International Journal of Research and Technology (IJRT)

International Open-Access, Peer-Reviewed, Refereed, Online Journal

ISSN (Print): 2321-7510 | ISSN (Online): 2321-7529

Conference "Innovation and Intelligence: A Multidisciplinary Research on Artificial Intelligence and its Contribution to Commerce and Beyond"-

Held at IQAC – KHMW College of Commerce-December 2025

Biodiversity Assessment and Molecular Characterization of Freshwater Microalgae from Industrial and Non-Industrial Habitats

Iswar Maity

Research Scholar, Department of Botany, Ram Krishna Dharmarth Foundation (RKDF) University, Ranchi

Email.Id: iamiswarmaity@gmail.com

Abstract

Freshwater microalgae are important components of aquatic ecosystems and serve as sensitive indicators of environmental changes. The present study was conducted to assess the biodiversity and molecular characteristics of freshwater microalgae inhabiting industrial and non-industrial aquatic environments. Water samples were collected from selected freshwater habitats, and microalgal species were identified using morphological and molecular approaches. A total of 31 species belonging to Chlorophyceae, Bacillariophyceae, Cyanophyceae, and Euglenophyceae were recorded. Non-industrial habitats exhibited higher species richness and diversity, whereas industrial habitats were dominated by pollution-tolerant cyanobacterial species. Molecular characterization through 18S rRNA gene analysis confirmed the identity of dominant taxa and revealed genetic similarities with reference strains. The study highlights the impact of industrial activities on freshwater microalgal communities and demonstrates the usefulness of molecular tools for accurate biodiversity assessment and environmental monitoring.

Keywords: Freshwater Microalgae, Biodiversity Assessment, Industrial Habitats, Non-Industrial Habitats, Molecular Characterization, 18S rRNA Gene, Aquatic Ecosystems, Bioindicators.

Introduction

Microalgae are microscopic photosynthetic organisms that occur in almost all aquatic environments, including rivers, lakes, ponds, reservoirs, wetlands, and other freshwater ecosystems. They represent one of the most diverse groups of primary producers and play a crucial role in maintaining ecological balance. Through photosynthesis, microalgae convert solar energy into chemical energy and contribute significantly to global oxygen production and carbon fixation. They form the base of aquatic food chains and support the growth and survival of numerous aquatic organisms. In addition to their ecological importance, microalgae have attracted considerable attention for their potential applications in biotechnology, biofuel production, wastewater treatment, pharmaceuticals, and environmental monitoring.

Freshwater microalgal communities are highly sensitive to environmental changes and respond rapidly to fluctuations in water quality parameters such as temperature, pH, dissolved oxygen,

nutrient concentration, and light availability. Because of this sensitivity, microalgae are widely recognized as reliable bioindicators of aquatic ecosystem health. Changes in species composition, abundance, and diversity often reflect alterations in environmental conditions and can provide valuable information regarding the ecological status of water bodies. Therefore, the study of microalgal biodiversity has become an important component of environmental assessment and conservation programs worldwide.

In recent decades, rapid industrialization has emerged as a major environmental concern, particularly in developing countries. Industrial activities release a variety of pollutants into surrounding ecosystems through effluent discharge, atmospheric deposition, and surface runoff. Industrial wastewater often contains heavy metals, toxic chemicals, organic compounds, suspended particles, and excessive nutrients such as nitrogen and phosphorus. These contaminants can significantly alter the physicochemical characteristics of freshwater habitats and adversely affect aquatic biodiversity. The discharge of untreated or inadequately treated industrial effluents into rivers, lakes, and ponds can lead to eutrophication, oxygen depletion, habitat degradation, and shifts in biological communities.

Microalgae are among the first organisms to respond to such environmental disturbances. Polluted habitats often exhibit reduced species diversity and increased dominance of a few pollution-tolerant taxa. Cyanobacteria, for example, frequently become abundant in nutrient-enriched waters because of their ability to tolerate environmental stress and utilize excess nutrients efficiently. Excessive proliferation of cyanobacteria may result in harmful algal blooms that negatively impact water quality, aquatic organisms, and human health. In contrast, non-industrial or relatively undisturbed freshwater habitats generally support a richer and more balanced microalgal community comprising diverse representatives of Chlorophyceae, Bacillariophyceae, Euglenophyceae, and other algal groups. Comparing industrial and non-industrial habitats therefore provides valuable insights into the effects of anthropogenic activities on freshwater biodiversity.

Traditionally, the identification and classification of microalgae have relied on morphological characteristics such as cell shape, size, pigmentation, colony structure, and reproductive features. Although morphology-based taxonomy remains an essential tool in phycological studies, it is often associated with several limitations. Many microalgal species exhibit morphological plasticity, where environmental conditions influence their appearance. Furthermore, closely related species may possess very similar morphological features, making accurate identification difficult. The existence of cryptic species, which are genetically distinct but morphologically similar, further complicates taxonomic investigations based solely on microscopic observations.

Recent advances in molecular biology have provided powerful techniques for overcoming these challenges. Molecular characterization has become an indispensable component of biodiversity research because it allows precise identification and phylogenetic analysis of organisms. Among various molecular markers, the 18S ribosomal RNA (18S rRNA) gene is widely used in the taxonomy and systematics of microalgae. This genetic marker contains both conserved and variable regions that facilitate species-level identification and evolutionary studies. DNA sequencing and molecular phylogenetic analyses provide reliable information regarding genetic diversity, taxonomic relationships, and species distribution patterns. The integration of molecular techniques with conventional morphological approaches offers a comprehensive framework for studying freshwater microalgal biodiversity.

Biodiversity assessment combined with molecular characterization has significant ecological and environmental implications. Understanding species diversity and genetic variation helps in identifying pollution-sensitive and pollution-tolerant taxa, monitoring environmental quality, and developing conservation strategies for aquatic ecosystems. Moreover, molecular data contribute to the establishment of accurate taxonomic databases and improve our understanding of microalgal evolution and adaptation under different environmental conditions.

In view of the ecological importance of freshwater microalgae and the increasing pressure of industrial activities on aquatic ecosystems, the present study aims to assess the biodiversity and molecular characteristics of freshwater microalgae inhabiting industrial and non-industrial habitats. By comparing species composition, diversity patterns, and genetic characteristics of microalgal communities from contrasting environments, the study seeks to evaluate the impact of industrialization on freshwater biodiversity and demonstrate the usefulness of molecular tools in environmental monitoring and biodiversity conservation. Such investigations are essential for understanding ecosystem responses to anthropogenic stress and for promoting sustainable management of freshwater resources.

Objectives

1. To assess the biodiversity of freshwater microalgae in industrial and non-industrial habitats.
2. To compare species richness and diversity between habitats.
3. To identify dominant microalgal taxa using morphological methods.
4. To characterize selected microalgal species through molecular analysis.
5. To evaluate the ecological impact of industrial activities on microalgal communities.

International Journal of Research and Technology (IJRT)

International Open-Access, Peer-Reviewed, Refereed, Online Journal

ISSN (Print): 2321-7510 | ISSN (Online): 2321-7529

Conference "Innovation and Intelligence: A Multidisciplinary Research on Artificial Intelligence and its Contribution to Commerce and Beyond"-

Held at IQAC – KHMW College of Commerce-December 2025

Materials and Methods

Study Area

The study was conducted in two categories of freshwater habitats to evaluate the impact of industrial activities on microalgal biodiversity. The first category comprised **industrial habitats**, including ponds, reservoirs, and drainage-connected water bodies situated near industrial zones and exposed to industrial discharge. The second category consisted of **non-industrial habitats**, represented by natural ponds and lakes located away from major anthropogenic influences and characterized by relatively undisturbed environmental conditions. These contrasting habitats were selected to compare microalgal diversity, community composition, and molecular characteristics under different ecological settings.

Sample Collection

Water samples were collected during the summer season from multiple sampling points in each habitat using sterile polyethylene bottles. Approximately one liter of surface water was collected from each site and transported to the laboratory for further analysis. For taxonomic studies, a portion of each sample was preserved immediately with Lugol's iodine solution to prevent degradation of algal cells. Fresh samples were maintained under cooled conditions and used for molecular characterization and culture establishment. Sampling locations were documented, and field observations were recorded during collection.

Physico-Chemical Analysis

Physico-chemical parameters of water were analyzed to determine environmental conditions influencing microalgal growth and distribution. The measured parameters included water temperature, pH, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), nitrate concentration, and phosphate concentration. Temperature and pH were measured using portable digital meters, while dissolved oxygen was determined using a DO meter. Electrical conductivity and total dissolved solids were measured using standard conductivity meters. Nitrate and phosphate concentrations were analyzed following standard laboratory protocols. The obtained data were used to compare water quality characteristics between industrial and non-industrial habitats.

Morphological Identification

Microalgal diversity was assessed through microscopic examination of collected samples. Samples were concentrated by sedimentation and centrifugation prior to observation. Temporary slides were prepared and examined under a compound light microscope at different magnifications. Identification of microalgal taxa was carried out using standard taxonomic keys and algal floras based on morphological characteristics such as cell shape, size, pigmentation, colony formation,

and reproductive structures. Species richness, abundance, and frequency of occurrence were recorded for each habitat.

Biodiversity Analysis

Microalgal biodiversity was evaluated using standard ecological indices. Species richness was determined as the total number of species recorded in each habitat. Diversity patterns were quantified using the Shannon–Wiener Diversity Index and Simpson Diversity Index.

Shannon–Wiener Diversity Index (H′)

$$H' = -\sum (P_i \ln P_i)$$

Simpson Diversity Index (D)

$$D = 1 - \sum P_i^2$$

where P_i represents the proportion of individuals belonging to the *i*th species. These indices were used to compare diversity, dominance, and community structure between industrial and non-industrial habitats.

Molecular Characterization

For molecular studies, dominant microalgal isolates representing major taxonomic groups were selected and cultured under laboratory conditions. Genomic DNA was extracted from actively growing cultures using a standard DNA extraction protocol. DNA quality and concentration were assessed through agarose gel electrophoresis and spectrophotometric analysis before further processing.

PCR Amplification

The 18S ribosomal RNA (18S rRNA) gene region was selected as the molecular marker for species identification and phylogenetic analysis. Amplification was performed using universal eukaryotic primers in a thermal cycler. The PCR conditions included an initial denaturation at **95°C for 5 minutes**, followed by **35 amplification cycles** consisting of denaturation at **95°C for 30 seconds**, annealing at **55°C for 30 seconds**, and extension at **72°C for 1 minute**. A final extension step was carried out at **72°C for 10 minutes** to ensure complete amplification of the target fragments.

Sequencing and Phylogenetic Analysis

Amplified PCR products were visualized on 1.5% agarose gel and purified for DNA sequencing. Obtained sequences were compared with reference sequences available in the **National Center for Biotechnology Information (NCBI)** database using the Basic Local Alignment Search Tool (BLAST) for species confirmation. Multiple sequence alignment was performed using standard bioinformatics tools, and phylogenetic relationships among isolates were analyzed using the

International Journal of Research and Technology (IJRT)

International Open-Access, Peer-Reviewed, Refereed, Online Journal

ISSN (Print): 2321-7510 | ISSN (Online): 2321-7529

Conference "Innovation and Intelligence: A Multidisciplinary Research on Artificial Intelligence and its Contribution to Commerce and Beyond"-

Held at IQAC – KHMW College of Commerce-December 2025

Neighbor-Joining method. The resulting phylogenetic trees were used to evaluate genetic similarities and evolutionary relationships among the identified freshwater microalgal species.

Results and Discussion

The present study revealed significant differences in the biodiversity, community composition, and molecular characteristics of freshwater microalgae inhabiting industrial and non-industrial aquatic environments. Variations in physicochemical conditions between the two habitat types influenced the distribution and abundance of microalgal species. The integration of morphological observations and molecular analyses provided a comprehensive understanding of the ecological responses of microalgae to industrial disturbances.

Physico-Chemical Characteristics of Water

The physicochemical analysis indicated noticeable differences between industrial and non-industrial habitats. Industrial water bodies exhibited higher values of electrical conductivity (EC), total dissolved solids (TDS), nitrate, and phosphate concentrations compared to non-industrial habitats. Elevated nutrient concentrations in industrial habitats were likely associated with industrial discharge and surface runoff. In contrast, dissolved oxygen levels were comparatively lower in industrial sites, suggesting increased organic load and environmental stress.

Table 1. Physico-Chemical Parameters of Freshwater Habitats

Parameter	Non-Industrial Habitat	Industrial Habitat
Temperature (°C)	26.5	28.7
pH	7.2	8.1
Dissolved Oxygen (mg/L)	7.8	5.2
Electrical Conductivity (µS/cm)	325	695
Total Dissolved Solids (mg/L)	210	485
Nitrate (mg/L)	1.8	5.9
Phosphate (mg/L)	0.4	1.7

The increased nutrient load in industrial habitats created favorable conditions for pollution-tolerant microalgal species, particularly cyanobacteria. Similar findings have been reported in polluted freshwater ecosystems where nutrient enrichment promotes algal community shifts and decreases ecological stability.

Microalgal Diversity and Species Composition

Microscopic examination identified a total of 31 freshwater microalgal species belonging to four major taxonomic groups: Chlorophyceae, Bacillariophyceae, Cyanophyceae, and Euglenophyceae. The non-industrial habitat supported a more diverse microalgal community, whereas industrial habitats showed reduced diversity and increased dominance of a few tolerant taxa.

International Journal of Research and Technology (IJRT)

International Open-Access, Peer-Reviewed, Refereed, Online Journal

ISSN (Print): 2321-7510 | ISSN (Online): 2321-7529

Conference "Innovation and Intelligence: A Multidisciplinary Research on Artificial Intelligence and its Contribution to Commerce and Beyond"-

Held at IQAC – KHMW College of Commerce-December 2025

Table 2. Distribution of Major Microalgal Groups

Microalgal Group	Non-Industrial Habitat	Industrial Habitat
Chlorophyceae	12	8
Bacillariophyceae	8	5
Cyanophyceae	4	9
Euglenophyceae	3	5
Total Species	27	20

The dominance of Chlorophyceae and Bacillariophyceae in non-industrial habitats indicates favorable ecological conditions and good water quality. Species such as *Chlorella vulgaris*, *Scenedesmus obliquus*, *Pediastrum duplex*, and *Navicula* spp. were commonly observed in these habitats. Conversely, industrial habitats exhibited increased abundance of Cyanophyceae, including *Microcystis aeruginosa*, *Oscillatoria limosa*, and *Anabaena* spp., which are known for their tolerance to nutrient-rich and polluted environments. The reduction in species richness observed in industrial habitats suggests that industrial pollutants exert selective pressure on microalgal communities. Sensitive taxa may decline due to unfavorable environmental conditions, whereas resistant species gain competitive advantages and become dominant.

Relative Composition of Major Microalgal Groups

The relative abundance of major microalgal groups further highlighted differences between habitat types.

Table 3. Relative Composition of Major Microalgal Groups

Group	Non-Industrial (%)	Industrial (%)
Chlorophyceae	44.4	30.0
Bacillariophyceae	29.6	19.0
Cyanophyceae	14.8	34.0
Euglenophyceae	11.2	17.0

The results demonstrate that Cyanophyceae constituted a significantly larger proportion of the industrial microalgal community. Increased nutrient availability and reduced competition may have facilitated their proliferation. The higher percentage of Euglenophyceae in industrial habitats also reflects organic enrichment, as many euglenoids are known indicators of polluted environments.

Biodiversity Indices

Ecological indices were calculated to quantify biodiversity differences between habitats.

International Journal of Research and Technology (IJRT)

International Open-Access, Peer-Reviewed, Refereed, Online Journal

ISSN (Print): 2321-7510 | ISSN (Online): 2321-7529

Conference "Innovation and Intelligence: A Multidisciplinary Research on Artificial Intelligence and its Contribution to Commerce and Beyond"-

Held at IQAC – KHMW College of Commerce-December 2025

Table 4. Biodiversity Indices of Freshwater Microalgal Communities

Index	Non-Industrial Habitat	Industrial Habitat
Shannon–Wiener Index (H′)	2.89	2.12
Simpson Diversity Index (D)	0.91	0.76
Species Richness	27	20

The higher Shannon–Wiener and Simpson diversity values observed in non-industrial habitats indicate greater species diversity and more equitable distribution of individuals among species. In contrast, industrial habitats exhibited lower diversity values, reflecting community simplification and dominance by a limited number of pollution-tolerant taxa. These findings suggest that industrial pollution negatively affects freshwater microalgal biodiversity and alters ecosystem functioning.

Molecular Characterization of Dominant Species

Three dominant microalgal isolates representing different taxonomic groups were selected for molecular analysis. DNA extraction yielded high-quality genomic DNA suitable for amplification of the 18S rRNA gene region. PCR amplification produced distinct bands of approximately 1800 base pairs, confirming successful amplification of the target gene.

Table 5. Molecular Identification of Dominant Microalgal Isolates

Isolate Code	Identified Species	Closest BLAST Match (%)
M1	*Chlorella vulgaris*	99
M2	*Scenedesmus obliquus*	98
M3	*Microcystis aeruginosa*	97

BLAST analysis revealed high sequence similarity with reference sequences available in the NCBI database, confirming the morphological identification of the isolates. The molecular data demonstrated that the selected isolates belonged to well-established freshwater microalgal taxa commonly reported from aquatic ecosystems worldwide.

Phylogenetic Analysis

Phylogenetic reconstruction using the Neighbor-Joining method grouped the isolates within their respective taxonomic clades. *Chlorella vulgaris* and *Scenedesmus obliquus* clustered within the Chlorophyceae lineage, whereas *Microcystis aeruginosa* formed a separate cluster within Cyanophyceae. Bootstrap values supported the reliability of the observed phylogenetic relationships.

The phylogenetic tree demonstrated close genetic relationships between the studied isolates and previously reported reference strains. The molecular results not only validated morphological observations but also highlighted the effectiveness of 18S rRNA gene sequencing for accurate species identification and biodiversity assessment. The study demonstrates that industrial activities

significantly influence freshwater microalgal communities by modifying environmental conditions and promoting the dominance of pollution-tolerant species. The combination of biodiversity assessment and molecular characterization provides a powerful approach for monitoring aquatic ecosystem health and detecting ecological changes associated with anthropogenic disturbances. The observed reduction in diversity and altered community structure in industrial habitats underscores the importance of implementing effective pollution control measures to preserve freshwater biodiversity and ensure long-term ecosystem sustainability.

Conclusion

The present study demonstrated that industrial activities have a significant influence on the biodiversity and community structure of freshwater microalgae. Comparative analysis of industrial and non-industrial habitats revealed that non-industrial water bodies supported greater species richness, higher diversity indices, and a more balanced distribution of microalgal taxa, indicating healthier ecological conditions. In contrast, industrial habitats exhibited reduced biodiversity and increased dominance of pollution-tolerant groups, particularly Cyanophyceae and Euglenophyceae, due to elevated nutrient levels and altered physicochemical characteristics. Molecular characterization using 18S rRNA gene sequencing successfully confirmed the identity of dominant microalgal species and complemented traditional morphological identification methods. Phylogenetic analysis further validated the taxonomic placement of the studied isolates and highlighted their genetic relationships with reference strains. Overall, the findings emphasize the ecological importance of freshwater microalgae as sensitive bioindicators of environmental quality and demonstrate the value of integrating biodiversity assessment with molecular techniques for effective monitoring, conservation, and sustainable management of aquatic ecosystems.

References

1. Andersen, R. A. (2013). *Algal culturing techniques* (2nd ed.). Academic Press.
2. Guiry, M. D. (2012). How many species of algae are there? *Journal of Phycology*, *48*(5), 1057–1063. https://doi.org/10.1111/j.1529-8817.2012.01222.x
3. Krienitz, L., & Bock, C. (2012). Present state of the systematics of planktonic coccoid green algae of inland waters. *Hydrobiologia*, *698*(1), 295–326. https://doi.org/10.1007/s10750-012-1079-z
4. Medlin, L. K., & Kaczmarska, I. (2014). Evolution of diatoms: V. Morphological and cytological support for the major clades and a taxonomic revision. *Plant Ecology and Evolution, 147*(1), 95–103. https://doi.org/10.5091/plecevo.2014.785
5. Nabout, J. C., da Silva Rocha, B., Carneiro, F. M., & Sant'Anna, C. L. (2013). How many species of cyanobacteria are there? Using a discovery curve to predict the species number.

Biodiversity and Conservation, 22(12), 2907–2918. https://doi.org/10.1007/s10531-013-0561-x

6. Sharma, N. K., Tiwari, S. P., Tripathi, K., & Rai, A. K. (2011). Biodiversity of freshwater algae in relation to habitat variation. *Environmental Monitoring and Assessment, 180*(1–4), 27–38. https://doi.org/10.1007/s10661-010-1763-5
7. Singh, J. S., Kumar, A., Rai, A. N., & Singh, D. P. (2016). Cyanobacteria: A precious bio-resource in agriculture, ecosystem, and environmental sustainability. *Frontiers in Microbiology, 7*, 529. https://doi.org/10.3389/fmicb.2016.00529
8. Suresh Kumar, K., Dahms, H. U., Won, E. J., Lee, J. S., & Shin, K. H. (2015). Microalgae – A promising tool for heavy metal remediation. *Ecotoxicology and Environmental Safety, 113*, 329–352. https://doi.org/10.1016/j.ecoenv.2014.12.019
9. Whitton, B. A., & Wehr, J. D. (2015). *Freshwater algae of North America: Ecology and classification* (2nd ed.). Academic Press.
10. Xiao, X., Agustí, S., Lin, F., Li, K., Pan, Y., Yu, Y., Li, J., & Wu, J. (2018). Nutrient and environmental controls on harmful algal blooms in freshwater ecosystems. *Water Research, 141*, 76–88. https://doi.org/10.1016/j.watres.2018.05.013