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REVIEW ARTICLE

Forensic Diatomological Study of Different Water Bodies, Contemporary Developments and Future Trajectories: A Review

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ABSTRACT

Forensic diatomology is a specialized branch of forensic science has ascertain the drowning cases for medico legal purpose. If any body of the deceased found in the water bodies i.e. river, Lake, Pond etc then the law enforcement agency police has to find out whether the person died due to drowning in water or someone killed him and threw him in the river. Diatom test is conducted in forensic science laboratory to find out. Diatoms are microscopic algae with silica based cell walls called frustule abundant in freshwater and marine environments; serve as crucial indicators for distinguishing between different water bodies. This comprehensive review explores the current state of forensic diatomology research, emphasizing its practical application in analysing water samples from diverse sources, including rivers, lakes, ponds, and coastal regions. The methodologies discussed range from light microscopy and scanning electron microscopy to molecular techniques. The paper also delves into the significance of diatom taxonomy, ecology, and distribution in forensic investigations, addressing challenges and outlining future directions in the field. In drowning cases, diatoms provide valuable insights into ante-mortem and post-mortem evidence, aiding in the determination of geographical locations. As unicellular aquatic organisms representing a significant taxonomic division of phytoplankton, diatoms exhibit diversity influenced by geographical and environmental conditions, as well as the physicochemical properties of their habitat. The study further explores diatom diversity in various water bodies across

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India, aiming to create a diatomological map that facilitates forensic analyses. The diversity analysis reveals unique diatom characteristics in each water body, influenced by physicochemical parameters promoting growth. However, elevated levels of heavy metals may impede growth and alter biochemical compositions. Overall, this study provides valuable insights into regional patterns of diatom diversity, establishing reference conditions for water bodies in the specified region, thereby contributing to the broader field of forensic diatomology.

KEYWORDS

• Forensic Diatomology • Diatom Analysis • Forensic Investigations • Taxonomy • Ecology

INTRODUCTION

Forensic diatomology has become an indispensable technique in investigating water related crimes, accidents. Diatoms are microscopic single-celled algae prevalent in aquatic environments, offer valuable insights into the origin of water samples through their species-specific distributions. This review explores the application of forensic diatomology in studying various water bodies and molecular technique to identify diatoms underscores its significance in forensic science, diatomology is a specialized field, involves the comprehensive examination and analysis of diatoms for diverse practical applications, including environmental monitoring, water resource management, paleoclimatology, bio indication, and forensic sciences. Diatoms, characterized by their unique silica based cell walls, thrive in diverse aquatic settings such as rivers, lakes, ponds, and oceans. This distinctive feature plays a pivotal role in forensic investigations. Forensic diatomology primarily concentrates on assessing diatoms found in different types of forensic evidence, including lung tissues, internal organs, and bones, particularly in cases of drowning or recovering bodies from aquatic environments. Diatoms enter to our body during submersion through water, disperse across various body tissues and bone marrow, offering crucial insights into the circumstances surrounding the death or immersion location. The historical timeline of diatom research dates back to 1777, with formal recognition and description by the French Naturalist Jean Baptiste Lamarck in 1783. Notably, Hofmann, a distinguished professor of Forensic Medicine in Vienna, identified diatoms in pulmonary fluid in 1896, establishing their significance in drowning-related deaths. Subsequent studies on diatom identification and analysis in forensic investigations commenced in the early nineteenth century, with Rudolf von Hassling proposing their application in forensics in 1913. Advancements in forensic diatomology during the mid-twentieth century included methodological and technical innovations, facilitating the extraction and analysis of diatoms from various biological samples.

Recognized as a valuable tool since the 1970s, forensic diatomology has benefited from standardized protocols and guidelines, becoming the preferred method for investigating suspicious drownings. Recent developments in DNA technology have further influenced the field, with researchers exploring diatom DNA extraction to enhance analysis accuracy, potentially identifying specific bodies of water where victims were submerged.

Ongoing research review in forensic diatomology focuses on advanced imaging techniques like scanning electron microscopy to strengthen diatom identification and classification. Efforts are also underway to enhance understanding of diatom ecology and distribution, contributing to more precise forensic findings.

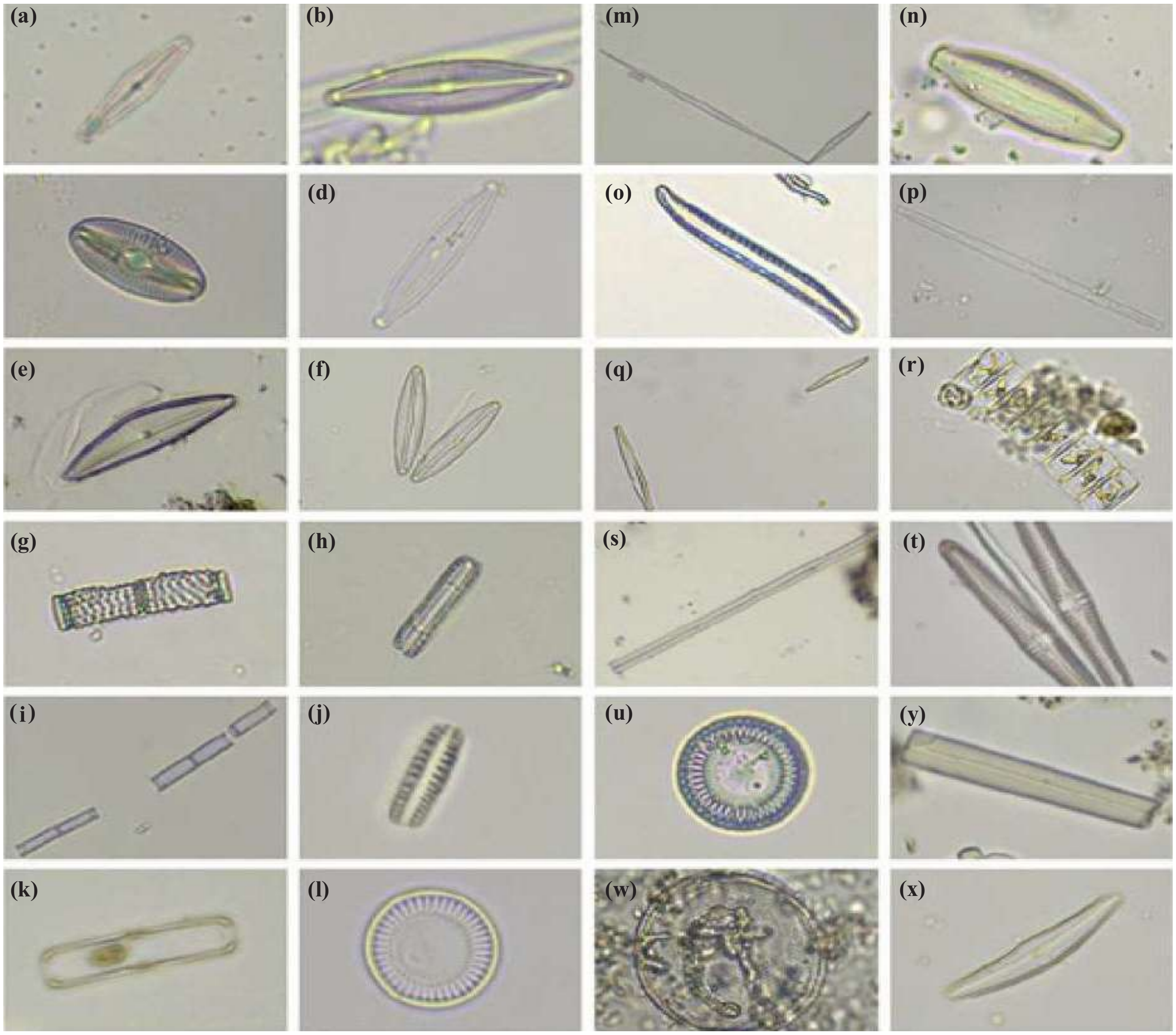
DIATOM TAXONOMY, MORPHOLOGY AND ECOLOGY

A comprehensive understanding of diatom taxonomy and ecology is imperative for the accurate interpretation of forensic diatomological data. Diatoms, characterized by diverse morphological features and ecological preferences, enable forensic scientists to deduce the origin of water samples by analysing diatom assemblages and species distributions. Diatoms, members of the class Bacillariophyta, are unicellular microscopic algae prevalent in both marine and freshwater habitats, distinguished by their silica-based cell walls known as frustules. These frustules exhibit intricate patterns and markings, consisting of two overlapping halves or valves. Diatoms play a pivotal role in primary production and the global carbon cycle, owing to their photosynthetic nature (d'Ippolito *et al.,* 2015). They possess the capability to absorb and store various nutrients, including silica, nitrogen, phosphorus, essential elements, fatty acids, and lipids, which can be utilized for biofuel production (Martin *et al.,* 2011; Pajunen *et al.,* 2020; Dahiya *et al.,* 2022).

Preserved as fossils due to their siliceous cell walls, diatoms function as indicators of past environmental conditions and climate (Rivera *et al.,* 2019). Their sensitivity to environmental changes makes them valuable bioindicator for assessing ecological health and water quality in aquatic ecosystems (Medley and Clements, 1998; Garrido *et al.,* 2016; Gogoi *et al.,* 2019). Abundance and diversity of diatoms are influenced by factors such as water quality, nutrient levels, pollution, temperature, pH, and total dissolved solids (Farrugia and Ludes, 2011; Armstrong and Erskine, 2018). Additionally, heavy metal contamination can impact diatoms' growth and biochemical composition (Piette and Letter, 2006; Armstrong and Erskine, 2018).

ROLE OF DIATOMS IN FORENSIC INVESTIGATIONS

Drowning, a significant cause of mortality worldwide, results in respiratory impairment and asphyxiation due to complete submersion in liquid. Forensic diatomology relies heavily on the role of diatoms, which are omnipresent in aquatic environments. When an individual is submerged in water, inadvertently ingesting or inhaling diatoms dispersed in the environment, these microscopic organisms travel through various body tissues and fluids, including bone tissues via the bloodstream. The presence and distribution of diatoms offer crucial insights into the timing of submersion relative to death, thereby assisting forensic investigations. Drowning incidents are categorized into primary and secondary drowning. Primary drowning, also known as typical or wet drowning, further divides into freshwater and saltwater drowning, where ventricular fibrillation and pulmonary edema, respectively, lead to fatality (Taylor *et al.,* 2007; Levin *et al.,* 2017). On the other hand, secondary drowning, referred to as atypical drowning, occurs within 30 minutes to several days after submersion and artificial respiration. In these cases, victims may succumb to conditions such as pulmonary edema, acidosis, or pneumonitis due to microbial infection. Secondary drowning includes dry drowning, where no water enters the gastrointestinal or respiratory tract, resulting in mechanisms of death like cardiac arrest, vagal reflex, or circulatory collapse. Diatom analysis proves to be a critical tool in differentiating between primary and secondary drowning cases. In primary drowning, significant diatom quantities are typically found in the respiratory system due to water inhalation, detectable through specialized microscopic techniques. Conversely, secondary drowning often lacks significant diatoms in the lungs, indicating the absence of water intake and coinciding with the cessation of blood circulation due to the victim's pre-existing deceased state. Drowning carries significant medico-legal implications, especially in homicide cases. Forensic pathologists must meticulously determine drowning as the cause of death while excluding alternative causes. Scene investigation, including signs of struggle, witness statements, and explanations for the victim's presence in the water, provides crucial evidence. Autopsy findings and toxicology tests aid in identifying trauma, injury, drug or alcohol presence, and potential homicidal drowning. Expert testimony and collaboration among forensic specialists are essential in determining the true nature of drowning incidents, aiding the justice system in holding individuals accountable when necessary. Examination of diatom concentrations and distribution patterns in tissues and fluids is crucial for distinguishing between drowning categories. Post-mortem methods, such as CT scanning, MRI scanning, toxicology, biomarker analysis, and VR simulation, supplement the investigation. Diatoms function as excellent biomarkers, offering essential information about the cause of death, whether the individual was alive or dead at the time of immersion, and potentially identifying the immersion location.



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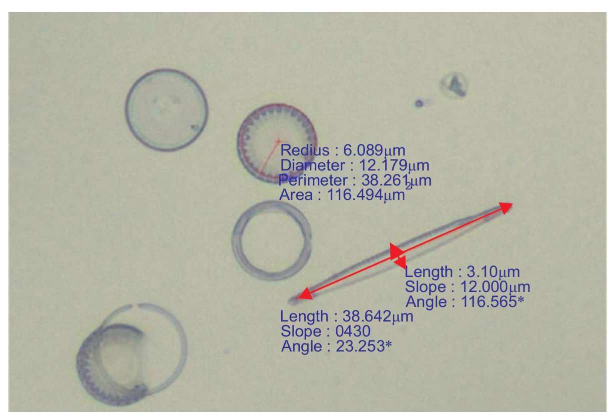

Figure: Centric

Figure 1: Morphology of some diatom found in India, **a:** Frustulia (SMB), **b:** Proschkinia (SMB), **c:** Diplonesis (SMB), **d:** Staurophora (SMB), **e:** Afrocymbella (ASMB), **f:** Envekadea (SMB), **g:** Guinardia (CNT), **h:** Scolioplura (SMB), **i:** Aulacoseria (CNT), **j:** Rhiocosphenia (ASMB), **k:** Navicula virdis (SMB), **l:** Cyclostephanos (CNT), **m:** Haslea (SMB), **n:** Adlafia (SMB), **o:** Gyrosigma (SMB), **p:** Bacillaria (NTZ), **q:** Tabularia (ARP), **r:** Diprora (ARP), **s:** Synedra (ARP), **t:** Encyonopsis (ASMB), **u:** Cyclotella (CNT), **v:** Thallasionema Nitschzoids (NTZ), **w:** Coscinodiscus (CNT) and **x:** Cymbella (ASMB).

Note: CNT: Centric, **SMB:** Symmetric Biraphid, **ASMB:** Asymmetric Biraphid, **ARP:** Araphid, **NTZ:** Nitzschioid.

FORENSIC DIATOM ANALYSIS METHODOLOGIES

Various techniques are employed in forensic diatom analysis, encompassing light microscopy, scanning electron microscopy (SEM), and molecular methods like DNA sequencing. The choice of method is influenced by unique advantages and limitations, with considerations based on forensic context and sample characteristics. To accurately determine the cause of death and crime location, meticulous sampling and analysis methods with minimal human error are essential.

Sample Collection:

The initial step in diatom testing involves careful sample collection by the doctor during post-mortem, generally sternum bone or femur bone has been collected in drowning cases. Precise examination and proper sampling techniques are crucial to avoid contamination. Collecting reference water samples from drowning sites are also taken by crime scene officer during crime scene examination.

Filtration:

To isolate diatoms from water samples, a commonly used method employs a membrane filter with a pore size of 0.45 µm or 1.0 µm. This filter retains diatoms while allowing non-diatomaceous substances and debris to pass through.

EXTRACTION METHODS

Various extraction methods are employed for samples containing solid particles or sediments to obtain diatoms for analysis. Diatom colonies from body tissues such as the liver, bone and lungs can serve as evidence in investigations. Extraction techniques include:

Acid digestion method: Involves treating the sample with acids like a mixture of hydrochloric acid and nitric acid to dissolve organic matter and non-diatomaceous substances. Diatoms are then isolated through filtration and centrifugation.

Enzyme digestion method: Involves the addition of a buffer solution and proteinase-K enzyme to the sample, followed by incubation and centrifugation to isolate diatoms.

Oxidation methods: Treat the sample with strong oxidizing agents such as hydrogen peroxide or a mixture of hydrogen peroxide and sulphuric acid to break down organic matter while leaving diatoms unaffected.

CHALLENGES AND LIMITATIONS

Despite its utility, forensic diatomology faces challenges such as sample contamination, diatom identification errors, and the need for standardized protocols. Addressing these challenges is crucial for enhancing the reliability and accuracy of forensic diatom analysis. Factors like the duration between death and sample collection, body condition, and sample quality can impact diatom detection and preservation. Limited reference databases in certain regions pose difficulties in accurately associating samples with crime scenes. Contamination is a significant concern, leading to false positives and inaccurate results.

FUTURE DIRECTIONS / ADVANCEMENTS IN DIATOM TESTING

Advancements in technology and interdisciplinary collaborations offer promising avenues for the future of forensic diatomology. Integrating molecular techniques, developing automated identification tools, and establishing comprehensive databases are essential for advancing the field and addressing emerging forensic challenges. Molecular approaches, such as species DNA barcoding, Next Generation Sequencing (NGS), Fluorescence in Situ Hybridization (FISH), and Metabarcoding or Environmental DNA (e-DNA) Barcoding, offer more efficient species identification compared to traditional morphology-based methods.

Methodologies in Forensic Diatom Analysis:

Different techniques are employed in forensic diatom analysis, including light microscopy, scanning electron microscopy (SEM), and molecular methods such as DNA sequencing. Each method offers unique advantages and limitations, influencing the choice of technique based on the forensic context and sample characteristics to accurately determine the cause of death and crime location, meticulous sampling and analysis methods with minimal human error are essential.

Challenges and Limitations

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COMMON MOLECULAR METHODS IN DIATOM TESTING

Species DNA Barcoding:

This method involves sequencing a standardized section of DNA from the genome to identify and differentiate diatom species. The ribosomal DNA (rDNA) region, particularly the small subunit (SSU) or the 18S rDNA gene, is commonly used as the DNA barcode marker. DNA is extracted from diatom samples and amplified using polymerase chain reaction (PCR) techniques. The amplified DNA is then sequenced and compared to reference databases to identify species. However, some species may have similar DNA codes, and this method may not capture the full extent of diatom diversity.

Next Generation Sequencing (NGS):

NGS platforms enable high-throughput sequencing of DNA samples, generating millions to billions of short DNA sequence reads in a single run. These short reads are aligned to a reference genome or assembled de novo to reconstruct genetic sequences. NGS can also perform whole genome synthesis (WGS), RNA sequencing, exome sequencing, nucleotide sequencing, and epigenetic analysis.

Diatom DNA Metabarcoding:

Diatom DNA Metabarcoding Metabarcoding identifies multiple species in an environment by sequencing complex mixtures of samples from soil and water. It uses high-throughput sequencing to simultaneously identify many taxa within a sample for ecological assessment, providing quick and efficient analysis of ecosystem biodiversity.

Automated Diatom Identification Software:

Advancements in computer vision and image analysis have led to automated methods for diatom identification using machine learning algorithms. These methods process images of diatoms, extract morphological and textural features, and apply machine learning algorithms for classification, enhancing the objectivity and efficiency of diatom testing.

DISCUSSION

Forensic diatomology, while extending beyond forensic applications, faces challenges such as time consumption, potential damage, variability, and lack of standardization in current methods. Ongoing global research aims to develop new techniques and technologies to address these challenges and evolve the field. Contamination poses a significant risk in diatom testing, leading to erroneous results. Mitigation strategies involve rigorous quality control, validation procedures, and collaboration to enhance analysts' skills and ensure quality. Molecular methods, particularly DNA barcoding, offer enhanced efficiency and accuracy compared to traditional techniques. Despite challenges like DNA degradation and contamination, automation reduces risks and improves efficiency. Integration of molecular techniques with traditional methods strengthens forensic evaluations, expands diatoms' role as forensic evidence, and contributes to criminal case resolution and environmental studies as extraction and analytical methods advance.

CONCLUSION

Forensic diatomology serves as a valuable tool for forensic investigations involving water samples from diverse environments, by combining knowledge of diatom taxonomy, ecology, and distribution with advanced analytical techniques, forensic scientists can provide valuable insights into the origin and source of water samples, contributing to the resolution of criminal cases. Forensic diatomology emerges as a powerful tool in aquatic death investigations and drowning-related cases, supported by diverse ecological distributions of diatoms. This review emphasizes the methodologies, applications, and recent advancements in forensic diatom analysis, highlighting its immense potential as a forensic tool. As the field progresses, promoting diatoms as reliable evidence contributes to forensic science's advancement, ultimately serving justice and truth in society. With ongoing developments, forensic diatomology is positioned to drive further advancements in forensic science, serving as a cornerstone of objective and reliable evidence analysis.

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