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Department of Forensic Medicine & Toxicology, Third Floor, Mortuary Building, Lokmanya Tilak Municipal Medical College & LTMG Hospital, Sion, Mumbai, Maharashtra, India. Pin-400 022. Email id: mlameditor@gmail.com Phone: 022-68660000/ +91-9423016325.



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Original Research Article

Forensic Limnological Profile of Water Bodies in Marathwada Region of Maharashtra, India

Miriam Reji Puthenpurackal,^a Sandeep G. Sangle,^b Malla Bharadwaj Sai Satya Murthy^{c*}, Kattamreddy Ananth Rupesh^d

^aGovernment institute of forensic science, Chhatrapati Sambhajanagar, Maharashtra, India. ^bAssistant Professor, Forensic Biology, Serology and DNA fingerprinting, Government institute of forensic science, Chhatrapati Sambhajanagar, Maharashtra, India. ^cResearch Intern, GITAM school of sciences, Department of Chemistry, GITAM University, Visakhapatnam, Andhra Pradesh, India. ^dAssistant Professor of Forensic Medicine and Toxicology, Visakhapatnam, Andhra Medical College, India. Pin-530002.

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Abstract

Introduction: Drowning is one of the most common forms of unnatural death encountered in Forensic Medicine practice in India. Although considered obsolete by many practitioners, the diatom test still plays a role in linking the site of drowning with the recovered body to some extent. The availability of a diatom database for individual water bodies can expedite forensic investigations and assist in confirming the location of drowning incidents. This study aimed to analyze the diversity of diatoms from 14 water samples collected from the Marathwada region of Maharashtra by identifying diatoms at the genus level. **Methods:** Diatoms were extracted from the water samples using a standardized aqua regia method. Slides were prepared, mounted, and examined under a phase-contrast microscope. Classification was performed using the *Diatoms of North America* database. **Findings:** More than 50 different genera of diatoms were identified which included both common and unique diatoms. These findings provide valuable insights into the ecological characteristics of each water body and their distinctive flora. **Conclusion:** The data generated in this research will aid in drowning investigations in Marathwada region of Maharashtra and contribute to establishing a regional database on diatom diversity.

1. Introduction

Diatoms (Kingdom: *Protista*, Class: *Bacillariophyceae*) are unicellular, photosynthetic eukaryotic microalgae ¹⁻³ that are among the most prevalent and ecologically significant primary producers in aquatic ecosystems. They occur in marine, freshwater, terrestrial, and artificial or

man-made habitats.^{1,4} The diversity of diatoms is extensive, with current estimates indicating that approximately 100,000 to 200,000 species have been identified, exhibiting a wide range of sizes and morphological characteristics.² Their distinctive features, such as silica-based cell walls

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***Corresponding author:** Malla Bharadwaj Sai Satya Murthy, Research Intern, GITAM school of sciences, Department of Chemistry, GITAM university, Visakhapatnam, Andhra Pradesh, India. Email: satishmalla4153@gmail.com

or frustules, have intricate and species-specific designs.¹ Diatoms function as primary producers that convert sunlight into biomass, thereby sustaining aquatic food webs and contributing significantly to global carbon and silicon cycles, as well as influencing climate dynamics and nutrient cycling processes.¹⁻⁶

Drowning, a form of asphyxial death in which access of air to the lungs is prevented by the submersion of the body in water or another fluid medium. It accounts for approximately 320,000 deaths globally each year. The diatom test is based on the principle that, during drowning, water containing microscopic diatoms enters the lungs and may subsequently enter the bloodstream. If circulation is active at the time of submersion, these diatoms can be transported to distant organs such as the brain, kidneys, or bone marrow, where they can later be detected microscopically. The presence of diatoms in these internal organs supports a diagnosis of ante-mortem drowning. However, the reliability and diagnostic value of this test remain a subject of debate among forensic experts.⁵⁻¹²

The primary objective of the present study was to collect and analyze water samples from selected locations in the Marathwada region. The samples were processed, examined microscopically, and photographed to identify genus-level morphological characteristics of diatoms and to assess the diversity of diatom assemblages in each water body.

2. Methodology:

Study Area: The field locations for the study were chosen from the Marathwada region of Maharashtra, India. A total of 14 samples were collected from variety of diverse water bodies such as rivers, lakes, ponds, and dams. The geographical location of the field spots are as follows; 1. Aurangabad caves (Chhatrapati Sambhajinagar), 2. Bindusara Lake (Beed), 3. Hiranya Lake (Chhatrapati Sambhajinagar), 4. Jalna Ghanewadi Reservoir (Jalna), 5. Ladgao Lake (Chhatrapati Sambhajinagar), 6. Jayakwadi Dam (Chhatrapati Sambhajinagar), 7. Majalgaon Dam (Beed), 8. MIDC phase III pond (Jalna), 9. Salim Ali Lake (Chhatrapati Sambhajinagar), 10. Panasnala River (Shingnapur), 11. Siddheshwar Dam (Hingoli), 12. Terna Dam (Dharashiv), 13. Vilasnagar Ambad Well (Jalna), 14. Vishnupuri Dam (Nanded).

Sample collection: Water samples for the diatom study were collected primarily from riffle zones, where water flows over rocks or stones. Sampling from riffles provides optimal results, as continuous water movement minimizes chemical accumulation

and sedimentation, ensuring the collection of representative local biota.¹³ For still or deepwater bodies such as wells and ponds, samples were taken near the shore after gentle agitation to prevent the inclusion of organic debris or sediments.¹⁴

In all cases, samples were collected from depths of less than one meter, where sunlight penetration and substrate conditions best support diatom growth.¹³ Samples were collected in sterilized 250 ml screw cap glass bottles with tightly fitted caps and labeled with the location, date and time of collection, site coordinates, temperature, and humidity.

Sample Preparation: The agitated water samples were transferred to Falcon tubes and centrifuged using an Eppendorf Centrifuge 5810 R at 8000 rpm for 30 minutes.^{14, 15} The resulting pellet was subjected to acid digestion using the aqua regia method by adding equal volumes of HNO₃ and HCl to 2 mL of the sample under a fume hood. This process removed organic and unwanted materials from the sample. The digested mixture was then incubated at 30°C for 24 hours to facilitate complete digestion.^{5, 11, 14, 15, 17-19}

Slides were prepared by placing 40 µL of the digested sample on clean, labeled glass slides. A drop of DPX (Dibutyl Phthalate Polystyrene Xylene) mounting medium was added, and a coverslip was carefully placed on top. The slides were left undisturbed until completely dried. Observations were made under a phase-contrast microscope at 10×, 40×, and 100× magnifications. Images were captured using Mosaic 2.3 software with a GT 5.0 (5 MP) color microscope camera attached to the microscope. The captured images were labeled, saved, and compared with reference images from the *Diatoms of North America* database (diatoms.org) for genus-level identification. The above procedure is followed for all the collected samples to examine the diatoms morphologically under microscope.

This study was conducted at Forensic Biology Lab, Government Institute of Forensic Science, Aurangabad (GIFSA), Maharashtra, India. All the chemicals used in the study are quality grade. Proper safety precautions are taken to avoid any harm and the procedure is done in a clean work place to avoid contamination.

3. Results:

The results of the present study provide an overview of the diatom diversity observed in water samples collected from various locations in the Marathwada region of Maharashtra, India. Based on

morphological characterization, more than 50 diatom genera were identified across 14 water bodies. These findings highlight the ecological diversity of the sampled aquatic habitats. The observed genera and their distribution across sampling sites are summarized in **Figure 1**.

Figure 01: The observed genera and their distribution- 1-Afrocybella; 2-Amphora; 3-Aneumastus; 4-Aulacoseria; 5-Bacillaria; 6-Brebissonia; 7-Boreozonacola; 8-Cymbella; 9-Cyclotella; 10-Cyclostephanos; 11-Craticula; 12-Crenotia; 13-Cosmioneis; 14-Campylodiscus; 15-Diploneis; 16-Envekadea; 17-Encyonema; 18-Fragilariforma; 19-Gato ; 20-Grammatophora; 21-Hantzschia; 22-Homeocladia; 23-Iconella; 24-Lacustriella; 25-Mastoglia; 26-Navicula; 27-Navicymbula; 28-Neidiomorpha; 29-Nitzschia; 30-Nupela; 31-Nanofrustulum; 32-Oricymba; 33-Pinnularia; 34-Plagiotropis; 35-Rexlowea; 36-Stenopterobia; 37-Seminais; 38-Stephanocylus; 39-Stauroneis; 40-Surirella; 41-Sellaphora; 42-Spicaticribra; 43-Tabularia; 44-Tetramphora; 45-Thalassionema; 46-Tryblionella; 47-Tibetiella; 48-Ulnaria; 49-62: Unidentified.

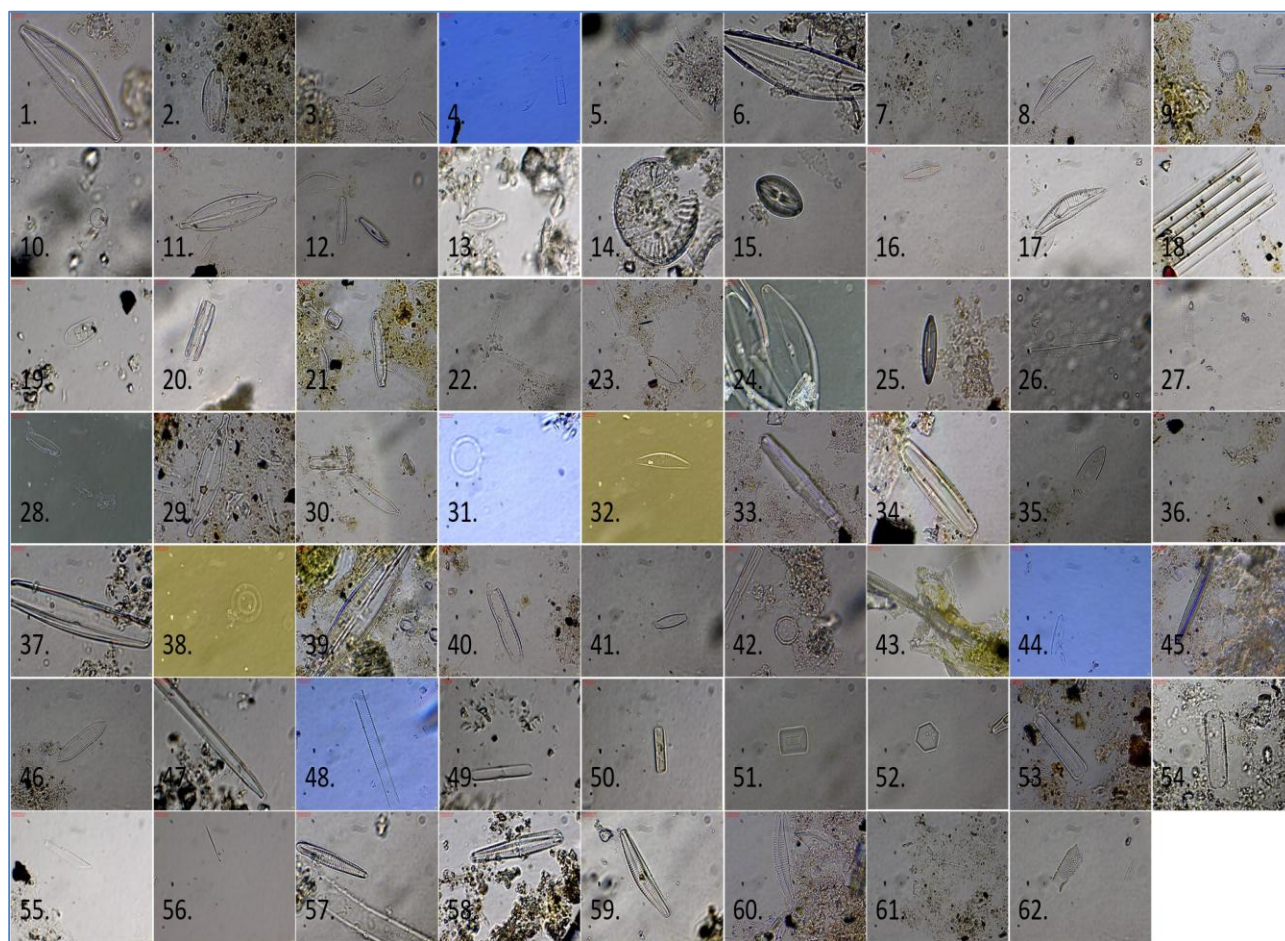


Table 1: Prevalence of Diatoms in Selected Water Bodies.

Sr. No.	Diatom	Water Samples														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1	Afrocybella						+		+							2
2	Amphora												+			1
3	Aneumastus						+									1
4	Aulacoseria			+	+											2
5	Bacillaria													+		1
6	Brebissonia		+													1
7	Boreozonacola												+			1

8	Cymbella			+			+	+	+	+	+	+				7
9	Cyclotella	+		+	+	+			+	+			+		+	8
10	Cyclostephanos			+					+	+						3
11	Craticula					+			+							2
12	Crenotia	+		+												2
13	Cosmioneis	+														1
14	Campylodiscus												+			1
15	Diploneis						+		+						+	3
16	Diatomella								+							1
17	Envekadea	+														1
18	Encyonema	+		+	+		+						+			5
19	Fragilariforma	+				+										2
20	Gato	+														1
21	Grammatophora		+	+				+				+				4
22	Hantzschia					+			+							2
23	Homeocladia												+			1
24	Iconella					+										1
25	Lacustriella										+					1
26	Mastoglia											+				1
27	Navicula	+	+	+	+	+			+	+	+	+			+	10
28	Navicymbula											+				1
29	Neidiomorpha					+										1
30	Nitzschia	+		+		+	+		+	+	+			+	+	9
31	Nupela	+														1
32	Nanofrustulum									+						1
33	Oricymba				+											1
34	Pinnularia			+	+				+	+		+				5
35	Plagiotropis	+		+									+		+	4
36	Rexlowea		+													1
37	Stenopterobia		+			+										2
38	Seminavis			+	+		+		+			+				5
39	Stephanocylus		+		+										+	3
40	Stauroneis								+			+				2
41	Surirella					+			+					+		3
42	Sellaphora					+			+				+			3
43	Spicaticribra								+							1
44	Strelnikoviella								+							1
45	Tabularia	+														1
46	Tetramphora				+											1
47	Thalassionema											+				1
48	Tryblionella								+						+	2
49	Tibetiella	+			+								+			3
50	Ulnaria	+	+	+	+	+	+		+		+	+				9

51	Unidentified	2	0	2	0	1	0	0	1	0	2	5	1	1	0	15
		16	7	15	11	13	8	2	20	7	7	15	8	6	7	

Note: “+” symbol indicates the presence of particular diatom in the respective water bodies.

Morphological analysis revealed variation in the predominant diatom genera across the 14 water samples from the Marathwada region. *Encyonema* was the most abundant genus in samples 1, 3, 4, 6, and 12, while *Ulnaria* predominated in sample 5. *Cymbella* was dominant in samples 7 and 11. Sample 8 showed the highest frequency of *Pinnularia*, followed by *Cymbella*, and *Pinnularia* was also abundant in sample 9. In sample 10, *Navicula* and *Ulnaria* occurred with equal frequency. *Campylodiscus* was predominant in sample 13, and *Nitzschia* was dominant in sample 14.

Across all sites, the most frequently occurring genera were *Navicula*, followed by *Nitzschia*, *Ulnaria*, *Cyclotella*, *Cymbella*, *Encyonema*, *Pinnularia*, and *Seminavis*. Several genera were found to be unique to specific water bodies, including *Amphora* and *Boreozonacola* in Terna Dam; *Aneumastus* in Jayakwadi Dam; *Bacillaria*, *Campylodiscus*, and *Homeocladia* in Vilasnagar Well (Ambad); *Brebissonia* and *Rexlowea* in Bindusara Lake; *Cosmioneis*, *Envekadea*, *Gato*, *Nupela*, and *Tabularia* in Aurangabad Caves; *Diatomella*, *Spicaticribra*, and *Strelnikoviella* in the MIDC Pond; *Iconella* in Ladgaon Lake; *Lacustriella* in Panasnala River (Shingnapur); *Mastoglia*, *Navicymbula*, and *Thalassionema* in Siddheshwar Dam; *Nanonfrustulum* in Salim Ali Lake; and *Oricymba* and *Tetramphora* in the Jalna Ghanewadi Reservoir.

4. Discussion:

As shown in the **Table 1 and Figure 01**, a total of more than 50 identified genera of diatoms and 15 unidentified forms were recorded from 14 water samples collected across the Marathwada region, based on morphological analysis. Although 50 different genera of diatoms were identified using existing databases, several specimens remained unidentified due to the absence of an indigenous diatom database. While reference literature and international databases aided in the identification process, no comprehensive regional database currently exists for the Marathwada region or the state of Maharashtra. At a broader level, India also lacks an authentic and centralized database documenting algal diversity. The establishment of such a database would significantly enhance the accuracy of forensic investigations, particularly in

drowning cases. Similar observations have been reported by Pandey LK.²⁰

Seasonal variations can significantly influence diatom diversity and abundance, as changes in temperature, light availability, and nutrient concentration affect their growth dynamics. Periods of increased nutrient availability, such as during dry or mixing seasons, may promote higher diatom productivity and morphological variability.²¹ Previous studies, such as that by Dahiya P.⁵, have shown that physicochemical parameters and heavy metal concentrations significantly influence diatom diversity and growth. However, the present study did not include an analysis of environmental or nutrient factors affecting diatom population dynamics.

Recent studies suggest that the analysis of diatom presence, growth behavior, and succession patterns, when combined with modern techniques such as water elemental profiling and phytoplankton examination from dental samples, may enhance the understanding of post-mortem changes in aquatic environments. However, the application of these methods for post-mortem submersion interval (PMSI) estimation remains exploratory and has not yet been fully validated for routine forensic use.^{22, 23} Some studies show increase in the species diversity of diatoms on the decaying carcasses in particular time frames during the petrification in waterbodies, which can help in estimating the PMSI of the submerged dead body.²⁴ This is innovative field and more research will enhance the accuracy.^{25,26}

By comparison of diverse species of diatoms within the victim's body with those in the nearby water helps in determining the drowning site, as each water body has its distinct community of diatoms. Diversity in diatoms flora across micro-habitat such as benthic, floating, surfaces zones²⁷ should be considered during sampling to improve accuracy. Elemental and nutritional analysis of samples support understanding of diatom distribution. Further research on these factors can refine interpretation of diatoms prevalence and enhances the precision of forensic investigations.

5. Conclusion:

Forensic diatomology plays a vital role in drowning investigations by aiding in determining cause of death and site of drowning. Although considered a traditional method, it remains useful in

localizing the drowning site, particularly when a body is displaced from its original location. The detection of diatoms in vital organs supports the diagnosis of ante-mortem drowning. Additionally, diatom analysis can assist in post-mortem submersion interval (PMSI) estimation. Establishing limnological profiles of regional water bodies, including their seasonal variations, would further enhance the accuracy and efficiency of such forensic determinations.

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