugie et al. 2020), Fragilaria to indicate changes in water quality such as eutrophication, A study conducted that the presence of metal contamination in waters proved to be a strong driver of the formation of diatom community structure, and allowed the identification of tolerant species such as *Cocconeis placentula* var. euglypta, *Eolimna minima*, *Fragilaria gracilis*, *Nitzschia sociabilis*, *Pinnularia parvulissima*, and *Surirella angusta* (Fernández et al. 2012; Brown et al. 2017; Mori et al. 2017). The use of periphytic diatoms as environmental bioindicators can provide more detailed and accurate information about environmental conditions in fresh waters, so it is important to conduct research on periphytic diatoms as environmental bioindicators.

Drafting a checklist of aquatic organisms is very important, especially those whose role is as bioindicators of

aquatic environmental conditions (Bilanovic et al. 2016; Islamy and Hasan 2020; Isroni et al. 2023). The checklist can help researchers and environmentalists to obtain data on the diversity of periphytic diatom species in certain fresh waters so that it can be a reference for determining which species are present or absent in fresh waters (Fortes et al. 2010). By obtaining accurate and detailed information about the condition of the freshwater environment, the periphytic diatom checklist can assist in environmental conservation efforts and improve the sustainability of freshwater management (Wang et al. 2012; Pattanayak et al. 2020).
One location that can be used as a case study to determine water quality is the midstream of Brantas River. This river is one of the rivers that has an important role in supporting the lives of the surrounding community, both as a source of irrigation water, a means of transportation, and as a tourist spot. However, the environmental conditions around the river are getting worse due to domestic and industrial waste pollution, as well as increasing human activities. Therefore, research on periphytic sampling of freshwater diatoms in midstream of Brantas River is important to do. This study aims to provide a checklist of the diversity of freshwater periphytic diatom species and an overview of the environmental conditions of the midstream of Brantas River. The research results can be used as a reference in making policies to maintain the quality of the water environment in the midtsream of river. In addition, this research can also provide a better understanding of the use of periphytic diatoms as an indicator of the environmental quality of water in the tropics (Hariyadi et al. 1992; Mattson 1999; Mason 2010; Martin et al. 2012; Schulte 2015; Sudrajat et al. 2016).

MATERIALS AND METHODS

Study area
This research was conducted at the midstream of Brantas River, Kedungkandang District, Malang City, East Java, Indonesia. Determination of periphytic freshwater diatom sampling locations based on different land uses (Soraya and Islamy 2022). First, various types of land use in the study area, such as agricultural land, residential areas, industrial areas, or natural areas were mapped and then a representative sampling location was determined for each type of land use to provide a comprehensive assessment of the diatom community in the study area.

Figure 3. Location of sampling sites at midstream of Brantas River, Kedungkandang District, Malang City, East Java, Indonesia

Sampling site 1: The sampling site 1 is located Rolak Kedungkandang DAM (7°59'21.9"S 112°39'11.4"E). Ecological and substrate conditions in this area are affected by the presence of dams, which provide a unique

habitat for a variety of freshwater organisms. The water inside the dam is relatively clear and shallow, with a depth of about 1-3 meters. The substrate in this area is dominated by rock and gravel, which provide suitable attachment sites for periphytic diatoms. On the other side of the dam is plantation land, with plants such as vegetables and fruits being cultivated in the surrounding area. This can allow the entry of water streams containing pesticides and agricultural fertilizers which can affect water quality and aquatic ecology.
Sampling site 2: This station is located in Coban Amprong which is a low waterfall tourist spot located in Kedungkandang District, Malang City, East Java, Indonesia. The ecology and substrate in this area are influenced by waterfall flows, vegetation, and other environmental factors. The water in Coban Amprong tends to be cold and fresh with a swift waterfall flow and the natural rocks around it form very different substrate conditions compared to the conditions at the Rolak Kedungkandang Dam. The substrate in this area is dominated by granite and other rocks, which are the attachment sites for periphytic diatoms and other microorganisms. The vegetation around the waterfall consists of plants such as pine trees, teak trees, bamboo, and other shrubs. Around Coban Amprong there are several plantation areas that are used as agricultural land for the cultivation of plants such as vegetables, fruits, and flowers.
Sampling site 3: This station is located in a river branching area near the Kedungkandang bridge, Malang, East Java, Indonesia, which is an area that has a unique ecology and substrate. This spot has a calm and slow water flow, as well as differences in elevation which can affect the condition of the substrate. Due to the calm water conditions, the substrate in these areas tends to be softer and easier to settle. The substrate around the river branching area is dominated by silt and sand. In addition, around this area there is also quite abundant vegetation, such as shrubs and trees. These plants provide shelter for many types of animals, such as fish and insects.
Sampling site 4: This sampling site is located in a residential area near Kedungkandang Sub-District, Malang, East Java, Indonesia. It is an area that has a different environment and substrate than the natural river area. In these areas, the dominant substrate is soil that has been altered by human activities to build houses, roads, and other infrastructure. The river which flows around this residential area has more polluted water conditions compared to the natural area of the river, due to domestic and industrial waste being dumped into the river. Even so, around this residential area, there are still a number of green plants such as trees and shrubs that can provide shelter for various types of animals and insects. This area can also still be a habitat for periphytic diatoms and other microorganisms that can live and thrive on substrates that have been altered by human activities.

Sample collecting

Water quality, Freshwater periphytic diatom sampling, and identification were carried out at the Laboratory of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya. This study was undertaken once a week in a month (May 2020) between 9 and 13 h (GMT + 7). Identification and Freshwater periphytic diatom samples were collected by scraping the substrate (stones/rocks/aquatic plant) in and around the respective water sampling stations.

Freshwater periphytic diatom sampling

The procedure of Freshwater periphytic diatom sampling is based on Freshwater periphytic diatom Collection Protocols (West Virginia Department of Environmental Protection 2018). Sample collected during stable flow conditions and streams are not turbid (i.e., the substrate is visible). Label the sample container with Stream Name, AN-Code, date, and collector. Sample only be collected from rocks (epilithic habitat) from riffle/run areas of the streams. Collect five separate cobble-sized rocks that are exposed to varying light conditions and contain varying Freshwater periphytic diatom communities (brown vs. green). Rinse the PVC ring, toothbrush, micro-spatula, and squirt bottle thoroughly with stream water at the site before each sampling event to avoid contamination from a prior sampling of subsequent collections.
Using the PVC ring to delimit the sample area (12.56 cm^2), use the micro-spatula to scrape all algae from the upper surface of rocks into the sample jar. Use the toothbrush to loosen any remaining Freshwater periphytic diatom. Remove the sampler and rinse loosened algae into the sample jar using clear stream water collected from that sampling site in the squirt bottle. Repeat Step 5 until all the Freshwater periphytic diatom from the five rocks (representing 62.8 cm^2 of the sampled area) is composited into one sample jar.
Rinse the micro-spatula, toothbrush, and PVC ring into the sample, removing as much of the lingering Freshwater periphytic diatom as possible. Snap the labeled lid onto the container. Rinse the PVC ring, toothbrush, micro spatula, and squirt bottle thoroughly with stream water at the site after each sampling event to avoid contamination of subsequent collections. For preservation, we assume that the sample jar is about 33 ml full, preserve with an adequate amount of 1 drop Lugol's solution (West Virginia Department of

Environmental Protection 2018).

Identification
Freshwater periphytic diatom identification was performed using an Olympus Light Microscope (model CX 40) to determine the types of Freshwater periphytic diatom that had been collected. Identification sampling based on Freshwater periphytic diatom Collection Protocols (West Virginia Department of Environmental Protection 2018). A drop of the sample was placed on glass slides. These were examined at different magnifications using an Olympus light microscope (model CX 40) and illustrate the Freshwater periphytic diatom that is seen in the microscope. Most taxa within samples were identified to species level by reference to standard works, such as identification books and numerous journals (Alika and Akoma 2012).

Freshwater periphytic diatom algae abundance
The calculation of Freshwater periphytic diatom abundance is carried out according to the published procedure (APHA 1985), with the formula:

$$N = \frac{n\ xA_t\ xV_t}{A_c\ x\ V_s\ x\ A_s}$$

Where N = density of Freshwater periphytic diatom algae (ind / mm^2); n = Number of organisms found; At = area of the cover glass (mm^2); Vt = volume of sample accommodated in sample bottle (ml), Ac = area of the field of view multiplied by the number of fields of view observed (mm^2); Vs = volume of water drops used in the observation (ml).

Freshwater periphytic diatom diversity index
Diversity assessment was carried out for analysis of the richness of elements, taxonomic diversity (by the number of a taxons in hydrobiological groups), expressed by the number of taxons, and 2) for the relative representation of populations in communities (by abundance or biomass). The indices are as follows (Protasov et al. 2019):

$$\mathrm{H} = -?\frac{\mathrm{ni}}{\mathrm{N}}\ln\frac{\mathrm{ni}}{\mathrm{N}}$$

Where: N = common organism abundance; s = species number; ni = species number of each species; H, Shannon diversity index.

Dominance Index
The dominance index was assessed by formula/equation as the Shannon Index. Simpson index is calculated as follows Simpson Dominance Index (D):

$$D = \ ?\left(\frac{ni}{n}\right)^2$$

Where D = Dominance index; ni = number of individuals of species i; n = total number of individuals.

RESULTS AND DISCUSSION

Composition and abundance of Freshwater periphytic diatom
Data from the composition determination and the results of the average Freshwater periphytic diatom abundance during the study at each sampling site are presented in Table 1. The total abundance of Freshwater periphytic diatom ranges from 938,905 Ind / mm^2 – 1,597,758 Ind / mm^2. The lowest total abundance is at station 1 with a value of 938,905 Ind / mm^2 and consists of 17 genera. The highest total abundance is at station 4 with a value of 1,597,758 Ind / mm^2 and consists of 17 genera. In this study, 27 genera were found spread across 3 phylum. Freshwater periphytic diatom composition and abundance data can be seen in Table 1.
Based on Table 1, at station 1 to station 3, the dominant Freshwater periphytic diatom division is Chrysophyta with relative abundance values of 96%, 91%, and 75%, respectively. Besides, their high abundance, at each station, species from this Division are also more numerous and varied than those from the Chlorophyta, Chyanophyta, and Euglenophyta Divisions that are found in the waters of the Bango River. The Chrysophyta is

a group of algae that are qualitatively and quantitatively found in various river type waters (Adjie et al. 2003; Kristiansen and Škaloud 2017). Golden algae are one of the most critical functional components in freshwater microalgae. They are indicators of changes in environmental parameters such as pH, salinity, and climate (Korneva and Solovyeva 2017; Kristiansen and Škaloud 2017). The Chrysophyta, a group of protists containing single-celled individuals as well as quite complex colonial forms, can briefly be defined by the following biochemical and structural criteria: chloroplasts with chlorophylls a and c but lacking b, fucoxanthin as the most critical accessory pigment, β-1, 3-glucan as a storage product, swarmers with heterokont flagella (i.e., one long hairy and one shorter smooth, the latter in many cases only to be detected by EM). Endogenous silicate cysts (stomatocysts) are present throughout the class (Kristiansen and Škaloud 2017).
Meanwhile, at sampling site 4, the dominant Freshwater periphytic diatom was from the Chlorophyta division with a relative abundance value of 63%. Chlorophyta is usually found in stagnant waters and is planktonic. However, Chlorophyta of the Genus Oedogonium and Ulothrix are not. Oedogonium and Ulothrix more often live attached either to plants, rocks, or other surfaces (García et al. 2012). In the Chlorophyta division, at stations 1, 2, and 4, Ulotrhix is more able to grow than other Chlorophyta genera, which was found probably due to the ability of Ulotrhix to be more tolerant of environmental conditions. At station 3, the abundance of Ulothrix is slight due to the condition of station three, which has lower levels of phosphate and nitrate than other stations.
Cyanophyta and Euglenophyta divisions were found at each station. The Euglenophyta are a heterogeneous group of freshwaters. Some of them are naked, covered only by the periplasm, and others have an external lorica (envelope), which encloses the cell (Poniewozik 2017). Euglenophyta has a flagellum that functions as a means of movement in the water. Hence the little Euglenophyta Division is found as Freshwater periphytic diatom attached to the substrate. Meanwhile, benthic cyanophytes or those that become Freshwater periphytic diatoms are less likely to bloom or be abundant. Species that are not planktonic are generally species that rarely result in population explosions (blooming) due to eutrophication (nutrient enrichment) (Prihantini et al. 2010).

Table 3. Data on Composition and Abundance of Freshwater periphytic diatom in the river during the Study

	Freshwater Periphytic Diatom	**SAMPLING SITES**							
		1		**2**		**3**		**4**	
		(Ind/mm^2)	**(%)**	**(Ind/mm^2)**	**(%)**	**(Ind/mm^2)**	**(%)**	**(Ind/mm^2)**	**(%)**
No	**Chlorophyta**	**33295**	**100%**	**17757**	**1**	**68440**	**1**	**999198**	**1**
1	*Cosmarium*					370	0,5%		
2	*Entransia*					54751	80,0%		
3	*Oedogonium*					9249	13,5%	69548	7,0%
4	*Ulotrhix*	33295	100,0%	14798	83,3%	2590	3,8%	929650	93,0%
5	*Cenedesmus*			2959	16,7%	1480	2,2%		
	Cyanophyta	**3700**	**100%**	**85826**	**100%**	**186818**	**100%**	**62890**	**100%**
6	*Spirulina*	370	10,0%			370	0,2%		
7	*Oscillatoria*	3330	90,0%	75098	87,5%	179049	95,8%	61410	97,6%
8	*Chroococcus*			10728	12,5%	7399	4,0%	1480	2,4%
	Chrysophyta	**901910**	**100%**	**1042113**	**100%**	**763182**	**100%**	**535670**	**100%**

9	*Navicula*	106912	11,9%	207904	20,0%	169061	22,2%	127628	23,8%
10	*Pinnularia*	41063	4,6%	85825	8,2%	32924	4,3%	45132	8,4%
11	*Terpsinoe*					370	0,05%	370	0,07%
12	*Nitzschia*	44763	5,0%	105432	10,1%	133547	17,5%	89155	16,6%
13	*Stauroneis*			7769	0,7%	4070	0,5%		
14	*Cymbella*	27746	3,1%	58080	5,6%	92854	12,2%	41433	7,7%
15	*Placoneis*	1110	0,1%	2220	0,2%	8509	1,1%	1110	0,2%
16	*Cocconeis*	215673	23,9%	81756	7,8%	73988	9,7%	43653	8,1%
17	*Gomphomena*	270424	30,0%	355509	34,1%	130218	17,1%	117270	21,9%
18	*Aulacoseira*			7029	0,7%	740	0,1%	740	0,1%
19	*Diploneis*	1850	0,2%	1110	0,1%	740	0,1%	4809	0,9%
20	*Achnanthes*	153524	17,0%	100993	9,7%	79166	10,4%	11838	2,2%
21	*Gyrosigma*	370	0,0%	7029	0,7%	7769	1,0%	1110	0,2%
22	*Surirella*				0,0%	8139	1,1%	1480	0,3%
23	*Rhopalodia*	370	0,0%	370	0,04%				
24	*Synedra*	27746	3,1%	14798	1,4%	18497	2,4%	12578	2,3%
25	*Chlorobotrys*	9989	1,1%			370	0,0%	37364	7,0%
26	*Frustulia*	370	0,0%	6289	0,6%	1480	0,2%		
27	*Cyclotella*					740	0,1%		
	TOTAL	**938905**		**1145696**		**1018440**		**1597758**	

In the Cyanophyta Division, the genus Oscillatoria is more dominant at each station than the other genera. Cyanobacteria are a common component of the Freshwater periphytic diatom (the ensemble of microorganisms attached to submerged surfaces), forming crusts and films over rocks (epilithon), plants (epiphytes), sand (epipsam-mon), sediments (epipelon), and other substrates. In many environments, these biofilms accumulate from millimeters to centimeters in thickness as vertically structured, microbial mats that form a benthic layer at the bottom of the water column, or that detach and float at the surface (Vincent 2009).

Sampling site 1

During the study at sampling site 1, the total abundance of freshwater periphytic diatom is 938,905 Ind/mm^2, and 17 genera were found, namely Spirulina, Ulotrhix, Navicula, Pinnularia, Nitzschia, Cymbella, Placoneis, Cocconeis, Gomphomena, Diploneis, Achnanthes, Oscillatoria, Gyrosigma, Rhopalodia, Synedra, Chlorobotrys, and Frustulia. In this sampling site 1, of all genera found at the time of observation, the Genus with the highest abundance was Gomphonema (270,424 Ind/mm^2), Cocconeis (215,673 Ind/mm^2), and Achnanthes (153,524 Ind/mm^2).

Studies show that diatoms such as Cocconeis are sensitive to changes in pH, temperature, salinity, water quality, nutrient availability, and even bathymetry (Martín and Fernandez 2012; Minelgaite et al. 2020). Another study discovered that Achnanthes and Gomphonema are types of periphytic microalgae that live in non-polluted waters (Novais et al. 2015; Noga et al. 2018). Based on the high abundance of Cocconeis, Achnanthes, and Gomphonema, we assume that the waters at sampling station 1 are waters that are still not polluted. Besides, this Genus that only lives in clean waters and has a low tolerance for changes in environmental conditions can be used as an indicator of non-polluted waters.

Sampling site 2

The total average abundance of Freshwater periphytic diatom at sampling station 2 was 1,146,436 Ind / mm^2. At this sampling site, we found 21 genera were found, namely Ulotrhix, Scenedesmus, Chroococcus, Navicula,

Pinnularia, Nitzschia, Stauroneis, Cymbella, Placoneis, Cocconeis, Gomphomena, Aulacoseira, Achnanthes, Oscillatoria, Gyrosigma, Rhopalodia, Synedra, and Frustulia. The Chrysophyta Division also dominates this sampling site with the Genus with the highest abundance are Gomphonema (355,509 Ind / mm^2), and Navicula (207,904 Ind / mm^2).
Similar to sampling site 1, Gomphonema at sampling site 2 still dominates. However, the abundance of unpolluted water Freshwater periphytic diatom (Cocconeis and Achnanthes) has decreased. Besides, the abundance of polluted water Freshwater periphytic diatom such as Navicula and Nitzschia at this station is starting to increase. Navicula and Nitzchia are known as microalgae whose existence can indicate that the waters where they live are subject to anthropogenic pollution (sources of unnatural pollution arise due to human influence or intervention or human activities) (Sawaiker and Rodrigues, 2017). We assume that sampling site 2 shows symptoms of increased pollution.

Sampling site 3
Data on the average abundance of Freshwater periphytic diatom at point 3 total sampling was 1,018,440 Ind / mm^2, and 22 genera were found, namely Spirulina, Cosmarium, Entransia, Oedogonium, Ulotrhix, enedesmus, Chroococcus, Navicula, Pinnularia, Terpsinoe, Nitzschia, Stauroneis, Cymbella, Placoneis, Cocconeis, Gomphomena, Aulacoseira, Diploneis, Achnanthes, Oscillatoria, Gyrosigma, Surirella, Synedra, Chlorobotrys, Frustulia, and Cyclotella. The Chrysophyta Division also dominates this station with the Genus with the highest abundance Nitzschia (133.547 Ind/mm^2) dan Navicula (169.061 Ind/mm^2), Oscillatoria (179.049 Ind/mm^2).
The total average abundance of Freshwater periphytic diatom at sampling site 3, it is dominated by the Genus Oscillatoria, Nitzchia, and Navicula. The Genus Oscillatoria is known as a type of microalgae that is very tolerant of organic matter contamination (Salem et al. 2017). However, the Freshwater periphytic diatom of other eutrophic water types such as Navicula and Nitzschia have increased and started to dominate. We assume that the waters at sampling site 3 are currently happening eutrophication or enrichment of organic polluting materials. Besides, Gomphonema, which is an indicator of unpolluted waters, has begun to decline. Likewise, for the types of Cocconeis and Achnanthes.

Sampling site 4
The total average abundance of freshwater periphytic diatom at sampling site 4 was 1,597,758 Ind/$mm^{2,}$ and 20 genera were found, namely Oedogonium, Ulotrhix, Oscillatoria, Chroococcus, Navicula, Pinnularia, Terpsinoe, Nitzschia, Cymbella, Placoneis, Cocconeis, Gomphomena, Aulacoseira, Diploneis, Achnanthes, Gyrosigma, Surirella, Synedra, and Chlorobotrys. At sampling site 4 here it is dominated by the Chlorophyta Division with the highest abundance of Genus is Ulotrhix (130,218 Ind / mm^2).
The Genus Ulothrix dominated in sampling site 4. It is suspected that in such water conditions, only Ulothrix can tolerate high water environments at sampling site 4. Genus Ulothrix is tolerant of organic pollution and is sometimes used as an indicator of heavily polluted water (Yusuf 2020). Hydrobiota that have high tolerance will be able to survive in polluted ecosystems, while those with low tolerance have a low abundance and eventually disappear (Ramakrishnan et al. 2010). Based on the data above, it can be concluded that at station 4, there has been pollution, which is thought to be due to the high input of household waste and domestic waste because it is in the residential area.

Diversity and Dominance Index
The value of Freshwater periphytic diatom diversity in the Bango river ranged from 0.91 to 2.44 (table 3). The lowest diversity occurred at sampling site 4 with a value of 0.91, and the highest diversity occurred at station 3 with a value of 2.44. This classification of the Shannon-Wiener diversity index value can be used to determine the distribution of each species and the stability of the community: H> 3 = high diversity, high distribution of individual numbers of each species, and high community stability; 1 <H <3 = Moderate diversity, moderate distribution of the number of individuals per species and moderate community stability; H <1 = low diversity, low distribution of the number of individuals per species, and low community stability. According to the data, Bango River is categorized into a small diversity category.

Table 4. Diversity and dominance index during the study

Sampling site	Dominance index (D)	Diversity Index (H)
1	0.18275	1.94
2	0.15468	2.18
3	0.10909	2.44
4	0.66237	0.91

Based on the data, we assume that the Bango River has a low to moderate Freshwater periphytic diatom diversity, individual distribution of each species, and moderate community stability. Sampling site 1 to Sampling site 3 can still be categorized into moderate diversity, moderate distribution of the number of individuals of each species, and moderate community stability. Whereas sampling site 4 has low diversity, the distribution of the number of individuals of each species is low, and the stability of the community is low.
In terms of the dominance index, a study states that the dominance value ranges between 0 and 1 (Hossain et al. 2017). If the dominance value is close to 0, it means that almost no individuals dominate. In contrast, if the dominance is close to 1, it means that there are individuals who dominate the population. According to the dominance index value in sampling site 1 to sampling site 3, the index value is close to the value of 0, so we assume that there is no species dominance. While at station 4, the dominance index value is close to the value 1, so we assume that there is species dominance at station 4.

The Checklist

The periphytic diatom checklist (figure 2) is a list of diatom species found on periphytic substrates, namely on the surface of solid objects such as rocks, wood, or leaves in the aquatic environment of the river. This periphytic diatom checklist is usually used to study the biological and ecological diversity of diatoms in a body of water. This checklist includes the identification of diatom species that can be found in various types of periphytic substrates in the waters. In addition, this checklist can also be used as a basis for further research on the ecology and dynamics of diatoms in waters, as well as an important source of information for biologists and managers of the aquatic environment.

Cosmarium
Cosmarium is a genus of blue-green algae belonging to the division Chlorophyta. Genus Cosmarium is worldwide recorded in many taxonomic surveys (Fadul-Souza et al., 2022). These algae can be found in a variety of freshwater habitats such as rivers, lakes and ponds. Cosmarium has a distinctive and unique cell shape, which is shaped like a half ball or semi-lunar with a hollow in the middle. Each Cosmarium cell consists of a tough cell wall and two semivacuoles, which are internal structures that look like the bubbles in an algal cell. This algae has chloroplasts which play a role in the process of photosynthesis. Cosmarium usually grows attached to substrates such as rocks, leaves or aquatic plants. Cosmarium is an important type of algae in aquatic ecosystems. This algae can be used as an indicator of water quality, because tolerance to different environmental conditions can distinguish one species from another. Apart from that, Cosmarium also plays a role in the food chain in the waters, because it is a food source for the higher organisms in it.

Entransia
Entransia is a genus of green algae belonging to the Selenastraceae family. These algae can be found in a variety of freshwater habitats such as rivers, lakes, and ponds (Kitzing & Karsten, 2015). Entransia have a distinctive and unique cell shape, which is round or oval in shape and looks like a small bottle or tube. Each Entransia cell consists of a tough cell wall and one or two chloroplasts located near the base of the cell. This algae also has additional pigments such as carotenoids and xanthophylls which play a role in photosynthesis. Usually, Entransia grows attached to substrates such as rocks or aquatic plants. Entransia is an important type of algae in aquatic ecosystems, because it is a source of food for the higher organisms in it and also plays a role in the water nutrient cycle. In addition, Entransia can also be used as an indicator of water quality, because tolerance to different environmental conditions can differentiate one species from another.

Oedogonium
Oedogonium is a genus of filamentous green algae found in freshwater environments such as rivers, lakes, and ponds. It typically grows in dense mats on rocks or other submerged surfaces. Oedogonium can serve as a

bioindicator of water quality (Tiwary et al., 2022) because it is sensitive to changes in environmental conditions such as water temperature, nutrient levels, and water flow. High levels of nutrients like nitrogen and phosphorus can cause excessive growth of Oedogonium and other algae, leading to eutrophication and a decrease in dissolved oxygen levels, which can negatively impact aquatic life. Therefore, the presence of Oedogonium in a water body may indicate high nutrient levels, poor water quality, and a potential risk to aquatic life. Its abundance and distribution can be used as a tool for monitoring and assessing the ecological health of freshwater environments.

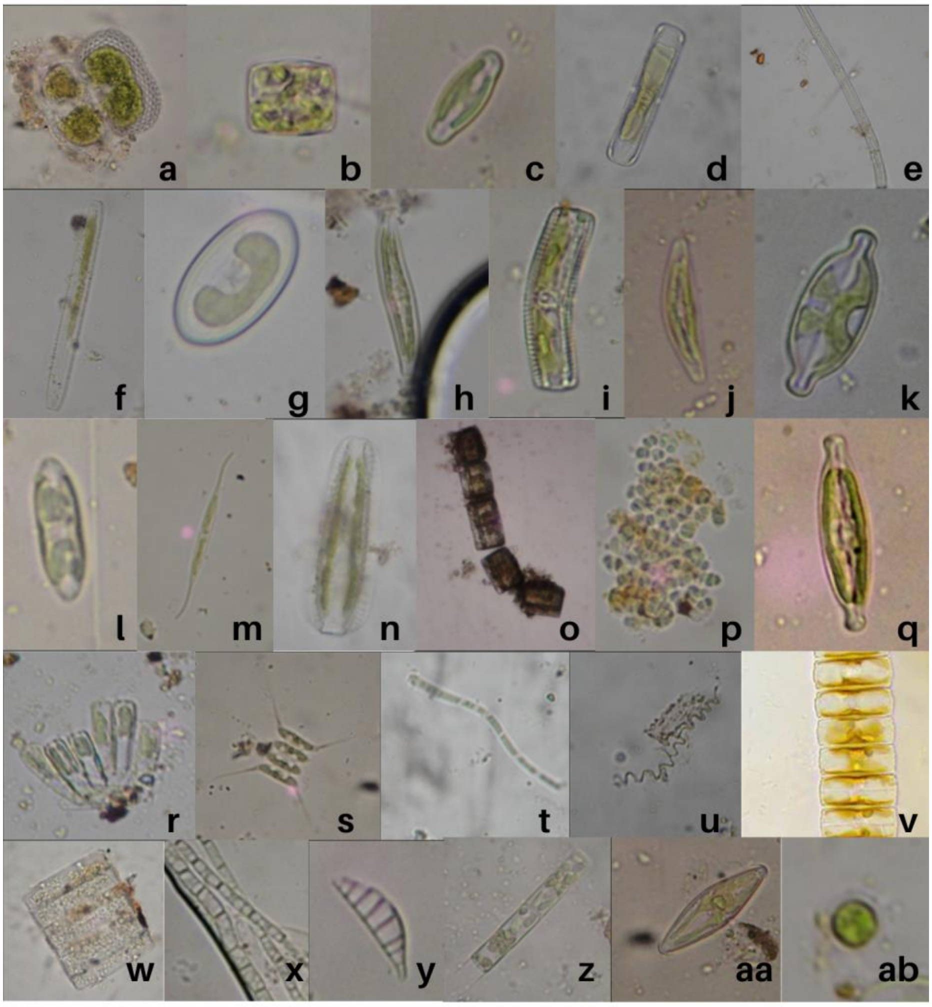

Figure 4. The checklist of freshwater periphytic diatom in stream of the brantas river during the research. (a) Cosmarium; (b) Cyclotella; (c) Navicula; (d) Pinnularia; (e) Oedogonium; (f) Synedra; (g) Cocconeis; (h) Gyrosigma; (i) Achnanthes; (j) Cymbella; (k) Placoneis; (l) Diploneis; (m) Nitzschia; (n) Surirella; (o) Melosira; (p) Chroococcus; (q) Stauroneis; (r) Gomphonema; (s) Scenedesmus; (t) Oscillatoria; (u) Spirulina; (v) Fragilaria; (w) Terpsinoe; (x) Entransia; (y) Rhopalodia; (z) Aulacoseira; (aa) Frustulia; and (ab) Chlorobotrys.

Ulotrhix
Ulothrix is a genus of filamentous green algae that are commonly found in freshwater habitats such as rivers, streams, ponds, and lakes. They are free-floating and can form mats or colonies on submerged surfaces. Ulothrix is known for its ability to fix carbon dioxide and produce oxygen through photosynthesis. As a bioindicator, Ulothrix is useful for monitoring water quality in freshwater systems. High levels of Ulothrix in a water body may indicate nutrient pollution, such as excess nitrogen and phosphorus, which can lead to harmful algal blooms and degraded water quality (Ghali et al., 2020). In addition, changes in the morphology of Ulothrix colonies, such as a decrease in size or branching, can indicate changes in water chemistry or environmental stressors, making it a valuable tool in assessing the health of aquatic ecosystems.

Scenedesmus
Scenedesmus is a genus of green algae that has a characteristic cell shape (Akgül et al., 2017), where its cells are arranged in groups called colonies or coenobia. Each Scenedesmus colony consists of several cells that are well-connected and are triangular or circular in shape, with the outermost cell having small protrusions called lobes. Each cell has chloroplasts that function in photosynthesis. Scenedesmus can reproduce asexually by cell division or sexually through the formation of zoospores. In aquatic environments, Scenedesmus is an important primary producer and serves as a food source for many aquatic organisms. Additionally, its abundance and diversity can be used as an indicator of water quality and environmental health.

Spirulina
Spirulina is a type of blue-green algae that belongs to the phylum Cyanobacteria (Nege et al., 2020). It is characterized by its spiral shape and blue-green color. Spirulina can be found in both freshwater and marine environments, and it is known for its ability to perform photosynthesis and fix atmospheric nitrogen. As a bioindicator, Spirulina can be used to monitor water quality. It is particularly sensitive to changes in nutrient levels and can indicate the presence of pollutants, such as heavy metals and organic compounds. Spirulina is also used in aquaculture as a source of food for fish and other aquatic animals due to its high protein content.

Oscillatoria
Oscillatoria is a type of cyanobacteria (blue-green algae) which has the form of single-celled filaments or many cells arranged in an unbranched manner. The main feature of the Oscillatoria is that it forms long, thin filaments with tapered ends. The cells usually have photosynthetic pigments such as chlorophyll a, phycocyanin, and phycoerythrin which give the filaments a greenish or bluish color. Oscillatoria can be found in fresh water, marine and wet land with high humidity. Oscillatoria's natural habitats include stagnant fresh water, brackish water, sea water, and wetlands on the banks of rivers or lakes. Oscillatoria usually lives attached to substrates such as rocks, gravel, or aquatic plants. Oscillatoria plays an important role in the carbon and nitrogen cycles in aquatic ecosystems (Morales et al. 2017). As a primary producer, Oscillatoria can convert sunlight into energy through the process of photosynthesis, so that it becomes a food source for other organisms in the aquatic food chain. In addition, Oscillatoria can also act as a bioindicator for water quality, because its presence can show the level of pollution and nutrient content in the waters. However, some Oscillatoria species can also be a problem in the environment. Some species can produce toxins that are harmful to human and animal health (Gupta, 2015; Haschek et al., 2013). In addition, Oscillatoria can also thrive in eutrophic conditions (too many nutrients), causing the growth of other invasive algae and damaging the balance of aquatic ecosystems.

Chroococcus
Chroococcus is a genus of non-chlorophyll photosynthetic bacteria belonging to the Cyanobacteria group. These bacteria are usually spherical or nearly spherical, about 1-10 micrometers in diameter and usually live together in dense colonies. The cells are protected by a thick, bluish-green cell wall. Chroococcus habitat is very diverse, it can be found in fresh water, sea water or wet soil environments and they highly tolerant of habitat pollution (Al-Mayaly et al., 2012; Fitri et al., 2021; Wood et al., 2017). These bacteria can live in highly acidic or alkaline water and are able to survive in extreme environmental conditions. Chroococcus has an important role as an oxygen producer in biogeochemical cycles, and is able to fix atmospheric nitrogen into ammonia which is used by plants. In addition, Chroococcus can also be used as a water quality bioindicator, because its presence is sensitive to environmental changes such as the level of water pollution or water acidity.

If the Chroococcus population decreases drastically, this can be a bad indication for the health of the aquatic ecosystem.

Navicula
Navicula is a type of diatom that belongs to the Bacillariophyceae family. The characteristics of Navicula are its flat and sharp cell shape, and it has a special structure called a raphe which functions to control cell movement. Navicula generally live as periphyton on rock, sand, or aquatic plant substrates in fresh or seawater. In addition, Navicula can also be found in polluted waters. Navicula has an important role as a water quality bioindicator (Barinova & Mamanazarova, 2021; Khalil et al., 2021). Its presence can indicate the ecological condition of a waters. Navicula is often used as a tool to monitor water quality and heavy metal pollution, because of its ability to absorb these metals. In addition, Navicula is also used as a bioindicator of water temperature and acidity, as well as changes in water flow patterns in the waters that affect the condition of the substrate.

Pinnularia
Pinnularia is a genus of diatoms that generally live in fresh water. The characteristics of Pinnularia are the shape of the cells which are long and flat like ribbons, and have a special structure called a raphe which functions to control cell movement. Pinnularia can be found in a variety of freshwater habitats such as rivers, lakes, ponds, and swamps. Pinnularia has an important role as a water quality bioindicator. Its presence can indicate the ecological condition of waters and can be used to monitor organic and inorganic pollutants in waters. Pinnularia can also be used to indicate water quality (Nasery et al., 2020) in waters affected by industrial waste, such as waste from agriculture, livestock, and sewage treatment. In addition, Pinnularia can also show good water quality and stable ecosystem conditions, which are needed by other organisms in it.

Terpsinoe
Terpsinoe is a genus of diatoms that generally live in fresh water (Jiménez et al., 2017), especially in shallow waters such as lakes, ponds and rivers. The defining characteristic of Terpsinoe is the shape of the cells which are flat, round, and convex in the center, giving it a hat- or umbrella-like appearance when viewed from the side. Terpsinoe also have a raphe which functions to control cell movement. Terpsinoe can have an important role as a water quality bioindicator, especially in monitoring changes in the ecological conditions of waters and the impact of pollution on waters. Its presence can indicate the ecological conditions of water, including the level of acidity, nutrient levels, and the level of water pollution. Terpsinoe can also show good water quality and stable ecosystem conditions, which are needed by other organisms in it.

Nitzschia
Nitzschia is a genus of diatoms that have a long, cylindrical shape. These diatoms belong to the pennate group which means they have two valves and generally have bilateral symmetry. Nitzschia can be found in both fresh and salt water (Alakananda et al., 2015; Thessen et al., 2005). Several Nitzschia species can be used as bioindicators of water conditions because their presence is highly dependent on water quality. For example, Nitzschia palea is an indicator of a state of eutrophication or excessive increase in nutrients in the waters. The presence of excessive Nitzschia in water can be a sign that the water is experiencing eutrophication. In addition, several Nitzschia species can also be used in the cosmetic and pharmaceutical industries due to their natural fatty acid and pigment content.

Stauroneis
Stauroneis is a genus of diatoms that have a shape similar to an ax or knife. These diatoms belong to the pennate group which means they have two valves and generally have bilateral symmetry. Stauroneis can be found in fresh waters, both cold and warm waters. Several Stauroneis species can also be used as bioindicators of water conditions because their presence is highly dependent on water quality. For example, Stauroneis anceps is often used as a bioindicator of clean and good quality natural water conditions. The presence of other Stauroneis species may indicate water conditions that are more acidic or alkaline. In addition, several Stauroneis species can also be used in biogeochemical studies due to their ability to absorb heavy metals from the aquatic environment.

Cymbella

Cymbella is a genus of diatoms that have a cylindrical or ellipsoidal shape and are generally small to medium in size. These diatoms belong to the pennate group which means they have two valves and generally have bilateral symmetry. Cymbella can be found in freshwater, in both cold and warm water environments. They are often found on the surface of moist substrates such as rocks, wood, or leaves. Several Cymbella species can be used as bio-indicators of water conditions because their presence is highly dependent on water quality (Barinova & Mamanazarova, 2021; Khalil et al., 2021). For example, Cymbella cistula is often used as a bioindicator of clean and good quality water conditions. The presence of other Cymbella species may indicate water conditions that are more acidic or alkaline. In addition, several Cymbella species can also be used in biogeochemical studies because of their ability to absorb heavy metals from the aquatic environment.

Placoneis

Placoneis is a genus of diatoms belonging to the family Naviculaceae. The distinctive feature of this genus is the shape of the cells which are flat and tight, and have transverse raphe bands. In addition, in the cell there are chloroplasts which are flat and numerous. The habitat of Placoneis is in clean fresh water, such as rivers, lakes and swamps. This genus can be found on the surface of waterlogged substrates such as rocks, sand, and other organic matter. As a bioindicator, the presence of Placoneis can be an indicator of the condition of a clean freshwater ecosystem (Kezlya et al., 2020). Its dominant presence in an environment that is kept clean indicates good water quality and is not polluted by chemicals that are harmful to life in it. Apart from that, Placoneis can also be used to measure the level of water pollution by heavy metals, because its cells are able to absorb heavy metals and store them in the cells.

Cocconeis

Cocconeis is a genus of diatoms that have a vessel-like cell shape with two valves or valves, usually symmetrical. These cells are usually small, about 20-200 micrometers. Consistent shape and size make this genus an easy species to identify and measure, so it is often used as a bioindicator in fresh and marine waters. Cocconeis usually live in relatively calm waters, such as lakes, ponds and rivers that are not too fast. They can attach to different substrates, such as rocks, wood, and aquatic plants. As a bioindicator, Cocconeis can provide information about water conditions and pollution levels (Majewska et al., 2014). Different species of the Cocconeis genus have different tolerances to changing environmental conditions, such as temperature, pH, and nutrient content. Therefore, by studying the presence and abundance of Cocconeis species in a body of water, it can provide an overview of the water quality and environmental conditions that exist there.

Gomphomena

Gomphonema is a genus of diatoms that are commonly found in freshwater environments. They are characterized by their elongated shape and their ability to form chains of cells. Gomphonema cells are usually attached to substrates such as rocks, sediments, or aquatic plants by a mucilaginous stalk that is secreted by the cell. The cells are usually rectangular or trapezoidal in shape and have a raphe, a slit-like opening that runs the length of the cell. The raphe is used for movement and attachment to substrates. Gomphonema species are known to be indicators of environmental conditions, such as nutrient levels and water flow rates. They are commonly used as bioindicators for water quality assessments and are sensitive to changes in water chemistry and pollution levels (Prasertsin et al., 2021). Some species of Gomphonema are also known to produce bioactive compounds with potential uses in the pharmaceutical and biotechnology industries.

Aulacoseira

Aulacoseira is a genus of freshwater diatoms that are commonly found in lakes and rivers. They are characterized by their distinctive circular or elliptical shape and their radial symmetry. Aulacoseira cells are enclosed in a siliceous frustule, which consists of two overlapping halves. Aulacoseira species are important indicators of past and present environmental conditions, especially in lake sediments. Their abundance and composition can provide information about past nutrient levels, climate change, and other environmental factors. Aulacoseira is also important primary producers in freshwater ecosystems, contributing to the food web and supporting aquatic life. Some species of Aulacoseira are known to produce toxins, which can have harmful effects on aquatic organisms and human health.

Diploneis

Diploneis is a genus of freshwater and marine diatoms that are commonly found in a variety of aquatic habitats, such as lakes, rivers, and estuaries. They are characterized by their distinctive boat-shaped or elliptical frustules, which are composed of overlapping silica valves. Diploneis species play an important role in aquatic ecosystems as primary producers, contributing to the food web and supporting the growth of other organisms. They are also important indicators of environmental conditions, as their abundance and species composition can provide information about water quality and nutrient levels. Some species of Diploneis have been found to produce toxins that can have harmful effects on aquatic organisms and human health. Additionally, they are often used in environmental monitoring and assessment programs to evaluate the health of aquatic ecosystems and the effectiveness of management strategies.

Achnanthes
Achnanthes is a genus of diatoms with a distinctive feature of this genus is the shape of the cells which are lancet or elliptical with tight lines located in the middle of the cell. These cells have many microscopic pits called areolae which form a characteristic pattern and are often used for identification of diatoms. Achnanthes habitat is fresh water, whether in rivers, lakes or swamps. Some species can be found in nutrient-rich substrates, such as in water polluted by organic wastes or in eutrophic waters. However, most Achnanthes species are considered indicators of a relatively clean environment. Achnanthes has an important role as a water quality bioindicator. Some species are sensitive to environmental changes, such as increased nutrients, increased temperature, or decreased pH, so their presence can indicate environmental problems in these waters.

Gyrosigma
Gyrosigma is a genus of freshwater diatoms that are commonly found in streams, rivers, and lakes. They have a unique sigmoid shape that resembles a human ear or a boomerang, and are often used as bioindicators of water quality due to their sensitivity to environmental changes. In terms of ecology, Gyrosigma is an important part of the food chain in freshwater ecosystems, as they are a primary producer and provide a source of nutrition for other organisms such as zooplankton and small fish. They also play a role in nutrient cycling, helping to regulate levels of nitrogen and phosphorus in the water. Some species of Gyrosigma have been found to produce toxins that can have harmful effects on aquatic organisms and even humans. As such, monitoring populations of Gyrosigma can provide valuable information about the health of freshwater systems and potential risks to human health.

Surirella
Surirella is a type of diatom that can be found in both freshwater and marine environments. This diatom has a curved shape and consists of two cells tightly packed together so that it looks like a small cylindrical box. The cells have fine fibers or regular stripes, and there are many different species. Surirella's natural habitat includes clean and sufficiently flowing fresh water, and can occasionally be found in brackish or seawater environments that enter estuaries. Surirella can act as a water quality bioindicator because its existence is very sensitive to environmental changes caused by pollution, eutrophication, and other factors.

Rhopalodia
Rhopalodia is a genus of a group of diatoms that can be found in fresh waters, including rivers, lakes, and swamps. Diatoms of the genus Rhopalodia have general characteristics such as a flat or elliptical shape, and sizes that vary from microscopic to several millimeters. Rhopalodia is often found in waters with eutrophic or nutrient-abundant conditions, where high levels of phosphorus and nitrogen lead to algal overgrowth. As a bioindicator, Rhopalodia can be used to indicate the eutrophication level of water. If Rhopalodia is found in large numbers, it indicates a eutrophication problem. On the other hand, Rhopalodia can provide ecological benefits, including as a food source for aquatic organisms such as plankton, fish, and other aquatic animals.

Synedra
Synedra is a genus of diatoms that are commonly found in fresh and marine water. The distinguishing feature of this genus is its long, cylindrical shape and the transverse central slit (raphe). The natural habitats of Synedra vary from fresh water, such as lakes and rivers, to marine environments, such as estuaries and estuaries. Some Synedra species can also be found in muddy substrates or in nutrient-rich waters. Synedra can act as a bioindicator in assessing water quality because of its sensitivity to various environmental parameters, such as temperature, brightness, pH, and nutrients. The presence or abundance of Synedra species in an aquatic ecosystem can provide information about water quality and environmental conditions that may affect the life of

other organisms in it.

Chlorobotrys
Chlorobotrys is a genus of green algae that is commonly found in freshwater environments. It is a unicellular organism that has a distinctive shape, with two long flagella that are used for movement. Chlorobotrys is often found in stagnant or slow-moving water bodies such as ponds, swamps, and wetlands. As a bioindicator, Chlorobotrys is particularly useful for monitoring changes in water quality and nutrient levels. This is because it is highly sensitive to changes in the environment, and can quickly respond to changes in nutrient levels, temperature, and other environmental factors. In polluted water bodies, Chlorobotrys can be used to detect the presence of excess nutrients such as nitrogen and phosphorus, which can lead to eutrophication and harmful algal blooms. By monitoring the presence and abundance of Chlorobotrys, researchers can gain insights into the overall health of freshwater ecosystems, and take steps to mitigate the effects of pollution and other environmental stressors.

Frustulia
Frustulia is a genus of diatoms that are commonly found in freshwater environments. They are characterized by having a high valve density and an elongated cell shape. Several species of Frustulia can be found in clean waters with good water quality. Frustulia can be used as a water quality bioindicator because of its sensitivity to environmental changes. Several species of Frustulia can indicate the presence of pollutant substances in water such as heavy metals and pesticides, so that they can be used to monitor pollution levels in waters. In addition, a decrease in the number of species and density of Frustulia can also be an indicator of a decrease in water quality and disruption of aquatic ecosystems. Therefore, Frustulia can be used as a tool for monitoring water quality and environmental sustainability.

Cyclotella
Cyclotella is a genus of diatoms belonging to the centric group of diatoms. A distinctive feature of Cyclotella is the shape of the flattened round cells, with many apexes surrounding the cells. In nature, Cyclotella can be found in a variety of freshwater environments, such as lakes, rivers, and marshes. Cyclotella is used as a bioindicator because of its sensitivity to changes in the aquatic environment. Nutrient concentration, temperature, pH, and water clarity can affect the presence and abundance of Cyclotella in a body of water. Therefore, changes in Cyclotella populations can provide clues to the current state of the aquatic environment. In addition, several Cyclotella species are also used as indicators to monitor heavy metal pollutants in waters, because they can absorb these metals and concentrate in their cells.

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3. Halaman bukti revisi ke-2
13 uni 2023

A. Reviewer meminta melakukan revisi pada pendahuluan, pembahasan dan daftar pustaka melalui komentar langsung di dalam draft **(instruksi revisi 2)**

B.

Rebuttal Letter

endang-dm
2023-06-13 06:03 AM

Dear Editor,

All the corrections suggested by the reviewer were included in the new version of the manuscript.

Reviewer:

1. Change to Italic for all Genus
2. Change the name of the river according to the title
3. Add references in discussion

C. Author merevisi semua arahan reviewer langsung didalam draft dan mengirim *Rebuttal Letter* melalui sistem **(perbaikan revisi 2)**

Rebuttal Letter

endang-dm
2023-06-13 06:03 AM

Dear Editor,

All the corrections suggested by the reviewer were included in the new version of the manuscript.

Reviewer:

1. Change to Italic for all Genus
2. Change the name of the river according to the title
3. Add references in discussion

Answer:

1. We changed the writing of all Genus to italic
2. "Bango River" changed to "Brantas River"
3. We added references in Introduction and discussion

All the best,

Mashitah and Islamy

C. Hasil revisi ke-2

Checklist of Freshwater Periphytic Diatoms in the Midstream of Brantas River, East Java, Indonesia

Abstract. Periphytic diatoms are a group of microalgae that live attached to the surface of the substrate such as rocks, aquatic plants, or other objects in the water. This group has potential as an indicator of environmental quality of water because its existence is influenced by water quality, such as nutrient levels and pollution levels. They are also useful in describing the ecological state, performances, and sustainability of ecosystems because of their ability to measure various environmental parameters and correlate them with diversity, evenness, and richness. This study provides a checklist of freshwater periphytic diatoms in the midstream of Brantas River, East Java, Indonesia. The sampling and identification according to published methods were carried out at the Laboratory of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya. This study was undertaken once a week in a month (May 2022) between 9 a.m. and 1 p.m (GMT + 7). Determination of sampling sites based on differences in land use. Sampling site 1 is located at the Kedungkandang Dam, sampling site 2 is located at the Waterfall Amprong tourist area, sampling site 3 is located at the river branch below the Kedungkandang bridge, and sampling site 4 is located in a residential and agricultural area. The total abundance of freshwater periphytic diatom ranges from 938,905 Ind / mm^2 – 1,597,758 Ind / mm^2. The lowest total abundance is at station 1 with a value of 938,905 Ind / mm^2 and consists of 17 genera. The highest total abundance is at station 4 with a value of 1,597,758 Ind / mm^2 and consists of 17 genera. In this study, 27 genera were found spread across 3 phylum.

Commented [VH1]: "Rolak" is the local name of Dam. Use only common words

Commented [A2R1]: Already changed

Commented [VH3]: "Coban" is the local name of the waterfall. Please delete it

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Keywords: Epilithic, freshwater periphytic diatom, microalgae, water quality

Running title: Biodiversity of Freshwater periphytic diatom and Environmental Condition

INTRODUCTION

Diatoms are an important group of microalgae and are the largest group of periphytic algae in fresh waters. The diatom group is characterized by a cell body covered with a silica shell, which can take various shapes and have distinctive patterns on its surface (Danielson et al. 2012; Mayzel et al. 2021). The abundance of diatoms in fresh waters is very high and often becomes dominant in fresh waters because of their good ability to utilize nutrients in the waters and their high tolerance for various environmental conditions in fresh waters (De Nicola et al. 2014; Camas-Anzueto et al. 2015; Soraya and Islamy 2022). Therefore, Diatoms are an important group of algae as bioindicators of freshwater environmental conditions (Costache et al. 2013; Charles et al. 2019; Kazbar et al. 2019).

Freshwater periphytic diatoms have an important role as environmental bioindicators (Park et al. 2016; Rivera et al. 2019; Schmidt et al. 2019). The presence or absence of periphytic diatom species can provide very useful information to identify disturbances or changes in fresh waters. Some examples of periphytic diatom and their role as bioindicators are Nitzschia which is used to indicate organic contamination (Ahn et al. 2013; Rovira et al. 2015; Sugie et al. 2020), Fragilaria to indicate changes in water quality such as eutrophication, A study conducted that the presence of metal contamination in waters proved to be a strong driver of the formation of diatom community structure, and allowed the identification of tolerant species such as *Cocconeis placentula* var. euglypta, *Eolimna minima*, *Fragilaria gracilis*, *Nitzschia sociabilis*, *Pinnularia parvulissima*, and *Surirella angusta* (Fernández et al. 2012; Brown et al. 2017; Mori et al. 2017). The use of periphytic diatoms as environmental bioindicators can provide more detailed and accurate information about environmental conditions in fresh waters (Lobo et al., 2016), so it is important to conduct research on periphytic diatoms as environmental bioindicators.

Drafting a checklist of aquatic organisms is very important, especially those whose role is as bioindicators of aquatic environmental conditions (Bilanovic et al. 2016; Islamy and Hasan 2020; Isroni et al. 2023). The checklist can help researchers and environmentalists to obtain data on the diversity of periphytic diatom species in certain fresh waters so that it can be a reference for determining which species are present or absent in freshwaters (Fortes et al. 2010). By obtaining accurate and detailed information about the condition of the freshwater environment, the periphytic diatom checklist can assist in environmental conservation efforts and improve the sustainability of freshwater management (Wang et al. 2012; Pattanayak et al. 2020).

One location that can be used as a case study to determine water quality is the midstream of Brantas River, East Java. This river is one of the rivers that has an important role in supporting the lives of the surrounding community, both as a source of irrigation water, a means of transportation, and as a tourist spot. However, the environmental conditions around the river are getting worse due to domestic and industrial waste pollution, as well as increasing human activities (Akhtar et al. 2021; Kardono 2018; Pardamean et al. 2021). Therefore, research on periphytic sampling of freshwater diatoms in midstream of Brantas River is important to do. This study aims to provide a checklist of the diversity of freshwater periphytic diatom species and an overview of the environmental conditions of the midstream of Brantas River. The research results can be used as a reference in making policies to maintain the quality of the water environment in the midtsream of river. In addition, this research can also provide a better understanding of the use of periphytic diatoms as an indicator of the environmental quality of water in the tropics (Hariyadi et al. 1992; Mattson 1999; Mason 2010; Martin et al. 2012; Schulte 2015; Sudrajat et al. 2016).

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MATERIALS AND METHODS

Study area

This research was conducted at the midstream of Brantas River, Kedungkandang District, Malang City, East Java, Indonesia. Determination of periphytic freshwater diatom sampling locations based on different land uses (Soraya and Islamy 2022). First, various types of land use in the study area, such as agricultural land, residential areas, industrial areas, or natural areas were mapped and then a representative sampling location was determined for each type of land use to provide a comprehensive assessment of the diatom community in the study area.

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Figure 5. Location of sampling sites at midstream of Brantas River, Kedungkandang District, Malang City, East Java, Indonesia

Sampling site 1: Sampling site 1 is located Kedungkandang Dam (7°59'21.9"S, 112°39'11.4"E). Ecological and substrate conditions in this area are affected by the presence of dams, which provide a unique habitat for a variety of freshwater organisms. The water inside the dam is relatively clear and shallow, with a depth of about 1-3 meters. The substrate in this area is dominated by rock and gravel, which provide suitable attachment sites for periphytic diatoms. On the other side of the dam is plantation land, with plants such as vegetables and fruits being cultivated in the surrounding area. This can allow the entry of water streams containing pesticides and agricultural fertilizers which can affect water quality and aquatic ecology.
Sampling site 2: This station is located in Amprong Waterfall which is a low waterfall tourist spot located in Kedungkandang District, Malang City, East Java, Indonesia. The ecology and substrate in this area are influenced by waterfall flows, vegetation, and other environmental factors. The water in Amprong tends to be cold and fresh with a swift waterfall flow and the natural rocks around it form very different substrate conditions compared to the conditions at the Kedungkandang Dam. The substrate in this area is dominated by granite and other rocks, which are the attachment sites for periphytic diatoms and other microorganisms. The vegetation around the waterfall consists of plants such as pine trees, teak trees, bamboo, and other shrubs. Around Amprong Waterfall there are several plantation areas that are used as agricultural land for the cultivation of plants such as vegetables, fruits, and flowers.
Sampling site 3: This station is located in a river branching area near the Kedungkandang bridge, Malang, East Java, Indonesia, which is an area that has a unique ecology and substrate. This spot has a calm and slow water flow, as well as differences in elevation which can affect the condition of the substrate. Due to the calm water conditions, the substrate in these areas tends to be softer and easier to settle. The substrate around the river branching area is dominated by silt and sand. In addition, around this area there is also quite abundant vegetation, such as shrubs and trees. These plants provide shelter for many types of animals, such as fish and insects.
Sampling site 4: This sampling site is located in a residential area near Kedungkandang Sub-District, Malang, East Java, Indonesia. It is an area that has a different environment and substrate than the natural river area. In these areas, the dominant substrate is soil that has been altered by human activities to build houses, roads, and other infrastructure. The river which flows around this residential area has more polluted water conditions compared to the natural area of the river, due to domestic and industrial waste being dumped into the river. Even so, around this residential area, there are still a number of green plants such as trees and shrubs that can provide shelter for various types of animals and insects. This area can also still be a habitat for periphytic diatoms and other microorganisms that can live and thrive on substrates that have been altered by human

activities.

Sample collecting

Water quality, freshwater periphytic diatom sampling, and identification were carried out at the Laboratory of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya. This study was undertaken once a week in a month (May 2020) between 9 and 13 h (GMT + 7). Identification and freshwater periphytic diatom samples were collected by scraping the substrate (stones/rocks/aquatic plant) in and around the respective water sampling stations.

Freshwater periphytic diatom sampling

The procedure of freshwater periphytic diatom sampling is based on freshwater periphytic diatom Collection Protocols (West Virginia Department of Environmental Protection 2018). Sample collected during stable flow conditions and streams are not turbid (i.e., the substrate is visible). Label the sample container with Stream Name, AN-Code, date, and collector. Sample only be collected from rocks (epilithic habitat) from riffle/run areas of the streams. Collect five separate cobble-sized rocks that are exposed to varying light conditions and contain varying Freshwater periphytic diatom communities (brown vs. green). Rinse the PVC ring, toothbrush, micro-spatula, and squirt bottle thoroughly with stream water at the site before each sampling event to avoid contamination from a prior sampling of subsequent collections.

Using the PVC ring to delimit the sample area (12.56 cm^2), use the micro-spatula to scrape all algae from the upper surface of rocks into the sample jar. Use the toothbrush to loosen any remaining Freshwater periphytic diatom. Remove the sampler and rinse loosened algae into the sample jar using clear stream water collected from that sampling site in the squirt bottle. Repeat Step 5 until all the Freshwater periphytic diatom from the five rocks (representing 62.8 cm^2 of the sampled area) is composited into one sample jar.

Rinse the micro-spatula, toothbrush, and PVC ring into the sample, removing as much of the lingering Freshwater periphytic diatom as possible. Snap the labeled lid onto the container. Rinse the PVC ring, toothbrush, micro spatula, and squirt bottle thoroughly with stream water at the site after each sampling event to avoid contamination of subsequent collections. For preservation, we assume that the sample jar is about 33 ml full, preserve with an adequate amount of 1 drop Lugol's solution (West Virginia Department of Environmental Protection 2018).

Identification

Freshwater periphytic diatom identification was performed using an Olympus Light Microscope (model CX 40) to determine the types of Freshwater periphytic diatom that had been collected. Identification sampling based on Freshwater periphytic diatom Collection Protocols (West Virginia Department of Environmental Protection 2018). A drop of the sample was placed on glass slides. These were examined at different magnifications using an Olympus light microscope (model CX 40) and illustrate the Freshwater periphytic diatom that is seen in the microscope. Most taxa within samples were identified to species level by reference to standard works, such as identification books and numerous journals (Alika and Akoma 2012).

Freshwater periphytic diatom algae abundance

The calculation of Freshwater periphytic diatom abundance is carried out according to the published procedure (APHA 1985), with the formula:

$$N = \frac{n \, x A_t \, x V_t}{A_c \, x \, V_s \, x \, A_s}$$

Where N = density of Freshwater periphytic diatom algae (ind / mm^2); n = Number of organisms found; At = area of the cover glass (mm^2); Vt = volume of sample accommodated in sample bottle (ml), Ac = area of the field of view multiplied by the number of fields of view observed (mm^2); Vs = volume of water drops used in the observation (ml).

Freshwater periphytic diatom diversity index

Diversity assessment was carried out for analysis of the richness of elements, taxonomic diversity (by the number of a taxons in hydrobiological groups), expressed by the number of taxons, and 2) for the relative representation of populations in communities (by abundance or biomass). The indices are as follows (Protasov et al. 2019):

$$H = -?\frac{\mathrm{ni}}{\mathrm{N}}\ln\frac{\mathrm{ni}}{\mathrm{N}}$$

Where: N = common organism abundance; s = species number; ni = species number of each species; H, Shannon diversity index.

Dominance Index

The dominance index was assessed by formula/equation as the Shannon Index. Simpson index is calculated as follows Simpson Dominance Index (D):

$$D = ?\left(\frac{ni}{n}\right)^2$$

Where D = Dominance index; ni = number of individuals of species i; n = total number of individuals.

RESULTS AND DISCUSSION

Composition and abundance of Freshwater periphytic diatom

Data from the composition determination and the results of the average freshwater periphytic diatom abundance during the study at each sampling site are presented in Table 1. The total abundance of Freshwater periphytic diatom ranges from 938,905 Ind / mm^2 – 1,597,758 Ind / mm^2. The lowest total abundance is at station 1 with a value of 938,905 Ind / mm^2 and consists of 17 genera. The highest total abundance is at station 4 with a value of 1,597,758 Ind / mm^2 and consists of 17 genera. In this study, 27 genera were found spread across 3 phylum. Freshwater periphytic diatom composition and abundance data can be seen in Table 1.

Based on Table 1, at station 1 to station 3, the dominant Freshwater periphytic diatom division is Chrysophyta with relative abundance values of 96%, 91%, and 75%, respectively. Besides, their high abundance, at each station, species from this Division are also more numerous and varied than those from the Chlorophyta, and Chyanophyta Divisions that are found in the waters of the midstream of Brantas River. The Chrysophyta is a group of algae that are qualitatively and quantitatively found in various river type waters (Adjie et al. 2003; Kristiansen and Škaloud 2017). Golden algae are one of the most critical functional components in freshwater microalgae. They are indicators of changes in environmental parameters such as pH, salinity, and climate (Korneva and Solovyeva 2017; Kristiansen and Škaloud 2017). The Chrysophyta, a group of protists containing single-celled individuals as well as quite complex colonial forms, can briefly be defined by the following biochemical and structural criteria: chloroplasts with chlorophylls a and c but lacking b, fucoxanthin as the most critical accessory pigment, β-1, 3-glucan as a storage product, swarmers with heterokont flagella (i.e., one long hairy and one shorter smooth, the latter in many cases only to be detected by EM). Endogenous silicate cysts (stomatocysts) are present throughout the class (Kristiansen and Škaloud 2017).

Meanwhile, at sampling site 4, the dominant Freshwater periphytic diatom was from the Chlorophyta division with a relative abundance value of 63%. Chlorophyta is usually found in stagnant waters and is planktonic. However, Chlorophyta of the Genus *Oedogonium* and *Ulothrix* are not. *Oedogonium* and *Ulothrix* more often live attached either to plants, rocks, or other surfaces (García et al. 2012). In the Chlorophyta division, at stations 1, 2, and 4, *Ulotrhix* is more able to grow than other Chlorophyta genera, which was found probably due to the ability of Ulotrhix to be more tolerant of environmental conditions. At station 3, the abundance of *Ulothrix* is slight due to the condition of station three, which has lower levels of phosphate and nitrate than other stations.

Cyanophyta divisions were found at each station. Benthic cyanophytes or those that become Freshwater periphytic diatoms are less likely to bloom or be abundant. Species that are not planktonic are generally species that rarely result in population explosions (blooming) due to eutrophication (nutrient enrichment) (Prihantini et al. 2010). Further, algal communities in rivers are a diverse assemblage of Cyanobacteria and diatoms. Harmful and nuisance algal blooms are often dominated by cyanobacteria with diatoms are dominant in lower nutrient headwater systems, considered one of the most sensitive classes of organisms to pollution, while also showing great variation in community composition (Fenoglio et al., 2020; Schmidt et al., 2019).

Table 5. Data on Composition and Abundance of Freshwater periphytic diatom in the river during the Study

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No	Freshwater Periphytic Diatom	SAMPLING SITES 1 (Ind/mm²)	1 (%)	2 (Ind/mm²)	2 (%)	3 (Ind/mm²)	3 (%)	4 (Ind/mm²)	4 (%)
	Chlorophyta	**33295**	**100%**	**17757**	**1**	**68440**	**1**	**999198**	**1**
1	*Cosmarium*					370	0,5%		
2	*Entransia*					54751	80,0%		
3	*Oedogonium*					9249	13,5%	69548	7,0%
4	*Ulotrhix*	33295	100,0%	14798	83,3%	2590	3,8%	929650	93,0%
5	*Cenedesmus*			2959	16,7%	1480	2,2%		
	Cyanophyta	**3700**	**100%**	**85826**	**100%**	**186818**	**100%**	**62890**	**100%**
6	*Spirulina*	370	10,0%			370	0,2%		
7	*Oscillatoria*	3330	90,0%	75098	87,5%	179049	95,8%	61410	97,6%
8	*Chroococcus*			10728	12,5%	7399	4,0%	1480	2,4%
	Chrysophyta	**901910**	**100%**	**1042113**	**100%**	**763182**	**100%**	**535670**	**100%**
9	*Navicula*	106912	11,9%	207904	20,0%	169061	22,2%	127628	23,8%
10	*Pinnularia*	41063	4,6%	85825	8,2%	32924	4,3%	45132	8,4%
11	*Terpsinoe*					370	0,05%	370	0,07%
12	*Nitzschia*	44763	5,0%	105432	10,1%	133547	17,5%	89155	16,6%
13	*Stauroneis*			7769	0,7%	4070	0,5%		
14	*Cymbella*	27746	3,1%	58080	5,6%	92854	12,2%	41433	7,7%
15	*Placoneis*	1110	0,1%	2220	0,2%	8509	1,1%	1110	0,2%
16	*Cocconeis*	215673	23,9%	81756	7,8%	73988	9,7%	43653	8,1%
17	*Gomphonema*	270424	30,0%	355509	34,1%	130218	17,1%	117270	21,9%
18	*Aulacoseira*			7029	0,7%	740	0,1%	740	0,1%
19	*Diploneis*	1850	0,2%	1110	0,1%	740	0,1%	4809	0,9%
20	*Achnanthes*	153524	17,0%	100993	9,7%	79166	10,4%	11838	2,2%
21	*Gyrosigma*	370	0,0%	7029	0,7%	7769	1,0%	1110	0,2%
22	*Surirella*				0,0%	8139	1,1%	1480	0,3%
23	*Rhopalodia*	370	0,0%	370	0,04%				
24	*Synedra*	27746	3,1%	14798	1,4%	18497	2,4%	12578	2,3%
25	*Chlorobotrys*	9989	1,1%			370	0,0%	37364	7,0%
26	*Frustulia*	370	0,0%	6289	0,6%	1480	0,2%		
27	*Cyclotella*					740	0,1%		
	TOTAL	**938905**		**1145696**		**1018440**		**1597758**	

In the Cyanophyta Division, the genus *Oscillatoria* is more dominant at each station than the other genera. Cyanobacteria are a common component of the Freshwater periphytic diatom (the ensemble of microorganisms attached to submerged surfaces), forming crusts and films over rocks (epilithon), plants (epiphytes), sand (epipsam-mon), sediments (epipelon), and other substrates. In many environments, these biofilms accumulate from millimeters to centimeters in thickness as vertically structured, microbial mats that form a benthic layer at the bottom of the water column, or that detach and float at the surface (Vincent 2009).

Sampling site 1

During the study at sampling site 1, the total abundance of freshwater periphytic diatom is 938,905 Ind/mm^2, and 17 genera were found, namely Spirulina, *Ulotrhix, Navicula, Pinnularia, Nitzschia, Cymbella, Placoneis, Cocconeis, Gomphomena, Diploneis, Achnanthes, Oscillatoria, Gyrosigma, Rhopalodia, Synedra, Chlorobotrys, and Frustulia*. In this sampling site 1, of all genera found at the time of observation, the Genus with the highest abundance was *Gomphonema* (270,424 Ind/mm^2), *Cocconeis* (215,673 Ind/mm^2), and *Achnanthes* (153,524 Ind/mm^2).

Studies show that diatoms such as Cocconeis are sensitive to changes in pH, temperature, salinity, water quality, nutrient availability, and even bathymetry (Martín and Fernandez 2012; Minelgaite et al. 2020). Another study discovered that *Achnanthes* and *Gomphonema* are types of periphytic microalgae that live in non-polluted waters (Novais et al. 2015; Noga et al. 2018). Based on the high abundance of *Cocconeis*, *Achnanthes*, and *Gomphonema*, we assume that the waters at sampling station 1 are waters that are still not polluted. Besides, this Genus that only lives in clean to moderate polluted waters and has a low tolerance for changes in environmental conditions can be used as an indicator of moderate polluted waters (Prasertsin et al., 2021).

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Sampling site 2

The total average abundance of freshwater periphytic diatom at sampling station 2 was 1,146,436 Ind / mm^2. At this sampling site, we found 21 genera were found, namely *Ulotrhix, Scenedesmus, Chroococcus, Navicula, Pinnularia, Nitzschia, Stauroneis, Cymbella, Placoneis, Cocconeis, Gomphomena, Aulacoseira, Achnanthes, Oscillatoria, Gyrosigma, Rhopalodia, Synedra,* and *Frustulia*. The Chrysophyta also dominates this sampling site with the Genus with the highest abundance are *Gomphonema* (355,509 Ind / mm^2), and *Navicula* (207,904 Ind / mm^2).

Similar to sampling site 1, *Gomphonema* at sampling site 2 still dominates. However, the abundance of unpolluted water freshwater periphytic diatom (*Cocconeis* and *Achnanthes*) has decreased. Besides, the abundance of polluted water freshwater periphytic diatom such as *Navicula* and *Nitzschia* at this station is starting to increase. *Navicula* and *Nitzchia* are known as microalgae whose existence can indicate that the waters where they live are subject to anthropogenic pollution (sources of unnatural pollution arise due to human influence or intervention or human activities) (Sawaiker and Rodrigues, 2017). We assume that sampling site 2 shows symptoms of increased pollution.

Sampling site 3

Data on the average abundance of freshwater periphytic diatom at point 3 total sampling was 1,018,440 Ind / mm^2, and 22 genera were found, namely *Spirulina, Cosmarium, Entransia, Oedogonium, Ulotrhix, enedesmus, Chroococcus, Navicula, Pinnularia, Terpsinoe, Nitzschia, Stauroneis, Cymbella, Placoneis, Cocconeis, Gomphomena, Aulacoseira, Diploneis, Achnanthes, Oscillatoria, Gyrosigma, Surirella, Synedra, Chlorobotrys, Frustulia,* and *Cyclotella*. The Chrysophyta also dominates this station with the Genus with the highest abundance *Nitzschia* (133.547 Ind/mm^2), *Navicula* (169.061 Ind/mm^2), and *Oscillatoria* (179.049 Ind/mm^2). The total average abundance of freshwater periphytic diatom at sampling site 3, it is dominated by the Genus *Oscillatoria*, *Nitzchia*, and *Navicula*. The Genus *Oscillatoria* is known as a type of microalgae that is very tolerant of organic matter contamination (Salem et al. 2017). However, the freshwater periphytic diatom of other eutrophic water types such as *Navicula* and *Nitzschia* have increased and started to dominate. We assume that the waters at sampling site 3 are currently happening eutrophication or enrichment of organic polluting materials. Besides, *Gomphonema*, which is an indicator of unpolluted waters, has begun to decline. Likewise, for the types of *Cocconeis* and *Achnanthes*.

Sampling site 4

The total average abundance of freshwater periphytic diatom at sampling site 4 was 1,597,758 Ind/mm^{2,} and 20

genera were found, namely *Oedogonium, Ulotrhix, Oscillatoria, Chroococcus, Navicula, Pinnularia, Terpsinoe, Nitzschia, Cymbella, Placoneis, Cocconeis, Gomphomena, Aulacoseira, Diploneis, Achnanthes, Gyrosigma, Surirella, Synedra,* and *Chlorobotrys*. At sampling site 4 here it is dominated by the Chlorophyta with the highest abundance of Genus is *Ulotrhix* (130,218 Ind / mm^2).
The Genus *Ulothrix* dominated in sampling site 4. It is suspected that in such water conditions, only *Ulothrix* can tolerate high water environments at sampling site 4. Genus *Ulothrix* is tolerant of organic pollution and is sometimes used as an indicator of heavily polluted water (Yusuf 2020). Hydrobiota that have high tolerance will be able to survive in polluted ecosystems, while those with low tolerance have a low abundance and eventually disappear (Ramakrishnan et al. 2010). Based on the data above, it can be concluded that at station 4, there has been pollution, which is thought to be due to the high input of household waste and domestic waste because it is in the residential area.

Diversity and Dominance Index
The value of freshwater periphytic diatom diversity at the location ranged from 0.91 to 2.44 (table 3). The lowest diversity occurred at sampling site 4 with a value of 0.91, and the highest diversity occurred at station 3 with a value of 2.44. This classification of the Shannon-Wiener diversity index value can be used to determine the distribution of each species and the stability of the community: H> 3 = high diversity, high distribution of individual numbers of each species, and high community stability; 1 <H <3 = Moderate diversity, moderate distribution of the number of individuals per species and moderate community stability; H <1 = low diversity, low distribution of the number of individuals per species, and low community stability. According to the data, the midstream of Brantas River is categorized into a small diversity category.

Table 6. Diversity and dominance index during the study

Sampling site	Dominance index (D)	Diversity Index (H)
1	0.18275	1.94
2	0.15468	2.18
3	0.10909	2.44
4	0.66237	0.91

Based on the data, we assume that the the midstream of Brantas River has a low to moderate freshwater periphytic diatom diversity, individual distribution of each species, and moderate community stability. Sampling site 1 to Sampling site 3 can still be categorized into moderate diversity, moderate distribution of the number of individuals of each species, and moderate community stability. Whereas sampling site 4 has low diversity, the distribution of the number of individuals of each species is low, and the stability of the community is low.
In terms of the dominance index, a study states that the dominance value ranges between 0 and 1 (Hossain et al. 2017). If the dominance value is close to 0, it means that almost no individuals dominate. In contrast, if the dominance is close to 1, it means that there are individuals who dominate the population. According to the dominance index value in sampling site 1 to sampling site 3, the index value is close to the value of 0, so we assume that there is no species dominance. While at station 4, the dominance index value is close to the value 1, so we assume that there is species dominance at station 4.

The Checklist
The periphytic diatom checklist (figure 2) is a list of diatom species found on periphytic substrates, namely on the surface of solid objects such as rocks, wood, or leaves in the aquatic environment of the river. This periphytic diatom checklist is usually used to study the biological and ecological diversity of diatoms in a body of water. This checklist includes the identification of diatom species that can be found in various types of periphytic substrates in the waters. In addition, this checklist can also be used as a basis for further research on the ecology and dynamics of diatoms in waters, as well as an important source of information for biologists and managers of the aquatic environment.

Cosmarium
Cosmarium is a genus of blue-green algae belonging to the division Chlorophyta. Genus *Cosmarium* is worldwide recorded in many taxonomic surveys (Fadul-Souza et al. 2022). These algae can be found in a variety of freshwater habitats such as rivers, lakes and ponds. *Cosmarium* has a distinctive and unique cell

shape, which is shaped like a half ball or semi-lunar with a hollow in the middle. Each *Cosmarium* cell consists of a tough cell wall and two semivacuoles, which are internal structures that look like the bubbles in an algal cell. This diatom has chloroplasts which play a role in the process of photosynthesis. *Cosmarium* usually grows attached to substrates such as rocks, leaves or aquatic plants. *Cosmarium* is an important type of algae in aquatic ecosystems. This diatom can be used as an moderate to polluted waters indicator, because tolerance to different environmental conditions can distinguish one species from another. Apart from that, *Cosmarium* also plays a role in the food chain in the waters, because it is a food source for the higher organisms in it.

Entransia

Entransia is a genus of green algae belonging to the Selenastraceae family. These diatoms can be found in a variety of freshwater habitats such as rivers, lakes, and ponds (Kitzing and Karsten 2015). *Entransia* have a distinctive and unique cell shape, which is round or oval in shape and looks like a small bottle or tube. Each *Entransia* cell consists of a tough cell wall and one or two chloroplasts located near the base of the cell. This diatom also has additional pigments such as carotenoids and xanthophylls which play a role in photosynthesis. Usually, *Entransia* grows attached to substrates such as rocks or aquatic plants. *Entransia* is an important type of algae in aquatic ecosystems, because it is a source of food for the higher organisms in it and also plays a role in the water nutrient cycle. In addition, *Entransia* can also be used as an moderate to polluted waters indicator, because tolerance to different environmental conditions can differentiate one species from another.

Oedogonium

Oedogonium is a genus of filamentous green algae found in freshwater environments such as rivers, lakes, and ponds. It typically grows in dense mats on rocks or other submerged surfaces. *Oedogonium* can serve as a bioindicator of polluted waters (Tiwary et al. 2022) because it is sensitive to changes in environmental conditions such as water temperature, nutrient levels, and water flow. High levels of nutrients like nitrogen and phosphorus can cause excessive growth of *Oedogonium* and other algae, leading to eutrophication and a decrease in dissolved oxygen levels, which can negatively impact aquatic life. Therefore, the presence of *Oedogonium* in a water body may indicate high nutrient levels, poor water quality, and a potential risk to aquatic life. Its abundance and distribution can be used as a tool for monitoring and assessing the ecological health of freshwater environments.

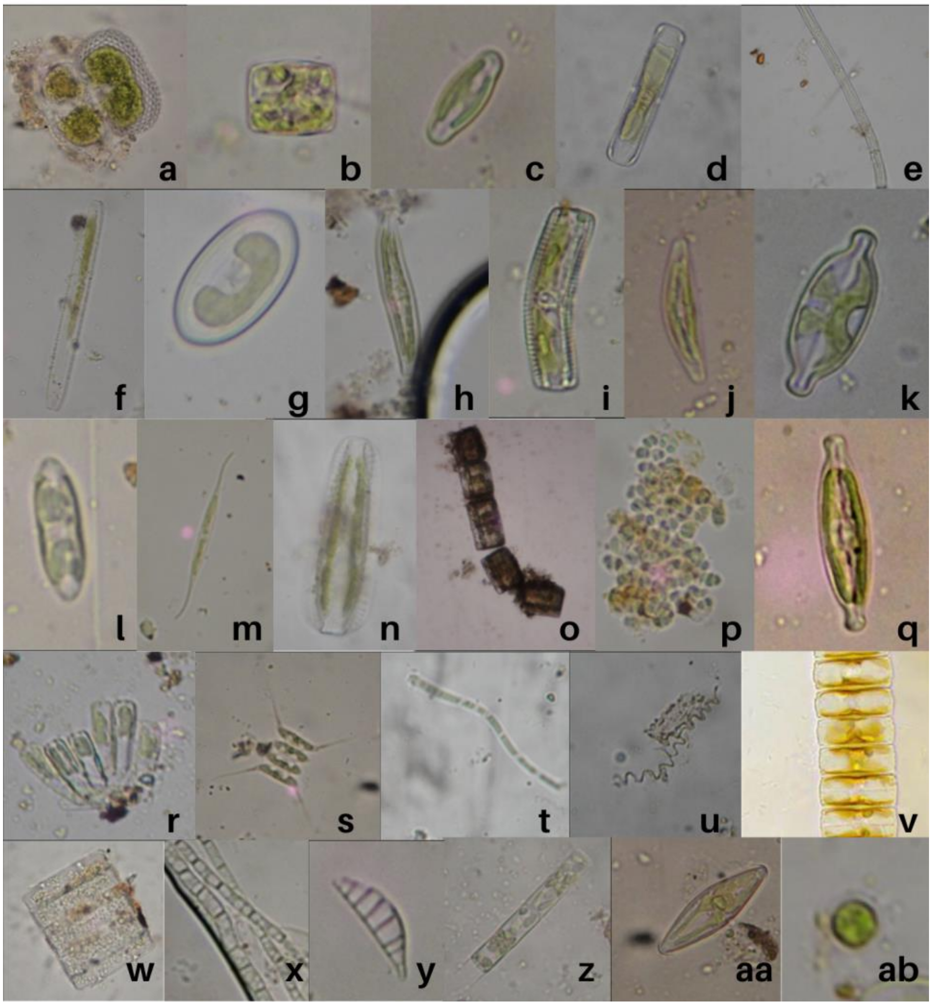

Figure 6. The checklist of freshwater periphytic diatom in stream of the brantas river during the research. (a) *Cosmarium*; (b) *Cyclotella*; (c) *Navicula*; (d) *Pinnularia*; (e) *Oedogonium*; (f) *Synedra*; (g) *Cocconeis*; (h) *Gyrosigma*; (i) *Achnanthes*; (j) *Cymbella*; (k) *Placoneis*; (l) *Diploneis*; (m) *Nitzschia*; (n) *Surirella*; (o) *Melosira*; (p) *Chroococcus*; (q) *Stauroneis*; (r) *Gomphonema*; (s) Scenedesmus; (t) *Oscillatoria*; (u) *Spirulina*; (v) *Fragilaria*; (w) *Terpsinoe*; (x) *Entransia*; (y) *Rhopalodia*; (z) *Aulacoseira*; (aa) *Frustulia*; and (ab) *Chlorobotrys*.

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Ulotrhix

Ulothrix is a genus of filamentous green algae that are commonly found in freshwater habitats such as rivers, streams, ponds, and lakes. They are free-floating and can form mats or colonies on submerged surfaces. *Ulothrix* is known for its ability to fix carbon dioxide and produce oxygen through photosynthesis. As a bioindicator, *Ulothrix* is useful for monitoring water quality in freshwater systems. High levels of *Ulothrix* in a water body may indicate nutrient pollution, such as excess nitrogen and phosphorus, which can lead to harmful algal blooms and degraded water quality (Ghali et al. 2020). In addition, changes in the morphology of *Ulothrix* colonies, such as a decrease in size or branching, can indicate changes in water chemistry or environmental stressors, making it a valuable tool in assessing the health of aquatic ecosystems.

Scenedesmus

Scenedesmus is a genus of green algae that has a characteristic cell shape (Akgül et al. 2017), where its cells are arranged in groups called colonies or coenobia. Each *Scenedesmus* colony consists of several cells that are well-connected and are triangular or circular in shape, with the outermost cell having small protrusions called lobes. Each cell has chloroplasts that function in photosynthesis. *Scenedesmus* can reproduce asexually by cell division or sexually through the formation of zoospores. In aquatic environments, *Scenedesmus* is an important primary producer and serves as a food source for many aquatic organisms. Additionally, its abundance and diversity can be used as an indicator of water quality and environmental health.

Spirulina

Spirulina is a type of blue-green algae that belongs to the phylum Cyanobacteria (Nege et al. 2020). It is characterized by its spiral shape and blue-green color. *Spirulina* can be found in both freshwater and marine environments, and it is known for its ability to perform photosynthesis and fix atmospheric nitrogen. As a bioindicator, *Spirulina* can be used to monitor water quality. It is particularly sensitive to changes in nutrient levels and can indicate the presence of pollutants, such as heavy metals and organic compounds. *Spirulina* is also used in aquaculture as a source of food for fish and other aquatic animals due to its high protein content.

Oscillatoria

Oscillatoria is a type of cyanobacteria (blue-green algae) which has the form of single-celled filaments or many cells arranged in an unbranched manner. The main feature of the *Oscillatoria* is that it forms long, thin filaments with tapered ends. The cells usually have photosynthetic pigments such as chlorophyll a, phycocyanin, and phycoerythrin which give the filaments a greenish or bluish color. *Oscillatoria* can be found in fresh water, marine and wet land with high humidity. The natural habitats of *Oscillatoria* include stagnant fresh water, brackish water, sea water, and wetlands on the banks of rivers or lakes. *Oscillatoria* usually lives attached to substrates such as rocks, gravel, or aquatic plants. *Oscillatoria* plays an important role in the carbon and nitrogen cycles in aquatic ecosystems (Morales et al. 2017). As a primary producer, *Oscillatoria* can convert sunlight into energy through the process of photosynthesis, so that it becomes a food source for other organisms in the aquatic food chain. In addition, *Oscillatoria* can also act as a bioindicator for water quality, because its presence can show the level of pollution and nutrient content in the waters. However, some *Oscillatoria* species can also be a problem in the environment. Some species can produce toxins that are harmful to human and animal health (Gupta, 2015; Haschek et al. 2013). In addition, *Oscillatoria* can also thrive in eutrophic conditions (too many nutrients), causing the growth of other invasive algae and damaging the balance of aquatic ecosystems.

Chroococcus

Chroococcus is a genus of non-chlorophyll photosynthetic bacteria belonging to the Cyanobacteria group. These bacteria are usually spherical or nearly spherical, about 1-10 micrometers in diameter and usually live together in dense colonies. The cells are protected by a thick, bluish-green cell wall. *Chroococcus* habitat is very diverse, it can be found in fresh water, sea water or wet soil environments and they highly tolerant of habitat pollution (Al-Mayaly et al. 2012; Fitri et al. 2021; Wood et al. 2017). These bacteria can live in highly acidic or alkaline water and are able to survive in extreme environmental conditions. *Chroococcus* has an important role as an oxygen producer in biogeochemical cycles, and is able to fix atmospheric nitrogen into ammonia which is used by plants. In addition, *Chroococcus* can also be used as a water quality bioindicator, because its presence is sensitive to environmental changes such as the level of water pollution or water acidity. If the *Chroococcus* population decreases drastically, this can be a bad indication for the health of the aquatic ecosystem.

Navicula

Navicula is a type of diatom that belongs to the Bacillariophyceae family. The characteristics of *Navicula* are its flat and sharp cell shape, and it has a special structure called a raphe which functions to control cell movement. *Navicula* generally live as periphyton on rock, sand, or aquatic plant substrates in fresh or seawater. In addition, *Navicula* can also be found in polluted waters. *Navicula* has an important role as a water quality bioindicator (Barinova and Mamanazarova, 2021; Khalil et al. 2021). Its presence can indicate the ecological condition of a waters. *Navicula* is often used as a tool to monitor water quality and heavy metal pollution, because of its ability to absorb these metals. In addition, *Navicula* is also used as a bioindicator of water temperature and acidity, as well as changes in water flow patterns in the waters that affect the condition of the substrate.

Pinnularia

Pinnularia is a genus of diatoms that generally live in fresh water. The characteristics of *Pinnularia* are the shape of the cells which are long and flat like ribbons, and have a special structure called a raphe which functions to control cell movement. *Pinnularia* can be found in a variety of freshwater habitats such as rivers, lakes, ponds, and swamps. *Pinnularia* has an important role as a water quality bioindicator. Its presence can indicate the ecological condition of waters and can be used to monitor organic and inorganic pollutants in waters. *Pinnularia* can also be used to indicate water quality (Nasery et al. 2020) in waters affected by industrial waste, such as waste from agriculture, livestock, and sewage treatment. In addition, *Pinnularia* can also show good water quality and stable ecosystem conditions, which are needed by other organisms in it.

Terpsinoe

Terpsinoe is a genus of diatoms that generally live in fresh water (Jiménez et al. 2017), especially in shallow waters such as lakes, ponds and rivers. The defining characteristic of *Terpsinoe* is the shape of the cells which are flat, round, and convex in the center, giving it a hat- or umbrella-like appearance when viewed from the side. *Terpsinoe* also have a raphe which functions to control cell movement. *Terpsinoe* can have an important role as a water quality bioindicator, especially in monitoring changes in the ecological conditions of waters and the impact of pollution on waters. Its presence can indicate the ecological conditions of water, including the level of acidity, nutrient levels, and the level of water pollution. *Terpsinoe* can also show good water quality and stable ecosystem conditions, which are needed by other organisms in it 9 Barinova and Mamanazarova 2021).

Nitzschia

Nitzschia is a genus of diatoms that have a long, cylindrical shape. These diatoms belong to the pennate group which means they have two valves and generally have bilateral symmetry. *Nitzschia* can be found in both fresh and salt water (Alakananda et al. 2015; Thessen et al. 2005). Several *Nitzschia* species can be used as bioindicators of water conditions because their presence is highly dependent on water quality. For example, *Nitzschia* palea is an indicator of a state of eutrophication or excessive increase in nutrients in the waters. The presence of excessive *Nitzschia* in water can be a sign that the water is experiencing eutrophication. In addition, several *Nitzschia* species can also be used in the cosmetic and pharmaceutical industries due to their natural fatty acid and pigment content.

Stauroneis

Stauroneis is a genus of diatoms that have a shape similar to an ax or knife. These diatoms belong to the pennate group which means they have two valves and generally have bilateral symmetry. *Stauroneis* can be found in fresh waters, both cold and warm waters. Several *Stauroneis* species can also be used as bioindicators of water conditions because their presence is highly dependent on water quality. For example, *Stauroneis* anceps is often used as a bioindicator of clean and good quality natural water conditions. The presence of other *Stauroneis* species may indicate water conditions that are more acidic or alkaline. In addition, several *Stauroneis* species can also be used in biogeochemical studies due to their ability to absorb heavy metals from the aquatic environment (Barinova and Mamanazarova 2021).

Cymbella

Cymbella is a genus of diatoms that have a cylindrical or ellipsoidal shape and are generally small to medium in size. These diatoms belong to the pennate group which means they have two valves and generally have bilateral symmetry. *Cymbella* can be found in freshwater, in both cold and warm water environments. They are often found on the surface of moist substrates such as rocks, wood, or leaves. Several *Cymbella* species can be used as bio-indicators of water conditions because their presence is highly dependent on water quality (Barinova and Mamanazarova 2021; Khalil et al. 2021). For example, *Cymbella cistula* is often used as a bioindicator of clean and good quality water conditions. The presence of other *Cymbella* species may indicate water conditions that are more acidic or alkaline. In addition, several *Cymbella* species can also be used in biogeochemical studies because of their ability to absorb heavy metals from the aquatic environment.

Placoneis

Placoneis is a genus of diatoms belonging to the family Naviculaceae. The distinctive feature of this genus is the shape of the cells which are flat and tight, and have transverse raphe bands. In addition, in the cell there are chloroplasts which are flat and numerous. The habitat of *Placoneis* is in clean fresh water, such as rivers, lakes and swamps. This genus can be found on the surface of waterlogged substrates such as rocks, sand, and other organic matter. As a bioindicator, the presence of *Placoneis* can be an indicator of the condition of a clean freshwater ecosystem (Kezlya et al. 2020). Its dominant presence in an environment that is kept clean indicates good water quality and is not polluted by chemicals that are harmful to life in it. Apart from that, *Placoneis* can also be used to measure the level of water pollution by heavy metals, because its cells are able to absorb heavy metals and store them in the cells.

Cocconeis
Cocconeis is a genus of diatoms that have a vessel-like cell shape with two valves or valves, usually symmetrical. These cells are usually small, about 20-200 micrometers. Consistent shape and size make this genus an easy species to identify and measure, so it is often used as a bioindicator in fresh and marine waters. *Cocconeis* usually live in relatively calm waters, such as lakes, ponds and rivers that are not too fast. They can attach to different substrates, such as rocks, wood, and aquatic plants. As a bioindicator, *Cocconeis* can provide information about water conditions and pollution levels (Majewska et al. 2014). Different species of the *Cocconeis* genus have different tolerances to changing environmental conditions, such as temperature, pH, and nutrient content. Therefore, by studying the presence and abundance of *Cocconeis* species in a body of water, it can provide an overview of the water quality and environmental conditions that exist there.

Gomphomena
Gomphonema is a genus of diatoms that are commonly found in freshwater environments. They are characterized by their elongated shape and their ability to form chains of cells. *Gomphonema* cells are usually attached to substrates such as rocks, sediments, or aquatic plants by a mucilaginous stalk that is secreted by the cell. The cells are usually rectangular or trapezoidal in shape and have a raphe, a slit-like opening that runs the length of the cell. The raphe is used for movement and attachment to substrates. *Gomphonema* species are known to be indicators of environmental conditions, such as nutrient levels and water flow rates. They are commonly used as bioindicators for water quality assessments and are sensitive to changes in water chemistry and pollution levels (Prasertsin et al. 2021). Some species of *Gomphonema* are also known to produce bioactive compounds with potential uses in the pharmaceutical and biotechnology industries.

Aulacoseira
Aulacoseira is a genus of freshwater diatoms that are commonly found in lakes and rivers. They are characterized by their distinctive circular or elliptical shape and their radial symmetry. *Aulacoseira* cells are enclosed in a siliceous frustule, which consists of two overlapping halves. *Aulacoseira* species are important indicators of past and present environmental conditions, especially in lake sediments. Their abundance and composition can provide information about past nutrient levels, climate change, and other environmental factors. *Aulacoseira* is also important primary producers in freshwater ecosystems, contributing to the food web and supporting aquatic life. Some species of *Aulacoseira* are known to produce toxins, which can have harmful effects on aquatic organisms and human health (Barinova and Mamanazarova 2021).

Diploneis
Diploneis is a genus of freshwater and marine diatoms that are commonly found in a variety of aquatic habitats, such as lakes, rivers, and estuaries. They are characterized by their distinctive boat-shaped or elliptical frustules, which are composed of overlapping silica valves. *Diploneis* species play an important role in aquatic ecosystems as primary producers, contributing to the food web and supporting the growth of other organisms. They are also important indicators of environmental conditions, as their abundance and species composition can provide information about water quality and nutrient levels. Some species of *Diploneis* have been found to produce toxins that can have harmful effects on aquatic organisms and human health. Additionally, they are often used in environmental monitoring and assessment programs to evaluate the health of aquatic ecosystems and the effectiveness of management strategies (Barinova and Mamanazarova 2021).

Achnanthes
Achnanthes is a genus of diatoms with a distinctive feature of this genus is the shape of the cells which are lancet or elliptical with tight lines located in the middle of the cell. These cells have many microscopic pits called areolae which form a characteristic pattern and are often used for identification of diatoms. *Achnanthes* habitat is fresh water, whether in rivers, lakes or swamps. Some species can be found in nutrient-rich substrates,

such as in water polluted by organic wastes or in eutrophic waters. However, most *Achnanthes* species are considered indicators of a relatively clean environment. *Achnanthes* has an important role as a water quality bioindicator. Some species are sensitive to environmental changes, such as increased nutrients, increased temperature, or decreased pH, so their presence can indicate environmental problems in these waters (Barinova and Mamanazarova 2021**).**

Gyrosigma

Gyrosigma is a genus of freshwater diatoms that are commonly found in streams, rivers, and lakes. They have a unique sigmoid shape that resembles a human ear or a boomerang, and are often used as bioindicators of water quality due to their sensitivity to environmental changes. In terms of ecology, *Gyrosigma* is an important part of the food chain in freshwater ecosystems, as they are a primary producer and provide a source of nutrition for other organisms such as zooplankton and small fish. They also play a role in nutrient cycling, helping to regulate levels of nitrogen and phosphorus in the water. Some species of *Gyrosigma* have been found to produce toxins that can have harmful effects on aquatic organisms and even humans. As such, monitoring populations of *Gyrosigma* can provide valuable information about the health of freshwater systems and potential risks to human health (Barinova and Mamanazarova 2021).

Surirella

Surirella is a type of diatom that can be found in both freshwater and marine environments. This diatom has a curved shape and consists of two cells tightly packed together so that it looks like a small cylindrical box. The cells have fine fibers or regular stripes, and there are many different species. The natural habitat of *Surirella* includes clean and sufficiently flowing fresh water, and can occasionally be found in brackish or seawater environments that enter estuaries. *Surirella* can act as a unpolluted water quality bioindicator because its existence is very sensitive to environmental changes caused by pollution, eutrophication, and other factors (Barinova and Mamanazarova 2021)..

Rhopalodia

Rhopalodia is a genus of a group of diatoms that can be found in fresh waters, including rivers, lakes, and swamps. Diatoms of the genus *Rhopalodia* have general characteristics such as a flat or elliptical shape, and sizes that vary from microscopic to several millimeters. *Rhopalodia* is often found in waters with eutrophic or nutrient-abundant conditions, where high levels of phosphorus and nitrogen lead to algal overgrowth. As a bioindicator, *Rhopalodia* can be used to indicate the eutrophication level of water. If *Rhopalodia* is found in large numbers, it indicates a eutrophication problem. On the other hand, *Rhopalodia* can provide ecological benefits, including as a food source for aquatic organisms such as plankton, fish, and other aquatic animals (Barinova and Mamanazarova 2021).

Synedra

Synedra is a genus of diatoms that are commonly found in fresh and marine water. The distinguishing feature of this genus is its long, cylindrical shape and the transverse central slit (raphe). The natural habitats of *Synedra* vary from fresh water, such as lakes and rivers, to marine environments, such as estuaries and estuaries. Some *Synedra* species can also be found in muddy substrates or in nutrient-rich waters. *Synedra* can act as a bioindicator in assessing water quality because of its sensitivity to various environmental parameters, such as temperature, brightness, pH, and nutrients. The presence or abundance of *Synedra* species in an aquatic ecosystem can provide information about water quality and environmental conditions that may affect the life of other organisms in it (Barinova and Mamanazarova 2021).

Chlorobotrys

Chlorobotrys is a genus of green algae that is commonly found in freshwater environments. It is a unicellular organism that has a distinctive shape, with two long flagella that are used for movement. *Chlorobotrys* is often found in stagnant or slow-moving water bodies such as ponds, swamps, and wetlands. As a bioindicator, *Chlorobotrys* is particularly useful for monitoring changes in water quality and nutrient levels. This is because it is highly sensitive to changes in the environment, and can quickly respond to changes in nutrient levels, temperature, and other environmental factors. In polluted water bodies, *Chlorobotrys* can be used to detect the presence of excess nutrients such as nitrogen and phosphorus, which can lead to eutrophication and harmful algal blooms. By monitoring the presence and abundance of *Chlorobotrys*, researchers can gain insights into the overall health of freshwater ecosystems, and take steps to mitigate the effects of pollution and other environmental stressors (Barinova and Mamanazarova 2021).

Frustulia

Frustulia is a genus of diatoms that are commonly found in freshwater environments. They are characterized by having a high valve density and an elongated cell shape. Several species of *Frustulia* can be found in clean waters with good water quality. *Frustulia* can be used as a water quality bioindicator because of its sensitivity to environmental changes. Several species of *Frustulia* can indicate the presence of pollutant substances in water such as heavy metals and pesticides, so that they can be used to monitor pollution levels in waters. In addition, a decrease in the number of species and density of *Frustulia* can also be an indicator of a decrease in water quality and disruption of aquatic ecosystems. Therefore, *Frustulia* can be used as a tool for monitoring water quality and environmental sustainability (Barinova and Mamanazarova 2021).

Cyclotella

Cyclotella is a genus of diatoms belonging to the centric group of diatoms. A distinctive feature of *Cyclotella* is the shape of the flattened round cells, with many apexes surrounding the cells. In nature, *Cyclotella* can be found in a variety of freshwater environments, such as lakes, rivers, and marshes. *Cyclotella* is used as a bioindicator because of its sensitivity to changes in the aquatic environment. Nutrient concentration, temperature, pH, and water clarity can affect the presence and abundance of *Cyclotella* in a body of water. Therefore, changes in *Cyclotella* populations can provide clues to the current state of the aquatic environment. In addition, several *Cyclotella* species are also used as indicators to monitor heavy metal pollutants in waters, because they can absorb these metals and concentrate in their cells (Barinova and Mamanazarova 2021).

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Checklist of freshwater periphytic diatoms in the midstream of Brantas River, East Java, Indonesia

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Abstract. *Masithah ED, Islamy RA. 2023. Checklist of freshwater periphytic diatoms in the midstream of Brantas River, East Java, Indonesia. Biodiversitas 24: xxxx*. Periphytic diatoms are a group of microalgae that live attached to the surface of the substrate such as rocks, aquatic plants, or other objects in the water. This group has potential as an indicator of environmental quality of water because its existence is influenced by water quality, such as nutrient levels and pollution levels. They are also useful in describing the ecological state, performances, and sustainability of ecosystems because of their ability to measure various environmental parameters and correlate them with diversity, evenness, and richness. This study provides a checklist of freshwater periphytic diatoms in the midstream of Brantas River, East Java, Indonesia. The sampling and identification according to published methods were carried out at the Laboratory of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya. This study was undertaken once a week in a month (May 2022) between 9 a.m. and 1 p.m (GMT + 7). Determination of sampling sites based on differences in land use. Sampling site 1 is located at the Kedungkandang Dam, sampling site 2 is located at the Waterfall Amprong tourist area, sampling site 3 is located at the river branch below the Kedungkandang bridge, and sampling site 4 is located in a residential and agricultural area. The total abundance of freshwater periphytic diatom ranges from 938,905 Ind / mm^2 - 1,597,758 Ind / mm^2. The lowest total abundance is at station 1 with a value of 938,905 Ind / mm^2 and consists of 17 genera. The highest total abundance is at station 4 with a value of 1,597,758 Ind / mm^2 and consists of 17 genera. In this study, 27 genera were found spread across 3 phylum.

Keywords: Epilithic, freshwater periphytic diatom, microalgae, water quality

INTRODUCTION

Diatoms are an important group of microalgae and are the largest group of periphytic algae in fresh waters. The diatom group is characterized by a cell body covered with a silica shell, which can take various shapes and have distinctive patterns on its surface (Danielson et al. 2012; Mayzel et al. 2021). The abundance of diatoms in fresh waters is very high and often becomes dominant in fresh waters because of their good ability to utilize nutrients in the waters and their high tolerance for various environmental conditions in fresh waters (DeNicola et al. 2014; Camas-Anzueto et al. 2015; Soraya and Islamy 2022). Therefore, Diatoms are an important group of algae as bioindicators of freshwater environmental conditions (Costache et al. 2013; Charles et al. 2019; Kazbar et al. 2019).

Freshwater periphytic diatoms have an important role as environmental bioindicators (Park et al. 2016; Rivera et al. 2019; Schmidt et al. 2019). The presence or absence of periphytic diatom species can provide very useful information to identify disturbances or changes in fresh waters. Some examples of periphytic diatom and their role as bioindicators are Nitzschia which is used to indicate organic contamination (Ahn et al. 2013; Rovira et al. 2015; Sugie et al. 2020), Fragilaria to indicate changes in water quality such as eutrophication, A study conducted that the presence of metal contamination in waters proved to be a strong driver of the formation of diatom community structure, and allowed the identification of tolerant species such as *Cocconeis placentula* var. euglypta, *Eolimna minima*, *Fragilaria gracilis*, *Nitzschia sociabilis*, *Pinnularia parvulissima*, and *Surirella angusta* (Fernández et al. 2012; Brown et al. 2017; Mori et al. 2017). The use of periphytic diatoms as environmental bioindicators can provide more detailed and accurate information about environmental conditions in fresh waters (Lobo et al. 2016), so it is important to conduct research on periphytic diatoms as environmental bioindicators.

Drafting a checklist of aquatic organisms is very important, especially those whose role is as

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bioindicators of aquatic environmental conditions (Bilanovic et al. 2016; Islamy and Hasan 2020; Isroni et al. 2023). The checklist can help researchers and environmentalists to obtain data on the diversity of periphytic diatom species in certain fresh waters so that it can be a reference for determining which species are present or absent in freshwaters (Fortes et al. 2010). By obtaining accurate and detailed information about the condition of the freshwater environment, the periphytic diatom checklist can assist in environmental conservation efforts and improve the sustainability of freshwater management (Wang et al. 2012; Pattanayak et al. 2020).

One location that can be used as a case study to determine water quality is the midstream of Brantas River, East Java. This river is one of the rivers that has an important role in supporting the lives of the surrounding community, both as a source of irrigation water, a means of transportation, and as a tourist spot. However, the environmental conditions around the river are getting worse due to domestic and industrial waste pollution, as well as increasing human activities (Kardono 2018; Akhtar et al. 2021; Pardamean et al. 2021). Therefore, research on periphytic sampling of freshwater diatoms in midstream of Brantas River is important to do. This study aims to provide a checklist of the diversity of freshwater periphytic diatom species and an overview of the environmental conditions of the midstream of Brantas River. The research results can be used as a reference in making policies to maintain the quality of the water environment in the midtsream of river. In addition, this research can also provide a better understanding of the use of periphytic diatoms as an indicator of the environmental quality of water in the tropics (Hariyadi et al. 1992; Mattson 1999; Mason 2010; Martin et al. 2012; Schulte 2015; Sudrajat et al. 2016).

MATERIALS AND METHODS

Study area

This research was conducted at the midstream of Brantas River, Kedungkandang District, Malang City, East Java, Indonesia. Determination of periphytic freshwater diatom sampling locations based on different land uses (Soraya and Islamy 2022). First, various types of land use in the study area, such as agricultural land, residential areas, industrial areas, or natural areas were mapped and then a representative sampling location was determined for each type of land use to provide a comprehensive assessment of the diatom community in the study area.

Sampling site 1

Sampling site 1 is located Kedungkandang Dam (7°59'21.9"S, 112°39'11.4"E). Ecological and substrate conditions in this area are affected by the presence of dams, which provide a unique habitat for a variety of freshwater organisms. The water inside the dam is relatively clear and shallow, with a depth of about 1-3 meters. The substrate in this area is dominated by rock and gravel, which provide suitable attachment sites for periphytic diatoms. On the other side of the dam is plantation land, with plants such as vegetables and fruits being cultivated in the surrounding area. This can allow the entry of water streams containing pesticides and agricultural fertilizers which can affect water quality and aquatic ecology.

Sampling site 2

This station is located in Amprong Waterfall which is a low waterfall tourist spot located in Kedungkandang District, Malang City, East Java, Indonesia. The ecology and substrate in this area are influenced by waterfall flows, vegetation, and other environmental factors. The water in Amprong tends to be cold and fresh with a swift waterfall flow and the natural rocks around it form very different substrate conditions compared to the conditions at the Kedungkandang Dam. The substrate in this area is dominated by granite and other rocks, which are the attachment sites for periphytic diatoms and other microorganisms. The vegetation around the waterfall consists of plants such as pine trees, teak trees, bamboo, and other shrubs. Around Amprong Waterfall there are several plantation areas that are used as agricultural land for the cultivation of plants such as vegetables, fruits, and flowers.

Sampling site 3

This station is located in a river branching area near the Kedungkandang bridge, Malang, East Java, Indonesia, which is an area that has a unique ecology and substrate. This spot has a calm and slow water flow, as well as differences in elevation which can affect the condition of the substrate. Due to the calm water conditions, the substrate in these areas tends to be softer and easier to settle. The substrate around the river branching area is dominated by silt and sand. In addition, around this area there is also quite abundant vegetation, such as shrubs and trees. These plants provide shelter for many types of animals, such as fish and insects.

Sampling site 4

This sampling site is located in a residential area near Kedungkandang Sub-district, Malang, East Java, Indonesia. It is an area that has a different environment

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and substrate than the natural river area. In these areas, the dominant substrate is soil that has been altered by human activities to build houses, roads, and other infrastructure. The river which flows around this residential area has more polluted water conditions compared to the natural area of the river, due to domestic and industrial waste being dumped into the river. Even so, around this residential area, there are still a number of green plants such as trees and shrubs that can provide shelter for various types of animals and insects. This area can also still be a habitat for periphytic diatoms and other microorganisms that can live and thrive on substrates that have been altered by human activities.

Sample collecting

Water quality, freshwater periphytic diatom sampling, and identification were carried out at the Laboratory of Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya. This study was undertaken once a week in a month (May 2020) between 9 and 13 h (GMT + 7). Identification and freshwater periphytic diatom samples were collected by scraping the substrate (stones/rocks/aquatic plant) in and around the respective water sampling stations.

Freshwater periphytic diatom sampling

The procedure of freshwater periphytic diatom sampling is based on freshwater periphytic diatom Collection Protocols (West Virginia Department of Environmental Protection 2018). Sample collected during stable flow conditions and streams are not turbid (i.e., the substrate is visible). Label the sample container with Stream Name, AN-Code, date, and collector. Sample only be collected from rocks (epilithic habitat) from riffle/run areas of the streams. Collect five separate cobble-sized rocks that are exposed to varying light conditions and contain varying Freshwater periphytic diatom communities (brown vs. green). Rinse the PVC ring, toothbrush, micro-spatula, and squirt bottle thoroughly with stream water at the site before each sampling event to avoid contamination from a prior sampling of subsequent collections.

Figure 7. Location of sampling sites at midstream of Brantas River, Kedungkandang District, Malang City, East Java, Indonesia

Using the PVC ring to delimit the sample area (12.56 cm^2), use the micro-spatula to scrape all algae from the upper surface of rocks into the sample jar. Use the toothbrush to loosen any remaining Freshwater periphytic diatom. Remove the sampler and rinse loosened algae into the sample jar using clear stream water collected from that sampling site in the squirt bottle. Repeat Step 5 until all the Freshwater periphytic diatom from the five rocks (representing 62.8 cm^2 of the sampled area) is composited into one sample jar. Rinse the micro-spatula, toothbrush, and PVC ring into the sample, removing as much of the lingering Freshwater periphytic diatom as possible. Snap the labeled lid onto the container. Rinse the PVC ring, toothbrush, micro spatula, and squirt bottle thoroughly with stream water at the site after each sampling event to avoid contamination of subsequent collections. For preservation, we assume that the sample jar is about 33 ml full, preserve with an adequate amount of 1 drop Lugol's solution (West Virginia Department of

Environmental Protection 2018).

Identification

Freshwater periphytic diatom identification was performed using an Olympus Light Microscope (model CX 40) to determine the types of Freshwater periphytic diatom that had been collected. Identification sampling based on Freshwater periphytic diatom Collection Protocols (West Virginia Department of Environmental Protection 2018). A drop of the sample was placed on glass slides. These were examined at different magnifications using an Olympus light microscope (model CX 40) and illustrate the Freshwater periphytic diatom that is seen in the microscope. Most taxa within samples were identified to species level by reference to standard works, such as identification books and numerous journals (Alika and Akoma 2012).

Freshwater periphytic diatom algae abundance

The calculation of Freshwater periphytic diatom abundance is carried out according to the published procedure (Lipps et al., 2022) with the formula:

$$N = \frac{n\ xA_t\ xV_t}{A_c\ x\ V_s\ x\ A_s}$$

Where:

N : Density of Freshwater periphytic diatom algae (ind / mm^2)

N : Number of organisms found

At : Area of the cover glass (mm^2)

Vt : Volume of sample accommodated in sample bottle (mL)

Ac : Area of the field of view multiplied by the number of fields of view observed (mm^2)

Vs : Volume of water drops used in the observation (mL)

Freshwater periphytic diatom diversity index

Diversity assessment was carried out for analysis of the richness of elements, taxonomic diversity (by the number of a taxons in hydrobiological groups), expressed by the number of taxons, and 2) for the relative representation of populations in communities (by abundance or biomass). The indices are as follows (Protasov et al. 2019):

$$\mathrm{H} = -\sum \frac{ni}{N}\ ln$$

Where:

N : Common organism abundance

S : Species number

Ni : Species number of each species

H : Shannon diversity index.

Dominance index

The dominance index was assessed by formula/equation as the Shannon Index. Simpson index is calculated as follows Simpson Dominance Index (D):

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

Where:

D : Dominance index

ni : Number of individuals of species i

N : Total number of individuals

RESULTS AND DISCUSSION

Composition and abundance of freshwater periphytic diatom

Data from the composition determination and the results of the average freshwater periphytic diatom abundance during the study at each sampling site are presented in Table 1. The total abundance of Freshwater periphytic diatom ranges from 938,905 Ind / mm^2 - 1,597,758 Ind / mm^2. The lowest total abundance is at station 1 with a value of 938,905 Ind / mm^2 and consists of 17 genera. The highest total abundance is at station 4 with a value of 1,597,758 Ind / mm^2 and consists of 17 genera. In this study, 27 genera were found spread across 3 phylum. Freshwater periphytic diatom composition and abundance data can be seen in Table 1.

Based on Table 1, at station 1 to station 3, the dominant Freshwater periphytic diatom division is Chrysophyta with relative abundance values of 96%, 91%, and 75%, respectively. Besides, their high abundance, at each station, species from this Division are also more numerous and varied than those from the Chlorophyta, and Chyanophyta Divisions that are found in the waters of the midstream of Brantas River. The Chrysophyta is a group of algae that are qualitatively and quantitatively found in various river type waters (Kristiansen and Škaloud 2017). Golden algae are one of the most critical functional components in freshwater microalgae. They are indicators of changes in environmental parameters such as pH, salinity, and climate (Korneva and Solovyeva 2017; Kristiansen and Škaloud 2017). The Chrysophyta, a group of protists containing single-celled individuals as well as quite complex colonial forms, can briefly be defined by the following biochemical and structural criteria: chloroplasts with chlorophylls a and c but lacking b, fucoxanthin as the most critical accessory pigment, β-1, 3-glucan as a storage product, swarmers with heterokont flagella (i.e., one long hairy and one shorter

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smooth, the latter in many cases only to be detected by EM). Endogenous silicate cysts (stomatocysts) are present throughout the class (Kristiansen and Škaloud 2017).

Meanwhile, at sampling site 4, the dominant Freshwater periphytic diatom was from the Chlorophyta division with a relative abundance value of 63%. Chlorophyta is usually found in stagnant waters and is planktonic. However, Chlorophyta of the Genus *Oedogonium* and *Ulothrix* are not. *Oedogonium* and *Ulothrix* more often live attached either to plants, rocks, or other surfaces (García-González et al. 2012). In the Chlorophyta division, at stations 1, 2, and 4, *Ulotrhix* is more able to grow than other Chlorophyta genera, which was found probably due to the ability of Ulotrhix to be more tolerant of environmental conditions. At station 3, the abundance of *Ulothrix* is slight due to the condition of station three, which has lower levels of phosphate and nitrate than other stations.

Cyanophyta divisions were found at each station. Benthic cyanophytes or those that become Freshwater periphytic diatoms are less likely to bloom or be abundant. Species that are not planktonic are generally species that rarely result in population explosions (blooming) due to eutrophication (nutrient enrichment) (Prihantini et al. 2010). Further, algal communities in rivers are a diverse assemblage of Cyanobacteria and diatoms. Harmful and nuisance algal blooms are often dominated by cyanobacteria with diatoms are dominant in lower nutrient headwater systems, considered one of the most sensitive classes of organisms to pollution, while also showing great variation in community composition (Schmidt et al. 2019; Fenoglio et al. 2020).

In the Cyanophyta Division, the genus *Oscillatoria* is more dominant at each station than the other genera. Cyanobacteria are a common component of the

Sampling site 2

The total average abundance of freshwater periphytic diatom at sampling station 2 was 1,146,436 Ind / mm^2. At this sampling site, we found 21 genera were found, namely *Ulotrhix, Scenedesmus, Chroococcus, Navicula, Pinnularia, Nitzschia, Stauroneis, Cymbella, Placoneis, Cocconeis, Gomphomena, Aulacoseira, Achnanthes, Oscillatoria, Gyrosigma, Rhopalodia, Synedra*, and *Frustulia*. The Chrysophyta also dominates this sampling site with the Genus with the highest abundance are *Gomphonema* (355,509 Ind / mm^2), and *Navicula* (207,904 Ind / mm^2).

Similar to sampling site 1, *Gomphonema* at sampling site 2 still dominates. However, the abundance of unpolluted water freshwater periphytic diatom (*Cocconeis* and *Achnanthes*) has decreased. Besides, the abundance of polluted water freshwater periphytic

Freshwater periphytic diatom (the ensemble of microorganisms attached to submerged surfaces), forming crusts and films over rocks (epilithon), plants (epiphytes), sand (epipsam-mon), sediments (epipelon), and other substrates. In many environments, these biofilms accumulate from millimeters to centimeters in thickness as vertically structured, microbial mats that form a benthic layer at the bottom of the water column, or that detach and float at the surface (Vincent 2009).

Sampling site 1

During the study at sampling site 1, the total abundance of freshwater periphytic diatom is 938,905 Ind/mm^2, and 17 genera were found, namely Spirulina, *Ulotrhix, Navicula, Pinnularia, Nitzschia, Cymbella, Placoneis, Cocconeis, Gomphomena, Diploneis, Achnanthes, Oscillatoria, Gyrosigma, Rhopalodia, Synedra, Chlorobotrys, and Frustulia*. In this sampling site 1, of all genera found at the time of observation, the Genus with the highest abundance was *Gomphonema* (270,424 Ind/mm^2), *Cocconeis* (215,673 Ind/mm^2), and *Achnanthes* (153,524 Ind/mm^2).

Studies show that diatoms such as Cocconeis are sensitive to changes in pH, temperature, salinity, water quality, nutrient availability, and even bathymetry (Martín and Fernandez 2012; Minelgaite et al. 2020). Another study discovered that *Achnanthes* and *Gomphonema* are types of periphytic microalgae that live in non-polluted waters (Novais et al. 2015; Noga et al. 2018). Based on the high abundance of *Cocconeis, Achnanthes*, and *Gomphonema*, we assume that the waters at sampling station 1 are waters that are still not polluted. Besides, this Genus that only lives in clean to moderate polluted waters and has a low tolerance for changes in environmental conditions can be used as an indicator of moderate polluted waters (Prasertsin et al. 2021).

diatom such as *Navicula* and *Nitzschia* at this station is starting to increase. *Navicula* and *Nitzchia* are known as microalgae whose existence can indicate that the waters where they live are subject to anthropogenic pollution (sources of unnatural pollution arise due to human influence or intervention or human activities) (Sawaiker and Rodrigues 2017). We assume that sampling site 2 shows symptoms of increased pollution.

Sampling site 3

Data on the average abundance of freshwater periphytic diatom at point 3 total sampling was 1,018,440 Ind / mm^2, and 22 genera were found, namely *Spirulina, Cosmarium, Entransia, Oedogonium, Ulotrhix, enedesmus, Chroococcus, Navicula, Pinnularia, Terpsinoe, Nitzschia,*

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Stauroneis, Cymbella, Placoneis, Cocconeis, Gomphomena, Aulacoseira, Diploneis, Achnanthes, Oscillatoria, Gyrosigma, Surirella, Synedra, Chlorobotrys, Frustulia, and *Cyclotella*. The Chrysophyta also dominates this station with the Genus with the highest abundance *Nitzschia* (133.547 Ind/mm^2), *Navicula* (169.061 Ind/mm^2), and *Oscillatoria* (179.049 Ind/mm^2).

The total average abundance of freshwater periphytic diatom at sampling site 3, it is dominated by the Genus *Oscillatoria*, *Nitzchia*, and *Navicula*. The Genus *Oscillatoria* is known as a type of microalgae that is very tolerant of organic matter contamination (Salem et al. 2017). However, the freshwater periphytic diatom of other eutrophic water types such as *Navicula* and *Nitzschia* have increased and started to dominate. We assume that the waters at sampling site 3 are currently happening eutrophication or enrichment of organic polluting materials. Besides, *Gomphonema*, which is an indicator of unpolluted waters, has begun to decline. Likewise, for the types of *Cocconeis* and *Achnanthes*.

Table 7. Data on Composition and Abundance of Freshwater periphytic diatom in the river during the study

Freshwater periphytic diatom	**Sampling sites**							
	1		**2**		**3**		**4**	
	(Ind/mm^2)	**(%)**	**(Ind/mm^2)**	**(%)**	**(Ind/mm^2)**	**(%)**	**(Ind/mm^2)**	**(%)**
Chlorophyta	33295	100%	17757	1	68440	1	999198	1
Cosmarium					370	0,5%		
Entransia					54751	80,0%		
Oedogonium					9249	13,5%	69548	7,0%
Ulotrhix	33295	100,0%	14798	83,3%	2590	3,8%	929650	93,0%
Cenedesmus			2959	16,7%	1480	2,2%		
Cyanophyta	3700	100%	85826	100%	186818	100%	62890	100%
Spirulina	370	10,0%			370	0,2%		
Oscillatoria	3330	90,0%	75098	87,5%	179049	95,8%	61410	97,6%
Chroococcus			10728	12,5%	7399	4,0%	1480	2,4%
Chrysophyta	901910	100%	1042113	100%	763182	100%	535670	100%
Navicula	106912	11,9%	207904	20,0%	169061	22,2%	127628	23,8%
Pinnularia	41063	4,6%	85825	8,2%	32924	4,3%	45132	8,4%
Terpsinoe					370	0,05%	370	0,07%
Nitzschia	44763	5,0%	105432	10,1%	133547	17,5%	89155	16,6%
Stauroneis			7769	0,7%	4070	0,5%		
Cymbella	27746	3,1%	58080	5,6%	92854	12,2%	41433	7,7%
Placoneis	1110	0,1%	2220	0,2%	8509	1,1%	1110	0,2%
Cocconeis	215673	23,9%	81756	7,8%	73988	9,7%	43653	8,1%
Gomphomena	270424	30,0%	355509	34,1%	130218	17,1%	117270	21,9%
Aulacoseira			7029	0,7%	740	0,1%	740	0,1%
Diploneis	1850	0,2%	1110	0,1%	740	0,1%	4809	0,9%
Achnanthes	153524	17,0%	100993	9,7%	79166	10,4%	11838	2,2%
Gyrosigma	370	0,0%	7029	0,7%	7769	1,0%	1110	0,2%
Surirella				0,0%	8139	1,1%	1480	0,3%
Rhopalodia	370	0,0%	370	0,04%				
Synedra	27746	3,1%	14798	1,4%	18497	2,4%	12578	2,3%
Chlorobotrys	9989	1,1%			370	0,0%	37364	7,0%
Frustulia	370	0,0%	6289	0,6%	1480	0,2%		
Cyclotella					740	0,1%		
Total	938905		1145696		1018440		1597758	

Sampling site 4

The total average abundance of freshwater periphytic diatom at sampling site 4 was 1,597,758 Ind/mm^{2,} and 20 genera were found, namely *Oedogonium, Ulotrhix, Oscillatoria, Chroococcus, Navicula, Pinnularia, Terpsinoe, Nitzschia, Cymbella, Placoneis, Cocconeis, Gomphomena, Aulacoseira, Diploneis, Achnanthes, Gyrosigma, Surirella, Synedra,* and *Chlorobotrys*. At sampling site 4 here it is dominated by the Chlorophyta with the highest abundance of Genus is *Ulotrhix* (130,218 Ind / mm^2).

The Genus *Ulothrix* dominated in sampling site 4. It is suspected that in such water conditions, only *Ulothrix* can tolerate high water environments at sampling site 4. Genus *Ulothrix* is tolerant of organic pollution and is sometimes used as an indicator of heavily polluted water (Yusuf 2020). Hydrobiota that have high tolerance will be able to survive in polluted ecosystems, while those with low tolerance have a low abundance and eventually disappear (Ramakrishnan et al. 2010). Based on the data above, it can be concluded that at station 4, there has been pollution, which is thought to be due to the high input of household waste and domestic waste because it is in the residential area.

Diversity and dominance index

The value of freshwater periphytic diatom diversity at the location ranged from 0.91 to 2.44 (table 3). The lowest diversity occurred at sampling site 4 with a value of 0.91, and the highest diversity occurred at station 3 with a value of 2.44. This classification of the Shannon-Wiener diversity index value can be used to determine the distribution of each species and the stability of the community: H> 3 = high diversity, high distribution of individual numbers of each species, and high community stability; 1 <H <3 = Moderate diversity, moderate distribution of the number of individuals per species and moderate community stability; H <1 = low diversity, low distribution of the number of individuals per species, and low community stability. According to the data, the midstream of Brantas River is categorized into a small diversity category.

Based on the data, we assume that the the midstream of Brantas River has a low to moderate freshwater periphytic diatom diversity, individual distribution of each species, and moderate community stability. Sampling site 1 to Sampling site 3 can still be categorized into moderate diversity, moderate distribution of the number of individuals of each species, and moderate community stability. Whereas sampling site 4 has low diversity, the distribution of the number of individuals of each species is low, and the stability of the community is low.

In terms of the dominance index, a study states that the dominance value ranges between 0 and 1 (Hossain et al. 2017). If the dominance value is close to 0, it means that almost no individuals dominate. In contrast, if the dominance is close to 1, it means that there are individuals who dominate the population. According to the dominance index value in sampling site 1 to sampling site 3, the index value is close to the value of 0, so we assume that there is no species dominance. While at station 4, the dominance index value is close to the value 1, so we assume that there is species dominance at station 4.

The checklist

The periphytic diatom checklist (Figure 2) is a list of diatom species found on periphytic substrates, namely on the surface of solid objects such as rocks, wood, or leaves in the aquatic environment of the river. This periphytic diatom checklist is usually used to study the biological and ecological diversity of diatoms in a body of water. This checklist includes the identification of diatom species that can be found in various types of periphytic substrates in the waters. In addition, this checklist can also be used as a basis for further research on the ecology and dynamics of diatoms in waters, as well as an important source of information for biologists and managers of the aquatic environment.

Cosmarium

Cosmarium is a genus of blue-green algae belonging to the division Chlorophyta. Genus *Cosmarium* is worldwide recorded in many taxonomic surveys (Fadul-Souza et al. 2022). These algae can be found in a variety of freshwater habitats such as rivers, lakes and ponds. *Cosmarium* has a distinctive and unique cell shape, which is shaped like a half ball or semi-lunar with a hollow in the middle. Each *Cosmarium* cell consists of a tough cell wall and two semivacuoles, which are internal structures that look like the bubbles in an algal cell. This diatom has chloroplasts which play a role in the process of photosynthesis. *Cosmarium* usually grows attached to substrates such as rocks, leaves or aquatic plants. *Cosmarium* is an important type of algae in aquatic ecosystems. This diatom can be used as an moderate to polluted waters indicator, because tolerance to different environmental conditions can distinguish one species from another. Apart from that, *Cosmarium* also plays a role in the food chain in the waters, because it is a food source for the higher organisms in it.

Entransia

Entransia is a genus of green algae belonging to the Selenastraceae family. These diatoms can be found in a variety of freshwater habitats such as rivers, lakes, and ponds (Kitzing and Karsten 2015). *Entransia* have a distinctive and unique cell shape, which is round or oval in shape and looks like a small bottle or tube. Each *Entransia* cell consists of a tough cell wall and one or two chloroplasts located near the base of the cell. This diatom also has additional pigments such as carotenoids and xanthophylls which play a role in photosynthesis. Usually, *Entransia* grows attached to substrates such as rocks or aquatic plants. *Entransia* is an important type of algae in aquatic ecosystems, because it is a source of food for the higher organisms in it and also plays a role in the water nutrient cycle. In addition, *Entransia* can also be used as an moderate to polluted waters indicator, because tolerance to different environmental conditions can differentiate one species from another.

Oedogonium

Oedogonium is a genus of filamentous green algae found in freshwater environments such as rivers, lakes, and ponds. It typically grows in dense mats on rocks or other submerged surfaces. *Oedogonium* can serve as a bioindicator of polluted waters (Dora et al. 2021) because it is sensitive to changes in environmental conditions such as water temperature, nutrient levels, and water flow. High levels of nutrients like nitrogen and phosphorus can cause excessive growth of *Oedogonium* and other algae, leading to eutrophication and a decrease in dissolved oxygen levels, which can negatively impact aquatic life. Therefore, the presence of *Oedogonium* in a water body may indicate high nutrient levels, poor water quality, and a potential risk to aquatic life. Its abundance and distribution can be used as a tool for monitoring and assessing the ecological health of freshwater environments.

Table 8. Diversity and dominance index during the study

Sampling site	Dominance index (D)	Diversity Index (H)
1	0.18275	1.94
2	0.15468	2.18
3	0.10909	2.44
4	0.66237	0.91

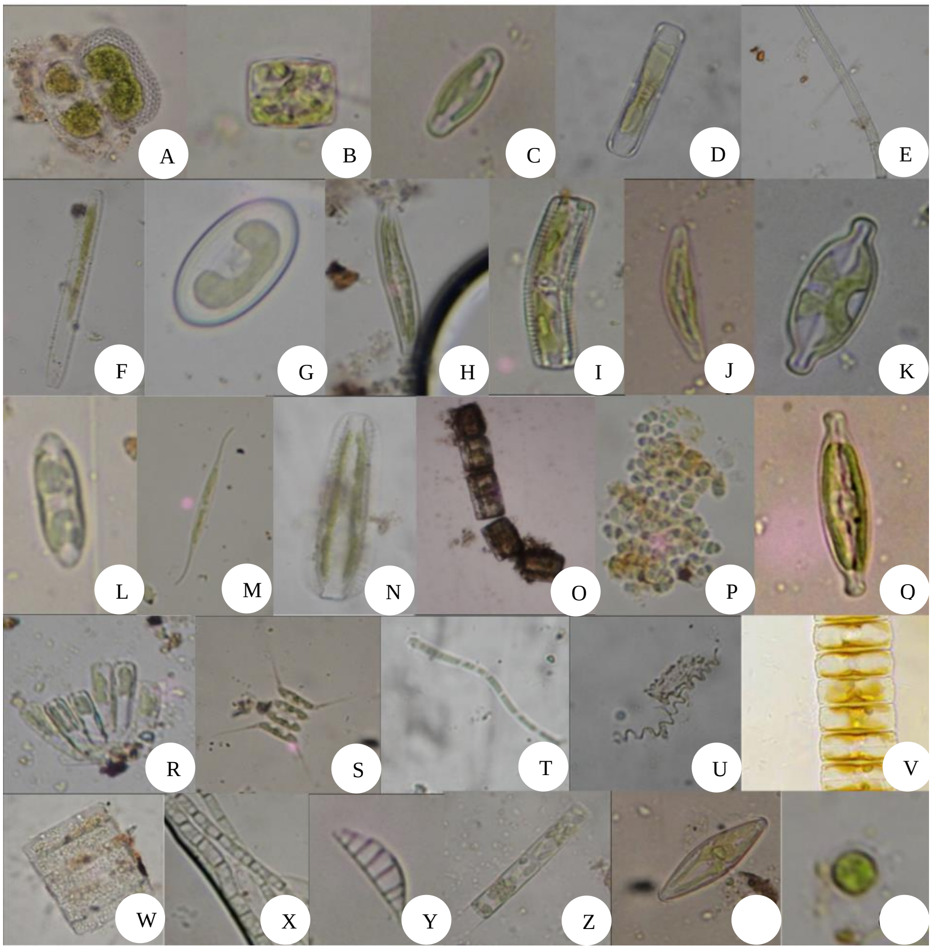

Figure 8. The checklist of freshwater periphytic diatom in stream of the brantas river during the research. A. *Cosmarium*, B. *Cyclotella*, C. *Navicula*, D. *Pinnularia*, E. *Oedogonium*, F. *Synedra*, G. *Cocconeis*, H. *Gyrosigma*, I. *Achnanthes*, J. *Cymbella*, K. *Placoneis*, L. *Diploneis*, M. *Nitzschia*, N. *Surirella*, O. *Melosira*, P. *Chroococcus*, Q. *Stauroneis*, R. *Gomphonema*, S. Scenedesmus, T. *Oscillatoria*, U. *Spirulina*, V. *Fragilaria*, W. *Terpsinoe*, X. *Entransia*, Y. *Rhopalodia*, Z. *Aulacoseira*, AA. *Frustulia*, and AB. *Chlorobotrys*

Ulotrhix

Ulothrix is a genus of filamentous green algae that are commonly found in freshwater habitats such as rivers, streams, ponds, and lakes. They are free-floating and can form mats or colonies on submerged surfaces. *Ulothrix* is known for its ability to fix carbon dioxide and produce oxygen through photosynthesis. As a bioindicator, *Ulothrix* is useful for monitoring water quality in freshwater systems. High levels of *Ulothrix* in a water body may indicate nutrient pollution, such as excess nitrogen and phosphorus, which can lead to harmful algal blooms and degraded water quality (Ghali et al. 2020). In addition, changes in the morphology of *Ulothrix* colonies, such as a decrease in size or branching, can indicate changes in water chemistry or environmental stressors, making it a valuable tool in assessing the health of aquatic ecosystems.

Scenedesmus

Scenedesmus is a genus of green algae that has a characteristic cell shape (Akgül et al. 2017), where its cells are arranged in groups called colonies or coenobia. Each *Scenedesmus* colony consists of several cells that are well-connected and are triangular or circular in shape, with the outermost cell having small protrusions called lobes. Each cell has chloroplasts that function in photosynthesis. *Scenedesmus* can reproduce asexually by cell division or sexually through the formation of zoospores. In aquatic environments, *Scenedesmus* is an important primary producer and serves as a food source for many aquatic organisms. Additionally, its abundance and diversity can be used as an indicator of water quality and environmental health.

Spirulina

Spirulina is a type of blue-green algae that belongs to the phylum Cyanobacteria (Nege et al. 2020). It is characterized by its spiral shape and blue-green color. *Spirulina* can be found in both freshwater and marine environments, and it is known for its ability to perform photosynthesis and fix atmospheric nitrogen. As a bioindicator, *Spirulina* can be used to monitor water quality. It is particularly sensitive to changes in nutrient levels and can indicate the presence of pollutants, such as heavy metals and organic compounds. *Spirulina* is also used in aquaculture as a source of food for fish and other aquatic animals due to its high protein content.

Oscillatoria

Oscillatoria is a type of cyanobacteria (blue-green algae) which has the form of single-celled filaments or many cells arranged in an unbranched manner. The main feature of the *Oscillatoria* is that it forms long, thin filaments with tapered ends. The cells usually have photosynthetic pigments such as chlorophyll a, phycocyanin, and phycoerythrin which give the filaments a greenish or bluish color. *Oscillatoria* can be found in fresh water, marine and wet land with high humidity. The natural habitats of *Oscillatoria* include stagnant fresh water, brackish water, sea water, and wetlands on the banks of rivers or lakes. *Oscillatoria* usually lives attached to substrates such as rocks, gravel, or aquatic plants. *Oscillatoria* plays an important role in the carbon and nitrogen cycles in aquatic ecosystems (Morales et al. 2017). As a primary producer, *Oscillatoria* can convert sunlight into energy through the process of photosynthesis, so that it becomes a food source for other organisms in the aquatic food chain. In addition, *Oscillatoria* can also act as a bioindicator for water quality, because its presence can show the level of pollution and nutrient content in the waters. However, some *Oscillatoria* species can also be a problem in the environment. Some species can produce toxins that are harmful to human and animal health (Haschek et al. 2013; Gupta 2015). In addition, *Oscillatoria* can also thrive in eutrophic conditions (too many nutrients), causing the growth of other invasive algae and damaging the balance of aquatic ecosystems.

Chroococcus

Chroococcus is a genus of non-chlorophyll photosynthetic bacteria belonging to the Cyanobacteria group. These bacteria are usually spherical or nearly spherical, about 1-10 micrometers in diameter and usually live together in dense colonies. The cells are protected by a thick, bluish-green cell wall. *Chroococcus* habitat is very diverse, it can be found in fresh water, sea water or wet soil environments and they highly tolerant of habitat pollution (Al-Mayaly et al. 2012; Wood et al. 2017; Fitri et al. 2021). These bacteria can live in highly acidic or alkaline water and are able to survive in extreme environmental conditions. *Chroococcus* has an important role as an oxygen producer in biogeochemical cycles, and is able to fix atmospheric nitrogen into ammonia which is used by plants. In addition, *Chroococcus* can also be used as a water quality bioindicator, because its presence is sensitive to environmental changes such as the level of water pollution or water acidity. If the *Chroococcus* population decreases drastically, this can be a bad indication for the health of the aquatic ecosystem.

Navicula

Navicula is a type of diatom that belongs to the Bacillariophyceae family. The characteristics of

Navicula are its flat and sharp cell shape, and it has a special structure called a raphe which functions to control cell movement. *Navicula* generally live as periphyton on rock, sand, or aquatic plant substrates in fresh or seawater. In addition, *Navicula* can also be found in polluted waters. *Navicula* has an important role as a water quality bioindicator (Barinova and Mamanazarova 2021; Khalil et al. 2021). Its presence can indicate the ecological condition of a waters. *Navicula* is often used as a tool to monitor water quality and heavy metal pollution, because of its ability to absorb these metals. In addition, *Navicula* is also used as a bioindicator of water temperature and acidity, as well as changes in water flow patterns in the waters that affect the condition of the substrate.

Pinnularia

Pinnularia is a genus of diatoms that generally live in fresh water. The characteristics of *Pinnularia* are the shape of the cells which are long and flat like ribbons, and have a special structure called a raphe which functions to control cell movement. *Pinnularia* can be found in a variety of freshwater habitats such as rivers, lakes, ponds, and swamps. *Pinnularia* has an important role as a water quality bioindicator. Its presence can indicate the ecological condition of waters and can be used to monitor organic and inorganic pollutants in waters. *Pinnularia* can also be used to indicate water quality (Nasery et al. 2020) in waters affected by industrial waste, such as waste from agriculture, livestock, and sewage treatment. In addition, *Pinnularia* can also show good water quality and stable ecosystem conditions, which are needed by other organisms in it.

Terpsinoe

Terpsinoe is a genus of diatoms that generally live in fresh water (Jiménez et al. 2017), especially in shallow waters such as lakes, ponds and rivers. The defining characteristic of *Terpsinoe* is the shape of the cells which are flat, round, and convex in the center, giving it a hat- or umbrella-like appearance when viewed from the side. *Terpsinoe* also have a raphe which functions to control cell movement. *Terpsinoe* can have an important role as a water quality bioindicator, especially in monitoring changes in the ecological conditions of waters and the impact of pollution on waters. Its presence can indicate the ecological conditions of water, including the level of acidity, nutrient levels, and the level of water pollution. *Terpsinoe* can also show good water quality and stable ecosystem conditions, which are needed by other organisms in it (Barinova and Mamanazarova 2021).

Nitzschia

Nitzschia is a genus of diatoms that have a long, cylindrical shape. These diatoms belong to the pennate group which means they have two valves and generally have bilateral symmetry. *Nitzschia* can be found in both fresh and salt water (Thessen et al. 2005; Alakananda et al. 2015). Several *Nitzschia* species can be used as bioindicators of water conditions because their presence is highly dependent on water quality. For example, *Nitzschia* palea is an indicator of a state of eutrophication or excessive increase in nutrients in the waters. The presence of excessive *Nitzschia* in water can be a sign that the water is experiencing eutrophication. In addition, several *Nitzschia* species can also be used in the cosmetic and pharmaceutical industries due to their natural fatty acid and pigment content.

Stauroneis

Stauroneis is a genus of diatoms that have a shape similar to an ax or knife. These diatoms belong to the pennate group which means they have two valves and generally have bilateral symmetry. *Stauroneis* can be found in fresh waters, both cold and warm waters. Several *Stauroneis* species can also be used as bioindicators of water conditions because their presence is highly dependent on water quality. For example, *Stauroneis* anceps is often used as a bioindicator of clean and good quality natural water conditions. The presence of other *Stauroneis* species may indicate water conditions that are more acidic or alkaline. In addition, several *Stauroneis* species can also be used in biogeochemical studies due to their ability to absorb heavy metals from the aquatic environment (Barinova and Mamanazarova 2021).

Cymbella

Cymbella is a genus of diatoms that have a cylindrical or ellipsoidal shape and are generally small to medium in size. These diatoms belong to the pennate group which means they have two valves and generally have bilateral symmetry. *Cymbella* can be found in freshwater, in both cold and warm water environments. They are often found on the surface of moist substrates such as rocks, wood, or leaves. Several *Cymbella* species can be used as bio-indicators of water conditions because their presence is highly dependent on water quality (Barinova and Mamanazarova 2021; Khalil et al. 2021). For example, *Cymbella cistula* is often used as a bioindicator of clean and good quality water conditions. The presence of other *Cymbella* species may indicate water conditions that are more acidic or alkaline. In addition, several *Cymbella* species can also be used in biogeochemical studies because of their ability to absorb heavy metals from the aquatic environment.

Placoneis

Placoneis is a genus of diatoms belonging to the family Naviculaceae. The distinctive feature of this genus is the shape of the cells which are flat and tight, and have transverse raphe bands. In addition, in the cell there are chloroplasts which are flat and numerous. The habitat of *Placoneis* is in clean fresh water, such as rivers, lakes and swamps. This genus can be found on the surface of waterlogged substrates such as rocks, sand, and other organic matter. As a bioindicator, the presence of *Placoneis* can be an indicator of the condition of a clean freshwater ecosystem (Kezlya et al. 2020). Its dominant presence in an environment that is kept clean indicates good water quality and is not polluted by chemicals that are harmful to life in it. Apart from that, *Placoneis* can also be used to measure the level of water pollution by heavy metals, because its cells are able to absorb heavy metals and store them in the cells.

Cocconeis

Cocconeis is a genus of diatoms that have a vessel-like cell shape with two valves or valves, usually symmetrical. These cells are usually small, about 20-200 micrometers. Consistent shape and size make this genus an easy species to identify and measure, so it is often used as a bioindicator in fresh and marine waters. *Cocconeis* usually live in relatively calm waters, such as lakes, ponds and rivers that are not too fast. They can attach to different substrates, such as rocks, wood, and aquatic plants. As a bioindicator, *Cocconeis* can provide information about water conditions and pollution levels (Majewska et al. 2014). Different species of the *Cocconeis* genus have different tolerances to changing environmental conditions, such as temperature, pH, and nutrient content. Therefore, by studying the presence and abundance of *Cocconeis* species in a body of water, it can provide an overview of the water quality and environmental conditions that exist there.

Gomphomena

Gomphonema is a genus of diatoms that are commonly found in freshwater environments. They are characterized by their elongated shape and their ability to form chains of cells. *Gomphonema* cells are usually attached to substrates such as rocks, sediments, or aquatic plants by a mucilaginous stalk that is secreted by the cell. The cells are usually rectangular or trapezoidal in shape and have a raphe, a slit-like opening that runs the length of the cell. The raphe is used for movement and attachment to substrates. *Gomphonema* species are known to be indicators of environmental conditions, such as nutrient levels and water flow rates. They are commonly used as bioindicators for water quality assessments and are sensitive to changes in water chemistry and pollution levels (Prasertsin et al. 2021). Some species of *Gomphonema* are also known to produce bioactive compounds with potential uses in the pharmaceutical and biotechnology industries.

Aulacoseira

Aulacoseira is a genus of freshwater diatoms that are commonly found in lakes and rivers. They are characterized by their distinctive circular or elliptical shape and their radial symmetry. *Aulacoseira* cells are enclosed in a siliceous frustule, which consists of two overlapping halves. *Aulacoseira* species are important indicators of past and present environmental conditions, especially in lake sediments. Their abundance and composition can provide information about past nutrient levels, climate change, and other environmental factors. *Aulacoseira* is also important primary producers in freshwater ecosystems, contributing to the food web and supporting aquatic life. Some species of *Aulacoseira* are known to produce toxins, which can have harmful effects on aquatic organisms and human health (Barinova and Mamanazarova 2021).

Diploneis

Diploneis is a genus of freshwater and marine diatoms that are commonly found in a variety of aquatic habitats, such as lakes, rivers, and estuaries. They are characterized by their distinctive boat-shaped or elliptical frustules, which are composed of overlapping silica valves. *Diploneis* species play an important role in aquatic ecosystems as primary producers, contributing to the food web and supporting the growth of other organisms. They are also important indicators of environmental conditions, as their abundance and species composition can provide information about water quality and nutrient levels. Some species of *Diploneis* have been found to produce toxins that can have harmful effects on aquatic organisms and human health. Additionally, they are often used in environmental monitoring and assessment programs to evaluate the health of aquatic ecosystems and the effectiveness of management strategies (Barinova and Mamanazarova 2021).

Achnanthes

Achnanthes is a genus of diatoms with a distinctive feature of this genus is the shape of the cells which are lancet or elliptical with tight lines located in the middle of the cell. These cells have many microscopic pits called areolae which form a characteristic pattern and are often used for identification of diatoms. *Achnanthes* habitat is fresh water, whether in rivers, lakes or swamps. Some species can be found in nutrient-rich substrates, such as in water polluted by organic wastes or in eutrophic waters. However, most *Achnanthes* species are considered indicators of a relatively clean environment. *Achnanthes* has an important role as a water quality bioindicator. Some species are sensitive to environmental changes, such as increased nutrients, increased temperature, or decreased pH, so their presence can indicate environmental problems in these waters (Barinova and Mamanazarova 2021).

Gyrosigma

Gyrosigma is a genus of freshwater diatoms that are commonly found in streams, rivers, and lakes. They have a unique sigmoid shape that resembles a human ear or a boomerang, and are often used as bioindicators of water quality due to their sensitivity to environmental changes. In terms of ecology, *Gyrosigma* is an important part of the food chain in freshwater ecosystems, as they are a primary producer and provide a source of nutrition for other organisms such as zooplankton and small fish. They also play a role in nutrient cycling, helping to regulate levels of nitrogen and phosphorus in the water. Some species of *Gyrosigma* have been found to produce toxins that can have harmful effects on aquatic organisms and even humans. As such, monitoring populations of *Gyrosigma* can provide valuable information about the health of freshwater systems and potential risks to human health (Barinova and Mamanazarova 2021).

Surirella

Surirella is a type of diatom that can be found in both freshwater and marine environments. This diatom has a curved shape and consists of two cells tightly packed together so that it looks like a small cylindrical box. The cells have fine fibers or regular stripes, and there are many different species. The natural habitat of *Surirella* includes clean and sufficiently flowing fresh water, and can occasionally be found in brackish or seawater environments that enter estuaries. *Surirella* can act as a unpolluted water quality bioindicator because its existence is very sensitive to environmental changes caused by pollution, eutrophication, and other factors (Barinova and Mamanazarova 2021).

Rhopalodia

Rhopalodia is a genus of a group of diatoms that can be found in fresh waters, including rivers, lakes, and swamps. Diatoms of the genus *Rhopalodia* have general characteristics such as a flat or elliptical shape, and sizes that vary from microscopic to several millimeters. *Rhopalodia* is often found in waters with eutrophic or nutrient-abundant conditions, where high levels of phosphorus and nitrogen lead to algal overgrowth. As a bioindicator, *Rhopalodia* can be used to indicate the eutrophication level of water. If *Rhopalodia* is found in large numbers, it indicates a eutrophication problem. On the other hand, *Rhopalodia* can provide ecological benefits, including as a food source for aquatic organisms such as plankton, fish, and other aquatic animals (Barinova and Mamanazarova 2021).

Synedra

Synedra is a genus of diatoms that are commonly found in fresh and marine water. The distinguishing feature of this genus is its long, cylindrical shape and the transverse central slit (raphe). The natural habitats of *Synedra* vary from fresh water, such as lakes and rivers, to marine environments, such as estuaries and estuaries. Some *Synedra* species can also be found in muddy substrates or in nutrient-rich waters. *Synedra* can act as a bioindicator in assessing water quality because of its sensitivity to various environmental parameters, such as temperature, brightness, pH, and nutrients. The presence or abundance of *Synedra* species in an aquatic ecosystem can provide information about water quality and environmental conditions that may affect the life of other organisms in it (Barinova and Mamanazarova 2021).

Chlorobotrys

Chlorobotrys is a genus of green algae that is commonly found in freshwater environments. It is a unicellular organism that has a distinctive shape, with two long flagella that are used for movement. *Chlorobotrys* is often found in stagnant or slow-moving water bodies such as ponds, swamps, and wetlands. As a bioindicator, *Chlorobotrys* is particularly useful for monitoring changes in water quality and nutrient levels. This is because it is highly sensitive to changes in the environment, and can quickly respond to changes in nutrient levels, temperature, and other environmental factors. In polluted water bodies, *Chlorobotrys* can be used to detect the presence of excess nutrients such as nitrogen and phosphorus, which can lead to eutrophication and harmful algal blooms. By monitoring the presence and abundance of *Chlorobotrys*, researchers can gain insights into the overall health of freshwater ecosystems, and take steps to mitigate the effects of pollution and other environmental stressors (Barinova and Mamanazarova 2021).

Frustulia

Frustulia is a genus of diatoms that are commonly found in freshwater environments. They are characterized by having a high valve density and an elongated cell shape. Several species of *Frustulia* can be found in clean waters with good water quality. *Frustulia* can be used as a water quality bioindicator because of its sensitivity to environmental changes. Several species of *Frustulia* can indicate the presence of pollutant substances in water such as heavy metals and pesticides, so that they can be used to monitor pollution levels in waters. In addition, a decrease in the number of species and density of *Frustulia* can also be an indicator of a decrease in water quality and disruption of aquatic ecosystems. Therefore, *Frustulia* can be used as a tool for monitoring water quality and environmental sustainability (Barinova and Mamanazarova 2021).

Cyclotella

Cyclotella is a genus of diatoms belonging to the centric group of diatoms. A distinctive feature of *Cyclotella* is the shape of the flattened round cells, with many apexes surrounding the cells. In nature, *Cyclotella* can be found in a variety of freshwater environments, such as lakes, rivers, and marshes. *Cyclotella* is used as a bioindicator because of its sensitivity to changes in the aquatic environment. Nutrient concentration, temperature, pH, and water clarity can affect the presence and abundance of *Cyclotella* in a body of water. Therefore, changes in *Cyclotella* populations can provide clues to the current state of the aquatic environment. In addition, several *Cyclotella* species are also used as indicators to monitor heavy metal pollutants in waters, because they can absorb these metals and concentrate in their cells (Barinova and Mamanazarova 2021).

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