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Extraction and Identification of Diatom Species in Local Water Bodies of Jaipur Region

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Abstract:
The present study entitled "Extraction and Identification of Diatom Species in Local Water Bodies of Jaipur Region" was conducted in the Department of Forensic Science, JECRC University, Jaipur, Rajasthan. Objective of the study was to determine the diatom species in different water bodies of Jaipur from Dravyavati River, Maota lake, Man Sagar lake, Jeerota lake, Tal Katora. Examination of diatoms leads to determine the cause of death i.e. anti-mortem or post-mortem drowning cases. Acid digestion method was used for the extraction of diatoms from the collected water sample. Planktonic algae communities, with total of 9 diatom genera were identified from 5 different water bodies of Jaipur, examined under light microscope at 10x and 40x magnification. The Specific species genera with selected water bodies are: Diatoma sp., Nitzschia, Actinella, Melosira have been identified in Dravyavati River. Diatom genera Navicula, Cylindrotheca closterium and Nitzschia are found in Man Sagar Lake (Jal Mahal). Diatom Genera Synedra, Fragilaria, Nitzschia, and Encyonema are found in Tal Katora. Diatom Genera Nitzschia , Navicula, Melosira are found in Maota Lake (Amer Fort) and finally the diatom genera Nitzschia is the only species found in Jeerota Lake. Major diatom genera that are frequently found in all five water samples from water bodies are Nitzschia, Navicula and Melosira.

Keywords: Diatoms, Forensic Diatomology, Acid Digestion Method, Chemical Extraction, Forensic Science

Introduction

Diatoms are unicellular, eukaryotic, and photosynthetic algae characterized by intricately patterned silica-based cell walls known as frustules [1]. These microscopic organisms, typically ranging from 2 to 200 µm in size, inhabit a wide range of moist and aquatic environments, including freshwater, marine ecosystems, and even damp soils [2]. Their occurrence is influenced by several physicochemical factors such as calcium, chloride, pH, alkalinity, silica, and ammonia, which shape the distribution and dominance of species in water bodies [3]. Diatoms are broadly classified into centric (radially symmetrical) and pennate (bilaterally symmetrical) forms, each exhibiting unique morphological features such as areolae, striae, raphe, nodules, and punctae [4,5].

The current study investigates the identification of various diatom species prevalently found in the local water bodies of Jaipur. It emphasizes their classification based on cell structure, size (ranging from 5–1000 µm), and ecological habitat [6,7]. Both centric and pennate types were observed with their respective morphological markers and environmental associations. The reproductive behavior of diatoms includes both asexual reproduction through binary fission and sexual reproduction, which is either oogamous in centric forms or isogamous in penates [6]. Shrinkage during vegetative division and size restoration through sexual reproduction are characteristic features of their life cycle.

Diatoms have extensive ecological and practical applications. They serve as bioindicators for environmental monitoring due to their sensitivity to ecological changes and long-term preservation of their frustules [8]. Their unique optical and mechanical properties make them suitable for emerging applications in nanotechnology, biosensors, and biotechnology [9, 10]. Additionally, diatoms contribute to the global carbon cycle and have potential use in biofuel production due to their lipid content accumulated during vegetative growth phases.

In forensic science, diatoms play a pivotal role, particularly in drowning investigations. The detection of diatoms in vital organs such as the brain, spleen, kidneys, liver, and bone marrow serves as strong evidence of ante-mortem drowning, as they can only reach these organs through circulation during active respiration [11, 4]. In contrast, post-mortem submersion yields little or no presence of diatoms beyond the lungs. Their species-specific presence can also aid in determining the exact location of drowning by comparing environmental diatom profiles with those extracted from the body [3].

The study used standard acid digestion techniques for extracting diatoms from both water samples and biological tissues, enabling microscopic identification and comparison. Challenges such as contamination, false positives, seasonal variability, and low diatom density in some environments are acknowledged as limitations in forensic diatomology [3]. However, the development of artificial intelligence for automated diatom identification and the use of diatom DNA barcoding are prospective advancements that promise increased reliability and precision in forensic investigations.

Furthermore, the research establishes a baseline for the diversity and forensic relevance of diatoms in the water bodies of Jaipur. It emphasizes their dual role as ecological indicators and forensic tools, advocating for their broader implementation in forensic protocols, particularly in drowning-related cases.

Numerous studies across India have investigated the diversity and applications of diatoms in freshwater ecosystems, with growing emphasis on their forensic utility. AS et al.[1] conducted a one-year study across three lakes in Kolhapur, identifying 18 diatom species and employing diversity indices such as Shannon-Wiener (H'), Evenness (J'), Species Richness (SR), and Index of Dominance. Their findings highlighted moderate species richness (1.73–2.27), with Kankala lake showing maximum richness and Kalamba lake displaying even species distribution. Similarly, Bhupender and Kumar[2] examined phytoplankton diversity in a Bikaner pond, documenting 14 species across Chlorophyceae, Cyanophyceae, and Bacillariophyceae using the Sedgwick-Rafter method. Dahiya et al.[3] advanced this by surveying 100 water samples from Gujarat, identifying 250 diatom species from 66 genera across various morphological types—centrics (e.g., *Melosira*, *Cyclotella*), araphids (e.g., *Ulnaria*, *Fragilaria*), and biraphids (e.g., *Cymbella*, *Gomphonema*, *Navicula*)—employing trinocular microscopy and ICP-MS for heavy metal detection.

In Rajasthan, Jyoti et al.[12] found 43 species from 28 genera in the Chandloi River over a two-year period, dominated by Chlorophyceae and Dinophyceae. Kumar and Singh[6] identified 32 Bacillariophyceae species in Hanumangarh, with *Gomphonema* being most prevalent. Narayan and Barupal[13] conducted a year-long study in Jodhpur's Kaylana Lake using acid digestion and recorded 32 species across 15 genera, again noting *Gomphonema* as dominant. In Jaipur, Pareek et al.[14] studied Galta Kund, reporting 24 species from 11 genera, applying indices like Shannon-Wiener and Berger Parker Dominance. Pareek et al.[15] also assessed Ghaggar River water using the Hasle method, identifying 27 species, prominently

Nitzschia, *Navicula*, and *Synedra*. Singh et al. [11]] expanded to Delhi, documenting 20 species, some site-specific, with a diatom database proposed for forensic reference.

In the Doon Valley of the Western Himalayas, Kadam et al. [5] sampled microalgae from rivers and lakes, reporting 33 taxa across 27 genera including Chlorophyta, Bacillariophyta, and Myxophyta. They utilized the Algal Genus Pollution Index to evaluate pollution levels, identifying the Rispana and Bindal rivers as most polluted. Rana and Bhandari [16] examined ten water bodies in Mumbai, isolating 35 diatom species via acid digestion to establish a regional database for ecological and forensic applications. Sharma et al, [17] identified 12 species from ten river sites in Punjab, using compound microscopy and photomicrography to document genera like *Achnanthes*, *Cyclotella*, *Craticula*, and *Diatoma*.

Tandon et al. [18] contributed data from Chhattisgarh, identifying 34 species using a digital binocular microscope, with species classified under seven morphological categories including *Actinella*, *Encyonema*, and *Nitzschia*. The forensic significance of diatoms is underscored by Patel [19], who explained that their presence in internal organs indicates ante-mortem drowning. Acid digestion and microscopic examination confirm diatom entry via inhalation during life, enabling differentiation between ante- and post-mortem drowning. Saini and Rohilla [4] emphasized diatom profiling for drowning investigations, highlighting data from Punjab and national drowning statistics. They noted that comparing diatom species in victim tissues and water bodies aids in locating the drowning site, with implications extending to botany, medico-legal science, and environmental monitoring.

Objectives

1. To extract diatoms from the local water bodies of Jaipur.
2. To identify different species prevalently found in the local water bodies of Jaipur.
3. To map the diatom species from the various water bodies in relation to the geographical origin.

Materials & Methods:

Sample Collection: For the identification of diatom species in water bodies, five major water bodies from Jaipur are chosen for the examination purpose. These water bodies are popular for tourism, where the number of drowning cases, including Homicide cases, suicides and accidents are reported over time. Here are the names of selected water bodies:

- Dravyavati River
- Jal Mahal
- Tal Katora
- Maota Lake
- Jeerota Lake

Figure 1 - Dravyavati River

Figure 2 - Man Sagar Lake (Jal Mahal)

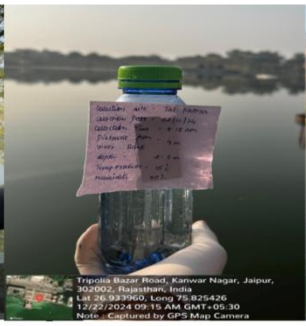


Figure 3 - Tal Katora

Figure 4 - Maota Lake(Amer lake)

Materials Required

For performing Acid digestion method to extract diatoms from collected water sample, few chemicals and apparatus are required namely:

Lab apparatus

- Beakers – 50 & 250 ml
- Test tubes
- Pipette
- Microscope slides
- Cover slips
- Test tube holder
- Aluminium foil
- Watch glasses to cover beakers to prevent contamination
- Light microscope
- Gloves
- Lab coat
- Spatula
- Eppendorf tubes (1.5-2ml)
- Measuring cyclinder
- Centrifugation machine

Chemicals

- Concentrated Nitric acid (HNO3)
- Formaldehyde
- Lugol's iodine solution
- Distilled water

Sample Preparation

Water sample for phytoplankton were directly collected from the water bodies in narrow mouthed polythene water bottle of 500ml. The sample preparation was done by using the standard protocol [2].

To prepare the sample, take 250ml beaker. Measure 50-100 ml of sample by measuring cyclinder and put water sample into beaker. Pour 2-3ml of 4% formaldehyde into sample for preservation of Sample over long period of time. Add 1-2 drops of Lugol's iodine solution into beaker containing water sample for breaking down organic matter before acid digestion. Measure 15ml of Concentrated Nitric Acid by measuring cyclinder and add into beaker containing water sample. Now, cover the beaker with a watch glass to avoid contamination and label each beaker sample with its specific location. Keep sample untouched for 24-48 hours [2]

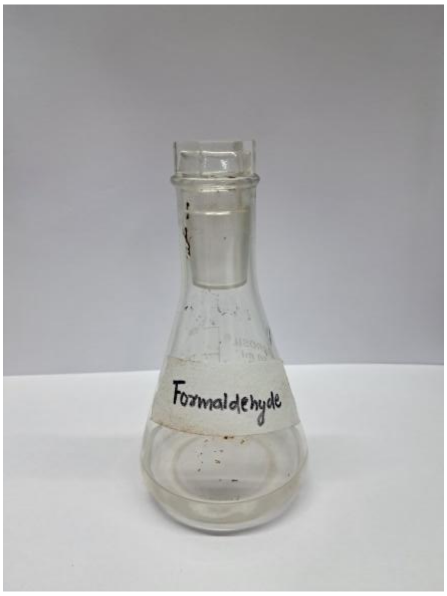


Figure 5 - Formaldehyde

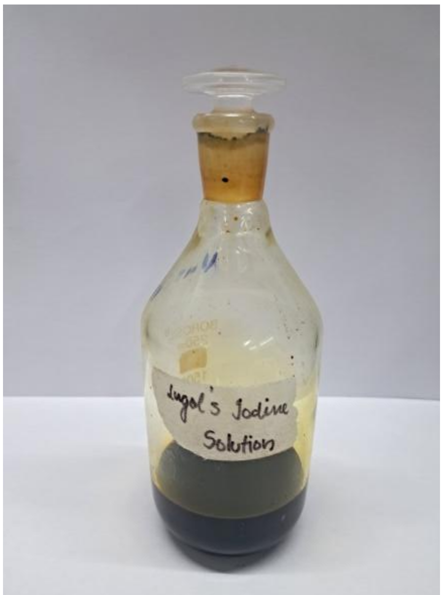


Figure 6 -Lugol's Iodine solution

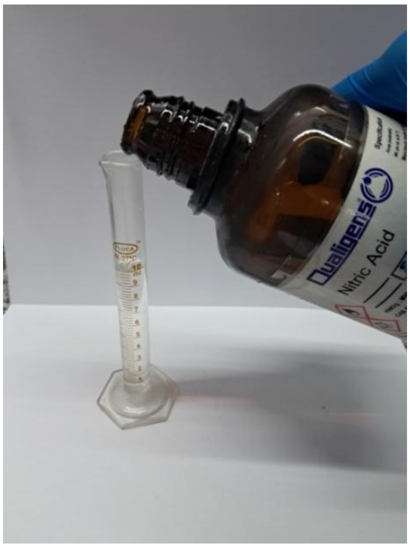


Figure 7 - Measuring of Conc. HNO3 into beaker

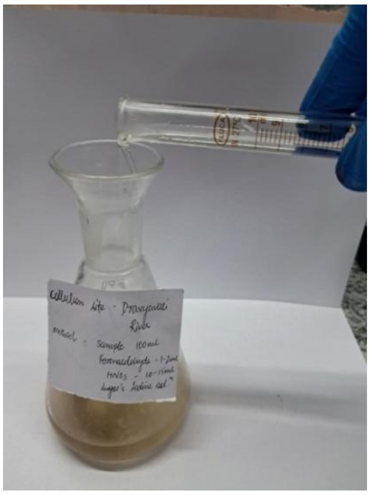


Figure 8 - Pouring of HNO3 into Water Sample

Sample Extraction:

The 10ml of sample prepared was taken from the beaker and transfered into test tube. Test tube was shaked over Vortex shaker for 1-2 min. The sample from a test tube of specific water body was transferred to Eppendorf tubes of approximately 2ml. The tubes are then centrifuged at 5000 rpm for 5 minutes. To obtain the pellet, the supernatant was discarded. The remaining pellet was then resuspended in distilled water and subjected to another round of centrifugation using the same parameters. The remaining pellet is kept safe, collected at the bottom of the Eppendorf tube and rest of all the supernatant left over to be discarded [3]

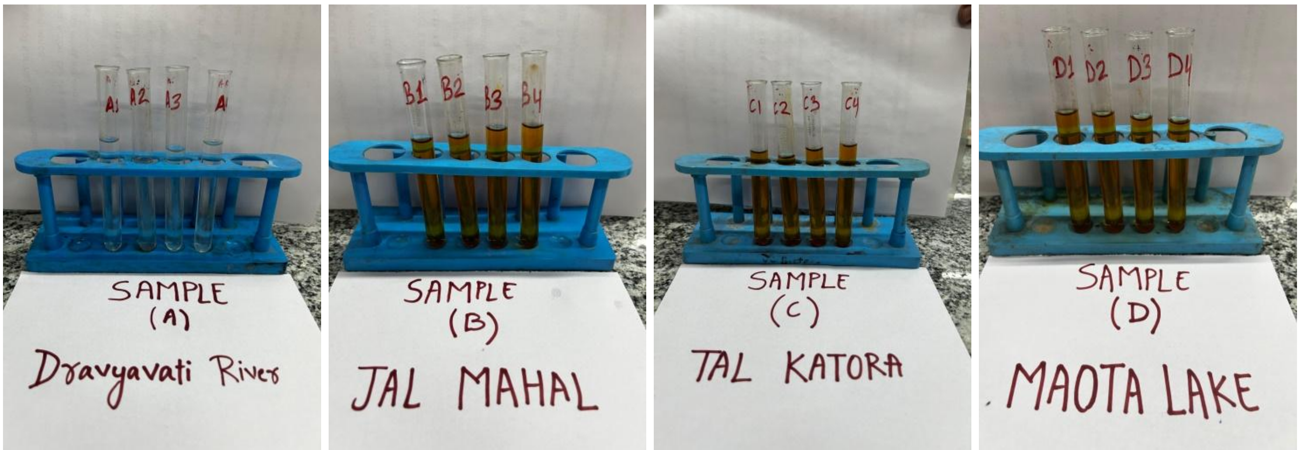


Figure 9 - Sample separated into test tubes for Vortex shaking

Figure 10 - Vortex shaker

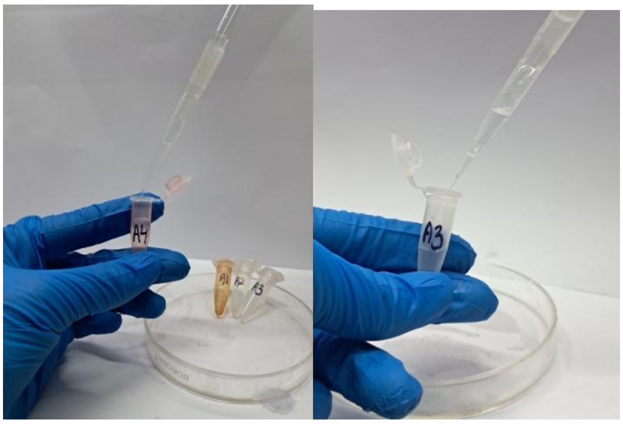


Figure 11 -Separation of Water Sample into Eppendorf Tubes

Figure 12 - Extracted Sample after Centrifugation

Figure 13 - Centrifuge Machine

Slide Preparation for Microscopic Examination:

Take a clean Microscope slide, put a drop of Centrifuged sample. Now, put a drop of methylene blue solution for pigmentation and the cover slip was placed over sample.

Microscopic Examination:

The prepared slides are then examined under a light microscope, applying immersion oil to achieve clear images at 10x and 40x magnifications. The Entire process of sample preparation, extraction, slide preparation and microscopic examination are conducted for each water body sample collected for diatom mapping individually.

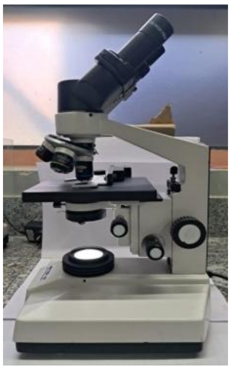


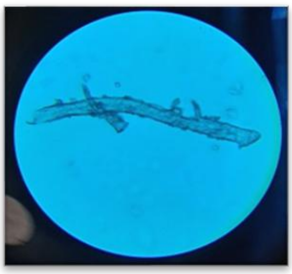
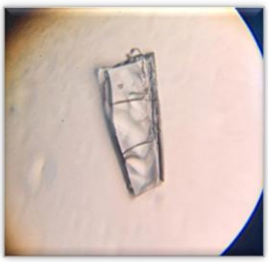
Figure 14 - Compound Microscope

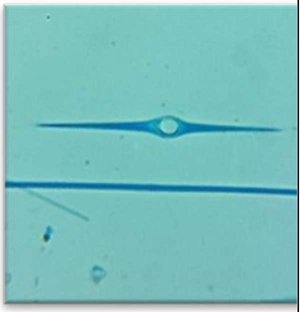
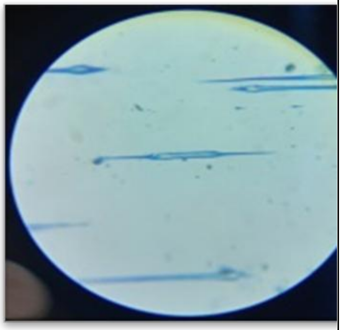
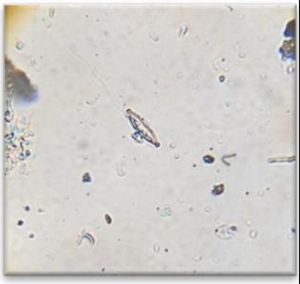
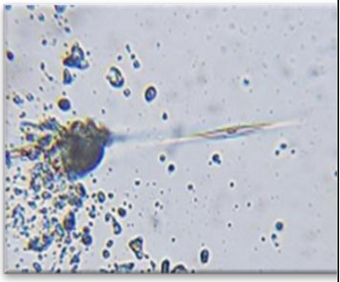
Result and Discussion

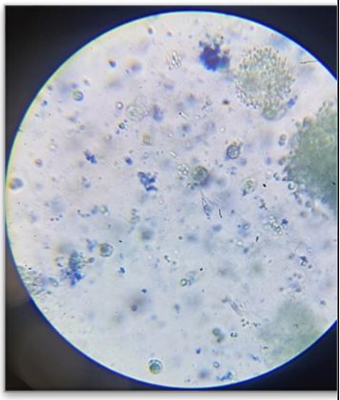
The present study was conducted at the department of Forensic Science, JECRC University, Jaipur in month of December,2024 & January,2025. The sampling was done from 5 different regions of Jaipur Rajasthan (Dravyavati River, Maota Lake, Tal Katora, Man Sagar Lake and Jeerota Lake). The sample extraction was done by using acid digestion method. And the examination was carried out using Compound and stereo microscope.

Table 1 represent the list of diatoms isolated and characterised from different water bodies of Jaipur. In the present study, 9 diatom genera were identified from 5 waterbodies of Jaipur, Rajasthan, India, examined under a light microscope at 10x and 40x. The Specific species genera with selected water bodies are: *Diatoma sp., Nitzschia, Actinella, Melosira* have been identified in Dravyavati River. Diatom genera *Navicula, Cylindrotheca closterium and Nitzschia* are found in Man Sagar Lake (Jal Mahal). Diatom Genera *Synedra, Fragilaria, Nitzschia, and Encyonema* are found in Tal Katora. Diatom Genera *Nitzschia , Navicula, Melosira* are found in Maota Lake (Amer Fort) and finally the diatom genera *Nitzschia* is the only one found in Jeerota Lake. Major diatom genera that are frequently found in all five water samples from water bodies are Nitzschia, Navicula and Melosira.

Table 1 - Analysis of Diatom Genera under Light Microscope:

<table>
<tr><td colspan="4">Below figures 1-3 shows Diatom Species Extracted from Water Sample Collected from Dravyavati River</td></tr>
<tr><td></td><td></td><td></td><td>NA</td></tr>
<tr><td>Fig. 1: Water Body:</td><td>Fig. 2: Water Body:</td><td>Fig. 3: Water Body:</td><td></td></tr>
</table>

Dravyavati River Diatom Genera: Nitzschia thermalis[6]	Dravyavati River Diatom Genera: Actinella[18]	Dravyavati River Diatom Genera: Melosira granulata[6]	
Below figures 4-7 shows Diatom Species Extracted from Water Sample Collected from Man Sagar Lake (Jal Mahal)			
			
Fig. 4: Water Body: Man Sagar Lake (Jal Mahal) Diatom Genera: Nitzschia thermalis[6]	Fig. 5: Water Body: Man Sagar Lake (Jal Mahal) Diatom Genera: Nitzschia closterium[6]	Fig. 6: Water Body: Man Sagar Lake (Jal Mahal) Diatom Genera: Cylindrotheca closterium[6]	Fig. 7: Water Body: Man Sagar Lake (Jal Mahal) Diatom Genera: Navicula sp. [18]
Below figures 8-11 shows Diatom Species Extracted from Water Sample Collected from Tal Katora			
			
Fig. 8: Water Body: Tal Katora Diatom Genera:	Fig. 9: Water Body: Tal Katora Diatom Genera:	Fig. 10: Water Body: Tal Katora	Fig. 11: Water Body: Tal Katora

Synedra acus[6]	Fragilaria intermedia[6]	Diatom Genera: Nitzschia palea[6]	Diatom Genera: Encyonema[14]
Below figures 12-15 shows Diatom Species Extracted from Water Sample Collected from Maota Lake (Amer Fort)			
			
Fig. 12: Water Body: Maota Lake (Amer Fort) Diatom Genera: Nitzschia palea[6]	Fig. 13: Water Body: Maota Lake (Amer Fort) Diatom Genera: Melosira varians [15]	Fig. 14: Water Body: Maota Lake (Amer Fort) Diatom Genera: Navicula cuspidate [18], [14]	Fig. 15: Water Body: Maota Lake (Amer Fort) Diatom Genera: Nitzschia linearis[14]
Below figures 16-19 shows Diatom Species Extracted from Water Sample Collected from Jeerota Lake			
			
Fig. 16: Water Body: Jeerota Lake Diatom Genera: Nitzschia linearis[14]	Fig. 17: Water Body: : Jeerota Lake Diatom Genera: Nitzschia palea[6]	Fig. 18: Water Body: Jeerota Lake Diatom Genera: Nitzschia kuetzingiana[16]	Fig. 19: Water Body: Jeerota Lake Diatom Genera: Nitzschia linearis[6]

The proposed study deals with the algological analysis of diatoms of Pond, River and Canal of Jaipur district. Planktonic algae communities, with total of 9 diatom genera were identified from 5 different water bodies of Jaipur, examined under light microscope at 10x and 40x magnification. The Specific species genera with selected water bodies are: *Diatoma sp., Nitzschia, Actinella, Melosira* have been identified in Dravyavati River. Diatom genera *Navicula, Cylindrotheca closterium and Nitzschia* are found in Man Sagar Lake (Jal Mahal). Diatom Genera *Synedra, Fragilaria, Nitzschia, and Encyonema* are found in Tal Katora. Diatom Genera *Nitzschia , Navicula, Melosira* are found in Maota Lake (Amer Fort) and finally the diatom genera *Nitzschia* is the only species found in Jeerota Lake. Major diatom genera that are frequently found in all five water samples from water bodies are Nitzschia, Navicula and Melosira.

Conclusion

Forensic Diatomology plays a vital role in resolving drowning cases mysteries. Drowning determine death cause and drowning location. Having enough diatoms in vital organs can confirm ante-mortem drowning. The scattering of diatoms in any body of water and their interrelation with those from the drowned body can help determine the site of drowning. This diatomological data provides a qualitative distribution of diatoms across various water bodies in the Jaipur region. In this study, Diatom diversity has been shown to be unique to locations important in the forensic investigation of drowning cases. Five water bodies of Jaipur have been effectively mapped for diatoms, and the following genera have been identified: Navicula, Melosira, and Nitzschia. The information obtained of Jaipur region can be an aid in creating a reference database or guide on diatoms for utilization in ecological assessment and identifying the location in the drowning cases.

For better results, 100x magnification should be used, more detailed information of the examined species will make more informative research. Methods Like SEM (Scanning electron microscope) & TEM (Transmission electron microscope) leads to study the inner structure of samples at high-resolution and 3D images of diatom frustules, revealing their fine structural details. Duration of collection and examination of Samples was of December,2024 i.e, of 1 month, Collection of samples from water bodies in different climatic conditions and time period will reveal more species and better results.

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