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Application of eDNA Technology to Assess Phytoplankton Diversity in the Ciliwung Downstream Area, Jakarta, Indonesia

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Abstract

Rivers have the potential for enormous flows of water, nutrients, and food that can create habitats for various living creatures. However, the flow of pollutants into estuaries and coastal areas can degrade environmental conditions in downstream areas of the river. Numerous studies have highlighted the loss of aquatic species due to increased stressors and pollutants in heavily contaminated rivers. This manuscript aims to analyze the phytoplankton diversity in three stations (natural riparian, concreted riparian, and estuary) of the Ciliwung River in Jakarta. Using environmental DNA (eDNA), we identified and compared the number of phytoplankton families and read sequences with various water quality parameters. The results showed that each station consists of 16, 13, and 20 families with 11,257; 75,963; and 37,339 read sequences, respectively. The study showed that phytoplankton family diversity in Stations 1 and 2 correlates with water pollution levels (lightly to extremely polluted). In contrast, Station 3 exhibited an unusual pattern, with high family diversity despite declining water quality.

Keywords: aquatic biota, habitat, riparian, water quality

1. Introduction

Rivers are aquatic ecosystems that serve various benefits to support human life. Various ecosystem services (ES), including food, water, water purification, microclimate regulation, tourism, and biodiversity, can be supported by riverine ecosystems [1]. River ecosystems consist of water bodies and riparian zones, which allow them to have aquatic, terrestrial, and transitional environments. This condition holds the potential to support biodiversity in providing biological natural resources and food for human needs [2]. In contrast to other aquatic ecosystems, rivers have the potential for enormous flows of water, nutrients, and food that can create habitats for various living creatures. Seasonal changes in river ecosystems also affect the fluctuation of water and nutrient flow, which provides a unique ecosystem for some species. This condition will not be found in lentic aquatic ecosystems with standing or slow-flowing water.

Rivers' ability to provide ES for humans decreases as the ecosystem is damaged. Habitat destruction due to environmental degradation reduces populations of native species and changes the conditions of the food chain [3]. Introducing invasive species also threatens the ecosystem balance and reduces the optimization of natural resource provision. The need for built-up land shrink riparian areas, leaving only aquatic ecosystems. The loss of vegetation strips in riparian areas also increases the pollutants flow into water bodies and affects the pollutant index [4]. Moreover, the flow of pollutants can decompose into estuaries and coastal areas, resulting in a decline in environmental conditions in the downstream areas of the river. Various studies show the loss of aquatic biota species due to increased stressors and pollutants in highly polluted rivers.

Land use changes highly threaten urban areas. The high demand for built-up land has an impact on river ecosystems. Rivers in urban areas have been urbanized, characterized by high built-up areas along the ecosystem [5]. This condition impacts low-absorption areas in river basins and increases the risk of flooding. In several segments, local governments modify the natural characteristics of meandering rivers through river normalization to speed upriver flow to the sea and reduce the risk of water stagnating for a long time. The high level of human modification in managing river ecosystems can change rivers' natural habitat and ability to support biodiversity [6].

Metropolitan cities are highly vulnerable to environmental degradation [7]. The high population and demand for built-up land impact the number of residential and industrial areas. Jakarta is Indonesia's capital and one of the largest megacities in Asia, and it has a high risk of increasing river pollutant flows from domestic and industrial waste. Improper household sanitation facilities impact increasing domestic pollutants such as coli bacteria in surface water. High levels of pollutants in waters reduce the concentration of dissolved oxygen, which results in oxygen depletion and hypoxia. As a result, various aquatic biota that are sensitive to oxygen content are at high risk of mortality [8]. The destruction of terrestrial and aquatic habitats in Jakarta River reduces the ability of this ecosystem to provide various ecosystem services for its residents.

In recent years, environmental DNA (eDNA) technology has been increasingly used to assess the diversity of biota in aquatic ecosystems, such as seas and rivers. This method allows researchers to monitor species distribution in each location without relying on conventional sampling techniques, which can pose risks to animal welfare and cause environmental damage. By extracting water and sediment samples that contain traces of skin, urine, feces, mucus, and reproductive cells, researchers can obtain genomic information and determine the taxonomic abundance of species [9]. The use of eDNA is also closely linked to the study of water pollution. Various aquatic species, including macroinvertebrates, serve as indicators of environmental contamination. Certain taxa exhibit high sensitivity to changes in environmental conditions, such as decreased dissolved oxygen levels or increased pollution.

Water parameters such as BOD, COD, and DO are often associated with aquatic biota survival. However, water's trophic status using some parameters like phosphate and nitrogen is believed to be the main factor influencing macroinvertebrate composition [10]. The availability of several macroinvertebrate taxa can determine the level of river pollution in urban areas. By utilizing the results of the phytoplankton eDNA inventory, this manuscript aims to analyze the phytoplankton diversity in three stations (natural riparian, concreted riparian, and estuary) of the Ciliwung River in Jakarta and see its relationship with water quality at the sampling point. Hopefully, the research results will provide an overview of the impact of urbanization on river pollution levels and the composition of aquatic biota in river ecosystems.

2. Methods

2.1. Study Area

Ciliwung River flows from the highlands of the Mount Pangrango and Mount Gede region and empties into Jakarta Bay. River conditions show a decline in water quality in downstream areas due to high urbanization, economic activities, and land conversion. This research was carried out at three different stations in Ciliwung Hilir (S1, S2, and S3), DKI Jakarta Province (Figure 1). Station selection is based on the location of sampling points for quarterly river water quality monitoring carried out by the Provincial Environmental Service. Three stations represent natural river conditions, normalized rivers, and estuary areas. In this research, a natural river is defined as a river with a natural landscape without alteration of its flow, and the riverbanks are still bordered by natural vegetation. A normalized river refers to a river segment modified, straightened, or engineered by human activities to control its flow and shape, typically for purposes such as flood prevention. This segment often features concrete embankments. Estuary areas are defined as partially enclosed coastal bodies of water where freshwater from rivers and streams meets and mixes with saltwater from the ocean. Tidal forces and fluctuations in water depth also influence these areas.

2.2. Field Sampling and eDNA Analysis

Field surveys collected water and sediment samples in water bodies and littoral zones to represent aquatic and terrestrial biota. At each station, three samples were taken with three replications per station. This research was carried out in August 2023 to see the worst conditions of the river's concentrated pollutants in the dry season. The samples were then analyzed using eDNA to determine potential diversity up to the species level.

Environmental DNA (eDNA) analysis involves several simple steps: first, collecting water samples from the environment; next, extracting DNA from these samples using gSYNC DNA extraction kits manufactured by Geneaid Biotech following the manufacturer's guidelines; then, using PCR to amplify specific DNA regions of interest (in this study using 12S-V5 primer sets); after that, sequencing the DNA fragments to identify the organisms present; following this, using bioinformatics tools to analyze the sequencing data and determine the types of organisms found; then, interpreting the results to understand the diversity and composition of the environmental community; ensuring quality control throughout the process; and finally, reporting and using the findings for various purposes such as biodiversity monitoring and conservation. Overall, eDNA analysis provides a straightforward and effective method for studying organisms in their natural habitats [6].

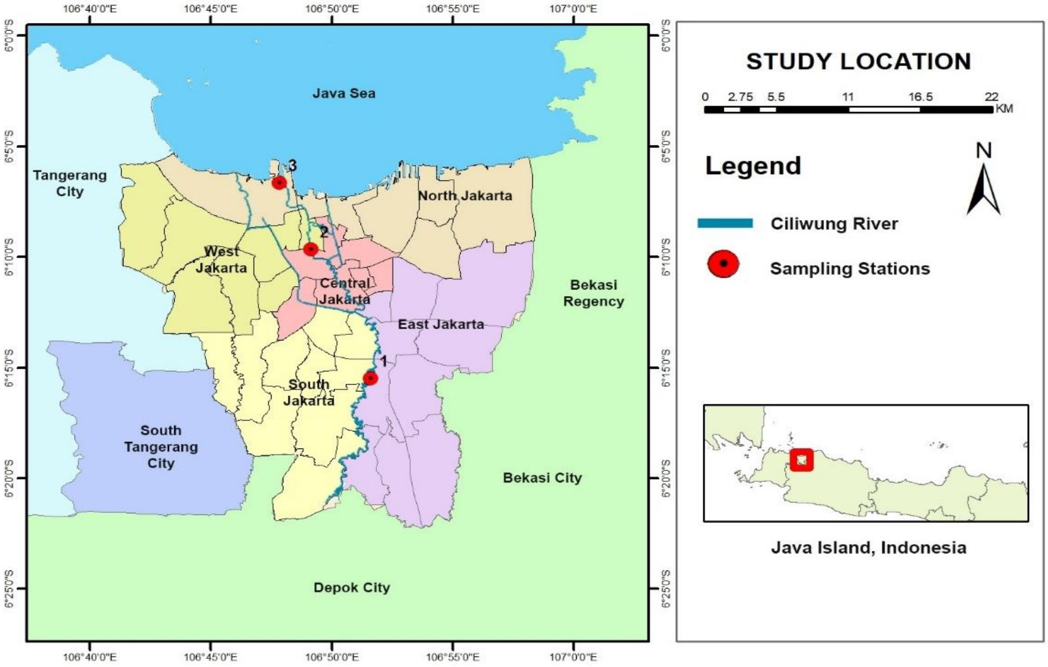


Figure 1. Ciliwung River which flows through several municipalities in the Jakarta Province.

2.3. Water Quality

Water quality data was obtained from monitoring conducted by the Jakarta Provincial Environmental Service in August 2023. The institution measured several *in situ* parameters, such as temperature, salinity, water clarity, turbidity, color, total dissolved solids (TDS), and total suspended solids (TSS). In contrast, the remaining parameters were analyzed in the laboratory. Water quality monitoring at each river point is carried out four times a year, covering different periods (rainy season, rainy-dry transition, dry season, and dry-rainy transition). This study's water quality data is focused on the dry season, as it was collected in August 2023, corresponding to the eDNA sampling period.

Water quality parameters collected included salinity, TSS, pH, Dissolved Oxygen (DO), ammonia, total phosphate (TP), Biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD). Several parameters were chosen due to the high relationship with the species abundance in water bodies. Household activities contribute significantly to organic pollutants such as BOD, COD, and TSS. Elevated levels of organic pollutants in water bodies can directly impact DO concentrations [11]. In addition to these parameters, other factors, such as pH and TP, play crucial roles in determining the presence of biota in limnic ecosystems. High levels of pollutants can have a stressful effect on aquatic life in river ecosystems [12].

2.4. Pollution Index

The Pollution Index (PI) is a comprehensive tool for assessing water quality in diverse aquatic environments, including rivers, lakes, coastal areas, and oceans. It is a vital metric for understanding the degree of contamination and degradation within aquatic ecosystems, informing management and mitigation strategies. Typically, the PI encompasses a range of parameters that reflect different aspects of water quality. In many regions, including Indonesia, the PI is crucial in guiding regulatory frameworks, shaping water management policies, and fostering stakeholder collaboration. The determination of water pollution status using the PI is stated in the Decree of the Minister of the Environment of Indonesia Number 115 of 2003.

$$\mathrm{PI} = \sqrt{\frac{(\mathrm{Ci/Lij})^2_{\mathrm{M}} + (\mathrm{Ci/Lij})^2_{\mathrm{R}}}{2}} \tag{1}$$

where Ci is the concentration of water quality parameters (i) analysis results, Lij is the concentration of water quality parameters (i) the quality standard for water allocation (j); (Ci/Lij) M is a maximum value of Ci/Lij; and (Ci/Lij) R is the average value of Ci/Lij.

The calculation of the Pollution Index (PI) involves comparing the water quality parameters measured against the class II river water quality standards outlined in Attachment VI to Government Regulation of Indonesia Number 22 of 2021. Through IP values, the state of water is categorized into four criteria, as outlined in Table 1.

Table 1. A classification system for water quality using the PI to evaluate and categorize water conditions

Class	Score	Criteria
1	$0 \leq \mathrm{PI} \leq 1.0$	Good water quality
2	$1.0 < \mathrm{PI} \leq 5.0$	Lightly polluted
3	$5.0 < \mathrm{PI} \leq 10$	Moderately polluted
4	$\mathrm{PI} > 10$	Extremely polluted

Source: Government Regulation of Indonesia Number 22 of 2021

3. Result and Discussion

3.1. Result

3.1.a. Water Quality

Water quality from Stations 1 to 3 generally shows a decrease in several parameters such as ammonia, BOD, and COD (Table 2). This indicates that water quality conditions are getting worse downstream. On the other hand, several parameters, such as TSS and TP, show that conditions are improving downstream.

Table 2. The results of water quality at each station, along with a comparison to the Class 2 water quality standards

Parameters	Unit	Standard	Station 1	Station 2	Station 3
TSS	mg L^{-1}	50	85.5*	67.8*	18.5
pH	-	6-9	7.2	7.4	7.4
DO	mg L^{-1}	4	2.3*	1.6*	2.1*
Ammonia	mg L^{-1}	0.2	0.4*	3.9*	9.3*
TP	mg L^{-1}	0.2	0.03	0.02	0.015
BOD	mg L^{-1}	3	6.4*	8.1*	17.4*
COD	mg L^{-1}	25	28.9*	31*	70.3*

*did not meet water quality standard.

3.1.b. Phytoplankton Composition

Each station shows a different phytoplankton family composition. At Station 1, 16 families were identified with read sequence dominance by Stephanodiscaceae (9,084) and Bacillariaceae (1,061) (Figure 2). These families were dominated by the same genus, Cyclotella. Among the Cyclotella, *Cyclotella meneghiniana* showed the highest sequence reads at all three stations. At Station 2, Chlorosarcinaceae family with the species *Neochlorosarcina* sp. dominates the phytoplankton composition (**Error! Reference source not found.**). In contrast to Station 2, the chlorellaceae family with the species *Micractinium pusillum* dominates the phytoplankton composition at Station 3.

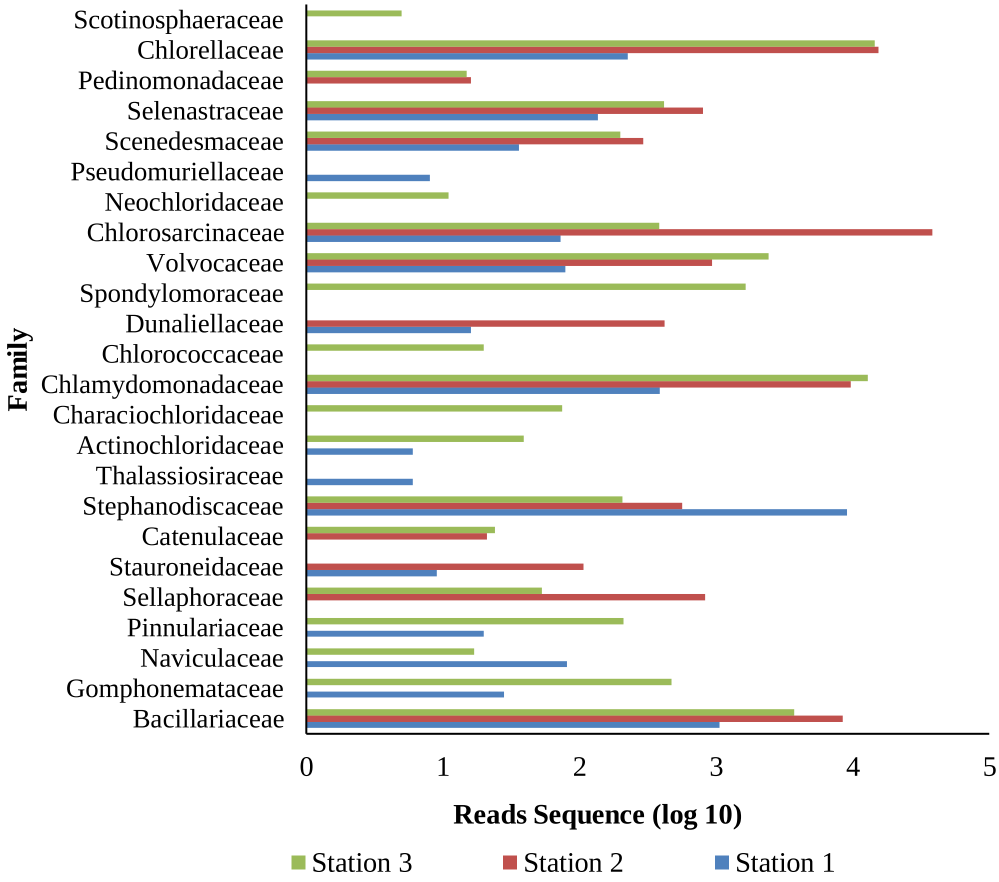
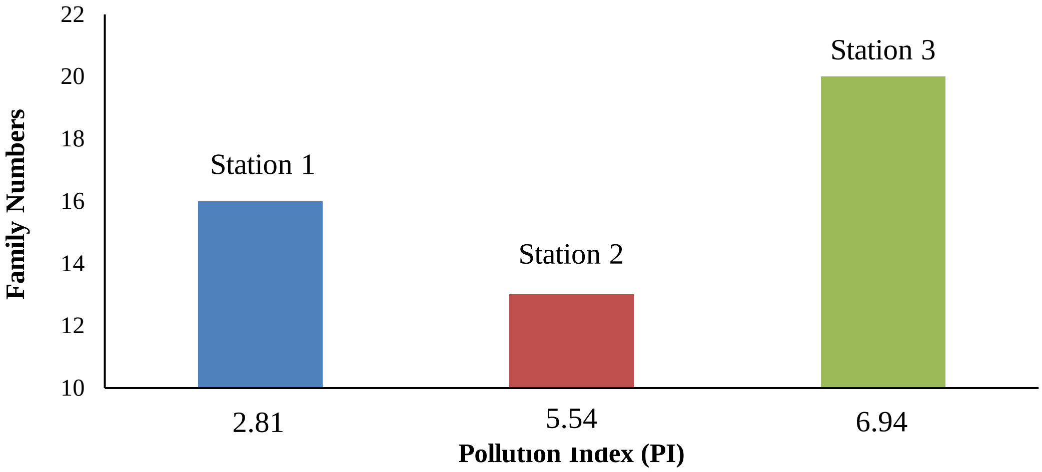


Figure 2. Phytoplankton composition determined by the sequence reads obtained from each station.

3.1.c. Correlation between Water Quality and Phytoplankton Composition

Based on the PI, the values range from 2.81 to 6.94, so the pollution level of the Ciliwung River is classified as lightly to moderately polluted condition. Pollution levels increase towards the downstream of the river (Figure). With increasing pollution levels, phytoplankton families decreased from Station 1 to Station 2. On the other hand, Station 3 shows anomalous conditions with high family diversity and declining water quality. The p-value of 0.731306 suggests that there is no significant relationship between pollution levels (x) and family numbers (y) (Figure). However, this result may be influenced by this study's very limited sample size, which includes only 3 stations.



	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	12.84247	8.202186	1.565737	0.361838	-91.3762	117.0611
PI	0.684932	1.525289	0.44905	**0.731306**	-18.6957	20.06557

Figure 3. Statistical analysis of PI and phytoplankton family numbers with low P-value, indicating the low relationship between two parameters.

3.2. Discussion

3.2.a. Water Quality

Heavy water discharge in upstream areas can trigger high levels of sedimentation which high TSS indicates [13]. The relationship between TSS and TP is always consistent in various hydrological conditions, is positively correlated, and is strongly influenced by turbidity [14]. Meanwhile, DO values vary at each station and are all below the quality standard. High concentrations of pollutants such as BOD and COD can reduce DO concentrations in waters because they require a lot of oxygen to break down pollutants.

Various studies link water quality to the abundance of phytoplankton. In the dry season, phosphate and nitrate concentrations are higher due to the accumulation of nutrients from sediments and riparian areas and high solar intensity. This has an impact on the high abundance and biomass production of phytoplankton, especially in tropical rivers [15]. Phytoplankton was positively correlated with high organic matter such as N-NH4^{+}, P-PO4 3^{-} , BOD, and TSS [16]. On the other hand, phytoplankton diversity has a positive correlation with DO, which is characterized by good water quality [17]. Anthropogenic activities related to decreasing water quality and changes in natural riparian areas can reduce phytoplankton diversity. Although phytoplankton diversity is associated with good water quality, there are several cases where diatoms can thrive in estuarine ecosystems. The presence of high nutrition means that several phytoplankton communities can grow well in this area. The higher salinity in the estuary compared to the freshwater ecosystem changes the composition of the area, which is characterized by a high abundance of species that are resistant to salinity [18].

3.2.b. Phytoplankton Composition

Seasonal changes could be the strong factor for phytoplanton composition. Research conducted in Japan shows that several diatoms (mainly the genus Cyclotella) bloom in summer [19]. The high composition of the Cyclotella is also often associated with the composition of nitrate and phosphate in water. This is highly correlated, considering that the TP concentration at the high station is the highest compared to the other two locations. *Neochlorosarcina* sp. is a certain operational taxonomic unit (OTU) that dominates in summer or autumn and peaks in July. Some species have good adaptability to various aquatic ecosystems [20]. Chlorellaceae is the family with the second largest composition with the dominance of the *Micractinium pusillum* species. The high presence of this species is often correlated with the nitrate and phosphate content in the water. *Micractinium pusillum* also has high productivity in nutrient removal and is recommended for wastewater treatment [21].

The high ammonia level at this station is thought to be the trigger for this dominance. Apart from Chlorellaceae, the Chlamydomonadaceae family with the species *Chlamydomonas* sp. became the OTU with the 2nd largest position. *Chlamydomonas* is often used in wastewater treatment because of its efficiency in nitrate, ammoniacal nitrogen, and phosphate removal [22]. Therefore, the existence of *Chlamydomonas* in water bodies is often correlated with high levels of nutrients such as nitrate and phosphate.

3.2.c. Correlation between Water Quality and Phytoplankton Composition

Pollution levels tend to escalate downstream in rivers due to various factors. Initially, as a river flows downstream, it gathers pollutants from multiple sources, including urban areas, industrial sites, and agricultural lands. These pollutants encompass various contaminants such as nutrients, heavy metals, pesticides, and organic compounds. Moreover, the dilution effect, which helps mitigate pollution in upstream regions through the influx of cleaner water from tributaries or groundwater, diminishes as the river progresses downstream. Consequently, downstream areas receive fewer dilution benefits, leading to higher concentrations of pollutants.

Additionally, the concentration of pollution is exacerbated by the presence of point sources like sewage treatment plants and industrial discharges, as well as non-point sources such as runoff from urban streets and agricultural fields [23]. Sediment transport further compounds the issue, as sediment particles can carry pollutants downstream, contributing to the accumulation of contaminants. Furthermore, downstream sections of rivers often exhibit reduced rates of natural degradation and dilution, as slower flow rates, diminished oxygen levels, and increased nutrient loads impede the river's ability to mitigate pollution naturally [24].

Pollution levels profoundly impact the abundance and distribution of phytoplankton in aquatic ecosystems. This influence stems from various pollution sources, such as agricultural runoff and industrial discharges, which introduce excess nutrients like nitrogen and phosphorus into the water, triggering eutrophication. Consequently, phytoplankton populations may experience rapid growth, leading to algal blooms. However, these blooms can become harmful as they deplete oxygen levels in the water, creating hypoxic conditions that can threaten marine life. Additionally, heavy metals, pesticides, and industrial chemicals can directly harm phytoplankton by inhibiting photosynthesis and disrupting cellular functions. Moreover, changes in water chemistry resulting from pollution, such as acidification and altered oxygen levels, can further impact phytoplankton abundance and diversity. These effects highlight the sensitivity of phytoplankton to environmental disturbances caused by pollution, emphasizing the importance of addressing pollution sources to maintain healthy aquatic ecosystems [25].

With such a small sample size, the statistical power of the analysis is likely too low to detect meaningful relationships, even if they exist. Therefore, for future research, collecting more data from additional stations is essential. A larger sample size would provide more reliable estimates, increase the statistical power of the analysis, and allow for more robust conclusions regarding the relationship between pollution levels and family numbers. This would help minimize the effect of random variability and increase confidence in the results.

When pollution levels rise, phytoplankton, tiny algae crucial for aquatic ecosystems, can decline for several reasons. First, excess nutrients from pollution can initially boost phytoplankton growth, causing harmful algal blooms. However, phytoplankton can struggle once these nutrients run out due to nutrient shortages. Second, pollutants like chemicals and heavy metals can directly harm phytoplankton, affecting their ability to grow and reproduce. Third, changes in water quality caused by pollution, like murky water from sediment runoff, can limit the sunlight phytoplankton need for photosynthesis. Finally, competition from pollution-tolerant species and shifts in predator-prey dynamics can further reduce phytoplankton populations [25–27].

Phytoplankton thrive in river mouths or estuaries primarily due to the abundant nutrient influx from rivers, fostering their growth. The convergence of freshwater from rivers and saline ocean water creates favorable conditions for phytoplankton, characterized by varying temperatures and ample nutrients. Additionally, the sediment transported by rivers contains essential nutrients required by phytoplankton. Moreover, due to reduced currents, the comparatively tranquil conditions in river mouths and estuaries provide an environment conducive for phytoplankton to flourish without the risk of displacement. Furthermore, the rich biodiversity in these areas relies on phytoplankton as a vital component of their diet, further facilitating their proliferation [28,29].

4. Conclusions

The phytoplankton composition varies at each station. At Station 1 (natural riparian), 16 families were identified, with Stephanodiscaceae and Bacillariaceae dominating in read sequence. The dominance of the Chlorosarcinaceae characterizes station 2 (concreted riparian). Station 3 (estuary) exhibits dominance of the Chlorellaceae. As pollution levels increase, there's a decrease in the number of phytoplankton families from Station 1 to Station 2. However, Station 3 presents anomalous conditions with high family diversity despite declining water quality.

Author Contributions

PAP: Conception and design of the study; Analysis, Drafting the manuscript; **MA**: Investigation, Analysis, Acquisition of data, Drafting the manuscript; **HE**: Drafting the manuscript, Critical review; **JZ**: Investigation, Drafting the manuscript; Interpretation of data.

Conflicts of interest

There are no conflicts to declare.

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References

1. Ashrafi, S.; Kerachian, R.; Pourmoghim, P.; Behboudian, M.; Motlaghzadeh, K. Evaluating and Improving the Sustainability of Ecosystem Services in River Basins under Climate Change. *Science of The Total Environment* **2022**, *806*, 1–13, doi:10.1016/j.scitotenv.2021.150702.
2. Cole, L.J.; Stockan, J.; Helliwell, R. Managing Riparian Buffer Strips to Optimise Ecosystem Services: A Review. *Agriculture, Ecosystems & Environment* **2020**, *296*, 1–12, doi:10.1016/j.agee.2020.106891.
3. Mishra, R.K. Fresh Water Availability and It's Global Challenge. *JMSR* **2023**, *2*, 1–3, doi:10.58489/2836-5933/004.
4. Nikolaus, R.; Schafft, M.; Maday, A.; Klefoth, T.; Wolter, C.; Arlinghaus, R. Status of Aquatic and Riparian Biodiversity in Artificial Lake Ecosystems with and without Management for Recreational Fisheries: Implications for Conservation. *Aquatic Conservation* **2021**, *31*, 153–172, doi:10.1002/aqc.3481.
5. Wei, W.; Gao, Y.; Huang, J.; Gao, J. Exploring the Effect of Basin Land Degradation on Lake and Reservoir Water Quality in China. *Journal of Cleaner Production* **2020**, *268*, 1–12, doi:10.1016/j.jclepro.2020.122249.
6. Aprilia, M.; Effendi, H.; Hariyadi, S.; Permatasari, P.A. Aquatic eDNA Metabarcoding Reveals Biodiversityand Plankton Composition in River Ecosystems. Pol. *J. Environ. Stud.* **2023**, *32*, 3491–3500, doi:10.15244/pjoes/163625.

7. Bera, B.; Shit, P.K.; Saha, S.; Bhattacharjee, S. Exploratory Analysis of Cooling Effect of Urban Wetlands on Kolkata Metropolitan City Region, Eastern India. *Current Research in Environmental Sustainability* **2021**, *3*, 1–13, doi:10.1016/j.crsust.2021.100066.
8. Kjelland, M.E.; Woodley, C.M.; Swannack, T.M.; Smith, D.L. A Review of the Potential Effects of Suspended Sediment on Fishes: Potential Dredging-Related Physiological, Behavioral, and Transgenerational Implications. *Environ Syst Decis* **2015**, *35*, 334–350, doi:10.1007/s10669-015-9557-2.
9. Effendi, H.; Aprilia, M.; Permatasari, P.A.; Amalo, L.F.; Hariyadi, S.; Wardiatno, Y. Environmental DNA Biomonitoring in Urban River Ecosystem: A Ciliwung River Case Study. *Pol. J. Environ. Stud.* **2024**, *33*, 3491–3500, doi: 10.15244/pjoes/184633.
10. Vander Zanden, M.J.; Vadeboncoeur, Y. Putting the Lake Back Together 20 Years Later: What in the Benthos Have We Learned about Habitat Linkages in Lakes?. *Inland Waters* **2020**, *10*, 305–321, doi:10.1080/20442041.2020.1712953.
11. Fashae, O,A.; Ayorinde, H.A.; Olusola, A.O.; Obateru, R.O. Landuse and Surface Water Quality in An Emerging Urban City. *Appl. Water Sci.* **2019**, *9*, 1–12, doi: 10.1007/s13201-019-0903-2.
12. Fan, J.; Wang, S.; Li, H.; Yan, Z.; Zhang, Y.; Zheng, X.; Wang, P. Modeling the Ecological Status Response of Rivers to Multiple Stressors Using Machine Learning: A Comparison of Environmental DNA Metabarcoding and Morphological Data. *Water Res.* **2020**, *183*, 1–12. doi: 10.1016/j.watres.2020.116004.
13. Namugize, J.N.; Jewitt, G.; Graham, M. Effects of Land Use and Land Cover Changes on Water Quality in the uMngeni River Catchment, South Africa. *Physics and Chemistry of the Earth* **2018**, *105*, 247–264, doi:10.1016/j.pce.2018.03.013.
14. Jones, A.S.; Stevens, D.K.; Horsburgh, J.S.; Mesner, N.O. Surrogate Measures for Providing High Frequency Estimates of Total Suspended Solids and Total Phosphorus Concentrations. *Journal of the American Water Resources Association* **2011**, *47*, 239–253, doi:10.1111/j.1752-1688.2010.00505.x.
15. Nwonumara, G.N. Water Quality and Phytoplankton as Indicators of Pollution in a Tropical River. In *P*roceedings of the Nigeria Chapter of Society for Conservation Biology (NSCB); University of Uyo: Akwa Ibom State, Nigeria, 2018; pp. 83–99.
16. Giao, N.T.; Nhien, H.T.H. Phytoplankton-Water Quality Relationship in Water Bodies in the Mekong Delta, Vietnam. *App. Envi. Res.* **2020**, *42*, 1–12, doi:10.35762/AER.2020.42.2.1.
17. Malik, D.S.; Sharma, M.K.; Sharma, A.K.; Kamboj, V.; Sharma, A.K. Anthropogenic Influence on Water Quality and Phytoplankton Diversity of Upper Ganga Basin: A Case Study of Ganga River and Its Major Tributaries. *World Water Policy* **2021**, *7*, 88–111, doi:10.1002/wwp2.12049.
18. Gong, W.; Hall, N.; Paerl, H.; Marchetti, A. Phytoplankton Composition in a Eutrophic Estuary: Comparison of Multiple Taxonomic Approaches and Influence of Environmental Factors. *Environmental Microbiology* **2020**, *22*, 4718–4731, doi:10.1111/1462-2920.15221.
19. Mikawa, M.; Sugimoto, K.; Amano, Y.; Machida, M.; Imazeki, F. Competitive Growth Characteristics between *Microcystis aeruginosa* and *Cyclotella* sp. Accompanying Changes in River Water Inflow and Their Simulation Model. *Phycological Research* **2016**, *64*, 123–132, doi:10.1111/pre.12129.
20. Shi, X.; Li, S.; Zhang, M.; Liu, C.; Wu, Q. Temperature Mainly Determines the Temporal Succession of the Photosynthetic Picoeukaryote Community in Lake Chaohu, a Highly Eutrophic Shallow Lake. *Science of The Total Environment* **2020**, *702*, 1–15, doi:10.1016/j.scitotenv.2019.134803.
21. Mehrabadi, A.; Farid, M.M.; Craggs, R. Potential of Five Different Isolated Colonial Algal Species for Wastewater Treatment and Biomass Energy Production. *Algal Research* **2017**, *21*, 1–8, doi:10.1016/j.algal.2016.11.002.
22. Kamyab, H.; Chelliapan, S.; Din, M.F.M.; Shahbazian-Yassar, R.; Rezania, S.; Khademi, T.; Kumar, A.; Azimi, M. Evaluation of Lemna Minor and Chlamydomonas to Treat Palm Oil Mill Effluent and Fertilizer Production. *Journal of Water Process Engineering* **2017**, *17*, 229–236, doi:10.1016/j.jwpe.2017.04.007.
23. Sarkar, A.; Pandey, P. River Water Quality Modelling Using Artificial Neural Network Technique. *Aquatic Procedia* **2015**, *4*, 1070–1077, doi:10.1016/j.aqpro.2015.02.135.
24. Costa, D.; Burlando, P.; Priadi, C. The Importance of Integrated Solutions to Flooding and Water Quality Problems in the Tropical Megacity of Jakarta. *Sustainable Cities and Society* **2016**, *20*, 199–209, doi:10.1016/j.scs.2015.09.009.

25. D’Costa, P.M.; D’Silva, M.S.; Naik, R.K. Impact of Pollution on Phytoplankton and Implications for Marine Econiches. In Marine Pollution and Microbial Remediation; Naik, M.M., Dubey, S.K., Eds.; Springer Singapore: Singapore, 2017; pp. 205–222 ISBN 978-981-10-1042-2.
26. Echeveste, P.; Dachs, J.; Berrojalbiz, N.; Agustí, S. Decrease in the Abundance and Viability of Oceanic Phytoplankton Due to Trace Levels of Complex Mixtures of Organic Pollutants. *Chemosphere* **2010**, *81*, 161–168, doi:10.1016/j.chemosphere.2010.06.072.
27. Häder, D.P.; Gao, K. Interactions of Anthropogenic Stress Factors on Marine Phytoplankton. *Front. Environ. Sci.* **2015**, *3*, 1–14, doi:10.3389/fenvs.2015.00014.
28. Rozirwan; Melki; Apri, R.; Nugroho, R.Y.; Iskandar, I. Assessment of Phytoplankton Community Structure in Musi Estuary, South Sumatra, Indonesia. *AACL Bioflux* **2021**, *14*, 1451–1463.
29. Zhong, Q.; Xue, B.; Noman, M.A.; Wei, Y.; Liu, H.; Liu, H.; Zheng, L.; Jing, H.; Sun, J. Effect of River Plume on Phytoplankton Community Structure in Zhujiang River Estuary. *J. Ocean. Limnol.* **2021**, *39*, 550–565, doi:10.1007/s00343-020-9213-7.