Forensic Analysis of Diatoms in different ponds at Durg Rural area of Chhattisgarh

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ABSTRACT

Main body Diatoms are eukaryotic, unicellular, photosynthetic (autotrophic) creatures that are frequently categorized as algae. The scientific name for them is Bacillariophyceae. **Background** Diatom research can be used for medical-legal purposes to resolve situations involving drowned or abandoned bodies that have not been claimed. The identification of diatoms within the organs can help a drowning death diagnosis, a procedure known as the "diatom test." This study was conducted in the department of Forensic Science student included the extraction and identification of diatoms from the collected water samples from different ponds at Durg Rural area in Chhattisgarh.

Conclusion This comprehensive review provides an in-depth analysis of the principles, methodologies, applications, and challenges associated with the field of forensic diatomology. Research in this area has led to the creation of a database of diatom species, which enhances the forensic potential of diatoms by providing a reference for identifying species found in forensic samples. The study of diatoms in the Durg rural area of Chhattisgarh not only enriches the existing database but also provides a valuable tool for forensic applications. This research supports the use of diatom analysis in forensic investigations, offering a scientific basis for linking individuals to specific water bodies and aiding in the determination of drowning as a cause of death.

Keywords: Diatoms, ponds, Durg Rural, Chhattisgarh, drowning death.

INTRODUCTIONS

Diatoms are unicellular, photosynthetic algae found widely in aquatic environments. They are significant in forensic science, especially for diagnosing drowning and identifying the site of drowning, because their presence in body tissues can indicate inhalation of water during submersion **Ludes et al., (2024)[1]; Bogusz et al., (2023)[2]; Girela-López et al., (2020)[3].** Diatoms are also used as environmental indicators due to their sensitivity to ecological conditions **(Bogusz et al., 2023)[2].**

Diatoms are microscopic, single-celled organisms. While specific size ranges are not detailed in the provided abstracts, they are consistently described as "unicellular" and "microscopic," requiring light or electron microscopy for observation and identification **Zhou et al., (2019)[4]; Tournois et al., (2023)[5]; Ludes et al.,(2024)[1]; Girela-López et al., (2020)[3]**. Their small size allows them to enter the bloodstream and organs during drowning, which is why they are useful in forensic investigations **Ludes et al., (2024)[1]; Girela-López et al., 2020)[3].**

Special Characteristics

- **Silica Frustules:** Diatoms possess unique, rigid cell walls called frustules made of silica, which are highly resistant to decay and can be identified even after significant decomposition **Jiang et al., (2020)[6]; Tournois et al., (2023)[5]; Ludes et al., (2024)[1].**
- **Species Diversity:** There are many species of diatoms, each with distinct morphological features. This diversity allows for site-specific identification in forensic cases **Jiang et al., (2020)[6]; Tournois et al., (2023)[5]; Bogusz et al., (2023)[2]; Li et al., (2019)[7].**
- **Environmental Indicators:** Diatoms' presence and species composition can reflect specific environmental conditions, making them valuable for linking a body to a particular water source **Bogusz et al., (2023)[2].**
- **Forensic Utility:** Their ability to persist in tissues and their species-specific distribution in different water bodies make diatoms reliable markers for drowning diagnosis and site identification **Ludes et al., (2024)[1]; Bogusz et al., (2023)[2]; Girela-López et al., (2020)[3]; Li et al., (2019)[7].**
- **Detection Methods:** Traditional identification relies on microscopy, but new methods include DNA barcoding and automated image analysis using deep learning, which improve accuracy and efficiency **Jiang et al., (2020)[6]; Zhou et al., (2019)[4]; Tournois et al., (2023)[5]; Ludes et al., (2024)[1]; Zhang et al., (2023)[8]; Li et al., (2019)[7].**

Forensic Diatomology:

Forensic diatomology is a specialized field within forensic science that utilizes the study of diatoms—unicellular, photosynthetic algae commonly found in aquatic environments—to aid

in the investigation of drowning cases and to help determine the site of drowning. Diatoms are valuable forensic indicators because their species composition and abundance can reflect specific environmental conditions, making them useful for linking a body to a particular water body or location **Bogusz et al., (2023)[1]; Zhang et al., (2020)[9]; Thakar & Singh, (2010)[10]; Zhuo et al., (2016)[11].**

Importance in Forensic Investigations

Diatom analysis is particularly significant in diagnosing drowning, as diatoms can enter the bloodstream and distant organs when water is inhaled during submersion. The presence and diversity of diatoms in body tissues, especially when matched with those from a suspected drowning site, can provide strong evidence for ante-mortem drowning and help identify the location where the incident occurred **Ludes et al., (2024)[1]; Thakar & Singh, (2010)[10]; Zhuo et al., (2016)[11]; Girela-López et al., (2020)[3].** This is especially useful when circumstantial evidence about the drowning site is unclear or disputed **Thakar & Singh, (2010)[10].**

Methodological Advances

Recent advances in forensic diatomology include the development of more sensitive and specific analytical techniques, such as microwave digestion, vacuum filtration, and automated scanning electron microscopy (MD-VF-Auto SEM), as well as the integration of DNA sequencing and deep learning for improved diatom detection and classification **Zhou et al., (2020)[9]; Ludes et al., (2024)[1]; Wu et al., (2024)[12].** These methods aim to enhance the reliability and efficiency of diatom testing, which has traditionally been time-consuming and labore-intensive **Wu et al., (2024)[12].**

Applications and Limitations

Forensic diatomology is not only used to confirm drowning as the cause of death but also to exclude or confirm specific sites of drowning by comparing diatom profiles from environmental samples and biological tissues **Bogusz et al., (2023)[2]; Zhang et al., (2020)[13]; Thakar & Singh, (2010)[10].** However, the method has limitations, including the need for standardized protocols, potential for false positives, and the requirement for close collaboration between forensic pathologists and diatomology experts **Bogusz et al., (2023)[2]; Ludes et al., (2024)[1]; Wu et al., (2024)[12].**

Relevance to Local Studies

Diatomological mapping—systematic documentation of diatom diversity across different water bodies and seasons—has been shown to be valuable for forensic investigations in various regions, including India and China. Such mapping helps forensic scientists characterize local water bodies and provides reference data for future drowning cases **Zhang et al., (2020)[9]; Thakar & Singh, (2010)[10]; Zhuo et al., (2016)[11].**

Forensic diatomology is a crucial tool in forensic investigations of drowning, offering insights into both the cause and site of death through the analysis of diatom species in biological and environmental samples. Continued methodological improvements and regional mapping efforts are enhancing its accuracy and applicability in forensic science.

BACKGROUND

Diatoms are microscopic, single-celled algae with siliceous cell walls, found ubiquitously in aquatic environments. Their unique species composition and distribution, which vary with environmental and geographical factors, make them valuable in forensic investigations, particularly in drowning cases. The presence of diatoms in body tissues can help establish drowning as the cause of death and link individuals to specific water bodies, as each site often has a distinct diatom assemblage shaped by local water chemistry and ecological conditions **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Dahiya et al., (2022)[16]; Sirmour & Naik, (2015)[17].**

In Chhattisgarh, research has focused on cataloging diatom diversity in various water bodies, including rivers and ponds, to build comprehensive regional databases. These databases are crucial for forensic scientists, as they enable the comparison of diatom profiles from crime scenes or victims with reference samples, aiding in the determination of the geographical origin of drowning incidents **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Sirmour & Naik, (2015)[17].** Studies in the region have identified numerous diatom species and highlighted the importance of detailed taxonomic and morphological analysis for accurate identification and ecological assessment **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Sirmour & Naik, (2015)[17].**

Environmental factors such as pH, dissolved oxygen, conductivity, and the presence of pollutants or heavy metals significantly influence diatom diversity and abundance. These parameters can affect the growth and composition of diatom communities, which in turn reflect

the ecological status of the water body and can serve as indicators of pollution or anthropogenic impact (Arumugham et al., 2022; Thacker & Karthick, 2022; Dahiya et al., 2022). Forensic limnology thus relies on both biological and physico-chemical analyses to interpret diatom evidence accurately **Arumugham et al., (2022)[18]; Thacker & Karthick, (2022)[19]; Dahiya et al., (2022)[16].**

The establishment of a diatom database specific to Chhattisgarh, including the Durg rural area, enhances the forensic potential of diatom analysis by providing a scientific basis for linking diatom evidence to particular locations. This approach supports more precise and reliable forensic investigations in cases of suspected drowning and contributes to broader ecological and environmental monitoring efforts in the region **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Sirmour & Naik, (2015)[17].**

Scope (Objective) of the Study

- To analyze and identify the diversity of diatom species present in different ponds of the Durg Rural area, Chhattisgarh, with the aim of creating a comprehensive database of local diatom assemblages for forensic applications, especially in drowning investigations **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Dahiya et al., (2022)[16]; Sirmour & Naik, (2015)[17].**
- To examine the relationship between diatom diversity and environmental factors (such as water chemistry and pollution levels) in these ponds, providing insights into how local conditions influence diatom populations and their forensic relevance **Kumar et al., (2024)[15]; Arumugham et al., (2022)[18]; Dahiya et al., (2022)[16].**
- To establish reference conditions and diatomological maps for the ponds in the Durg Rural area, which can assist forensic scientists in linking diatom evidence from drowning cases to specific water bodies, thereby aiding in the determination of the geographical origin of incidents **Kumar et al., (2024)[15]; Dahiya et al., (2022)[16]; Sirmour & Naik, (2015)[17].**
- To contribute to the broader understanding of diatom ecology in Chhattisgarh and enhance the scientific basis for using diatoms as trace evidence in forensic investigations **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Sirmour & Naik, (2015)[17].**

- Different ponds at Durg Rural area, located in Chhattisgarh, is a significant water body that supports various aquatic life forms, including diatoms. This study aims to explore the forensic potential of diatoms in different ponds at Durg Rural area. Cause they have a unique cell wall made of silica, making them an excellent tool for forensic analysis.

MATERIALS AND METHODS

Study Area

- The study area comprises various ponds located in the rural region of Durg, Chhattisgarh. This region is characterized by diverse aquatic environments, where diatom samples are collected from multiple ponds to analyze species diversity and distribution. The focus on Durg rural area allows for the creation of a localized diatom database, which is essential for forensic investigations, particularly in drowning cases, by linking diatom assemblages to specific water bodies within this geographic region **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Sirmour & Naik, (2015)[17].**The environmental and geographical characteristics of these ponds influence the diatom populations, making the study area significant for both ecological and forensic research **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Sirmour & Naik, (2015)[17].**

Materials Required for Conducting Experimental Work

- Water Sample Collection Bottles
- Laboratory Glassware (Beakers, Pipettes, Test Tubes)
- Centrifuge or Filtration Apparatus
- Glass Slides and Cover Slips
- Microscope (Trinocular or Compound)
- Chemical [2% Formalin solution, Conc. Nitric acid (HNO_3) or Conc. Sulfuric acid (H_2SO_4), Emersion Oil]

Collection of water samples

Total 21 samples were collected from various ponds located in the rural region of Durg, Chhattisgarh. In the month of January 2025. Samples were taken from surface , 1-2 feet deep inside ponds into plastic bottle (500ml).

Extraction of Diatoms from Water Samples

For extraction of diatoms following techniques are frequently used:

a) Acid digestion by conc. HCl and KMnO4

b) Acid digestion by conc. HNO3/H2SO4

c) H2O2 method

d) Incineration method

The acid digestion method is a widely accepted and effective technique for diatom extraction, known for its simplicity and reliability in producing high-quality results. **Caeiro, D. (2021)[21]; Mester et al. (1999)[22]; Walsh et al. (2017)[23]; Naidoo et al. (2017)[24].**

Nitric acid method

Using this procedure, a 10:1 ratio of concentrated HNO3 is applied to the obtained water sample.

1. 0.5 ml of concentrated HNO3 is added to 5 ml of water sample from the sample container in a small beaker after it has been well shaken for one to two minutes. Only the siliceous skeleton of diatoms remains when the concentrated acid breaks down the organic materials in the water.

2. After that, the beaker is wrapped in aluminum foil and left overnight to facilitate digestion.

3. The following day, the beaker's contents are moved into a centrifuge tube, and centrifugation is run for roughly 10 minutes at 3,000 rpm.

4. The pellet is obtained by discarding the supernatant. To eliminate any last traces of acid, these steps are carried out two or three times while adding distilled water to the pellet.

5. Analyze the diatoms morphologically under a microscope, the above process is repeated for additional water samples that were collected.

Preparation of Microscopic slide

- After final centrifugation, the pellet is diluted with 1 ml distilled water. The above solution is poured over 2 microscopic slides, covered with cover slip, and left it to air dry. The microscopic slides prepared above are then observed under Compound microscope at 40X & 100X magnifications. The diatoms were identified based on their morphological characteristics. **Taylor et al. (2009)[25]; Verma (2020)[26]; Verma K (2013)[27].**

Identification of Diatoms

For identifying the diatoms, photographs were taken from the microscopic slide and then the structure was matched with the standard database Diatoms of North America **Tandon et al., (2023)[14]; Kumari et al., (2023)[20].**

RESULTS

Following the collection, extraction, and isolation of diatoms from water samples, a standard online diatom database from the United States was used to identify different types. Identification was predicated on traits like;- *Raphe, Cell wall structure, Diameter, Shape.*

To verify the identities of the diatom species, these morphological characteristics were contrasted and matched with the standard online database.

According to the results, 12 genera of diatoms as well as 84 diatom were observed at various ponds located in the rural region of Durg, Chhattisgarh **Kumari et al., (2023)[20].**The following traits were used to identify diatom genera "*Cyclotella, Navicula, Pinnularia, Triceratium, Gomphonema, Stephanodiscus, Nitzschia Tabellaria, Fragilaria / Synedra etc".*

Table 1: Observation Table: Diatom Classification (Under Compound Microscope) at different ponds at Durg Rural area of Chhattisgarh

S. No.	Specimen Code	Shape & Symmetry	Valve Type	Surface Features	Notable Features	Tentative ID	No. of Diatom Observed
01	S-01	Rectangular/Irregular, Bilateral/Radial	Centric, Pennate	Smooth, fine striae, granular	Ribbon colonies	*Cyclotella*	02
02	S-02	Needle-like, Boat-shaped, Radial	Centric, Pennate	Striated, fibrous, clustered	Central line, possible raphe	*Cyclotella, Navicula, Pinnularia*	08
03	S-03	Elongated, Oval, Circular, Radial	Centric, Pennate	Smooth, translucent	Central thickening, faint striae	*Cyclotella, Navicula, Pinnularia*	06
04	S-04	Box-like, Elongated-curved, Radial	Centric, Pennate	Smooth, faint striae	Central raphe suspected	*Cyclotella, Navicula, Pinnularia*	02
05	S-05	Rectangular-Oval, Radial	Centric, Pennate	Semi-transparent, granular	Silica frustules	*Cyclotella, Navicula, Pinnularia*	01
06	S-06	Triangular/Wedge-like, Bilateral	Centric, Pennate	Faint surface	Fine striae	*Triceratium*	01
07	S-07	Elongated, Boat-shaped, Fusiform	Centric, Pennate	Granular, central thickening	No clear raphe, broken frustules	*Cyclotella, Navicula, Pinnularia*	05
08	S-08	Needle-like, Spindle, Boat-shaped	Centric, Pennate	Longitudinal ridges, ornamented	Central raphe, faint punctae	*Navicula, Gomphonema*	06
09	S-09	Needle-like, Elongated	Centric, Pennate	Smooth, refractile	Faint striae	*Navicula*	02
10	S-10	Rod/Boat-shaped, Bilateral	Centric, Pennate	Transparent, slight striae	Possible axial raphe	*Navicula*	02

11	S-11	Elliptical, Circular, Slightly Curved	Centric, Pennate	Longitudinal lines, radial ornamentation	Central raphe, fine striae	*Cyclotella, Navicula*	05
12	S-12	Bar, Disc-shaped, Polygonal cluster	Centric, Pennate	Radial striae, concentric ornamentation	Central raphe, punctae	*Cyclotella, Navicula, Stephanodiscus*	03
13	S-13	Rod-like, Wedge, Clustered	Centric, Pennate	Smooth, fine striae	Central raphe, central nodule	*Navicula, Pinnularia, Triceratium*	04
14	S-14	Lanceolate, Linear, Circular	Centric, Pennate	Symmetrical taper, radial/longitudinal striae	Central raphe, radial striae	*Navicula, Pinnularia*	03
15	S-15	Rod, Elliptical, Disc-like	Centric, Pennate	Smooth, granular, thickened center	Faint striae, unclear raphe	*Cyclotella, Navicula, Nitzschia/ Synedra*	03
16	S-16	Needle-like, Oval, Oblong	Centric, Pennate	Smooth with faint markings	Central raphe, faint striae	*Cyclotella, Navicula, Pinnularia*	02
17	S-17	Rod, Star, Chain	Centric, Pennate	Radiate pattern, translucent	Central raphe, striae, central nodule	*Cymbella, Gomphonema, Navicula*	07
18	S-18	Rod, Rectangular, Segmented	Centric, Pennate	Banded, transparent, colonial	No raphe, costae-like striae	*Tabellaria, Fragilaria*	09
19	S-19	Lanceolate, Rectangular chain	Centric, Pennate	Smooth, colonial	Central raphe, fine striae	*Cyclotella, Navicula, Pinnularia*	02
20	S-20	Boat-shaped, Elliptical, Linear	Centric, Pennate	Finely striated, punctate	Central raphe, fine striae	*Cyclotella, Navicula, Pinnularia*	06
21	S-21	Rod, Fusiform, Irregular	Centric, Pennate	Slightly granulated, translucent	Central raphe, axial structure, striae	*Cyclotella, Navicula, Pinnularia*	05

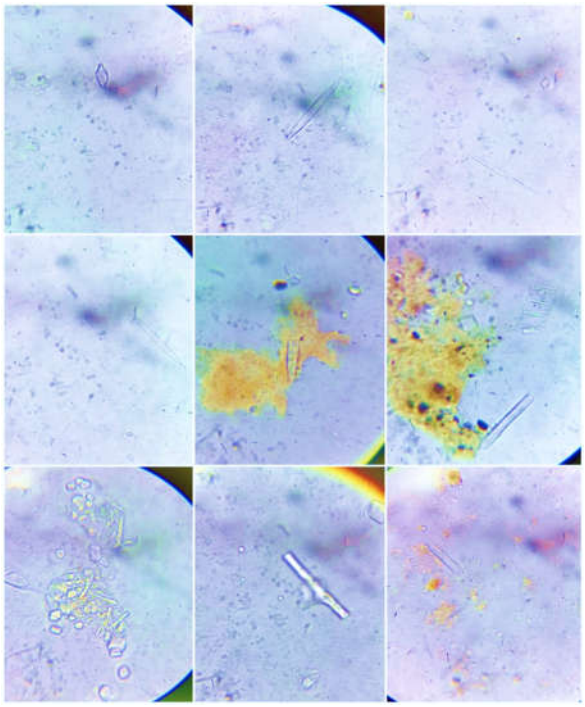

Fig.- 01

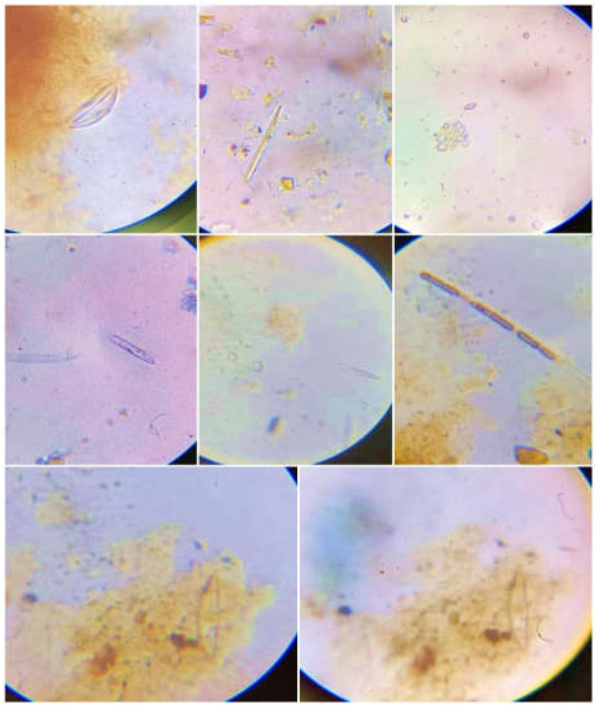

Fig.- 02

Fig-01 & Fig-02 different diatom observe in microscope at 100X.

According to Table No. 1, it was discovered that pennate diatoms, which are bilaterally symmetrical, were considerably more common than centric diatoms, which are radially symmetrical. About 84% of the 84 diatoms that were seen were classified as pennates, whereas just 16% were centrics. The dominance of pennate diatoms in the area was confirmed by the tentative identification of 21 diatom species at several ponds in the Durg Rural area of Chhattisgarh, of which 18 belonged to pennales and only 3 to centrales.

DISCUSSION

Forensic analysis of diatoms in ponds involves identifying and cataloging the microscopic algae present in different water bodies. Diatoms are highly diverse, and their species composition varies depending on environmental and geographical factors. This diversity makes them valuable in forensic investigations, especially in drowning cases, as the presence and type of diatoms found in a victim's tissues can be matched to those in specific water bodies, helping to determine the site of drowning **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Sirmour**

& Naik, (2015)[17]. The creation of a regional diatom database for Chhattisgarh enhances the ability of forensic scientists to use diatom evidence more accurately, supporting both legal investigations and ecological monitoring **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Sirmour & Naik, (2015)[17].**

The studies conducted in Chhattisgarh and similar regions revealed a rich diversity of diatom species across different ponds and water bodies. Each pond exhibited unique diatom assemblages, influenced by factors such as water chemistry, pollution, and seasonal changes **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Dahiya et al., (2022)[16]; Sirmour & Naik, (2015)[17].**The identification of 34 diatom species in Chhattisgarh's rivers and the establishment of a comprehensive database demonstrate the region's ecological complexity and the potential for precise forensic matching **(Tandon et al., (2023)[14]; Sirmour & Naik, (2015)[17].**The results also show that diatom analysis can provide insights into water quality, as certain species act as indicators of pollution or environmental change **Arumugham et al., (2022)[18]; Dahiya et al., (2022)[16].** This dual role supports both forensic and environmental applications.

The expectations for the forensic analysis of diatoms in Durg rural ponds were largely met. Researchers anticipated finding significant diatom diversity, which was confirmed by the identification of numerous species and genera unique to different water bodies **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Sirmour & Naik, (2015)[17].**The creation of a diatom database for the region was successfully achieved, providing a valuable tool for future forensic investigations and ecological studies **Sirmour & Naik, (2015)[17].**Additionally, the results validated the forensic utility of diatom analysis, as the unique assemblages in each pond allow for reliable matching in drowning cases **Tandon et al., (2023)[14]; Kumar et al., (2024)[15]; Sirmour & Naik, (2015)[17].**The findings also exceeded expectations by offering additional environmental insights, such as the ability to monitor water quality and detect pollution through diatom indicators **Arumugham et al., (2022)[18]; Dahiya et al., (2022)[16].**

Forensic diatom analysis in Durg rural ponds has proven effective for both forensic and ecological purposes, confirming the anticipated diversity and supporting the development of a regional database that enhances the accuracy and reliability of forensic investigations.

CONCLUSION

This study came to the conclusion that the acid digestion approach is appropriate for the extraction and isolation of diatom flora after a total of 12 diatom species were discovered from all 21 distinct ponds situated in the rural Durg, Chhattisgarh area. 5 common diatom genera were found from all 21 locations, whereas 7 site-specific diatom genera can be used for the analysis, evaluation, and identification of drowning death cases.

This study demonstrated that certain diatom populations are useful markers for distinguishing the decomposition phase of a body submerged in an aquatic setting and establishing whether submersion happened ante-mortem or post-mortem. In forensic investigations concerning crimes involving submersion in the different ponds in the Durg Rural area of Chhattisgarh, this is very crucial.

In medico-legal instances, diatom analysis has shown itself to be a valuable tool, particularly when handling unidentified drowned or discarded bodies. Diatoms can reveal important details about the cause of death, probable location, and even the likely season of death when they are found in biological tissues and water samples.

In forensic science, diatoms are frequently used to prove drowning as a cause of death. In order to create a new, region-specific database, water samples from 21 different ponds were gathered, and the diatom species were taxonomically examined. Since changes in diatom flora brought on by climatic variations improve the accuracy of drowning detection, it is imperative to continuously monitor local water bodies and their diatom populations.

When it came to solving drowning instances involving skeletonized or severely decomposed remains, diatom testing was particularly successful. When the results of a spot test or a conventional autopsy are unclear, this approach is quite helpful. Investigators can identify the probable location and season of drowning by keeping thorough records of site-specific and seasonal diatom flora. As a result, a diatom fingerprinting method based on commonly found species, arranged according to their size and shape, has been developed.

The study's conclusions demonstrate the creative uses of diatom biodiversity in environmental monitoring, forensic research, and upcoming biotechnological developments. Diatoms are important in industry outside of forensics because they can exude oils, which helps with the manufacturing of biofuel. Additionally, they are being utilized more and more in the creation of anti-proliferative agents, soaps, solar panels, cosmetics, and antibiotics.

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CONFLICT OF INTEREST: Nil.

ETHICAL CLEARANCE: Not Required.

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