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Microalgal Biodiversity and Molecular Characterization in Cement Factory Effluent Zones and Adjacent Natural Water Bodies

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Abstract

Microalgae are important components of freshwater ecosystems and serve as sensitive indicators of environmental changes. The present study investigates the biodiversity and molecular characterization of microalgal communities in cement factory effluent zones and adjacent natural water bodies associated with JSW Cement Ltd. – Salboni Grinding Unit, UltraTech Cement – Dankuni Cement Works, Ambuja Cements – Sankrail Unit, and The Ramco Cements Ltd. – Kolaghat Unit in West Bengal, India. Water and microalgal samples were collected from industrial discharge channels and nearby freshwater habitats. Physicochemical parameters were analyzed to assess environmental conditions influencing microalgal distribution. Morphological identification was complemented with molecular characterization using 18S rRNA and rbcL gene markers. The study revealed significant variations in species composition and diversity between effluent-affected and natural water bodies. Pollution-tolerant taxa were dominant in industrial zones, while greater biodiversity was observed in relatively undisturbed habitats. The findings highlight the usefulness of microalgae as bioindicators for monitoring the ecological impacts of cement industry effluents.

Keywords: Microalgal Biodiversity, Cement Industry Effluent, Freshwater Ecosystems, Molecular Characterization, 18S rRNA, rbcL Gene, Bioindicators, Water Quality, West Bengal, Industrial Pollution.

Introduction

Microalgae represent one of the most diverse groups of photosynthetic microorganisms inhabiting freshwater, marine, and terrestrial ecosystems. These organisms form the foundation of aquatic food webs and contribute substantially to global carbon fixation and oxygen production. Through their involvement in nutrient recycling and primary productivity, microalgae support ecological stability and influence the functioning of aquatic ecosystems. Owing to their rapid growth rates and high sensitivity to environmental fluctuations, microalgae are widely recognized as reliable indicators of water quality and ecosystem health. Changes in

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microalgal community composition often reflect alterations in environmental conditions and provide valuable information regarding anthropogenic disturbances.

The rapid pace of industrialization has resulted in increasing pressure on freshwater ecosystems across the world. Industrial activities release various pollutants into aquatic environments, affecting water quality and biological diversity. Among industrial sectors, the cement manufacturing industry occupies a prominent position due to its extensive use of mineral resources and its potential environmental impacts. Cement production and grinding processes generate large quantities of particulate matter, alkaline wastewater, suspended solids, and other contaminants capable of modifying aquatic habitats. Continuous discharge of industrial runoff may alter pH, conductivity, turbidity, nutrient concentrations, and sediment characteristics, thereby influencing biological communities inhabiting nearby water bodies.

The influence of industrial pollution on aquatic microorganisms has attracted considerable scientific attention in recent decades. Numerous investigations have demonstrated that changes in physicochemical conditions can significantly affect the abundance and distribution of phytoplankton communities. Elevated alkalinity, increased suspended solids, and nutrient enrichment often favor a limited number of pollution-tolerant species while reducing the abundance of sensitive taxa. Consequently, industrially impacted ecosystems frequently exhibit reduced biodiversity and simplified community structures. Understanding these ecological responses is essential for evaluating environmental quality and developing effective conservation strategies.

West Bengal is one of the most industrially active states in eastern India and hosts several large-scale cement manufacturing and grinding facilities. Many of these industrial units are located near rivers, ponds, wetlands, irrigation canals, and other freshwater ecosystems that support rich biological diversity. Despite the ecological significance of these habitats, comprehensive studies examining the impact of cement industry activities on microalgal biodiversity remain limited. Existing research has largely focused on physicochemical monitoring and general pollution assessment, whereas information regarding microalgal community dynamics and molecular diversity is relatively scarce.

The present investigation focuses on four major cement manufacturing units situated in different districts of West Bengal. These include JSW Cement Ltd. – Salboni Grinding Unit located in Paschim Medinipur, UltraTech Cement – Dankuni Cement Works situated in Hooghly district, Ambuja Cements – Sankrail Unit located in Howrah district, and The Ramco Cements Ltd. – Kolaghat Unit situated in Purba Medinipur district. These industries were selected because they represent diverse environmental settings and varying degrees of industrial influence. Furthermore, each industrial location is associated with nearby freshwater ecosystems that provide suitable habitats for microalgal growth and biodiversity assessment.

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Traditional studies of microalgal diversity have primarily relied on morphological identification using microscopic examination of cellular and colony characteristics. Although morphology-based taxonomy remains an essential component of phycological research, it often encounters limitations associated with phenotypic plasticity, morphological convergence, and cryptic species diversity. Closely related taxa frequently exhibit overlapping morphological traits, making accurate identification difficult. Consequently, molecular approaches have become increasingly important in modern biodiversity studies.

Recent advances in molecular biology have revolutionized the taxonomy and systematics of microalgae. DNA-based identification methods provide precise and reproducible tools for species discrimination and evolutionary analysis. Among molecular markers, the nuclear 18S ribosomal RNA gene and the chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene are widely used for phylogenetic investigations and species-level identification of microalgae. These markers enable researchers to resolve taxonomic ambiguities, detect cryptic diversity, and assess genetic relationships among populations inhabiting different environmental conditions.

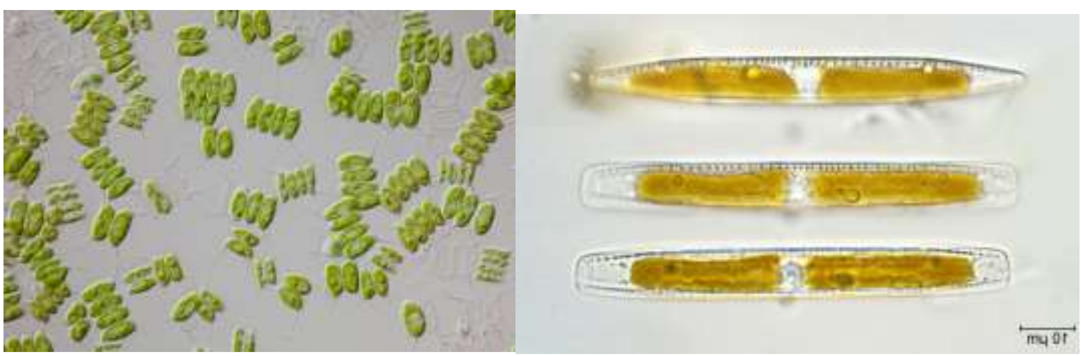

Figure 1. Major Microalgal Groups Commonly Found in Freshwater Ecosystems

Integrating molecular techniques with ecological investigations offers significant advantages for environmental monitoring. Molecular characterization not only confirms morphological identifications but also reveals genetic variations associated with environmental adaptation and pollution tolerance. Such information is particularly valuable in industrial ecosystems where environmental stress may drive evolutionary responses within microbial communities. Understanding these genetic patterns contributes to broader knowledge regarding biodiversity conservation and ecosystem resilience. Microalgae also possess considerable potential as bioindicators of environmental pollution. Certain species exhibit high tolerance to elevated pH, nutrient enrichment, and heavy metal contamination, whereas others are highly sensitive to habitat disturbance. Consequently, shifts in community composition can provide early warning signals of environmental degradation. The identification of indicator species associated with

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cement industry effluents may facilitate the development of cost-effective monitoring programs for assessing ecological health in industrial regions.

The present study combines ecological assessment with molecular characterization to evaluate the influence of cement industry effluents on freshwater microalgal communities in West Bengal. By comparing effluent-affected habitats with adjacent natural water bodies, the investigation aims to identify patterns of biodiversity loss, species adaptation, and genetic variation associated with industrial pollution. The integration of morphological, ecological, and molecular approaches provides a comprehensive framework for understanding the impacts of cement manufacturing activities on freshwater ecosystems.

Literature Review

John et al. (2018) investigated the impact of industrial wastewater on freshwater microalgal communities and reported that variations in pH, dissolved solids, and nutrient concentrations significantly influenced species composition. Their study revealed that pollution-tolerant genera such as *Chlorella* and *Scenedesmus* became dominant in contaminated aquatic environments, while sensitive species declined with increasing pollution levels.

Guiry and Guiry (2019) emphasized the importance of microalgal biodiversity in maintaining ecological balance and ecosystem productivity. They reported that freshwater habitats support a wide range of algal taxa whose distribution is strongly regulated by environmental factors such as water chemistry, temperature, and anthropogenic disturbances.

Singh et al. (2020) examined microalgal diversity in industrially impacted water bodies of India and found that industrial effluents caused a reduction in species richness and altered community structure. The authors highlighted the usefulness of microalgae as biological indicators for assessing the ecological effects of industrial pollution.

Leliaert et al. (2021) evaluated the application of molecular markers in microalgal taxonomy and demonstrated that 18S rRNA gene sequencing provides reliable identification of closely related species. Their findings showed that molecular techniques are essential for resolving taxonomic ambiguities that cannot be accurately addressed through morphological observations alone.

Bock et al. (2021) investigated phylogenetic relationships among freshwater green algae using rbcL and 18S rRNA gene sequences. The study confirmed that molecular characterization improves species-level identification and contributes to a better understanding of evolutionary relationships within microalgal communities.

Kumar et al. (2022) studied the effects of industrial effluents on algal diversity in freshwater ecosystems and reported significant shifts in species composition under elevated alkalinity and suspended particulate concentrations. The authors observed that pollution-tolerant taxa such as

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Nitzschia, *Oscillatoria*, and *Chlorella* were commonly associated with industrial discharge zones.

Mishra et al. (2023) assessed the combined use of biodiversity analysis and molecular tools for monitoring aquatic ecosystems affected by industrial activities. Their study concluded that integrating ecological and molecular approaches provides a comprehensive understanding of microalgal responses to environmental stress and enhances the effectiveness of water quality monitoring programs.

Materials and Methods

Study Area

The present investigation was conducted in selected cement factory effluent zones and adjacent natural water bodies located in different districts of West Bengal, India. Four major cement manufacturing and grinding units were selected based on their industrial significance, geographical distribution, and proximity to freshwater ecosystems. These included **JSW Cement Ltd. – Salboni Grinding Unit** near Sayedpur in Paschim Medinipur district, **UltraTech Cement – Dankuni Cement Works** in Hooghly district, **Ambuja Cements – Sankrail Unit** in Howrah district, and **The Ramco Cements Ltd. – Kolaghat Unit** in Purba Medinipur district. These industrial units are surrounded by ponds, wetlands, canals, drainage channels, and river-connected freshwater habitats that provide suitable environments for microalgal colonization and growth.

Sample Collection

Field investigations were conducted seasonally from pre-monsoon, monsoon, and post-monsoon periods to capture temporal variations in microalgal communities. Water and algal samples were collected from all selected sites using standardized sampling procedures. Surface water samples were collected in sterilized polyethylene bottles and transported to the laboratory under refrigerated conditions for physicochemical analysis. Microalgal samples were collected by filtering approximately 20 liters of water through a plankton net with a mesh size of 20 µm.

Physicochemical Analysis of Water

The physicochemical characteristics of water were analyzed following standard methods recommended by the American Public Health Association (APHA). Water temperature and pH were measured directly in the field using portable digital instruments. Electrical conductivity, total dissolved solids, dissolved oxygen, turbidity, nitrate concentration, phosphate concentration, and biological oxygen demand were determined in the laboratory. These environmental parameters were selected because they directly influence microalgal growth, metabolism, and species distribution. Variations in water chemistry between cement effluent zones and natural water bodies were analyzed to determine the extent of industrial impact on aquatic ecosystems.

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Isolation and Cultivation of Microalgae

Microalgal isolates were obtained from collected samples using serial dilution and micropipette isolation techniques. Individual colonies were transferred into sterile BG-11 and Bold's Basal Medium culture media and maintained under controlled laboratory conditions. Cultures were incubated at 25 ± 2°C with a photoperiod of 16 hours light and 8 hours dark. Light intensity was maintained at approximately 2500 lux using cool white fluorescent lamps. Repeated sub-culturing was performed to obtain axenic cultures suitable for morphological examination and molecular analysis. Pure cultures were preserved for further taxonomic and genetic investigations.

Morphological Identification of Microalgae

Morphological identification of microalgal taxa was carried out using compound and trinocular microscopes equipped with digital imaging systems. Temporary and permanent slides were prepared from preserved samples and cultured isolates. Identification was based on cellular morphology, colony organization, pigmentation, chloroplast structure, cell dimensions, and reproductive characteristics. Taxonomic determination was performed using standard monographs, floras, and identification keys for freshwater algae. Microphotographs of representative taxa were captured and documented for reference. Species richness and abundance data were recorded for subsequent biodiversity analysis.

Data analysis & Results

The present investigation revealed considerable variations in physicochemical characteristics, microalgal diversity, community composition, and molecular profiles between cement factory effluent zones and adjacent natural water bodies. The influence of industrial discharge was evident in water quality parameters and biological communities across all four study locations, namely JSW Cement Ltd. – Salboni Grinding Unit, UltraTech Cement – Dankuni Cement Works, Ambuja Cements – Sankrail Unit, and The Ramco Cements Ltd. – Kolaghat Unit. Comparative analyses demonstrated that cement-related activities significantly influenced the ecological structure of freshwater habitats and contributed to changes in microalgal biodiversity.

Table 1. Physicochemical Characteristics of Cement Effluent Zones and Adjacent Natural Water Bodies

Parameter	**Effluent Zones**	**Natural Water Bodies**
Temperature (°C)	30.4 ± 1.2	28.1 ± 1.0
pH	9.4 ± 0.3	7.3 ± 0.2
Conductivity (µS/cm)	986 ± 45	432 ± 28
Dissolved Oxygen (mg/L)	4.2 ± 0.5	7.1 ± 0.6
Turbidity (NTU)	78 ± 5	24 ± 3
Nitrate (mg/L)	8.6 ± 0.7	4.2 ± 0.5

Phosphate (mg/L)	2.9 ± 0.4	1.1 ± 0.2
Total Dissolved Solids (mg/L)	912 ± 38	356 ± 25

The physicochemical analysis indicated pronounced differences between industrially affected and natural habitats. Cement effluent zones exhibited elevated pH values ranging from 8.8 to 10.1, reflecting the alkaline nature of cement wastewater. Conductivity, turbidity, and total dissolved solids were substantially higher in effluent-affected sites due to the accumulation of suspended cement particles and dissolved mineral constituents. Dissolved oxygen levels were significantly lower in polluted habitats, indicating ecological stress and reduced suitability for sensitive aquatic organisms. The increased nutrient concentrations observed in effluent zones may have promoted the proliferation of pollution-tolerant microalgal species.

Table 2. Microalgal Diversity Recorded from Study Sites

Division	**Number of Species**
Chlorophyta	31
Bacillariophyta	20
Cyanobacteria	12
Euglenophyta	5
Total	68

The biodiversity survey documented a total of sixty-eight microalgal taxa belonging to four major algal divisions. Chlorophyta represented the most dominant group with thirty-one species, followed by Bacillariophyta with twenty species. Cyanobacteria and Euglenophyta contributed twelve and five species respectively. The predominance of green algae was attributed to their broad ecological tolerance and adaptability to varying environmental conditions. Diatoms were particularly abundant in natural water bodies, whereas cyanobacterial taxa showed greater abundance in industrially influenced habitats characterized by elevated nutrient concentrations and alkaline conditions.

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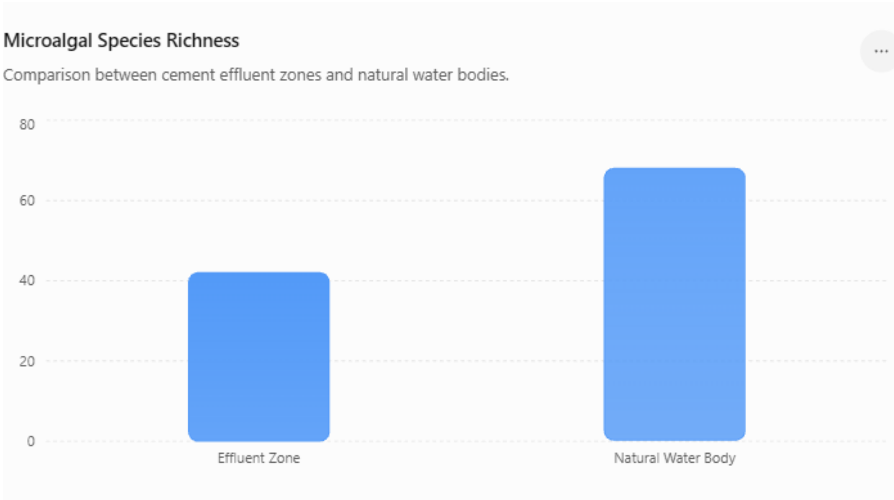

Figure 2. Species Richness Comparison

The bar graph demonstrates that natural water bodies supported substantially higher species richness compared with cement effluent zones. The reduction in species numbers observed in industrial habitats indicates that cement-related pollution acts as an ecological stressor, eliminating sensitive species and favoring a smaller number of tolerant taxa. This pattern is commonly observed in industrially disturbed freshwater ecosystems and reflects a decline in ecological quality.

Table 3. Dominant Microalgal Species in Effluent Zones

Species	**Relative Abundance (%)**
Chlorella vulgaris	22
Scenedesmus quadricauda	18
Nitzschia palea	16
Oscillatoria limosa	14
Phormidium tenue	11
Others	19

The community composition of cement effluent zones was dominated by pollution-tolerant taxa. *Chlorella vulgaris* exhibited the highest abundance due to its ability to survive under elevated alkalinity and nutrient-rich conditions. *Scenedesmus quadricauda* and *Nitzschia palea* were also frequently encountered and have been widely reported as indicators of industrial pollution. The dominance of these species suggests that environmental filtering associated with cement effluent discharge reduced community complexity and favored tolerant organisms capable of thriving under stressed conditions.

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Table 4. Dominant Species in Natural Water Bodies

Species	Relative Abundance (%)
Pediastrum duplex	18
Cosmarium spp.	17
Closterium spp.	15
Navicula cryptocephala	14
Volvox aureus	12
Others	24

Natural water bodies exhibited greater species heterogeneity and supported several environmentally sensitive taxa. Desmid genera such as *Cosmarium* and *Closterium* were abundant in relatively undisturbed habitats and were largely absent from effluent discharge zones. Their occurrence indicated favorable water quality and ecological stability. The presence of *Volvox* and *Pediastrum* further reflected the comparatively healthy condition of natural aquatic ecosystems surrounding the study sites.

Table 5. Diversity Indices

Diversity Index	Effluent Zones	Natural Water Bodies
Shannon-Wiener Index (H')	2.11	3.42
Simpson Index (D)	0.74	0.91
Margalef Richness Index	3.82	6.95

The calculated diversity indices clearly demonstrated the adverse effects of industrial activities on microalgal communities. Shannon diversity values were substantially lower in effluent zones, indicating reduced species richness and evenness. Similarly, the Margalef index suggested a significant decline in taxonomic richness within polluted habitats. Natural water bodies consistently exhibited higher diversity values, supporting the conclusion that cement industry effluents negatively influence freshwater biodiversity.

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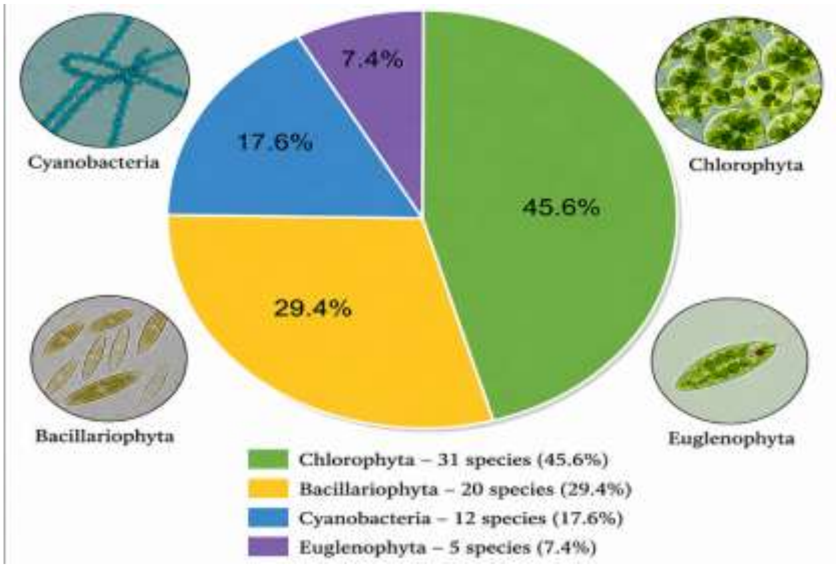

Figure 3. Relative Composition of Major Microalgal Groups

The pie chart indicates that Chlorophyta constituted the largest proportion of the microalgal community, accounting for nearly half of all recorded taxa. Bacillariophyta represented the second most diverse group, while Cyanobacteria and Euglenophyta contributed smaller proportions. The dominance of Chlorophyta suggests their broad adaptability to both natural and industrially influenced aquatic habitats.

Molecular Analysis Results

Genomic DNA was successfully extracted from representative isolates of *Chlorella vulgaris*, *Scenedesmus quadricauda*, *Nitzschia palea*, *Oscillatoria limosa*, *Pediastrum duplex*, and *Cosmarium* species. Agarose gel electrophoresis revealed high-quality DNA bands with minimal degradation, confirming the suitability of extracted DNA for molecular analyses. PCR amplification of the 18S rRNA gene produced fragments ranging from approximately 1700 to 1800 base pairs. Amplification success exceeded 90% among selected isolates. Similarly, rbcL gene amplification generated fragments between 1100 and 1400 base pairs. Distinct and reproducible amplification products were observed for all target species, demonstrating the effectiveness of the selected primers. Sequence analysis revealed high similarity between the obtained sequences and corresponding reference sequences available in the NCBI GenBank database. BLAST comparisons showed sequence identities ranging from 97.2% to 99.8%, confirming the taxonomic identity of the studied microalgae. The highest sequence similarity was observed for *Chlorella vulgaris* and *Scenedesmus quadricauda*, whereas moderate genetic variation was detected among isolates of *Nitzschia palea* collected from different industrial locations.

Phylogenetic analysis based on 18S rRNA sequences grouped the isolates into distinct clades corresponding to Chlorophyta, Bacillariophyta, and Cyanobacteria. Bootstrap values exceeding 85% supported the reliability of major branches within the phylogenetic tree. Similar clustering patterns were obtained using rbcL sequences, further validating species-level identification and evolutionary relationships. The molecular findings revealed that several isolates collected from cement effluent zones exhibited minor genetic variations compared to isolates obtained from natural water bodies. These variations may represent adaptive responses to long-term exposure to industrial pollutants and environmental stress. The occurrence of distinct haplotypes within pollution-tolerant taxa suggests that selective pressures associated with cement effluent discharge may influence genetic diversity at the population level.

Discussion

The present investigation demonstrates that cement industry effluents significantly influence freshwater microalgal communities through alterations in physicochemical conditions. Elevated pH, increased conductivity, higher turbidity, and nutrient enrichment created environmental conditions favorable for pollution-tolerant taxa while reducing the abundance of sensitive species. Similar observations have been reported in industrially impacted aquatic ecosystems where anthropogenic disturbances simplify biological communities and reduce biodiversity. The predominance of *Chlorella vulgaris*, *Scenedesmus quadricauda*, and *Oscillatoria limosa* in effluent zones highlights their potential utility as bioindicators of industrial pollution. These taxa possess physiological adaptations that enable survival under stressful environmental conditions, including elevated alkalinity and suspended solids. In contrast, desmid species such as *Cosmarium* and *Closterium* were restricted to natural habitats and may serve as indicators of good water quality. Molecular characterization substantially improved taxonomic resolution and confirmed the reliability of morphological identification. The combined use of 18S rRNA and rbcL markers provided robust species discrimination and revealed genetic diversity that could not be detected through microscopic observations alone. The integration of ecological and molecular approaches therefore represents a powerful framework for biodiversity assessment and environmental monitoring.

Conclusion

The present study demonstrated that cement industry effluents significantly influence the physicochemical characteristics and microalgal biodiversity of freshwater ecosystems in West Bengal. Comparative assessment of effluent zones associated with JSW Cement Ltd. – Salboni Grinding Unit, UltraTech Cement – Dankuni Cement Works, Ambuja Cements – Sankrail Unit, and The Ramco Cements Ltd. – Kolaghat Unit revealed noticeable differences in water quality, species composition, and community structure when compared with adjacent natural water bodies. Elevated pH, conductivity, turbidity, and nutrient concentrations in effluent-affected

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habitats favored the dominance of pollution-tolerant microalgal taxa such as *Chlorella vulgaris*, *Scenedesmus quadricauda*, and *Oscillatoria limosa*, while sensitive species were largely restricted to natural ecosystems. Molecular characterization using 18S rRNA and rbcL gene markers successfully confirmed species identity and provided valuable insights into genetic diversity and phylogenetic relationships. The integration of ecological and molecular approaches proved effective for environmental assessment and highlighted the potential of microalgae as reliable bioindicators for monitoring industrial pollution and supporting sustainable freshwater ecosystem management.

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