

Species diversity, canonical correspondence, and phenetic analysis of microalgae in the Tancak Maesan River, Bondowoso, Indonesia

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Abstract. Hariani SA, Kurniawati AR, Rohman A, Buana YC. 2025. *Species diversity, canonical correspondence, and phenetic analysis of microalgae in the Tancak Maesan River, Bondowoso, Indonesia. Biodiversitas 26: 5612-5625.* Microalgae are microscopic autotrophic organisms that inhabit various aquatic environments, including the Tancak Maesan River, Bondowoso, Indonesia. They play an essential role as primary producers in aquatic ecosystems and serve as bioindicators of water quality. Anthropogenic activities within the area may influence the microalgal community. The high diversity of microalgae also reflects considerable morphological variation among species. Due to their morphological plasticity and the limited availability of identification keys, morphological and anatomical characterization is crucial for phenetic analysis. This study aimed to analyze the diversity and phenetic characteristics of microalgae found in the Tancak Maesan River. Eleven species were identified, belonging to three divisions (Ochrophyta, Charophyta, and Chlorophyta). *Navicula* sp. had the highest density across all sampling sites. The diversity index indicated a moderate category ($H' = 2.0906$). The evenness index shows a high value ($E = 0.8386$), while the dominance index falls into a low category ($D = 0.1436$). Canonical Correspondence Analysis revealed that two microalgal species (*Cymbella* sp. and *Pinnularia* sp.) were strongly correlated with three abiotic parameters (pH, salinity, and Total Dissolved Solids). According to phenetic analysis, *Achnanthes inflata* and *Navicula* sp. cluster together with the highest similarity (Jaccard coefficient = 0.833). This study concludes that community activities and abiotic and anthropogenic conditions influence the presence of microalgae. The findings affirm the role of microalgae as sensitive bioindicators of water quality, responding to both natural and human-induced environmental changes. Consequently, this research establishes a crucial baseline for the river's microalgal diversity and underscores the importance of phenetic characterization for future ecological and monitoring studies.

Keywords: Bioindicator, canonical correspondence analysis, diatoms

INTRODUCTION

Microalgae possess exceptionally high biodiversity, estimated at between 200,000 and 800,000 species (Wu et al. 2021). This richness allows them to occupy a wide range of habitats and diverse species (Mallén-Ponce et al. 2022; Rojas-Vilalta et al. 2024). Microalgae function as the primary producers in aquatic food chains (Gogoi et al. 2019; Withana et al. 2025). Furthermore, they can provide a comprehensive picture of water quality, as they exhibit specific tolerance limits to various physical and chemical parameters of aquatic ecosystems. Their presence thus serves as a reliable bioindicator of water quality (Stantcheva and Sheath 2016; Kuturo et al. 2024). In addition, microalgae contribute to pollutant reduction and detoxification by acting as degradative organisms (bioremediation agents) in aquatic ecosystems (Goswami et al. 2022; Kumar et al. 2024; El-Sheekh et al. 2025). Microalgae can act as a CO₂ absorber for photosynthesis (Prasad et al. 2021; Iglina et al. 2022; Srimongkol et al. 2022). Based on these considerations, conducting an inventory of microalgae species is essential for assessing biodiversity in aquatic environments.

Microalgae exhibit a distinct preference for favorable environmental conditions, with one such habitat in the waters of the Tancak Maesan River in Bondowoso, East Java, Indonesia. Perhutani manages this strategic area and encompasses a complex ecosystem, including a protected forest zone and a limited-use area. This location provides an ideal habitat for microalgal community growth, as it offers stable physico-chemical water conditions and a balanced nutrient supply from decomposing leaves and organic matter from the surrounding forest. This combination of factors creates an ecological niche that fosters the diversity and productivity of microalgae in the river.

Several factors influence the microalgal community in this region, particularly the activities of tourists and plantation workers. Bathing activities involving the use of cleansing products, such as soap and shampoo, directly in the river can degrade water quality, leading to declines in microalgal populations. The area also serves as a utilization zone where tree replacement cycles occur, including *Hibiscus tiliaceus* (waru), *Falcataria moluccana* (sengon), and coffee plantations, following logging activities. Such practices may cause soil erosion, mud flooding, increased

surface runoff, and sedimentation. Enhanced sediment loads contribute to riverbed shallowing and water turbidity (Dalu et al. 2020; Hope et al. 2020). The decrease in water clarity negatively affects water quality and microalgal growth, as reduced light penetration hampers photosynthesis (Drylie et al. 2018; Abdelfattah et al. 2023). Given these circumstances, systematic monitoring of microalgae is essential as a baseline dataset, particularly since no prior studies have been conducted in this river system.

High species diversity of microalgae often results in considerable variation in morphological characteristics. Morphological identification remains the most commonly employed method in the phenotypic taxonomy of microalgae (Badr and Fouad 2023). However, this method faces several major constraints: the lack of comprehensive identification keys and the morphological plasticity of microalgae. Therefore, characterizing microalgae based on both morphological and anatomical traits is essential for analyzing their phenetic relationships. To date, more than 35,000 microalgae species have been identified (Borowitzka 2013), showcasing their immense taxonomic diversity. This vast number, however, presents a significant challenge for morphological identification methods. Phenetic analysis aids in classifying microalgae species based on morphological and anatomical data, although the complexity of this data often complicates the grouping process. This limitation underscores the need for a more reliable approach. Nevertheless, research on the phenetic analysis of microalgae, particularly in specific river ecosystems, remains scarce. Consequently, a more in-depth investigation into their characterization is essential.

To date, no study has integrated the community-environment relationship (canonical correspondence analysis) with the phenetic characterization of microalgae in this ecosystem. Abiotic gradients exert intense selective pressure on microalgal communities, directly shaping their species composition and abundance. Research by Kumar

and Thomas (2019) concluded that physico-chemical parameters significantly influence dominant microalgal taxa. Canonical Correspondence Analysis (CCA) statistically reveals the relationship between these environmental conditions and community composition. This pattern underpins their bioindicator function, where microalgae serve as a natural proxy for aquatic health.

Furthermore, forest management activities by Perhutani, such as logging and conservation practices, directly alter stream parameters by increasing phosphate and turbidity levels. These abiotic shifts subsequently reshape the microalgal community structure, establishing it as a sensitive indicator for monitoring the impact of land management on the health of aquatic ecosystems. Based on this rationale, this research investigates the species diversity, canonical correspondence, and phenetic analysis of microalgae in the Tancak Maesan River, Bondowoso, Indonesia.

MATERIALS AND METHODS

Study area

This research was carried out between August 2024 and January 2025 in the Tancak Maesan River, located in Dawuhan Hamlet, Suco Lor Village, Maesan Sub-district, Perhutani KPH Bondowoso, Bondowoso District, East Java Province, Indonesia (Figures 1 and 2; Table 1). The instruments and equipment used included an glass slides, cover slips, dropper pipettes, 30 mL sample bottles, 250 mL containers, stationery, refractometer, thermohygrometer TFA model 45.2007, lux meter (Eagletech digital LX1010B), TDS meter, pH meter (mediatech ph TDS EC Temp meter waterproof auto calibrate ATC 9908 merahabu-B1900134), laptop, Garmin GPSmap 60CSx, 30 μm plankton net, toothbrush, and camera.

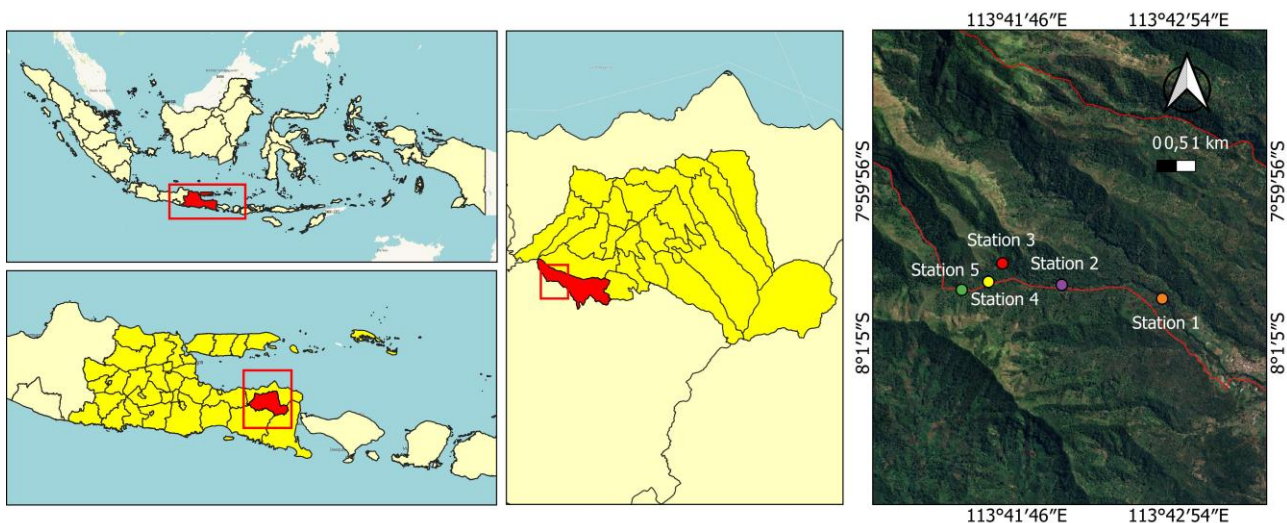


Figure 1. Research site at the Tancak River, Maesan Sub-district, Bondowoso District, East Java Province, Indonesia



Figure 2. Presents the study locations of: A. Station 1, B. Station 2, C. Station 3, D. Station 4, E. Station 5

Table 1. Description of the five research stations along the Tancak River, Maesan, Bondowoso District, East Java, Indonesia

Location	Coordinate points		Height (mdpl)	Description
	Latitude	Longitude		
Station 1	8°00'31.0" S	113°41'39.6" E	697	Moderate water flow; clear and shallow water; gravel and sand substrate; unshaded; located near zones of human utilization and activities.
Station 2	8°00'45.9" S	113°42'47.2" E	980	Strong water flow; clear and shallow water; rocky substrate; open area situated between two hill slopes with surrounding vegetation.
Station 3	8°00'38.8" S	113°41'33.7" E	1130	Strong water flow; clear and shallow water; large boulders, gravel, and sand substrate; unshaded; located within a utilization zone and distant from settlements.
Station 4	8°00'40.1" S	113°42'04.7" E	1080	Strong water flow; clear and shallow water; rocky substrate; open area situated between two hill slopes with surrounding vegetation.
Station 5	8°00'42.2" S	113°41'22.4" E	1117	Moderate flow; clear and shallow water; sandy rocky substrate; partially shaded, near waterfalls, and far from settlements.

Procedures

Determination of research stations

Sampling stations were determined using purposive sampling, in which five stations were selected based on specific criteria and research considerations. The stations encompassed areas ranging from upstream near the waterfall (Station 5) to downstream (Stations 1, 2, 3, and 4). These five stations were considered representative of the environmental variability within the study area. At each station, samples were collected at three distinct points as replicates: point a (yellow) located on the left riverbank, point b (green) located in the midstream, and point c (red) located on the right riverbank (Figure 3).

Microalgae data collection

Water sampling involved conducting 15 horizontal hauls with a 5000-mL bucket. Following collection, the water was filtered through a 30-micron plankton net. In addition, epiphytic microalgae were sampled by brushing

the surface of submerged stones with a toothbrush (Barinova and Smith 2019). The toothbrush was then rinsed in a 30 ml sample bottle to allow the attached microalgae to detach and accumulate in the container. Stones presumed to harbor microalgae were characterized by their slippery texture. Each sampling station was labeled, and the collected samples were preserved in a refrigerator.

The water samples were identified using an Olympus CX21 LEDFS1 microscope. A drop of the water sample was placed on a glass slide using a dropper pipette, covered with a cover slip, and observed under the Olympus CX21 LEDFS1 microscope. The findings were documented using a camera and subsequently identified with reference to Freshwater Algae: Identification and Use as Bioindicators by Bellinger and Sigeo (2010) and the online resource diatom.org.

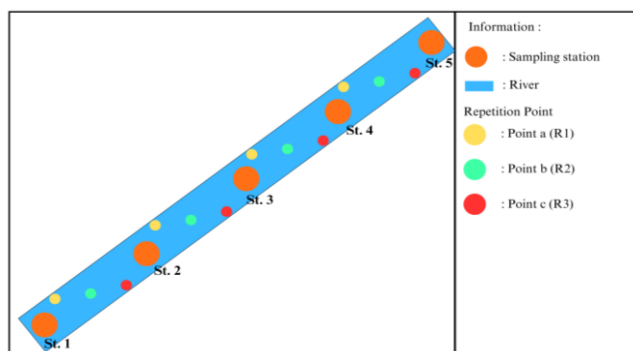


Figure 3. Sketch of the sampling station (St. 1: Stations 1, St. 2: Stations 2, St. 3: Stations 3, St. 4: Stations 4, St. 5: Stations 5)

Table 2. Phenotypic characteristics of microalgae

Characteristic	(0)	(1)
Oval shape	(0) absent	(1) present
Perfect oval shape	(0) absent	(1) present
Asymmetric oval shape	(0) absent	(1) present
Oval oblong	(0) absent	(1) present
Rod shape	(0) absent	(1) present
Elongated rod shape	(0) absent	(1) present
Round shape	(0) absent	(1) present
Perfect spherical shape	(0) absent	(1) present
Asymmetric spherical shape	(0) absent	(1) present
Polygonal shape	(0) absent	(1) present
Ultraplankton size (< 5 µm)	(0) absent	(1) present
Nanoplankton size (5-50 µm)	(0) absent	(1) present
Microplankton size (>50-500 µm)	(0) absent	(1) present
Macroplankton size (5000-50,000 µm)	(0) absent	(1) present
Spines	(0) absent	(1) present
Spine visibility	(0) not visible	(1) visible
Horns	(0) absent	(1) present
Number of horns 1-4	(0) absent	(1) present
Number of horns >4	(0) absent	(1) present
Cell wall	(0) thin	(1) thick
Cell wall edge	(0) regular	(1) wavy
Pyrenoids	(0) absent	(1) present
Motility	(0) nonmotile	(1) motile
Lifestyle	(0) solitary	(1) colony
Raphe	(0) absent	(1) present
Raphe visibility	(0) not visible	(1) visible
Central raphe (in the center)	(0) absent	(1) present
Eccentric raphe (at one edge of the cell)	(0) absent	(1) present
Marginal raphe (at both edges of the cell)	(0) absent	(1) present
Striae	(0) absent	(1) present
Striae visibility	(0) not visible	(1) visible
Central nodule	(0) absent	(1) present
Filaments	(0) absent	(1) present
Unbranched filament array	(0) absent	(1) present
Branched filament array	(0) absent	(1) present
Gelatinous stalk	(0) absent	(1) present

Abiotic parameters

We measured abiotic parameters at each station using three distinct points as replicates. We selected five key abiotic parameters based on their direct influence on microalgal communities. Water temperature governs metabolic rates, light intensity determines photosynthesis rates, and pH affects nutrient availability and metal toxicity. Salinity controls osmotic pressure and species distribution, while Total Dissolved Solids (TDS) influences light penetration and habitat conditions through turbidity.

Data analysis

Data analysis included the calculation of the Shannon-Wiener diversity index (H'), Evenness index (E'), and Simpson's dominance index (D) (Magurran 2004). The relationship between abiotic parameters and microalgae was analyzed using Canonical Correspondence Analysis (CCA), which was computed with the PAST 4.09 paleontological statistics software (Hammer et al. 2001).

Phenetic analysis was conducted using three software applications: Microsoft Excel for $n \times t$ data tabulation, Programmer's File Editor (PFE), and MVSP 3.1 for phenogram construction.

Microalgae characterization was carried out by compiling character data into an $n \times t$ matrix (strain \times character) using Microsoft Excel (Table 2). Binary coding was applied, with "0" denoting the absence and "1" denoting the presence of a given character. The coded $n \times t$ matrix was then copied and pasted into PFE for file formatting into .mvs format to ensure compatibility with MVSP 3.1. In MVSP, similarity indices for each strain were calculated using the Jaccard Coefficient, followed by phenogram construction through the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) clustering algorithm. Jaccard's Coefficient is one of the most prevalent methods for calculating similarity values in a similarity matrix, particularly for presence-absence data.

RESULTS AND DISCUSSION

Microalgal diversity in the Tancak Maesan River

The most abundant microalgae identified in the Tancak Maesan River, Bondowoso, belonged to the division Ochrophyta, predominantly comprising species from the class Bacillariophyceae. According to Marella et al. (2020), Blanco (2024) and Samudra et al. (2024), Bacillariophyceae, commonly referred to as diatoms, exhibit high tolerance to unstable environmental conditions and can adapt to both strong and weak water currents. This ability is attributed to their thick and rigid cell walls, which contain silica and are resistant to degradation, thereby enabling diatoms to thrive under various aquatic conditions (Pančić et al. 2019; Harmilia et al. 2022). Diatoms frequently dominate riverine ecosystems as plankton and periphyton, except in muddy rivers (Harmoko et al. 2019; B-Béres et al. 2023). Moreover, diatoms effectively utilize nutrients and exhibit high rates of reproduction and cell division (Zhou et al. 2022; Inomura et al. 2023; Arteaga-Castrejon et al. 2024). A total of eleven microalgal species,

representing eleven families, were identified in the Tancak Maesan River, Bondowoso (Table 3; Figure 4). Station 1 comprised seven species, while Station 3 comprised nine species, with *Navicula* sp. being the dominant species in both stations. The predominance of *Navicula* sp. in Stations 1 and 3 is presumed to be due to its high tolerance to diverse environmental conditions. Station 1 was characterized by moderate current velocity, clear water, and substrates consisting of gravel and sand. In contrast, Station 3 was characterized by fast-flowing, clear water and substrates composed of large rocks, gravel, and sand. *Navicula* exhibits high adaptability across various aquatic environments, which accounts for its wide distribution (Gong et al. 2024; Zheng et al. 2024; Cid-Rodríguez et al. 2024). Additionally, this genus possesses mucilaginous appendages that allow strong attachment to substrate surfaces, thereby supporting its abundance in both stations with differing flow regimes.

Synedra ulna was the most abundant species at Station 5, which may be explained by its ability to survive and grow under diverse environmental conditions with favorable light intensity. As a diatom, *S. ulna* possesses silica-based cell walls that are resistant to damage, thus enhancing its persistence in the conditions observed at Station 5. This station was characterized by moderate flow, clear water, dense riparian vegetation providing partial shading, substrates composed of gravel and sand, and abundant leaf litter and woody debris within the river. The presence of leaf litter and woody debris contributes to nutrient enrichment through decomposition processes, creating an optimal environment for *S. ulna*. Candra et al. (2022) reported that the abundance of *S. ulna* is strongly associated with nutrient availability, with higher nutrient

concentrations supporting increased growth. Similarly, Isti'anah et al. (2015) and Li et al. (2025) noted that *Synedra* demonstrates high tolerance to fluctuating environmental conditions, including nutrient-poor environments. This tolerance is supported by its multilayered cell walls and its capacity to accumulate and store reserve nutrients in the form of insoluble polymers.

Meanwhile, *Rhopalodia gibba* and *Achnanthes inflata* were not observed in Stations 1 and 3, which is likely due to higher Total Dissolved Solids (TDS) and light intensity in these stations compared with Stations 2, 4, and 5, rendering these species less tolerant to the prevailing abiotic conditions. According to Zhang et al. (2015), the absence of a species in a given habitat is generally attributable to environmental conditions exceeding its specific tolerance threshold, ultimately leading to its inability to persist within the community.

Analysis of the microalgae index in the Tancak Maesan River

Table 4 presents the Shannon-Wiener index values of the eleven microalgal species identified in the Tancak Maesan River. The table indicates that each species exhibited varying levels of diversity across different sampling stations. The Shannon-Wiener diversity index analysis across five stations in the Tancak Maesan River revealed values ranging from 1.83 to 2.25. According to Odum (1996), values within the range of $1 < H' < 3$ are categorized as moderate diversity. This finding suggests that the environmental conditions of the five stations remain sufficiently favorable to support microalgal communities.

Table 3. List of microalgae species in the Tancak Maesan River, Bondowoso District, East Java, Indonesia

Division	Family	Species	St					N
			1	2	3	4	5	
Ochrophyta	Cymbellaceae	<i>Cymbella</i> sp.	17	14	11	10	13	65
Ochrophyta	Surirellaceae	<i>Surirella</i> sp.	13	9	6	12	19	59
Ochrophyta	Naviculaceae	<i>Navicula</i> sp.	27	23	31	22	16	119
Ochrophyta	Catenulaceae	<i>Amphora ovalis</i> (Kützing) Kützing, 1844	2	6	5	4	8	25
Ochrophyta	Bacillariaceae	<i>Nitzschia palea</i> (Kützing) W.Smith, 1856	13	17	21	15	14	80
Ochrophyta	Fragilariaceae	<i>Synedra ulna</i> (Nitzsch) Ehrenberg, 1832	9	13	12	14	23	71
Ochrophyta	Pinnulariaceae	<i>Pinnularia</i> sp.	12	5	7	6	9	39
Ochrophyta	Rhopalodiaceae	<i>Rhopalodia gibba</i> (Ehrenberg) Otto Müller, 1895	0	3	0	2	3	8
Ochrophyta	Achnanthesaceae	<i>Achnanthes inflata</i> (Kützing) Grunow, 1867	0	2	0	2	2	6
Charophyta	Zygnemataceae	<i>Spirogyra</i> sp.	0	3	3	5	6	15
Chlorophyta	Microsporaceae	<i>Microspora</i> sp.	0	5	4	4	5	18
Total			93	100	100	96	118	507

Note: St: Station; N: Total; Verificator species: *Cymbella* sp., *Surirella* sp., *Navicula* sp., *Pinnularia* sp., *Spirogyra* sp., and *Microspora* sp.: Bellinger and Sigeo (2010); *Amphora ovalis*: Stepanek and Kociolek (2011); *Nitzschia palea*: Kociolek (2011); *Synedra ulna*: Spaulding and Edlund (2015); *Rhopalodia gibba*: Spaulding and Metzeltin (2011); *Achnanthes inflata*: Burge (2012)

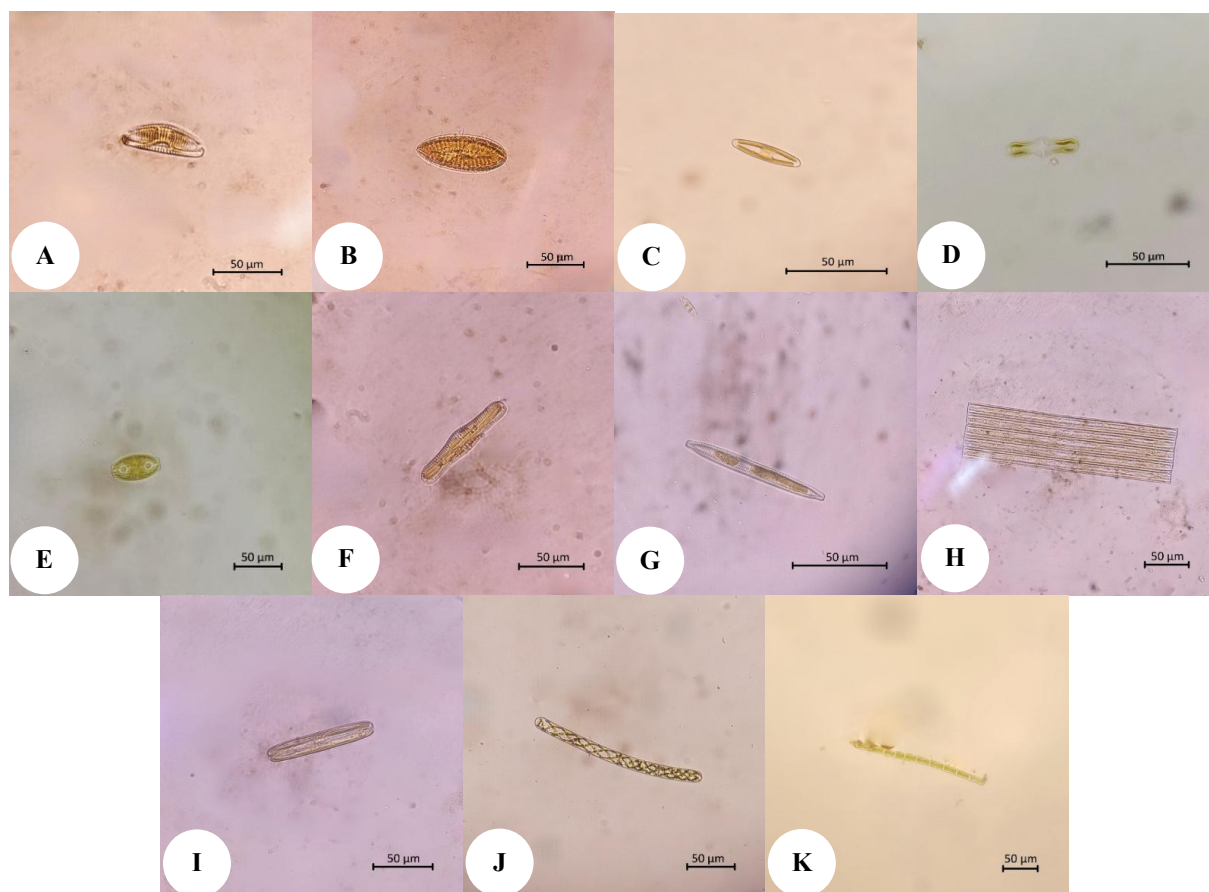


Figure 4. Microalgal in the Tancak Maesan River, Bondowoso, Indonesia: A. *Cymbella* sp., B. *Surirella* sp., C. *Navicula* sp., D. *Achnanthes inflata*, E. *Amphora ovalis*, F. *Rhopalodia gibba*, G. *Nitzschia palea*, H. *Synedra ulna*, I. *Pinnularia* sp., J. *Spirogyra* sp., K. *Microspora* sp.

A study by Dayana et al. (2022) reported that the Air Lais River exhibited diversity index values between 2.2 and 2.59, which similarly indicated that the ecosystem was under moderate ecological pressure. Among the five stations in the Tancak Maesan River, Station 5 exhibited the highest diversity index value ($H' = 2.25$), with a total of 118 individuals from 11 species. In contrast, Station 1 recorded the lowest diversity index value ($H' = 1.82$), with a total of 93 individuals from 7 species. The high diversity at Station 5 is attributable to the greater number and relatively even distribution of species, with no single species dominating the community. Conversely, the low diversity at Station 1 is associated with the lower species richness compared with Stations 2, 3, 4, and 5, alongside the dominance of *Navicula* sp.. Astuti et al. (2017) noted that the number of species present strongly influences diversity index values; the higher the species richness, the greater the diversity index. This pattern is also shaped by environmental conditions such as physico-chemical factors, biological parameters, and the water cycle (Swaleh et al. 2022; Delgado-Fernández et al. 2025). Unfavorable environmental conditions can act as a limiting factor, reducing species richness, decreasing individual abundance, and increasing the dominance of certain microalgae (Josephine et al. 2022; Kholssi et al. 2023). Therefore, microalgae diversity analysis aims to identify the types of microalgae present at the study site. This information is crucial for distinguishing

between adaptive species and those vulnerable to environmental changes. Since the abiotic conditions of the water strongly influence diversity, analyzing the abiotic parameters at the study site is essential for understanding the influence of environmental factors on microalgal distribution.

Analysis of the evenness index across five stations in the Tancak Maesan River showed values ranging from 0.8 to 0.89 (Table 4). According to Odum (1971), an E value > 0.6 indicates a high level of evenness. This high evenness signifies that nearly all microalgae species possess a similar abundance of individuals, preventing any single species from dominating the community (Ma et al. 2024). Such a condition typically occurs in stable aquatic ecosystems where the physico-chemical environment supports the balanced growth of various species (Gao et al. 2024; Hui et al. 2024). Station 1 exhibited the highest evenness value, while Station 3 showed the lowest. This disparity is likely influenced by environmental factors such as nutrient availability, light intensity, and current velocity. The environmental conditions at Station 1 supported the uniform growth of all present species, even though its species richness was lower than that of other stations. According to Estrada-Villegas et al. (2022), evenness can remain high despite low species richness if the distribution of individuals among species is relatively uniform.

Analysis of the Simpson dominance index across five stations in the Tancak Maesan River yielded values ranging from 0.12 to 0.17 (Table 4). Station 1 exhibited the highest dominance index value, while Station 5 showed the lowest. According to Odum (1971), a dominance value in the range of $0 < D \leq 0,5$ falls into the low dominance category. These low values indicate no single species dominated the community. The uniformly low index values result from the relatively even distribution of individuals among species, preventing any species from attaining a significantly larger population than others (see Table 4). A low dominance value signifies a healthy state of the aquatic ecosystem (Martsenyuk et al. 2016). Furthermore, the low dominance index aligns with the high values of the diversity index (Table 4) and evenness index (Table 4), indicating a relatively balanced distribution of individuals among species.

Abiotic parameters of the Tancak Maesan River

The abiotic parameters measured in the Tancak Maesan River included pH (7.1-7.33), salinity (0-0.33 ppt), Total Dissolved Solids (TDS; 52.33-57.67 ppm), water temperature (26.33-26.93°C), and light intensity (882.33-1364.67 lux) (Table 5). Abiotic factors play an important role in shaping microalgal distribution, resulting in variation in species composition across aquatic environments. The abiotic conditions in the Tancak Maesan River varied across sampling stations, yet provided a favorable environment for the 11 microalgal species identified, allowing them to survive and adapt to their habitat. The pH values ranged between 7.1 and 7.33, indicating slightly alkaline conditions, though still close to neutral. This condition can be attributed to the river's origin from mountain springs and the relatively minimal anthropogenic activities, which limit pollutant input. Station 1 recorded a slightly higher pH value of 7.33, which was likely influenced by soap use from plantation workers bathing in the river. Soap, being alkaline, can elevate pH levels and consequently affect microalgal communities. Mousavi and Khodadoost (2019) and Eraljita and Afdhal (2022) reported that household waste, including detergents and soap, can increase water pH, and excessively high pH values may endanger aquatic organisms by disrupting metabolic and respiratory processes. Non-tolerant species may decline in abundance, whereas tolerant species may persist or even increase. Furthermore, diurnal fluctuations in photosynthetic activity also influence pH: during daylight, photosynthesis reduces CO₂ concentrations, causing pH to rise, while at night respiration increases CO₂ concentrations, lowering pH (Vasker et al. 2021). Meier et al. (2022) emphasized that

most microalgae grow optimally within a pH range of 7 to 9, suggesting that the pH conditions of the Tancak Maesan River remain suitable for sustaining microalgal life.

Salinity values ranged between 0 and 0.33 parts per thousand (ppt), confirming that the river is a freshwater system. Freshwater salinity levels are typically less than 0.5 ppt (Castillo and De León 2021). The low salinity of the river reflects its location on the eastern slopes of Mount Argopuro, far from the coast and with minimal human activity, ensuring the water remains pristine with limited dissolved salts. Drushka et al. (2019) noted that salinity levels are influenced by geographical location, rainfall, and freshwater inflows, with decreases linked to precipitation and freshwater input, and increases caused by evaporation or seawater intrusion. Sun et al. (2022) highlighted that microalgal species exhibit varying salinity tolerances, which influence their distribution. Diatoms are commonly found in freshwater or low-salinity environments. Increased salinity raises osmotic pressure in diatom cells, reducing growth and nutrient uptake efficiency. Consequently, diatom richness and diversity typically decline as salinity increases (Frost et al. 2023). This was consistent with findings at Station 1, which had the highest salinity value (0.33 ppt) and hosted only seven microalgal species, fewer than Stations 2-5. Thus, even within a low range, elevated salinity may negatively affect species richness, particularly among diatoms.

The TDS values ranged from 52.33 to 57.67 ppm, indicating low concentrations of dissolved solids and confirming the river's classification as fresh and relatively pristine water. These values are considered favorable and within the normal range for supporting microalgal growth. Arfian et al. (2022) suggested that normal TDS levels should be below 500 ppm. Elevated TDS can reduce light penetration, thereby inhibiting photosynthesis in microalgae (Nurhidayah and Nirwansyah 2024). TDS levels are often influenced by household wastewater inputs, such as soap, detergents, and sewage (Anggrini et al. 2023; Adjovu et al. 2023). This corresponds with conditions at Station 1, which recorded the highest TDS value (57 ppm), likely due to soap use by residents.

Table 4. Microalgae diversity index in the Tancak Maesan River, Bondowoso District, East Java Province, Indonesia

Index	Station 1	Station 2	Station 3	Station 4	Station 5
Taxa_S	7	11	9	11	11
Individuals	93	100	100	96	118
Shannon_H	1.83	2.2	1.97	2.21	2.25
Evenness_e^H/S	0.89	0.82	0.8	0.83	0.86
Dominance D	0.17	0.13	0.17	0.13	0.12

Table 5. Abiotic parameters in the Tancak Maesan River, Bondowoso District, East Java Province, Indonesia

Location	Water pH	Salinity (ppt)	TDS (ppm)	Water temperature (°C)	Light intensity (lux)
Station 1	7.33 ± 0.12	0.33 ± 0.29	57.67 ± 0.58	26.67 ± 0.58	1188.33 ± 142.67
Station 2	7.25 ± 0.05	0 ± 0.00	56 ± 1	26.67 ± 0.58	945.33 ± 7.64
Station 3	7.1 ± 0.26	0.17 ± 0.29	55.67 ± 0.58	28.33 ± 0.58	1364.67 ± 71.94
Station 4	7.18 ± 0.02	0 ± 0.00	55 ± 0.82	26.67 ± 0.58	919.33 ± 30.34
Station 5	7.17 ± 0.25	0 ± 0.00	52.33 ± 0.58	26.33 ± 0.58	882.33 ± 56.12
Average	7.21 ± 0.09	0.1 ± 0.16	55.33 ± 2.05	26.93 ± 0.83	1060 ± 197.34

Water temperature is another critical factor affecting microalgal survival, as it regulates growth and metabolic processes. Temperature strongly influences biological activity and the distribution of aquatic organisms (Barten et al. 2021; Tascón-Peña et al. 2025). The optimal water temperature range for most microalgae lies between 20 and 30°C (Yu et al. 2024; Ugya et al. 2025). Measurements in the Tancak Maesan River revealed temperatures ranging from 26.33 to 28.33°C, values that fall within the optimal range for microalgal growth. Variations in water temperature across stations were associated with the time of sampling: approximately 10:00 a.m. at Stations 1 and 2, 12:30 p.m. at Stations 3 and 4, and 2:00 p.m. at Station 5. Water temperature tended to be higher during midday due to maximum solar radiation. Since microalgae possess specific thermal tolerance limits, only species capable of adapting to these temperature ranges were present in the river. Consequently, thermal variation across stations may influence the spatial distribution of microalgal species.

The measurement of light intensity in the Tancak Maesan River ranged between 882.33 and 1364.67 lux. These values indicate that illumination conditions at the site are relatively bright. The highest measurement was recorded at Station 3 (1364.67 lux), which received direct sunlight and was not shaded by surrounding vegetation. Conversely, Station 5 exhibited the lowest light intensity (882.33 lux), attributed to dense vegetation cover in the area and the timing of measurement, which was conducted in the late afternoon under shadier conditions. Variations in light intensity within the river system may influence species composition (Frost et al. 2023). According to Yusuf et al. (2020), the optimal light intensity required for microalgal growth ranges from 1000 to 10,000 lux, with adequate light enabling optimal proliferation. However, excessively high light intensity can cause inhibitory effects

(photoinhibition) on microalgal growth (Maltsev et al. 2021). Diatom assemblages, in particular, exhibit adaptive efficiency under fluctuating or high light conditions due to their capacity to optimize photosynthesis and regulate light-induced stress (Juffermans et al. 2025).

Canonical Correspondence Analysis (CCA)

Canonical correspondence analysis was employed to examine the relationship between abiotic parameters and microalgal assemblages in the Tancak Maesan River. CCA revealed that axis 1 and axis 2 accounted for 71.79% and 28.21% of the data variation, respectively. Quadrant I demonstrated that *Cymbella* sp. and *Pinnularia* sp. were correlated with pH, salinity, and Total Dissolved Solids (TDS) (Figure 5). This finding indicates that these species tend to occur in environments with relatively higher values of pH, salinity, and TDS, as recorded at Station 1. Station 1 exhibited a slightly alkaline pH (7.33), low salinity (0.33 ppt), and low TDS (57.67 ppm). Based on the classification of Callisto and De Leon (2021), salinity values < 0.5 ppt fall within freshwater categories. Furthermore, Purwanto et al. (2019) reported that low TDS reflects clear water conditions. The high abundance of *Cymbella* under such pH levels is consistent with Kumaraswamy et al. (2023), who reported that *Cymbella* populations tend to increase with rising pH. In addition, *Cymbella* is known to persist in oligotrophic freshwater environments (Zhao et al. 2025). Similarly, *Pinnularia* represents a freshwater diatom that typically inhabits oligotrophic waters (Goss et al. 2025) and can thrive under nutrient-poor conditions (Tarigas et al. 2020). Consequently, *Pinnularia* is considered a potential bioindicator of clean water quality (Arindri and Sulistyarsi 2018).

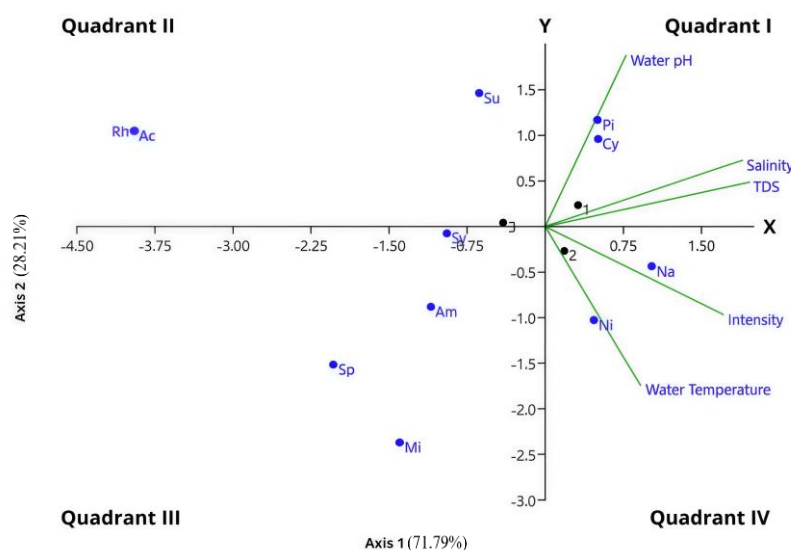


Figure 5. CCA graph of abiotic parameters with microalgae species in the Tancak Maesan River, Bondowoso District, East Java Province, Indonesia. Rh: *Rhopalodia gibba*, Ac: *Achnanthes inflata*, Sy: *Synedra ulna*, Am: *Amphora ovalis*, Sp: *Spirogyra* sp., Cy: *Cymbella* sp., Pi: *Pinnularia* sp., Na: *Navicula* sp., Ni: *Nitzschia palea*, Mi: *Microspora* sp., Su: *Surirella* sp.

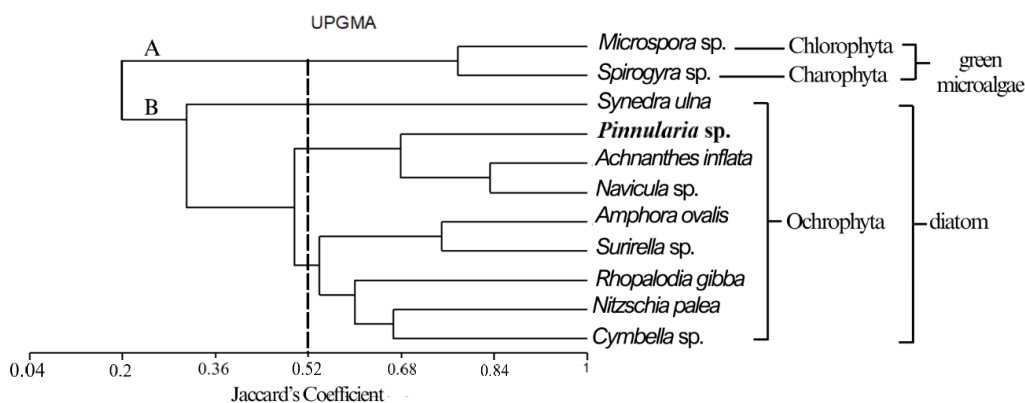


Figure 6. Phenogram of microalgae phenetics analysis in the Tancak Maesan River, Bondowoso District, East Java Province, Indonesia. A: Green microalgae cluster (Chlorophyta and Charophyta divisions), B: Diatom cluster (Ochrophyta division)

Quadrant IV demonstrated that *Nitzschia palea* and *Navicula* sp. were positively correlated with light intensity and temperature, suggesting their preference for environments characterized by elevated illumination and temperature. This indicates that both species exhibit enhanced photosynthetic efficiency under such conditions. Increasing light intensity is commonly associated with rising surface water temperature (Sinaga et al. 2020). According to Shi et al. (2016), *N. palea* demonstrates higher growth rates and photosynthetic activity under elevated light intensity, with stable growth occurring within the 28-35°C range (Zhang et al. 2020). Similarly, Mudrikah et al. (2024) reported that high light intensity promotes the growth of *Navicula* sp.. Telussa and Nurachman (2019) further observed that growth rates of *Navicula* sp. increase with rising temperature and illumination, resulting in higher population densities. The Canonical correspondence analysis results demonstrate that the presence and abundance of specific microalgae species can serve as an early warning system for detecting disturbances in ionic balance and increased dissolved pollutants, enabling more responsive and effective environmental management measures. While this phenetic analysis utilizes morphological data, future studies would significantly benefit from integrating molecular verification to strengthen taxonomic identifications.

Phenetic analysis of microalgae based on morphological characters

Phenetic analysis of 11 microalgal species from the Tancak Maesan River was conducted using morphological and anatomical character variations. The analysis was performed using MVSP 3.1 software, and the results were represented as a phenogram. This classification enabled the identification of interspecific similarities and differences based on cell morphology, size, and structural attributes. Species exhibiting numerous differences were grouped further apart, whereas species with greater morphological similarity were clustered more closely (Figure 6).

The phenogram demonstrated that at a similarity coefficient of 0.2 (20%), the assemblages were divided into two principal clusters, namely Cluster A and Cluster B. Cluster A comprised *Microspora* sp. and *Spirogyra* sp. At

the same time, Cluster B consisted of *S. ulna*, *A. inflata*, *Navicula* sp., *R. gibba*, *Pinnularia* sp., *Amphora ovalis*, *Surirella* sp., *N. palea*, and *Cymbella* sp. Jaccard similarity index analysis revealed that *A. inflata* and *Navicula* sp. exhibited the highest Similarity Index value (SI = 0.833; 83.3%). According to Danong et al. (2024), similarity indices are categorized as very close (≥ 0.75), close (0.51-0.74), not close (0.26-0.50), and very distant (≤ 0.25).

The species *A. inflata* was found to be closely related to *Navicula* sp. Based on the Jaccard coefficient analysis, *A. inflata* exhibited a high degree of similarity with *Navicula* sp. As presented in Table 6, the obtained Similarity Index (SI) value was 0.833. According to Danong et al. (2024), an SI value ≥ 0.75 indicates a very close relationship. Based on the observed morphological characters, *A. inflata* and *Navicula* sp. shared 34 characters (oval-shaped, not perfectly oval, not asymmetrically oval, elongated oval-shaped, not rod-shaped, not elongated rod-shaped, not spherical, not perfectly spherical, not asymmetrically spherical, not polygonal, not ultraplanktonic in size ($<5 \mu\text{m}$), nanoplanktonic in size ($>5-50 \mu\text{m}$), not microplanktonic ($>50-500 \mu\text{m}$), not macroplanktonic ($>500-50,000 \mu\text{m}$), spineless, spines not visible, hornless, horn number 1-4 absent, horn number >4 absent, thick cell wall, absence of pyrenoid, motile, solitary life form, presence of raphe, raphe visible, raphe positioned centrally, raphe not positioned eccentrically, raphe not positioned marginally, presence of striae, presence of a central nodule, absence of filaments, absence of unbranched filaments, absence of branched filaments, absence of gelatinous stalks) and differed in two characters. According to Hanurandi et al. (2022), the genus *Achnanthes* is classified as monoraphid diatoms with a concave raphe. The raphe is centrally located on the valve, and the striae are arranged in either uniseriate or biseriate rows (Majewska et al. 2017; You et al. 2019). In contrast, *Navicula* is a biraphid isopolar diatom characterized by a boat-like morphology. Its striae are spindle-shaped and uniseriate in arrangement. The raphe system of *Navicula* is relatively simple, accompanied by a central nodule, and its cell walls are composed of silica (Pane et al. 2023).

Table 6. Jaccard similarity index between microalgae species of the Tancak Maesan River, Bondowoso District, East Java Province, Indonesia

UPGMA Jaccard's Coefficient Similarity matrix											
	Cy	Su	Na	Am	Ni	Sy	Pi	Rh	Ac	Sp	Mi
Cy	1.000										
Su	0.500	1.000									
Na	0.500	0.429	1.000								
Am	0.571	0.750	0.400	1.000							
Ni	0.667	0.462	0.357	0.538	1.000						
Sy	0.286	0.417	0.133	0.385	0.455	1.000					
Pi	0.615	0.667	0.667	0.500	0.462	0.308	1.000				
Rh	0.615	0.667	0.429	0.500	0.583	0.308	0.667	1.000			
Ac	0.533	0.467	0.833	0.438	0.400	0.188	0.692	0.571	1.000		
Sp	0.056	0.125	0.000	0.118	0.133	0.364	0.059	0.059	0.000	1.000	
Mi	0.056	0.059	0.059	0.056	0.133	0.250	0.059	0.059	0.053	0.778	1.000
	Cy	Su	Na	Am	Ni	Sy	Pi	Rh	Ac	Sp	Mi

Note: Cy: *Cymbella* sp., Su: *Surirella* sp., Na: *Navicula* sp., Am: *Amphora ovalis*, Ni: *Nitzschia palea*, Sy: *Synedra ulna*, Pi: *Pinnularia* sp., Rh: *Rhopalodia gibba*, Ac: *Achnanthes inflata*, Sp: *Spirogyra* sp., Mi: *Microspora* sp.

The species *Microspora* sp. was closely related to *Spirogyra* sp. Based on the Jaccard coefficient analysis, *Microspora* sp. exhibited a high degree of similarity with *Spirogyra* sp., with an IS value of 0.778 (Table 6). According to Danong et al. (2024), an $SI \geq 0.75$ indicates a very close relationship. Based on the morphological characters observed, *Microspora* sp. and *Spirogyra* sp. shared 34 characters (not oval-shaped, not perfectly oval, not asymmetrically oval, not elongated oval, rod-shaped, elongated rod-shaped, not spherical, not perfectly spherical, not asymmetrically spherical, not polygonal, not ultraplanktonic in size ($<5 \mu\text{m}$), microplanktonic in size ($>50\text{-}500 \mu\text{m}$), not macroplanktonic ($>500\text{-}50,000 \mu\text{m}$), spineless, spines not visible, hornless, horn number 1-4 absent, horn number >4 absent, thin cell wall, regular cell wall margin, nonmotile, solitary life form, absence of raphe, raphe not visible, raphe not central, raphe not eccentric, raphe not marginal, absence of striae, striae not visible, absence of central nodule, presence of filaments, presence of unbranched filament chains, absence of branched filament chains, absence of gelatinous stalks) and differed in two characters. According to Attique et al. (2022) and Khaleel and Dwaish (2023), *Microspora* and *Spirogyra* are unbranched filamentous green algae. Green algae typically possess cell walls composed of cellulose and pectin (Domozych and LoRicco 2024).

The species *A. ovalis* was closely related to *Surirella* sp. Based on the Jaccard coefficient analysis, *A. ovalis* exhibited a high similarity to *Surirella* sp., with an IS value of 0.750 (Table 6). According to Danong et al. (2024), an $SI \geq 0.75$ indicates a very close relationship. Based on the morphological characters observed, *A. ovalis* and *Surirella* sp. shared 34 characters (oval-shaped, not asymmetrically oval, not rod-shaped, not elongated rod-shaped, not spherical, not perfectly spherical, not asymmetrically spherical, not polygonal, not ultraplanktonic in size ($<5 \mu\text{m}$), not nanoplanktonic in size ($>5\text{-}50 \mu\text{m}$), microplanktonic in size ($>50\text{-}500 \mu\text{m}$), not macroplanktonic ($>500\text{-}50,000 \mu\text{m}$), spineless, spines not visible, hornless, horn number 1-4 absent, horn number >4

absent, thick cell wall, regular cell wall margin, presence of pyrenoid, motile, solitary life form, presence of raphe, raphe not central, raphe not eccentric, raphe marginal, presence of striae, striae visible, presence of central nodule, absence of filaments, absence of unbranched filament chains, absence of branched filament chains, absence of gelatinous stalks) and differed in three characters. According to Wu et al. (2020), *Amphora* sp. possesses marginal raphes located along both sides of the cell, with striae arranged in uniseriate rows. In contrast, *Surirella* sp. is classified within the order Surirellales, characterized by solitary cells possessing a canal raphe system extending along the valve margin (Morais et al. 2018). *Surirella* exhibits heteropolar valves with marginal raphes that are often inconspicuous and faintly visible striae (Borgohain and Tanti 2018).

Nitzschia palea is phenetically related to *Cymbella* sp. Based on the Jaccard coefficient analysis, *N. palea* exhibits a moderate degree of similarity with *Cymbella* sp., as indicated in Table 6, where the Similarity Index (SI) obtained was 0.667. According to Danong et al. (2024), SI values ranging from 0.51 to 0.74 represent a close relationship. Based on the observed character data, *N. palea* and *Cymbella* sp. share 33 characters (not perfectly oval, not elongated oval, not rod-shaped elongated, not round, not perfectly round, not asymmetrically round, not polygonal, not ultraplanktonic ($<5 \mu\text{m}$), not nanoplanktonic ($>5\text{-}50 \mu\text{m}$), microplanktonic ($>50\text{-}500 \mu\text{m}$), not macroplanktonic ($>500\text{-}50,000 \mu\text{m}$), lacking spines, spines not visible, lacking horns, absence of 1-4 horns, absence of >4 horns, thick cell wall, regular cell wall margins, absence of pyrenoids, motile, solitary lifestyle, presence of raphe, visible raphe, raphe not centrally positioned, raphe located at one lateral margin (eccentric), raphe not situated along both margins (marginal), presence of striae, striae visible, absence of filaments, absence of unbranched filaments, absence of branched filaments, absence of gelatinous stalk) and 3 differing characters. According to Borgohain and Tanti (2018), *Cymbella* possesses uniseriate punctate striae arranged in a slightly radiate to parallel pattern, with a

raphe system that is slightly shifted towards the ventral or lateral side. Furthermore, the raphe in *Cymbella* may be either centrally or eccentrically positioned (Babu and Pauline 2018). By contrast, *N. palea* is characterized by the absence of a central nodule, very fine striae, a raphe canal surface that is flat and aligned with the valve surface, and an eccentrically positioned raphe (Barkia et al. 2019).

Pinnularia sp. is phylogenetically related to *A. inflata* and *Navicula* sp. Based on the Jaccard coefficient analysis, *Pinnularia* sp. exhibits a moderate similarity with *A. inflata* and *Navicula* sp., with similarity indices of 0.692 and 0.667, respectively, as presented in Table 6. According to Danong et al. (2024), SI values between 0.51 and 0.74 denote a close degree of similarity. Based on the observed character data, *Pinnularia* sp., *A. inflata*, and *Navicula* sp. share 32 characters (oval, not perfectly oval, not asymmetrically oval, elongated oval, not rod-shaped, not elongated rod, not round, not perfectly round, not asymmetrically round, not polygonal, not ultraplanktonic (<5 µm), not macroplanktonic (> 500-50,000 µm), lacking spines, spines not visible, lacking horns, absence of 1-4 horns, absence of >4 horns, thick cell wall, absence of pyrenoids, motile, solitary lifestyle, presence of raphe, raphe centrally located, raphe not eccentrically located, raphe not positioned along both margins, presence of striae, presence of central nodule, absence of filaments, absence of unbranched filaments, absence of branched filaments, absence of gelatinous stalk) and 5 differing characters. According to Goss et al. (2025), the genus *Pinnularia* comprises solitary pennate diatoms possessing a raphe system located centrally, with multiseriate and hollow striae.

Rhopalodia gibba is phylogenetically related to *N. palea* and *Cymbella* sp.. Based on the Jaccard coefficient analysis, *R. gibba* exhibits a moderate degree of similarity with *N. palea* and *Cymbella* sp., with similarity indices of 0.583 and 0.615, respectively, as indicated in Table 6. According to Danong et al. (2024), SI values between 0.51 and 0.74 indicate a close relationship. Based on the observed characters, *R. gibba*, *N. palea*, and *Cymbella* sp. share 30 characters (not perfectly oval, not elongated rod, not round, not perfectly round, not asymmetrically round, not polygonal, not ultraplanktonic (<5 µm), not nanoplanktonic (>5-50 µm), microplanktonic (>50-500 µm), not macroplanktonic (>500-50,000 µm), lacking spines, spines not visible, lacking horns, absence of 1-4 horns, absence of >4 horns, thick cell wall, absence of pyrenoids, motile, solitary lifestyle, presence of raphe, raphe not centrally located, raphe eccentrically positioned, raphe not marginal, presence of striae, striae visible, absence of filaments, absence of unbranched filaments, absence of branched filaments, absence of gelatinous stalk) and 7 differing characters. According to Satpati et al. (2021), *R. gibba* possesses distinctly visible striae, while in the genus *Rhopalodia*, the raphe is situated on the dorsal margin (Rybak et al. 2020).

Environmental conditions strongly influence morphological variation among microalgae. Microalgae exhibit a high capacity for adaptation in terms of morphology, physiology, and cellular structure, thereby

enabling survival under diverse environmental conditions. Such adaptation is achieved by modulating the ratio and composition of key cellular elements that govern structural and functional processes (Azaman et al. 2023). Variations in habitat conditions can thus trigger changes in morphological structures and functions. Previous research by Azaman et al. (2017) demonstrated that alterations in the biochemical composition of microalgae are correlated with their morphological characteristics. For instance, the accumulation of lipid bodies and carotenoids in *Chlorella sorokiniana* and *Chlorella zofingiensis* was shown to increase cell size. Similarly, Morabito et al. (2007), as cited in Neustupa and Woodard (2024), reported that seasonal temperature fluctuations can influence changes in the shape, size, and symmetry of microalgal cells. For example, *Micrasterias thomasi* exposed to elevated temperatures (>25°C) exhibited reduced cell size and increased asymmetry of cell lobes, particularly during summer (29°C). Such morphological changes occur as a direct response to environmental adaptation mechanisms.

This study successfully identified a sufficiently diverse microalgal community in the Tancak Maesan River, comprising 11 species from three different divisions (Ochrophyta, Charophyta, and Chlorophyta), with a clear dominance of diatoms (Ochrophyta). Environmental parameters such as pH, salinity, TDS, water temperature, and light intensity significantly influenced the distribution and abundance of microalgal species. Canonical correspondence analysis successfully identified specific correlations, revealing relationships between *Cymbella* sp. and *Pinnularia* sp. with pH, salinity, and TDS, as well as between *N. palea* and *Navicula* sp. with temperature and light. Phenetic analysis based on morphological characteristics grouped the species into two main clusters: green algae and diatoms. This research provides valuable baseline data on microalgal biodiversity and the aquatic environmental conditions of the Tancak Maesan River. This dataset can serve as a reference for future monitoring of the river ecosystem's health. The finding that the distribution of microalgal species strongly correlates with specific abiotic parameters reinforces the role of microalgae as effective bioindicators for assessing water quality and environmental conditions in an aquatic system. The dominance of diatoms, well-known indicators of water health, further supports this community's potential for use in the region's biomonitoring programs. This study provides a baseline for future biomonitoring and supports integrating morpho-taxonomic and environmental multivariate analyses in assessing tropical freshwater ecosystems.

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