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Detection and Quantification of Environmental DNA (e-DNA) as a Tool for Aquatic Ecosystem Monitoring

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Abstract: ***Environmental Deoxyribonucleic acid -(e-DNA) has emerged as a non-invasive and powerful tool for monitoring biodiversity in aquatic ecosystems. The present study is aimed at detecting and quantifying e-DNA in water samples using spectrophotometric analysis and electrophoretic separation following Polymerase Chain Reaction (PCR) amplification. Water samples were collected from three different locations of a highly polluted Hussainsagar lake and analyzed for e-DNA concentration with results expressed as mean ± standard error, the concentrations ranged from 0.35 to 0.60 mg/L. PCR amplification confirmed the presence of DNA fragments, as visualized through electropherograms. The extracted e-DNA was preserved for future sequencing and species identification, particularly focusing on fish species due to their ecological significance. The present findings demonstrate the feasibility of using e-DNA for ecological monitoring and contribute to the development of sustainable, non-invasive biodiversity assessment methods, study of interactions of species as well as ecosystem dynamics.***

Key Words: e -DNA, Electrophoresis, Biodiversity, Aquatic medium, Fish, Polymerase Chain Reaction

I. INTRODUCTION

Biodiversity assessment in aquatic environment is essential to monitoring the aquatic ecosystem health, conservation strategies and managing natural resources. There are several methods available for achieving the goal of biodiversity monitoring like sampling, visual surveys, trapping etc but these methods are laborious, invasive and have limitations with regard to environmental conditions. With the advancement in technology latest methods like molecular and remote sensing techniques are used. The molecular techniques using e-DNA extraction, sequencing and barcoding are promising tools. these methods can be individually used or in combination to monitor biodiversity effectively. The choice of method depends on the objective and target taxa of the study. The use of environmental DNA(e-DNA)has gained attention in the recent years as a sensitive and non-invasive tool for the detection of organisms in aquatic habitats*(Ashish sahu et al(2023).*

e-DNA refers to the genetic material released by organisms into their environment through various processes such as excretion, secretion, reproduction, or decomposition and that can be collected from the fields like water, soil, or air *(Taberlet et al., 2012).* Compared to conventional sampling methods, which may miss less abundant or elusive species, e-DNA techniques can detect organisms that are difficult to capture or observe directly *(Jerde et al., 2011;Rishan et al(2025).* Spectrophotometric quantification and PCR-based methods have enabled researchers to not only detect but also estimate relative abundance, although environmental variables such as temperature, pH, and microbial activity can influence e-DNA degradation and persistence *(Barnes et al., 2014).*

Once released, e-DNA can persist in water for days to years and can be identified from a sample of water first and then be analyzed by different techniques for various studies like genomic, taxonomic, distribution of species in a particular environment and pollutional effects *(Sakib et al.,2024).* Identification of e-DNA is a useful method of determining the organisms present in an aquatic environment like fishes and other organisms (amphibians, reptiles, insects and larval forms of some of these organisms.*) Helen et al(2014.)*The e-DNA is a useful tool in ecology and conservation biology *(Kingsly et al , 2020).* The integration of e-DNA with modern bioinformatics tools allows for accurate taxonomic identification through sequence comparison with genetic databases like GenBank ,particularly useful for fish species, which are often used as ecological indicators due to their role in trophic networks and ecosystem dynamics *(Evans et al., 2017*).This study contributes to a growing body of research demonstrating the utility of e-DNA for ecological monitoring and reinforces its potential for future applications in conservation biology, especially for aquatic ecosystems*(Batham and Garg,2024)*

The objective of this study was to detect and analyze e-DNA in water samples collected from different sources. Spectrophotometric analysis was used to quantify e-DNA concentration, while electrophoresis following PCR amplification for detecting the presence and quality of DNA. The e-DNA thus obtained can be preserved for further studies involving sequencing and taxonomic identification, with an emphasis on fish species, as they play a vital role in an aquatic food web and also serve as ecological indicators of water quality.

II. MATERIAL AND METHODS

A. Samples Collection

Water samples were collected from three different sites (referred to as Sample 1, Sample 2, and Sample 3) of a highly polluted Hussainsagar lake using sterile containers. One liter of water sample was collected from the sediments of each site as described by *Huston et al(2023).*Samples were transported to the laboratory on ice and processed within 24 hours of collection.

B. Extraction of e-DNA

e-DNA was extracted from water samples of three different sites using standard filtration followed by DNA precipitation techniques. Water was filtered through 0.45 µm pore-sized membrane filters to capture cellular and extracellular DNA. Filters were then subjected to chemical lysis, and DNA was isolated using a commercial DNA extraction kit(Bio-Rad), following the manufacturer's protocol

C. e-DNA Quantification

Spectrophotometric analysis was performed to quantify e-DNA concentrations in mg/L. Six replicates were measured per sample to ensure accuracy, and the results were expressed as mean ± standard error. The quantity of DNA by the spectrophotometric method was determined by calculating the purity of DNA by the ratio of absorbance at 260nm and 280nm. The absorbance determined is the purity of DNA i.e. 1.8.

Purity of the DNA A260: A280ratio = A260/ A280 = 1.8: pure DNA = 1.7 – 1.9; fairly pure DNA (acceptable ratio for PCR) = less than 1.8; presence of proteins. = greater than 1.8; presence of organic solvent The purity of e-DNA thus obtained was less than 1.8 *(R-sd function to calculate standard deviation.)*

D. PCR Amplification

The extracted e-DNA was used as a template for PCR amplification to confirm the presence of amplifiable DNA. Universal primers (COI-5'F:GGTCAACAAATCATAAAGATATTGG;COI-5'R:TAAACTTCAGGGTGAGACCAAAAAATCA)targeting conserved regions of aquatic vertebrate mitochondrial DNA were used to enhance the chances of detecting a broad range of species, particularly fish. Amplification was carried out in a thermal cycler under standard conditions with the following three steps: Denaturing (melting of the strandsat 92^0 to 96^0 Cannealing of primerto each strand at 40 ^{0}-60^0 C and extension by polymerase adding dNTPS complimentary at the 3'end of the primers.to the template strand Tin the thermal cycler.he steps are repeated 30- 40 times

E. Electrophoretic Analysis

PCR products were analyzed by agarose gel electrophoresis(0.8%) to visualize DNA bands as described by Meghan and Alexandria (2023).Electropherograms were generated to assess signal intensity, with the X-axis representing scan number (time) and the Y-axis indicating DNA signal strength.

A DNA ladder was not included, as size estimation was not the study's objective.Post-PCR, the amplified DNA fragments were preserved at -20°C for further sequencing and bioinformatics analysis using publicly available genetic databases to identify species composition, with a focus on fish biodiversity.

III. RESULTS

A. e-DNA Quantification

The concentration of environmental DNA (e-DNA) present in the water samples was measured spectrophotometrically.The data was analysed statistically. The results are shown in **Table 1**. Values are presented as the mean of six replicates ± standard deviation(SD), and are expressed in mg/L.

B. Electrophoretic Separation of Amplified e-DNA

Polymerase Chain Reaction (PCR) was used to successfully to amplify the extracted e-DNA. The amplified products were then subjected to electrophoretic separation, as shown in **Figure 1**.

The **X-axis** of the electropherogram represents scan numbers (time), while the **Y-axis** indicates signal intensity, corresponding to the presence of DNA fragments.. DNA ladder was excluded as the goal was detection rather than size estimation..From the electropherogram thus obtained in the present study consisted of three thick bands indicating the total e-DNA of particular organisms present in the water body..The separated e-DNA fractions were preserved for subsequent sequencing and species identification using reference databases. The focus is primarily on fish species, which are ecologically significant due to their role as dominant organisms in aquatic food chains.

Table 1. Statistical Analysis (Spectrophotometric Quantification) of e-DNA (mg/L)

Sample.	Mean	Standard Deviation(SD)
1	0.35	0.5
2	0.48	1.0
3	0.60	0.5

No significant differences between the sample means was found(p.0.05) while in terms of variability sample 2 has high standard deviation than the samples 1 and 3.

Note: Values represent the mean of six replicates ± standard deviation

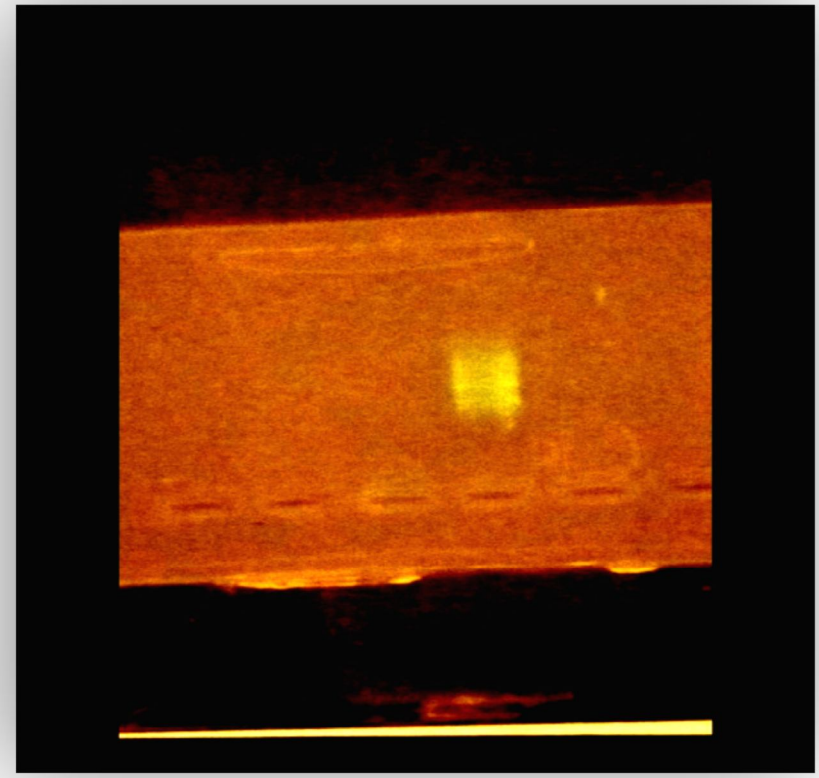

Figure 1. Electropherogram showing PCR-amplified e-DNA from water samples.

IV. DISCUSSION

The present study was aimed to detect and analyze environmental DNA (e-DNA) present in water samples through spectrophotometric quantification and electrophoretic separation following PCR amplification. The results indicate the successful extraction and detection of e-DNA from all three water samples.The water samples collected were from the sediments rather than from the surface water as sediments contain more DNA and settles at the bottom of the water body owing to its high molecular weight. The present study conforms to that done by Turner, et al (2015) which states that the environmental DNA is more concentrated in the sediments than in the surface waters. After extracting the e-DNA it may be subjected to metabarcoding i.e.,sequencing the DNA after amplifying the fragments using next generation sequencing techniques(NGS),the generated sequences are then analysed using bioinformatics tools comparing them with the reference databases of known DNA barcodes of various species,one can identify the actual species present in the water samples.

The spectrophotometric analysis shows measurable amounts of e-DNA across all samples, with values ranging from 0.35 mg/L to 0.60 mg/L**.** While Sample 3 recorded the highest e-DNA concentration (0.60 ± 0.5 mg/L), Sample 1 had the lowest (0.35 ± 0.5 mg/L). The standard deviations indicate a moderate degree of variability between replicates, particularly in Sample 2 (±1 mg/L), suggesting possible environmental or sampling inconsistencies, or variations in DNA degradation and distribution in the aquatic environment..

The electrophoretic separation confirmed the presence of amplifiable DNA fragments in all samples, demonstrating that the extracted e-DNA was of sufficient quality and quantity to serve as a template for PCR. Although a DNA ladder was not included, the primary purpose of this electrophoretic analysis was to verify the presence of DNA rather than determine its size. The electropherogram signal intensities support the spectrophotometric findings, further validating the presence of e-DNA. Environmental DNA also give insights into dynamics of ecosystem and species interactions.The organism's shed DNA is at a varying rate and it depends on ,its degradation over time and dispersal through various means like air,water etc that which can affect its detection.It can give predator -prey relationships,and detect symbiotic relationships too. (Maria et al(2023); Elizabeth et al(2024)).After electrophoretically separating e-DNA, species identity could be confirmed by sequencing methods,or probe based detection using species specific probes to hybridize with target DNA sequences. Fragment size determination to confirm species identity and to evaluate peak intensity to estimate DNA quantitatively are some of the methods for validating the results(Marc Kochzius et al(2010).

From the electropherogram thus obtained in the present study showed presence of thick bands indicating the e-DNA of total organisms present in the water body. The DNA fragments appear as bands after staining the gels with ethidium bromide(The stain is carcinogenic, used with precaution) which fluoresces under UV light The size of each band can be determined by its position relative with that of a DNA ladder.i.e., The fragments with known sizes which can give the information about particular organism's identity like fishes while the intensity of the band is indicative of the relative abundance of DNA of that species.

Roesma et al,(2021)reported studies involving e-DNA of different vertebrate animals of Maninjau lake. Environmental DNA(e-DNA) as a tool for assessing the fish biomass was reviewed by Meaghan et al (2022).These findings are significant as they establish the feasibility of using non-invasive e-DNA techniques for aquatic biodiversity monitoring.The available literature on the biomonitoring at the macro-organismal level was given by Takahashi et al (2023).The preserved e-DNA samples will allow for future sequencing and species-level identification, with a particular emphasis on fish species**,** which are not only dominant in aquatic ecosystems but also key indicators of ecological health.

V. CONCLUSION

The study successfully demonstrated the presence and amplifiability of e-DNA in water samples using spectrophotometry and PCR-based electrophoresis. The varying concentrations of e-DNA across samples suggest differences in biological activity or species distribution within the water body. These results support the utility of e-DNA as a reliable, non-invasive tool for aquatic biodiversity assessment. The stored DNA samples is a valuable resource for our future genetic analysis and species identification studies

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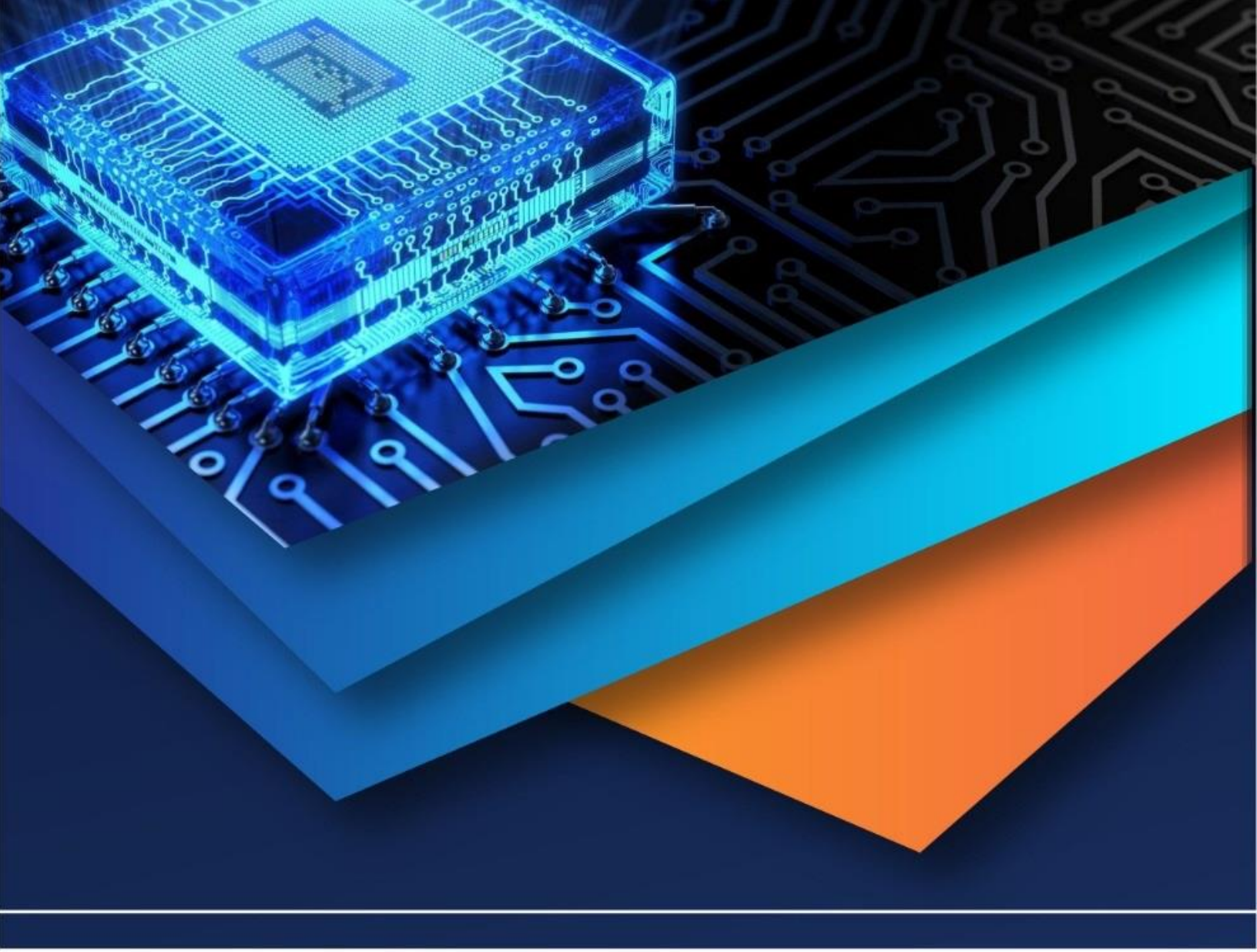

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