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ORIGINAL ARTICLE

A Pilot Study on Diatom Profiling from Stagnant Water Tanks Using Concentrated Hydrochloric Acid (37%) for Forensic Drowning Investigations

Amit Kumar Choudhary[1], Annwesha Mazumdar[2], Soma Roy[3], Ritika Roy[4], Vinod Dhingra[5], Ritika Gupta[6]

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ABSTRACT

Background: Diatom analysis is a significant forensic method for identifying antemortem drowning because it detects siliceous microalgae in internal tissues and ambient materials. While natural bodies of water have been widely researched, manufactured stagnant water tanks, which are widespread in cities, have received less attention. These tanks, which are frequently separated from active water supply systems, provide distinct biofilm-rich microhabitats that may support a wide range of diatom assemblages.

Aim: This study looks at the forensic possibilities of such surroundings utilising hydrochloric acid (37%) digestion to isolate diatoms.

Method: Microscopic investigation of samples from 48 stagnant water tanks in New Town, Kolkata revealed a wide range of diatom species.

Results: Dominant genera included *Achnanthidium minutissimum*, *Cymbella tumida*, and *Gomphonema sierrianum*, with site-specific occurrences of *Surirella*, *Coscinodiscus*, and *Cymbella auxospore*. The existence of both motile and non-motile groups (monoraphids, biraphids, and centric forms) suggests ecological resilience and biofilm-mediated survival. Spatial variation in species distribution was found throughout cardinal zones, indicating microclimatic and structural impacts.

AUTHOR'S AFFILIATION:

[1] Forensic Professional, Central Forensic Science Laboratory, Kolkata, West Bengal, India.

[2] Post-graduate, National Forensic Sciences University, India.

[3] Assistant Director & Scientist C, Central Forensic Science Laboratory, Kolkata, India.

[4] M.Sc. Student, The West Bengal National University of Juridical Sciences, India.

[5] S.S.O, PTS Tighra, Gwalior, Madhya Pradesh, India.

[6] S.S.A, Forensic Science Laboratory, NCT Government of Delhi.

CORRESPONDING AUTHOR:

Annwesha Mazumdar, Post-graduate, National Forensic Sciences University, India.

E-mail: annweshamazumdar334@gmail.com

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The acid digestion approach protected frustule integrity while also enabling semi-quantitative profiling suited for forensic comparison.

Conclusions: Stagnant water tanks provide biologically diverse and forensically relevant microhabitats for diatom analysis. Their incorporation in forensic methods improves drowning site localisation, particularly in metropolitan areas without natural water bodies. The result encourages further comparative research in isolated areas and suggests AI-assisted D-mapping as a scalable technique for automated diatom identification and geolocation. Standardised sampling and multidisciplinary collaboration are required to operationalise diatom evidence in medico-legal investigations.

KEYWORDS

• Autopsy • Diatoms • Drowning • Prima facia • Forensic limnology • Medico-legal cases • Acid – digestion method • Forensic microcosms

INTRODUCTION

Drowning is a complicated medico-legal diagnosis that forensic investigators frequently face, particularly when outward indications are equivocal or the circumstances surrounding the occurrence are unclear. Traditional autopsy findings, such as frothy fluid in the airways, water in the stomach, and washerwoman's skin, are insufficient to conclude a case.[1] Diatom analysis is one of the most scientifically sound procedures available to enhance drowning diagnosis. It uses the microscopic presence of diatoms unicellular, silica-walled algae in human tissues and ambient water samples to demonstrate antemortem water inhalation.[2] Other potential diatom species in the subjects or any other pertinent material may connect the crime scene to the prima facia.

The essential premise of this approach is simple yet powerful: diatoms, because to their nano-scale size and siliceous cell walls, may cross alveolar-capillary barriers during active drowning and reach systemic circulation.[3] Their presence in distant organs such as bone marrow, brain, and kidneys, when compatible with species discovered at the putative drowning location, supports a credible diagnosis of drowning as the cause of death.[4] Diatom Mapping (D-mapping) is a modern forensic technique that includes systematic cataloguing of diatom species across a variety of aquatic habitats.[5] D-mapping, which creates a geographic database of diatom assemblages, allows forensic professionals to compare diatoms retrieved from biological samples to those found in specific water bodies.[6] This approach improves the accuracy of drowning site identification, facilitates crime scene reconstruction, and distinguishes between antemortem and postmortem immersion. In urban and biologically varied areas, where water bodies differ in physicochemical qualities and human effect, D-mapping provides a scalable approach for forensic preparedness. It combines ecological profiling, seasonal variation tracking, and geotagged sampling to provide a strong reference framework.

Despite its importance, diatom analysis is underutilised and frequently poorly executed, especially in low-resource settings or in situations involving non-natural water bodies such as constructed tanks or urban reservoirs. The forensic utility of stationary water bodies such as rooftop tanks in homes, storage containers in office buildings, or attractive water features in parks has gotten little empirical scrutiny. These limited sources may become crucial in instances involving tiny children, the elderly, or suspected foul play where typical water supplies are not present. Under such cases, knowing diatom biodiversity and survival under stationary settings is critical to enhancing investigative accuracy. In many urban and peri-urban settings, stagnant water tanks are often disconnected from regular water supply systems due to disuse, neglect, or infrastructural limitations. Even where supply lines remain functional, these tanks frequently serve as secondary reservoirs accumulating rainwater, runoff, or intermittent municipal inflows. This irregular water input, coupled with prolonged stagnation, creates unique microhabitats rich in organic debris, algae, and microbial communities including diatoms. Their covert nature and intermittent accessibility make them potential sites for body disposal or accidental submersion. Investigative teams must therefore consider the possibility of involvement of isolated artificial aquatic environments when evaluating drowning cases, especially in the absence of proximate natural water bodies. Trace materials recovered from these locations including diatoms adhering to belongings like clothing, shoes, or accessories may provide critical circumstantial evidence in establishing scene reconstruction and postmortem submersion.

Incorporating these niche water bodies into forensic protocols not only enriches the ecological mapping of diatom species but also aids in tracing crime scene localization in resource-constrained or geographically complex environments. Such water bodies whether concealed on rooftops, basements, or within minimally accessible compounds pose a forensic significance that extends beyond ecological interest.

This pilot study focuses on characterizing the diatom assemblages present in stagnant water tanks an artificial and often overlooked aquatic environment. These tanks, commonly situated on the top of residential buildings, within office compounds, or in community parks, accumulate water that may remain unused for months, resulting in a confined ecological niche. Over time, these water bodies develop complex biofilms, which are structured communities of microorganisms including bacteria, algae, and diatoms encased in extracellular polymeric substances (EPS).[7] Biofilms adhere to submerged surfaces and often harbor both dominant and rare diatom taxa, influenced by factors such as nutrient limitation, light exposure, temperature fluctuations, and anthropogenic inputs.[8]

The presence of a biofilm alters species distribution in stagnant water systems by providing microsites for diatom colonization and resilience.[9] Certain species thrive within EPS-rich zones due to protective encasement and enhanced nutrient retention, while others appear only transiently, reflecting episodic changes in water chemistry or surface runoff.[10] Consequently, stagnant water tanks can exhibit high diatom biodiversity and distinct site-specific signatures making them potentially valuable for forensic source matching in localized drowning cases.[11]

The study used the acid digestion approach, namely concentrated hydrochloric acid (HCl 37%), which is designed to eliminate organic materials while conserving siliceous diatom frustules for microscopic identification. Acid digestion using HCl is a quick and cost-effective way to prepare environmental samples. While HCl digestion may not eliminate all organic residues as well as a mixed-acid approach, it does maintain the delicate frustule form, making it appropriate for qualitative and semi-quantitative surveys. The method was selected based on procedural reproducibility and its routine applicability. The acid digestion approach allows for both qualitative and semi-quantitative insights into species morphology and abundance across sampling sites. This approach was chosen for its accessibility and ease of analysis in forensic laboratories without specialised infrastructure.

Through this investigation, the study seeks to:

- Determine the presence, diversity, and morphological patterns of diatom species in stagnant tank water
- Establish a reference baseline for common and unique species found in such artificial water bodies
- Explore the viability of these findings for future forensic casework involving drowning in confined spaces
- Provide preliminary insights for police personnel and first responders on preserving water samples and recognizing sites with forensic relevance during early-stage investigations

Furthermore, this aims to study forensic limnology by contextualising how diatom populations endure and change in static settings across time. The capacity of some diatom species to live or thrive in isolated water systems may imply resistance to desiccation, poor nutrient flow, or algae competition. These elements are relevant to investigations, not just in drowning instances, but also in broader applications such as postmortem interval estimates, crime scene reconstruction, and event localisation. Forensic recovery must not be limited to biological samples from internal organs alone; belongings and exposed body surfaces offer vital clues, especially when systemic dissemination is not evident. The forensic implications of diatom diversity are strengthened when paired with variables such as geographical location, water pH, turbidity, and biofilm consistency, which can aid in species identification and the exclusion of alternate locations. In this regard, stagnant water bodies can serve as forensic microcosms, offering controlled ecological datasets that supplement broader environmental models.

Finally, the study emphasises the value of multidisciplinary collaboration among forensic pathologists, environmental scientists, law enforcement officers, and microbiologists. For diatom analysis to be effective, it must be integrated into operational workflows, with specific criteria for sample collection, storage, and analytical interpretation. The study's findings are expected to influence such practices and spur talks about incorporating environmental forensics into standard police processes, particularly in cases when traditional drowning signs are ambiguous.

Forensic insights for Law Enforcement in Drowning Cases

Drowning remains one of the most complex causes of death to establish, particularly when external signs are absent or decomposition has progressed. Law enforcement personnel play a critical role in the early stages of investigation, yet there is often a knowledge gap regarding the forensic indicators, evidentiary value, and procedural nuances surrounding drowning deaths. This section outlines practical insights informed by forensic hydrobiology and diatom analysis that can enhance police efficiency and accuracy in managing suspected drowning cases especially those occurring in stagnant or confined water sources like overhead household tanks, industrial reservoirs, septic systems, and other structures that contain stagnant water. Unlike open water basins, these sources have restricted circulation, low biological turnover, and typically protracted periods of water retention conditions that have a profound impact on microbial and diatom profiles. The current work focusses on stagnant domestic water tanks, which are important forensic substrates due to their accessibility, isolation from ambient factors, and usefulness in urban drowning diagnoses and diatomological profiling.

Scene Management and Sample Preservation:

Timely water sampling is critical. Temperature, light exposure, and biofilm activity can all have an impact on diatom populations in water. Delays in sample collection might jeopardise forensic matching. Maintaining the integrity of stagnant water tanks, including surface films, sediment layers, might aid in species stratification that may represent entrance dynamics or points of contact.[12] First responders should follow standardised sampling techniques, such as using clean, labelled containers and collecting samples at several depths.

Recognizing Potential Drowning Environments:

Law enforcement must broaden the scope of drowning investigations to include non-conventional aquatic settings: storage tanks, sumps, urban reservoirs, and decorative ponds. These sources may be overlooked yet offer high evidentiary yield. Localized water sources should be surveyed in cases involving children, elderly individuals, or persons with mobility impairments, especially when foul play is suspected.[13]

Diatom Analysis as Investigative Tool:

Diatom analysis can differentiate between drowning and postmortem immersion, particularly when internal tissues especially lungs and bone marrow from sternum and femur contain diatoms matching those from the suspected water source.[14] Forensic experts may collaborate with police to interpret species diversity, abundance, and unique taxa that suggest site-specific immersion. In ambiguous cases, the absence of diatoms in systemic circulation may support alternative causes of death, such as disposal after homicide.[15]

Operational Training and Cross-Sector Protocols:

Police personnel would benefit from basic training in forensic limnology, including the significance of microscopic algae, site mapping, and communication with forensic biologists.[16] Development of inter-agency SOPs between law enforcement, forensic laboratories, and medico-legal units can streamline evidence flow and reduce investigative delays. Routine inclusion of environmental forensics in postmortem protocols especially in water-associated deaths can improve conviction rates and reduce misclassification.

Ethical and Legal Considerations:

Proper handling of aquatic forensic evidence must comply with chain-of-custody standards, as diatom data may contribute to court proceedings. Clear documentation, including location photographs, sample volumes, and collection timestamps, enhances judicial admissibility and investigative transparency.

Forensic Relevance of Diatom Analysis in Water-Associated Cases

Water samples subjected to diatom profiling are typically collected from crime scenes that may represent primary, secondary, or tertiary locations. Common sample sources include waterlogged environments such as stagnant roadside puddles, sewer drains, urban water pools, and, most frequently, overhead or subterranean water storage tanks. These environments serve as potential reservoirs for diatom populations, which can provide valuable ecological and temporal markers for forensic investigation.

Diatom evidence has relevance in multiple forensic contexts:

- **Ante-Mortem Drowning Cases:** Diatoms present in internal tissues (e.g., lungs, bone marrow) can corroborate drowning as the cause of death. Such cases may involve homicidal drowning, accidental submersion, or homicidal acts not amounting to murder under legal classification.
- **Post-Mortem Disposal in Aquatic Environments:** When cadavers are disposed of in water postmortem, diatom presence may indicate contamination or environmental exposure. In select instances, cadaveric spasm or tightly clenched anatomical regions may retain clue material, allowing reconstruction of contact with specific water sources.
- **Accidental Death Scenarios:** Cases involving alcohol-induced drowning, misstep-related submersion, or vehicular accidents resulting in immersion can benefit from diatom analysis to determine aquatic exposure and assist in medico-legal reconstruction.
- **Crime Scene Traceability and Scene Transition Analysis:** Diatom species diversity and distribution can aid in tracking the transition between multiple crime scenes. Comparative profiling of water samples from suspected locations allows for inference of movement patterns and contact chronology, supporting investigative hypotheses regarding primary or secondary scene interactions.

METHODS

Diatom analysis was conducted using a trinocular compound microscope (Dewinter Optical Inc., Model DEW-2000) equipped with a high-resolution digital camera was employed for morphological identification and documentation of diatom frustules. Sample concentration and clarification were performed using a Sigma Laboratory Centrifuge (Model 2-16K), while acid digestion and incubation procedures were carried out in a Stuart Water Bath Incubator.

Glassware including borosilicate test tubes, and beakers were used for sample handling, digestion, and transfer. Reagents used for frustule isolation included hydrochloric acid (HCl, 37%, Empata Chemicals) for mineral dissolution and organic matter removal. HPLC grade distilled water was used. Ethanol (95%, analytical grade) was utilized for dehydration and cleaning of samples prior to mounting. Diatom frustules were mounted on glass slides (Blue Star) using Naphrax mounting medium, and cover slips were sealed using a slide warmer to ensure permanent fixation. Adjustable micropipettes (0.1–10mL) were used for precise reagent handling during all stages of sample preparation.

Sample Collection and Source Characterization

In the pilot study stagnant water samples were collected from overhead storage tanks located in residential and semi-public urban settings from 48 selected water tanks in the New Town area of Kolkata, West Bengal **(Table 1-4).** These tanks had remained unused for months, allowing for natural accumulation of organic matter, microbial biofilm, and potential diatom populations.

Approximately 20–30 mL of water was collected aseptically from the mid-depth zone of each tank using sterile polypropylene containers. To minimize external contamination, containers were sealed immediately and transported under refrigerated conditions (4–6°C) to the laboratory for further processing within 24 hours.

Diatom Extraction via Double Acid Digestion Method

The extraction protocol utilized a sequential hydrochloric acid digestion technique optimized for isolating siliceous diatom frustules from highly organic stagnant water samples. An overview of the entire methodological workflow is presented in **Figure 1**, is shown below.

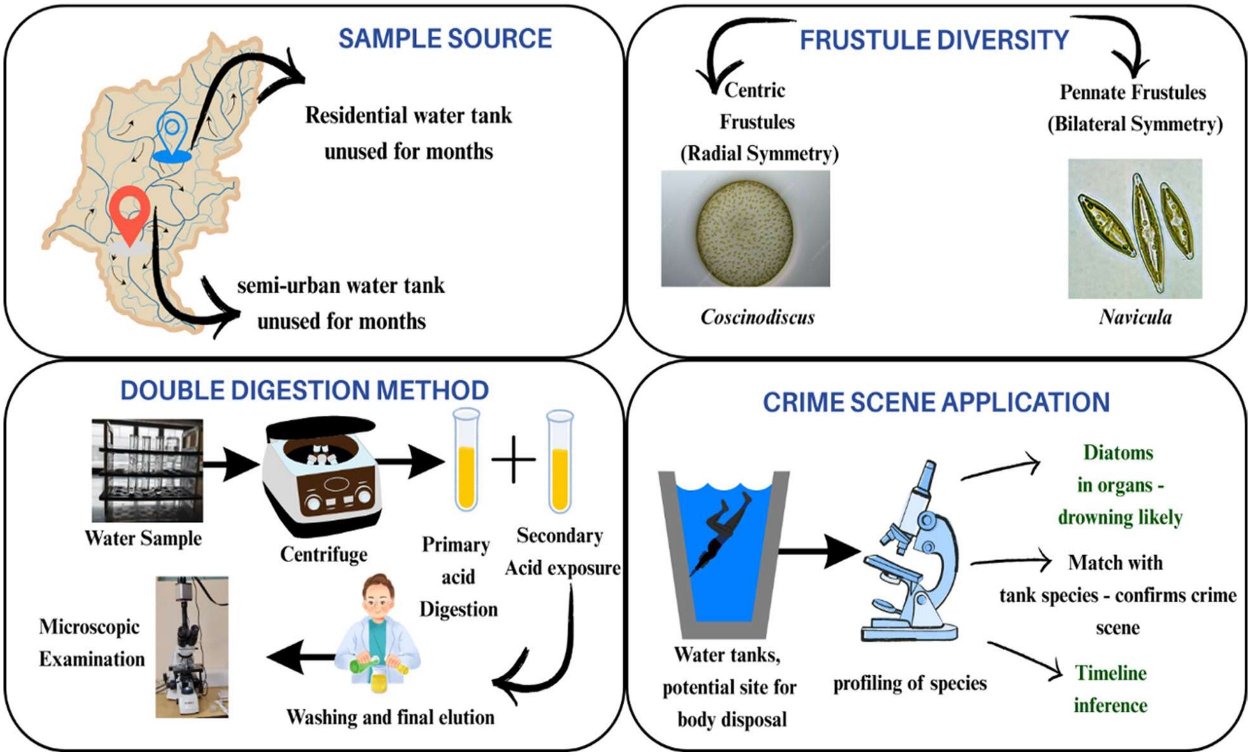


Figure 1: Encapsulates the full methodological approach employed in this study

Initial Preparation

- **Aliquot Volume:** 2–3 mL of water sample per test
- **Homogenization:** Each aliquot was vortexed vigorously for 30 seconds
- **Centrifugation:** Samples were centrifuged at 8000–10,000 rpm for 8–10 minutes, adjusted based on sediment load and visual turbidity
- **Supernatant Removal:** Carefully decanted to retain the pellet containing suspended particulates and possible diatom content

Primary Acid Digestion

- **Reagent:** Concentrated Hydrochloric Acid (HCl, 37%)
- **Volume:** Equal to initial sample (2–3 mL)
- **Action:** Vortexed until pellet fully dissolved
- **Incubation:** Left at ambient room temperature (25–27°C) for 12–16 hours overnight

Secondary Acid Exposure

- **Vortexing:** Re-homogenized after overnight digestion
- **Centrifugation:** Repeated at same speed and duration
- **Pellet Treatment:** Added 1–1.5 mL of HCl (37%), vortexed until fully dispersed
- **Thermal Incubation:** Maintained at 56°C for 5–6 hours in a calibrated water bath to enhance organic matrix breakdown without damaging siliceous frustules

Washing and Final Elution

Following digestion, the samples underwent a standardized washing protocol:

- **Water Type:** Distilled or HPLC-grade water

- **Number of Washes:** 2–3 cycles
- **Process:** Each wash cycle involved vortexing, centrifugation (8000–10,000 rpm, 8–10 mins), and supernatant removal
- **Final Volume:** 100–200 µL of distilled water added to the pellet
- **Clarification Spin:** Final centrifugation for 2 minutes to settle non-diatom debris
- **Storage:** Prepared samples were stored at 4°C until microscopic examination

Microscopic Examination and Morphological Profiling

Processed samples were mounted on glass slides and observed under bright field microscopy at 20× and 40× magnification. Diatom species were identified based on frustule morphology, symmetry, and ornamentation features. Diatom species identification is based on the qualities of diatoms, such as form, symmetry, pore arrangement, and other morphological traits listed in the accompanying table. Several keys have been produced, including an illustrated reference to common diatoms of Peninsular India, which has been linked with online keys for Diatoms of North America. For this investigation,[17] diatom identification keys were used. The information is presented in **Table 5.**

Table 1: Sampling sites in New Town Area East zone

Residential areas (RA)	Coordinates (Latitude, Longitude)
RA 1	
RA2	
RA3	
RA4	
RA5	
RA6	Starting point -22.588997, 88.522682 Ending point-12.22.652124, 88.546745
RA7	
RA8	
RA9	
RA10	
RA11	
RA12	

Table 2: Sampling sites in New Town Area West zone

Residential areas	Coordinates (Latitude, Longitude)
RA13	
RA14	
RA15	
RA16	
RA17	
RA18	**Starting point-** 13. 22.650759, 88.54335
RA19	**Ending point-** 22.615213, 88.448326
RA20	
RA21	
RA22	
RA23	
RA24	

Table 3: Sampling sites in New Town Area North zone

Residential areas	Coordinates (Latitude, Longitude)
RA25	
RA26	
RA27	
RA28	
RA29	
RA30	Starting point- 22.619761, 88.448334 Ending point- 22.582359, 88.458138
RA31	
RA32	
RA33	
RA34	
RA35	
RA36	

Table 4: Sampling sites in New Town Area South zone

Residential areas	Coordinates (Latitude, Longitude)
RA37	
RA38	
RA39	
RA40	
RA41	
RA42	Starting point- 22.598228, 88.483711 Ending point- 22.596878, 88.504555
RA43	
RA44	
RA45	
RA46	
RA47	
RA48	

Table 5: Morphological Features of Diatom Groups[17]

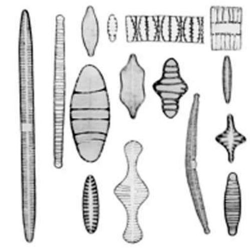
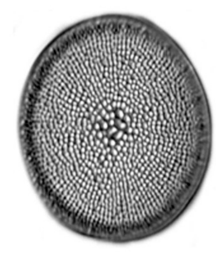
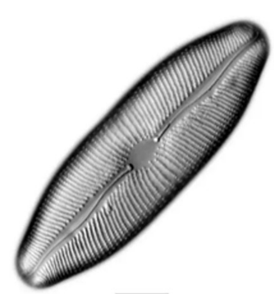
Sl. No.	Diatom Group	Symmetry	Raphe System	Other Features	Examples	Diagram
a.	Araphid	Bilateral (symmetric about a line)	Absent	Cells lack motility; rimoportulae may be present	*Diatoma, Fragilaria, Hannaea, Pseudostaurosira*	
b.	Asymmetrical 1. Biraphid	Asymmetrical to apical or transapical axis	Well developed	Some genera possess apical porefields that secrete mucilaginous stalks	*Afrocymbella, Amphora, Cymbella, Didymosphenia, Encyonema, Gomphonema*	
c	Centric	Radial	Absent	Cells lack significant motility	*Melosira, Aulacoseira, Cyclotella, Discostella*	
d.	Monoraphid	Bilateral	Present on one valve; absent on the other	Heterovalvar ornamentation	*Achnanthes, Achnanthidium, Cocconeis*	
e.	Symmetrical Biraphid	Bilateral; symmetrical to both apical and transapical axis	Well developed	Cells highly motile	*Amphipleura, Diatomella, Diploneis, Gyrosigma, Navicula, Pinnularia*	
f.	Nitzschioid	Bilateral; usually symmetrical to both axes	Well developed; near valve margin	Raphe enclosed within a canal; may be raised onto a keel	*Hantzschia, Nitzschia, Tryblionella*	

table cont...

Sl. No.	Diatom Group	Symmetry	Raphe System	Other Features	Examples	Diagram
g.	Surirelloid	Bilateral	Extremely well developed; enclosed in canal	Raphe positioned around entire valve margin and raised onto a keel	*Cymatopleura, Surirella*	
h.	Epithemioid	Bilateral; asymmetrical to apical axis	Well developed; enclosed in canal	Raphe positioned near valve margin	*Epithemia, Rhopalodia*	
i.	Eunotioid	Bilateral; often asymmetrical to apical axis	Short; weak motility	Raphe located on valve mantle and face; cells may have multiple rimoportulae	*Eunotia*	

RESULTS

Table 6: Distribution of diatoms found in Residential Areas at New Town, Kolkata

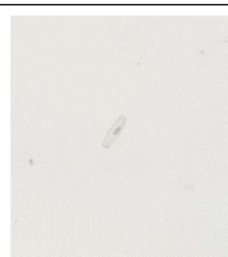
Sl. no.	Present in Samples	Enhanced images (For better comparison & studies)	Original pictures	Species	Class
I.	S1-S11, S-13, S15-S24, S28-34, S38-S41, S43-S49 (all most in all)			Achnanthidium minutissimum	Bacillariophyceae
II.	S1- S13, S15-S26, S28-S31, S33-S41, S43-S49 (almost in all)			Cymbella tumida	Bacillariophyceae

table cont...

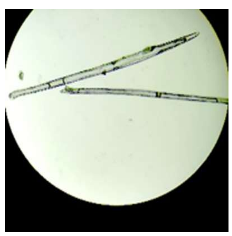
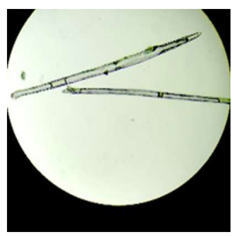
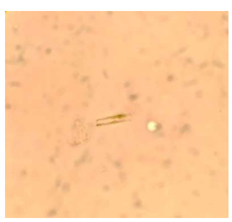
Sl. no.	Present in Samples	Enhanced images (For better comparison & studies)	Original pictures	Species	Class
III.	All most in all except for S37 & S49			Gomphonema sierrianum Stancheva & Kociolek, sp.	Bacillariophyceae
IV.	S7, S12, S13, S16, S17, S18, S20 S26, S27, S32, S35-47			Amphora	Bacillariophyceae
V.	All most in all except S48			Encyconema minimum	Bacillariophyceae
VI.	S13-S17, S-20-S24, S32-S40, (all most all in western side & Northen side)			Pseudo-nitzschia	Bacillariophyceae
VII.	S13-S18, S21-S24 (more dominant in western side)			Eunotia	Bacillariophyceae
VIII.	All most in all (missing in western side in three samples)			Eunotia (baccilariophyta)	Bacillariophyceae
IX.	Only specifically found in southern samples			Cymbella auxospore	Bacillariophyceae

table cont...

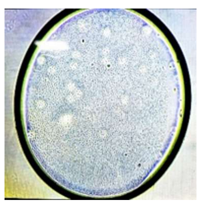
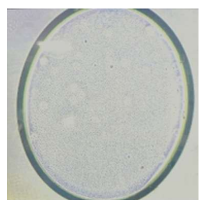
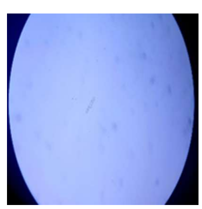
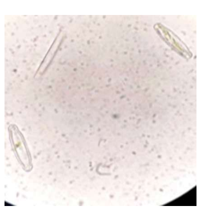
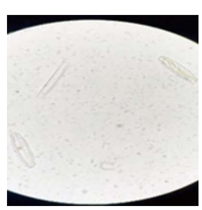
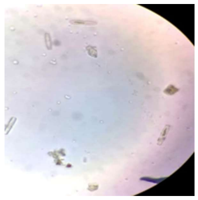
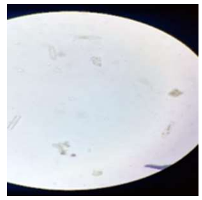
Sl. no.	Present in Samples	Enhanced images (For better comparison & studies)	Original pictures	Species	Class
X.	All most in all except for S49 & S50			Nitzschia amphibia	Bacillariophyceae
XI.	Dominately present in Eastern side but still present in all samples			Pinnularia	Bacillariophyceae
XII.	Present in mostly in Northern side			Surirella	Bacillariophyceae
XIII.	Unique one found in three samples (S11, S27, S28)			Coscinodiscus	Coscinodiscophyceae
XIV.	Dominately found in southern samples			Nitzchia cf. lorenziana	Bacillariophyceae
XV.	Found in southern samples			Synedra	Bacillariophyceae
XVI.	Found in all most all the samples			Eunotia	Bacillariophyceae
XVII.	Picture depicting mixed diatoms.			Amphora, Eunotia, etc.	Bacillariophyceae

table cont...

Sl. no.	Present in Samples	Enhanced images (For better comparison & studies)	Original pictures	Species	Class
XVIII.	Picture depicting mixed diatoms.			Amphora, etc.	Bacillariophyceae

DISCUSSION

This pilot research reveals the forensic use of stagnant water tanks as sources for diatom profiling in drowning cases. Due to privacy considerations, detailed geospatial coordinates of sample collection sites are not disclosed. The acid digestion approach, which uses hydrochloric acid (37%), was efficient in separating siliceous frustules from highly organic water samples while retaining morphological integrity for microscopic identification. Despite the simplicity of the methodology, the approach produced a rich collection of diatom species from all four cardinal zones in the New Town region. Following acid digestion, diatom samples were examined microscopically. The original photographs were subsequently processed and enhanced to improve visual clarity, thereby facilitating accurate comparison and detailed morphological analysis. In **Table 6** the observed dominance of *Achnanthidium minutissimum*[I], *Cymbella tumida*[II], and *Gomphonema sierrianum*[III] across multiple samples suggests ecological resilience and adaptability to stagnant, nutrient-variable conditions. The presence of *Pseudo-nitzschia*[VI] and *Eunotia*[VII/VIII/XVI] in western and northern zones, respectively, indicates spatial heterogeneity in species distribution, likely influenced by tank exposure, biofilm development, and microclimatic factors. Notably, *Surirella*[XII] and *Coscinodiscus*[XIII] appeared in limited southern samples, suggesting niche colonization or episodic influx. The discovery of both motile and non-motile diatom groups including symmetrical biraphids[e], monoraphids[d], and centric[c] forms confirms the concept that stationary tanks can maintain complex biofilm ecosystems capable of sustaining different diatom populations over long periods of time. These findings align with previous study on biofilm-mediated diatom survival in low-flow aquatic systems.[8] From a forensic aspect, the existence of site-specific diatom markers increases the usefulness of D-mapping in urban drowning cases. The capacity to match diatom species from water tanks to those obtained from biological samples or items can help with crime scene reconstruction, particularly when natural water bodies are not present.

The study also underscores the importance of timely sample collection and standardized processing protocols. Delays in sampling or improper handling can alter diatom composition, compromise forensic matching, and reduce evidentiary value. The integration of GPS (global positioning system)-tagged site data with morphological profiling offers a scalable framework for forensic limnology in resource-constrained settings.

CONCLUSIONS

This pilot research identifies stagnant water tanks as ecologically and forensically important microhabitats for diatom profiling in drowning cases. The effective use of hydrochloric acid (37% concentration) for frustule isolation illustrates the method's practicality in low-resource forensic laboratories while maintaining morphological integrity for qualitative and semi-quantitative analysis. The study discovered a varied array of diatom taxa, including both ubiquitous and site-specific species, in residential areas in New Town, Kolkata.

The presence of dominant genera such as *Achnanthidium*[I], *Cymbella*[II], and *Gomphonema*[III], alongside rarer taxa like *Surirella*[XII] and *Coscinodiscus*[XIII], underscores the ecological complexity of stagnant tanks shaped by biofilm formation, nutrient stratification, and microhabitat variability. These findings support the forensic value of artificial aquatic habitats, especially in metropolitan areas where natural water bodies may be missing or unavailable.

Importantly, the study emphasises the possibility of future comparison research using stagnant water samples from remote or non-urbanized areas. Due to changes in biomagnification processes and biofilm architecture, such settings may include both overlapping and distinct diatom assemblages when compared to urban water tanks, clogged drainage systems, or semi-natural water bodies such as ponds and riverbanks. These ecological differences can improve forensic differentiation and the precision of drowning site identification.

Looking ahead, the combination of Diatom Mapping (D-mapping) and artificial intelligence (AI) platforms represents a potential future in forensic limnology. AI-driven picture identification and pattern-matching algorithms may automate the comparison of diatom morphotypes to curated databases, allowing for quick estimation of likely sampling locations. When combined with geotagged biological metadata, such methods can help with geolocation prediction, scene reconstruction, and the exclusion of alternative immersion sites particularly in remote or disguised aquatic habitats. This combination of ecological profiling, digital automation, and forensic inquiry has the potential to elevate diatom research from a specialised approach to a scalable, operational tool for law enforcement and medico-legal practitioners. Future research should concentrate on increasing regional diatom databases, testing AI-assisted identification methods, and standardising sample collecting and interpretation protocols across a variety of aquatic environments.

List of Abbreviations:

HCl - Hydrochloric acid
D-mapping - Diatom mapping
EPS - Extracellular Polymeric Substances
RA - Residential areas
GPS - Global positioning system
AI - Artificial intelligence

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