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Review Article

Diatom analysis in forensic investigation: A review of techniques, AI progress, and limitations

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Abstract

This study underscores the forensic importance of diatom testing in drowning investigations, highlighting their ecological specificity and structural resilience as biomarkers. By correlating diatom presence in internal organs with environmental species profiles, it reinforces diagnostic criteria for distinguishing antemortem drowning characterized by active inhalation from postmortem submersion, where translocation is minimal. This paper reviews the multifaceted applications of diatoms in forensic science, including drowning diagnostics, trace evidence analysis, and environmental crime investigations. Techniques such as acid digestion, microscopic identification, and advanced molecular methods like DNA barcoding are examined for their efficacy in diatom detection and classification. Recent advancements, such as automated identification systems and interdisciplinary methodologies, are also discussed, showcasing the expanding role of diatom analysis in modern forensics. Comparative examination of diatom species found in victim tissues and aquatic environs allows for the localisation of drowning locations as well as the distinction between antemortem and postmortem submersion. Technological improvements, notably AI-driven categorisation and genetic diagnostics, have greatly increased species identification accuracy while reducing dependence on professional taxonomists. However, constraints exist in contamination control, database completeness, and procedure standardisation. Diatom-based forensic analysis presents unparalleled advantages in cases of aquatic deaths and environmental investigations. Despite challenges like contamination risks and limited reference databases, advancements in technology and standardization efforts hold promise for enhancing its reliability and accessibility.

Keywords: Diatoms, Environmental evidence, Forensic science, Forensic biomarkers, Molecular techniques.

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1. Introduction

Forensic biology is an interdisciplinary field that integrates biological sciences into criminal investigations to provide objective, scientific evidence. Among the various tools and methods used, diatoms—unicellular microalgae belonging to the class Bacillariophyta—have proven to be invaluable in forensic investigations, particularly in cases involving drowning.[1] Diatoms are microscopic, unicellular algae classified under the class Bacillariophyta.[2] They are renowned for their siliceous frustules, or cell walls, which display intricate and species-specific patterns. These organisms are fundamental to aquatic ecosystems, functioning as primary producers and contributing significantly to global photosynthesis and carbon cycling. Found in both marine and freshwater environments, diatoms are highly diverse, with thousands of species adapted to various ecological niches. The resilience and ecological importance of diatoms have made them a subject of interest across multiple disciplines, including forensic science. Forensic diatomology is the application of diatoms in criminal investigations, particularly in cases involving water-related deaths.[3] The unique distribution of diatom species in different water bodies enables investigators to link individuals, objects, or biological evidence to specific aquatic locations. In drowning investigations, diatoms act as indicators of antemortem water inhalation. **Figure 1** provides an overview of the diatom testing process, illustrating the procedural workflow from sample collection and preparation to analysis and interpretation. The diagram encapsulates both

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foundational methodologies and recent technological advancements, highlighting the integration of microscopic examination, species identification, and advanced molecular techniques within forensic investigations. Their detection in the internal organs of victims, such as bone marrow or lungs, provides valuable evidence to differentiate between drowning and postmortem submersion. The field continues to evolve with advancements in detection technologies and interdisciplinary approaches. The use of diatoms in forensic investigations dates back to the late 19th century when Rudolf Virchow first described their diagnostic significance in drowning cases.[4] Over the decades, forensic diatomology has progressed from simple microscopic examinations to more sophisticated techniques such as scanning electron microscopy (SEM), molecular analysis, and automated identification systems.[5] Notable advancements include acid digestion techniques for diatom extraction and the integration of molecular approaches such as DNA barcoding for precise species identification. These advancements have established diatom analysis as a reliable tool in forensic investigations, despite ongoing challenges like contamination and species identification. Diatoms' morphology is characterised by their siliceous frustules, which comprise of two overlapping valves.[6] These frustules are remarkably durable, allowing diatoms to persist in sediments and biological samples under various conditions. The diverse morphological features of frustules, including their size, shape, and ornamentation, are key to species identification. Ecologically, diatoms are sensitive to environmental factors such as salinity, pH, temperature, and nutrient availability[7] This sensitivity makes them excellent bioindicators for assessing water quality and environmental changes, further enhancing their forensic utility in tracing crime scenes and analysing aquatic environments.

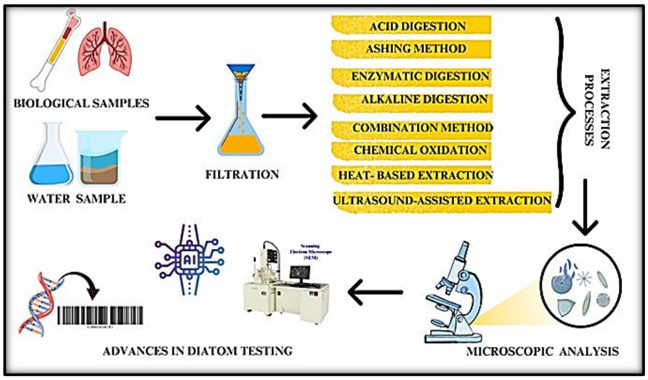

Figure 1: Workflow of diatom testing in forensic investigations, illustrating processes from sample preparation to analysis and incorporating advancements in technology like DNA barcoding and automated identification systems.

1.1. Role of diatoms in forensic investigation

Diatoms serve as indispensable forensic biomarkers, offering unique insights into aquatic crime scenes, drowning cases, and environmental conditions. Their diverse applications highlight their versatility and reliability as forensic tools. Below are detailed roles that diatoms play in forensic investigations:

1.1.1. Drowning diagnosis

Drowning is one of the most common water-related deaths, but determining whether death occurred before or after submersion can be challenging. Diatom analysis provides a critical solution to this forensic problem:

1. Antemortem vs. Postmortem Submersion: In cases of drowning, water inhaled during the victim's struggle introduces diatoms into the bloodstream, which are eventually deposited in organs like the lungs, kidneys, and bone marrow. The presence of diatoms in these organs strongly indicates antemortem drowning, as opposed to postmortem submersion (**Table 1**).
2. Correlation with Water Bodies: By comparing the diatom species found in a victim's body with those from a suspected water source, forensic experts can identify the location of drowning or establish the site of death.
3. Case Significance: This analysis is especially useful in homicides disguised as accidental drownings, providing critical evidence to support criminal investigations.

1.1.2. Trace evidence

Diatoms' remarkable ability to adhere to objects and surfaces makes them valuable trace evidence:

1. Linking Suspects to Crime Scenes**:** Diatoms can attach to clothing, footwear, vehicles, and other personal belongings. Diatom presence can tie suspects to specific aquatic sites, which can be designated as ideal geolocations for the particular species of diatoms, even if the crime occurred days or weeks ago.[8]

2. Durability as Evidence**:** The siliceous structure of diatom frustules allows them to persist under harsh conditions, ensuring their utility even when other biological evidence degrades.[9]

3. Environmental Profiling: Diatom samples can aid to reconstruct movement patterns including entry point like diatoms found on exterior surfaces (such as shoes or clothes) may correspond to the collection of a particular body of water, assisting in identifying the point of entry for the person or item into the aquatic environment [10], diatom exposure may be increased by active movement, but passive drift may concentrate diatoms in places that face the flow[11], by reflecting the direction of the water current, asymmetrical diatom deposition can reveal whether the corpse was passively carried, actively moving, or immobile[12].

Moreover, spatial distribution of diatoms on body surface or clothing can indicate the flow direction, postmortem drift or immersion orientation [13]. Such information may aid in reconstruction particularly in staged crime scene, accidental drowning or body disposal.

1.1.3. Environmental profiling and scene reconstruction

The ecological characteristics of diatoms provide a wealth of information about the crime scene:

1. Water Quality Assessment**:** The species composition of diatoms reflects water quality parameters such as salinity, pH, temperature, and nutrient levels [14]. This information can help forensic experts determine the conditions at the time of the incident and infer potential pollution or disturbances in the ecosystem.
2. Locational Fingerprinting: Specific diatom species have localized distributions due to environmental factors.[15] By analysing the diatom profile of water samples, investigators can narrow down the geographic location of the crime scene or drowning site.
3. Criminal and Environmental Investigations: Beyond personal crime cases, diatoms are used in environmental forensics to track industrial pollution, assess ecosystem damage, or investigate illegal dumping activities.

1.1.4. Supporting forensic scenarios

Diatoms complement traditional forensic techniques in various scenarios:

1. Establishing Time of Death: The diatom content in tissues, combined with environmental and biological factors, can provide understandings into the timeline of death.[16] Diatoms in deep tissues for example bone marrow indicate drowning occurred while the heart was still beating,[17] the extent and depth of diatom penetration may correlate with time spent underwater[18], presence of seasonal diatom blooms may narrow down the death timeframe[19], condition of diatom frustule (degraded or intact) and their location in decomposed tissues suggest the postmortem interval.[20]

2. Adulteration and Contamination: In cases involving food adulteration or water contamination, diatom analysis can help trace the source of pollution or determine the timeline and extent of contamination.[21]

3. Interdisciplinary Integration: Diatoms are increasingly being used alongside forensic techniques such as isotopic analysis and environmental DNA (eDNA) to provide comprehensive investigative evidence.

Table 1: The characteristic properties that help distinguish between antemortem drowning and postmortem drowning

Characteristic Property	Antemortem (True Drowning)	Postmortem (False Drowning)
Presence of water in lungs	Significant amount of water is found in the lungs due to active inhalation during the victim's struggle to breathe[22].	Minimal or no water present; water may only enter passively due to immersion.
Diatoms in internal organs	Diatoms are transported through the bloodstream and may be found in distant organs like bone marrow, kidneys, and liver, indicating water inhalation.[23]	Absence or minimal presence of diatoms in internal organs, as water is not inhaled actively postmortem.
Foam in airways and mouth	Frothy or blood-stained foam is commonly found in the respiratory tract, mouth, or nostrils, resulting from pulmonary edema caused by water inhalation.[24]	Foam is typically absent as there is no active breathing or inhalation during immersion
Signs of struggle	Abrasions, bruising, or cuts on hands, feet, or nails (e.g., defense wounds) may indicate efforts to escape drowning or struggle in the water.	Typically absent; no signs of struggle, especially if the body was deliberately submerged postmortem.
Hemodilution (diluted blood)	Hemodilution due to water entering the bloodstream via alveolar walls; can be confirmed by a reduced chloride ion concentration in the blood in freshwater drowning.[25]	Not observed, as there is no active exchange of water into the bloodstream postmortem.
Overinflated lungs	Lungs may appear overexpanded and waterlogged due to inhalation of water, with increased weight compared to normal lungs.	Lungs retain normal size and weight unless passive water entry occurs after death.
Vomiting or regurgitation	Stomach contents may be found in the mouth and airways, resulting from involuntary muscle spasms during drowning.	Typically absent, as there is no physiological response postmortem.

Skin wrinkling and discoloration	Skin wrinkling (e.g., "washerwoman hands") occurs on prolonged immersion, but this feature is not exclusive to drowning and is seen postmortem as well [26].	Similar wrinkling may occur with prolonged immersion; no distinction specific to true drowning.
Pupillary response	Pupils may show a dilated, non-reactive response immediately after death; however, it is not exclusive to drowning and could result from asphyxiation.	Similar dilation is observed, but it is not specific to the cause of drowning and depends on other factors like time since death or cause of postmortem submersion.
Histological changes in organs	Specific histological findings, such as alveolar rupture and water in the lungs, may be present, confirming active water inhalation.	Histological findings related to drowning are typically absent; the lungs show no evidence of water exchange.
Other physical evidence	Indicators such as mud, plant material, or debris consistent with the suspected water body are often found in the respiratory tract or clothing of the victim [27].	Such evidence may be absent unless artificially introduced postmortem to mimic drowning.
Postmortem lividity (hypostasis)	Lividity patterns may correspond to a body floating in water; this evidence alone is not definitive of drowning and requires correlation with other findings [27].	Lividity patterns are consistent with the position of the body during submersion, often masking signs of true cause of death.

2. Materials and Methods

2.1 Sample collection for diatom analysis

The first step towards diatom analysis is collection of samples and in the case of drowning, diatoms existing in the water surrounding the victim at the scene of crime might be present in body tissues, clothing etc.

Biological Samples such as tissues such as the lungs and bone marrow are collected during postmortem examinations to detect diatoms inhaled during drowning.[28] Other organs like the liver and kidney may also be sampled in cases where systemic circulation has transported diatoms. Water samples are taken from the suspected drowning site to establish a reference diatom profile. Sediments from the waterbed are collected as they often harbor a high concentration of diatoms. Control samples from nearby locations help verify that diatoms found in the victim's body match those at the scene.[29]

2.2. Collection method

Biological Samples are collected using sterilized tools to collect tissues (e.g., lungs or bone marrow) and store them in sterile containers to prevent contamination.[30] Extract around 10 grams of bone marrow from large bones, such as the femur or sternum. Ensure proper labeling and immediate refrigeration if transport is required.

Water Samples are collected at least 500 ml of water from various depths and areas around the suspected site of drowning,[27] Add a preservative, such as 5 ml of iodine solution, to prevent microbial growth. Scoop sediment from the bottom of the water body using clean, non-reactive tools. Store sediments in sealed containers for laboratory analysis.

2.3. Filtration methods

Filtration is a key step for isolating diatoms from both biological and environmental samples. For water sample pass water through a fine-mesh membrane filter of pore size 0.45 µm or 1.0 µm is used commonly to trap diatoms.[31] Rinse the filter with distilled water to dislodge diatoms into a sterile container. This filter has the property of retaining diatoms and allowing only non-diatomaceous substances and other debris to pass through. For Sediment Samples mix the sediment with distilled water, allow it to settle, and centrifuge the supernatant to concentrate diatoms. Alternatively, use direct filtration to remove large debris, retaining fine particles containing diatoms.

2.4. Extraction methods

Diatoms can be extracted from solid particles or sediments using a variety of ways. Extraction and examination of diatom colonies from bodily tissues such as the heart, liver, bone marrow, and lungs can provide evidence during investigations.

1. Acid Digestion: Tissue is treated with concentrated acids like nitric acid to dissolve organic material, leaving behind diatom frustules.[32] The sample is then neutralized with water and centrifuged to isolate diatoms.
2. Enzymatic Digestion: Proteolytic enzymes (e.g., Proteinase K) are added to break down proteins and tissues.[33] The mixture is incubated and then filtered to recover diatom frustules.
3. Ashing Method: Samples are heated in a muffle furnace at 450–550°C to incinerate organic material.[34] The residue is mixed with water, centrifuged, or filtered to collect diatoms.
4. Combination Method: Enzymatic digestion is used for initial tissue breakdown, followed by acid digestion for complete organic removal. This ensures thorough extraction, especially for complex samples.

5. Chemical oxidation: This method uses oxidizing agents to break down organic matter in biological samples.[35] Hydrogen peroxide (H_2O_2) or other oxidants are added to the sample to degrade organic material. The sample is then gently heated to accelerate oxidation. After digestion, the solution is centrifuged to concentrate the diatom frustules. Preferred when acid use is impractical or when mild digestion is sufficient. Less harsh than acid digestion and preserves diatom structures well.
6. Alkaline Digestion: This approach uses alkaline solutions to degrade proteins and organic materials in tissue samples.[36] Sodium hydroxide (NaOH) or other alkaline solutions are applied to the sample and incubated at 40–60°C for several hours. The digested mixture is then neutralized with an acid, rinsed with water, and centrifuged to recover diatoms. Suitable for protein-rich tissues, such as liver or kidney. Effective for dissolving tough tissues without the need for acids or high temperatures.
7. Heat-based extraction: This method relies on controlled heating to break down soft tissues.[37] The sample is gently heated to low temperatures to soften or dissolve tissues. Water is added to the digested sample, and centrifugation is used to isolate diatoms.
8. Ultrasound- assisted extraction: This method uses ultrasonic waves to facilitate the dislodging of diatoms from tissues or surfaces.[38] The sample is placed in an ultrasonic water bath, where high-frequency sound waves are used to dislodge diatoms. Afterward, the diatoms are filtered or centrifuged for collection. Non-destructive and enhances recovery without the need for harsh chemicals.

3. Results

3.1. Microscopic analysis

Microscopic analysis of diatoms involves examining prepared samples under light microscopes to identify their morphological features, such as size, shape, and ornamentation of frustules. The species identification process relies on comparing observed diatom structures to reference atlases or established databases[27,39] This step is critical for classifying diatom species and understanding their ecological significance. Following identification, data correlation is performed to match the diatom species found in a victim's biological samples or on evidence with those present at the suspected crime scene.[29] Such analysis helps establish connections between the victim, objects, or suspects and specific aquatic environments, providing vital evidence in forensic investigations.[2,3]

3.2. Advances in diatom testing

3.2.1. Automated identification systems

Automation has revolutionized diatom analysis by reducing the dependency on human expertise and minimizing the risk of error.

1. Machine Learning and Artificial Intelligence (AI): Algorithms trained on large datasets of diatom images can rapidly and objectively classify diatom species based on their morphological features.[40] AI also accelerates analysis, making diatom testing feasible in time-sensitive forensic cases.
2. Image Recognition Software: High-throughput imaging tools equipped with AI can scan thousands of samples quickly, highlighting potential matches for further investigation.[41]

3.2.2. Molecular techniques

Traditional diatom identification relies heavily on morphological analysis, which demands extensive expertise. Molecular methods such as DNA barcoding and next-generation sequencing (NGS) now allow species identification at the genetic level, overcoming the limitations of morphological variability.

1. DNA Barcoding involves sequencing specific genetic markers, such as the rbcL or 18S rRNA genes, to identify diatom species.[42] This approach is faster, highly accurate, and does not rely on intact frustules for identification.
2. Next-Generation Sequencing (NGS) permits the comprehensive analysis of diatom communities, enabling researchers to identify multiple species simultaneously [43]. This is especially useful in complex crime scenes where diatom diversity can indicate different environmental conditions or locations.

3.2.3. Advanced microscopy techniques

High-resolution imaging tools have become central to modern diatom analysis.

1. Scanning Electron Microscopy (SEM) offers detailed visualization of diatom frustules, allowing species identification even in degraded samples [44]. SEM is particularly effective for analysing minute morphological differences that distinguish closely related diatom species.
2. Confocal Laser Scanning Microscopy (CLSM) enables 3D imaging of frustules, providing intricate structural details that enhance the identification process.[45]

3.2.4. Integration with environmental DNA (eDNA)

Environmental DNA (eDNA) profiling allows diatoms and other microorganisms to be detected in water samples without physically collecting the organisms.[46] eDNA is non-invasive and can be applied to historical samples, helping in cases where direct evidence is compromised or unavailable.[47] It complements diatom analysis by confirming the presence of specific diatom species in both the victim’s body and the water body. This method analyses the genetic material present in the environment, providing clues about the species composition in the water.

4. Limitation in Forensic Diatom Analysis

Sample collection and processing are highly susceptible to contamination[39] from external diatoms, particularly during outdoor investigations or in shared laboratory facilities. Contaminated samples can lead to false positives or erroneous conclusions.[5] Comprehensive diatom reference databases are still under development. This limitation can hinder the precise identification of diatom species, especially in less-studied geographical regions, reducing the reliability of comparative analysis. Distinguishing between diatoms inhaled during antemortem drowning and those that adhered to the body postmortem remains difficult.[29] This ambiguity can complicate interpretations of cause and manner of death. Accurate diatom identification often relies on skilled taxonomists with significant experience in recognizing species-specific morphological features.[48] The reliance on human expertise introduces potential biases and delays in investigations. In cases where biological tissues are highly decomposed, diatom recovery may be compromised.[49] Poor preservation of samples can affect the integrity of the analysis and the ability to obtain meaningful results. Techniques like Scanning Electron Microscopy (SEM) and DNA barcoding require advanced equipment, skilled operators, and substantial funding, limiting their accessibility in resource-constrained settings. The seasonal and temporal variation in diatom communities within the same water body can complicate species matching.[50] Such ecological dynamics must be accounted for during analysis. There is a lack of universally accepted protocols for sample collection, preparation, and analysis, resulting in inconsistencies and reducing reproducibility across forensic laboratories.

5. Discussion

Forensic diatomology focusses on the study of diatoms. Moreover, the persistence of diatoms on clothing or personal belongings enables forensic experts to establish spatial and temporal links to aquatic environments, aiding in crime scene reconstruction.

Methods which are used are time-consuming, can cause harm, have variable efficiency, and lack standardisation. Contamination is a significant issue in diatom testing, which can lead to inaccurate findings. Recent advancements in molecular techniques and automated systems have addressed some of these challenges by increasing accuracy and reducing reliance on human expertise. DNA barcoding and next-generation sequencing (NGS) have revolutionized species identification, while automated machine learning systems have streamlined morphological analysis. Furthermore, the integration of diatom analysis with environmental DNA (eDNA) profiling and isotopic studies has opened new avenues for interdisciplinary research, enhancing forensic reliability. Artificial intelligence in diatom analysis can transform forensic diatomology. It can be trained to detect diatom species using microscopic photos with excellent accuracy and speed. Deep learning systems can recognise complicated diatom characteristics and patterns, simplifying the identification procedure. It swiftly processes and analyses massive datasets of diatom samples, allowing researchers to uncover trends, patterns, and correlations. Additionally, it can detect and flag possible contamination in diatom samples. Proper training and algorithm evaluation are crucial for ensuring the ethical and reliable application of AI in forensic diatomology. The integration of emerging technologies, combined with efforts to standardize and refine methodologies, will strengthen the role of diatoms as pivotal forensic biomarkers, ensuring their continued relevance in criminal investigations and environmental forensics.

6. Conclusions

Diatoms hold an exceptional place in forensic biology, serving as pivotal biomarkers for determining drowning cases, crime scene reconstruction, and environmental analysis. Their ecological diversity, species specificity, and resilience in diverse conditions make them a reliable tool in linking victims, suspects, and locations. The integration of molecular approaches, such as DNA barcoding and next-generation sequencing, as well as advancements in automated identification systems, has significantly enhanced the precision and efficiency of diatom analysis. This paper summarises the current state of forensic diatomology and highlights its potential as a significant forensic tool. Promoting the use of diatoms as objective evidence advances forensic science in general. Forensic diatomology has the potential to enhance forensic science, promoting justice and truth for society.

7. List of Abbreviations

1. H_2O_2: Hydrogen Peroxide
2. NaOH: Sodium Hydroxide
3. AI: Artificial Intelligence
4. NGS: Next-Generation Sequencing
5. SEM: Scanning Electron Microscopy
6. CLSM: Confocal Laser Scanning Microscopy
7. eDNA: Environmental DNA

8. Source of Funding

None.

9. Conflict of Interest

None.

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