Dahiya *et al.*
Egyptian Journal of Forensic Sciences (2024) 14:2
https://doi.org/10.1186/s41935-023-00378-7

Egyptian Journal of
Forensic Sciences

REVIEW **Open Access**

A comprehensive review of forensic diatomology: contemporary developments and future trajectories

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Abstract

Background Forensic diatomology is a rapidly progressing domain that involves the examination of microscopic algae known as diatoms for forensic purposes. Diatoms are microscopic, single-celled, eukaryotic algae that exist in diverse aquatic environments such as rivers, lakes, ponds, and oceans. They are characterized by their rigid cell wall made up of silica, which is a unique morphological character, making them useful for forensic investigations.

Main body Diatoms are a type of unicellular microscopic algae that belong to the class *Bacillariophyta*. They are one of the most common phytoplankton found in all aquatic environments, including marine and freshwater habitats. Diatoms have proven to be valuable evidence in various forensic investigations, particularly in cases involving drowning or bodies recovered from aquatic environments.

Conclusion This comprehensive review provides an in-depth analysis of the principles, methodologies, applications, and challenges associated with the field of forensic diatomology. It emphasizes the importance of diatoms as trace evidence and discusses their potential to establish critical associations between the victim and the surroundings. This review also explores some recent advancements in diatom analysis techniques, including molecular approaches and automated identification methods. Finally, the paper outlines future directions for research and underlines the necessity for standardized protocols and interdisciplinary collaborations to enhance the reliability and validity of forensic diatomology.

Keywords Diatomology, Forensic, Environment, Diatom, Molecular

Background

Diatomology is a branch of study that involves the examination and analysis of diatoms for various practical applications (Rabiee et al. 2021) like environmental monitoring (Smol and Stoermer 2010) water resource management (Tiwari and Marella 2019), palaeoclimatology, bioindication (Lobo et al. 2016), and forensic sciences (Verma 2013; Levkov et al. 2017a). Diatoms are microscopic, single-celled, eukaryotic algae that exist in diverse aquatic environments such as rivers, lakes, ponds, and oceans. They are characterized by their rigid cell wall made up of silica, which is a unique morphological character, making them useful for forensic investigations (Hofmann 1878; Verma et al. 2020).

Forensic diatomology focuses mainly on assessing and interpreting diatoms identified in different types of evidence like lung tissues, internal organs, and bone marrow, particularly in cases involving drowning or bodies recovered from aquatic environments. In such situations, the diatoms may get ingested or inhaled by the person before or during water immersion. Their existence and distribution throughout various body

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tissues and fluids can provide valuable information for determining the circumstances surrounding the death or the location of immersion of the deceased individual.

Diatoms were first observed in 1777, but it was not until 1783 that the French Naturalist, Jean Baptiste Lamarck, formally recognized and described their existence. Subsequently, in 1896, Hofman, a renowned professor of Forensic Medicine in Vienna, was the first to detect and identify the diatoms in pulmonary fluid. He elaborated on their significance and relevance in asphyxia-related drowning deaths (Porawski 1966). In the early nineteenth century, initial research concerning the identification and analysis of diatoms in forensic investigations (Timperman 1972; Karkola and Neittaanmäki 1981; Pollanen 1998; Andresen and Edlund 2001; Hu et al. 2013; Marshall et al. 2023) began. Rudolf von Hösslin, an Austrian scientist, was the first to propose the application of diatoms in forensics in 1913. Throughout the mid-twentieth century, forensic diatomology made significant advancements in terms of methodology and techniques (Uitdehaag et al. 2010; Scott et al. 2014). To extract and analyze the diatoms from different types of biological samples, such as lung tissues (Horton 2007), bone marrow (Lunetta 2016; Levkov et al. 2017a), and from clothing (Zhou et al. 2020), scientists developed a variety of extraction and analysis techniques, which included techniques like chemical digestion, filtration, and microscopic examination. Since the 1970s, forensic diatomology has been acknowledged as a valuable tool for criminal investigations. The development of standardized protocols and guidelines for diatom analysis helped in establishing it as a legitimate forensic discipline. The standardization of diatom analysis has become the method of choice for investigating cases involving suspicious drownings (Zgłobicka et al. 2021; Wagner et al. 2022; Arumugham et al. 2023). Recent advances in DNA technology have also had an impact on forensic diatomology (Tantanasarit et al. 2013; Rai et al. 2022).

Researchers have explored the possibility of extracting diatom DNA from samples to improve the accuracy and reliability of diatom analysis. This may provide additional information regarding the origin and source of diatoms, thereby facilitating the identification of a specific body of water where a victim may have been submerged.

Researchers are exploring the use of advanced imaging techniques, such as scanning electron microscopy (Fields et al. 2014), to strengthen diatom identification and classification. In addition, research is being carried out to improve the understanding of ecology and the distribution of diatoms, which can contribute to making forensic findings more accurate.

Main text

Diatoms: morphology and ecology

Diatoms are a type of unicellular microscopic algae that belong to the class *Bacillariophyta*. They are one of the most common phytoplankton found in all aquatic environments, including marine and freshwater habitats. Diatoms have a unique cell structure characterized by a rigid cell wall made of silica, known as a frustule, which exhibits intricate patterns and markings. The frustule consists of two overlapping halves, often referred to as valves, which fit together like a pillbox or petri dish. Diatoms being photosynthetic organisms play a significant role in primary production and the global carbon cycle (d'Ippolito et al. 2015). They can readily take up and store large amounts of nutrients, such as silver (Reid et al. 1995; Desianti et al. 2019), silica, and essential elements like nitrogen, phosphorus, and hydrogen within their cell walls. They can also store notable amounts of fatty acids and lipids which can be extracted and converted to biofuels (Martin et al. 2011; Pajunen et al. 2020; Dahiya et al. 2022).

Due to their siliceous cell wall, they remain well preserved as fossils and serve as an indicator of past environmental conditions and climate (Rivera et al. 2019). Apart from being highly sensitive to changes in environmental conditions, including water quality, nutrient levels, and pollution, they are often used as bioindicators to assess the ecological health and water quality of aquatic ecosystems (Medley and Clements 1998; Garrido et al. 2016; Gogoi et al. 2019). Diatoms are also sensitive to physicochemical parameters like temperature, pH, and TDS such that these factors affect the diversity of diatoms by altering their growth (Farrugia and Ludes 2011; Armstrong and Erskine 2018). Higher loads of heavy metals may affect the diatoms by reducing their growth and altering their biochemical compositions (Piette and Letter 2006; Armstrong and Erskine 2018).

Role of diatoms in forensic investigations

Drowning refers to the state of respiratory impairment and asphyxiation resulting from complete submersion or immersion in liquid. This condition arises when the airway becomes filled with water or any other fluid, preventing the normal exchange of oxygen and carbon dioxide in the lungs (Auer and Möttönen 1988; Leth and Madsen 2017; Levkov et al. 2017b). This oxygen deprivation ultimately leads to asphyxia, respiratory failure, and potentially cardiac arrest. The seriousness of this situation is highlighted by the substantial worldwide consequences, leading to approximately 320,000 deaths every year making it the third most common cause of death, thus increasing the necessity of employing appropriate methodologies to conduct meticulous forensic investigations.

Diatoms, being ubiquitously present in almost all aquatic environments, play a critical role in forensic diatomology. When an individual is submerged in a water body, the diatoms present in the surrounding environment can inadvertently be ingested or inhaled. These diatoms are then dispersed throughout various body tissues and fluids and even into the bone tissues through the bloodstream. The presence of these diatoms therein can serve as a determinant of whether the submersion was before or after the death, hence giving insights into the timeline of the victim immersion.

The drowning incidents are classified into primary drowning and secondary drowning. Primary drowning, also known as typical/wet drowning is further categorized into freshwater, where ventricular fibrillation is the mechanism of death and saltwater drowning, where pulmonary oedema leads to fatality (Taylor et al. 2007; Levin et al. 2017). On the other hand, secondary drowning, also referred to as atypical drowning, refers to the death occurring within 30 min to several days after submersion and artificial respiration. In these cases, victims often succumb to conditions like pulmonary oedema, acidosis, or pneumonitis due to microbial infection. Secondary drowning is subdivided into dry drowning, where no water enters the gastrointestinal or respiratory tract, leading to mechanisms of death such as cardiac arrest, vagal reflex, and circulatory collapse.

Diatom analysis forms a keystone for differentiating primary and secondary drowning cases. In primary drowning, diatoms are typically detected in significant quantities within the respiratory system due to water inhalation and the subsequent movement of water and diatoms into the lungs which can be identified through specialized microscopic techniques. Conversely, in secondary drowning, the lack of significant diatoms in the lungs aligns with the absence of actual water intake and also coincides with the cessation of blood circulation due to an individual's pre-existing deceased state.

Drowning holds significant medico-legal implications, particularly in homicide cases. When a person is found dead due to drowning, it's essential to carefully investigate the cause and manner of death. The forensic pathologist's primary task is to establish that drowning was indeed the cause of death while ruling out alternative causes. In cases of suspected homicide, scene investigation is critical, as it can yield evidence such as signs of struggle, witness statements, and the absence of reasonable explanations for the victim's presence in the water. Autopsy findings play a pivotal role in determining whether trauma, injury, or pre-existing medical conditions may suggest foul play. Toxicology tests are conducted to detect the presence of drugs or alcohol that could have impaired the victim's judgment. Moreover, physical injuries indicative of a struggle or resistance may raise suspicion of a homicidal drowning. Expert testimony, collaboration among forensic specialists, and meticulous evidence collection are key in determining the true nature of the drowning incident and its potential criminal aspect, aiding the justice system in holding individuals accountable when warranted.

Careful examination of diatom concentrations and distribution patterns in various tissues and fluids can thus yield essential information to distinguish between these two categories of drowning incidents. While investigating drowning cases, many post (Ludes et al. 1994; Yange et al. 1999; Singh et al. 2006; Wagner et al. 2022)-mortem methods like CT scanning, MRI scanning, toxicology, biomarker analysis, and VR simulation are useful to gather evidence and make informed conclusions about the cause of death. Among all these techniques, diatoms serve as excellent biomarkers and play a key role in determining the real cause of death (Yange et al. 1999; Singh et al. 2006; Rácz et al. 2016; Wagner et al. 2022). By examining the diatom evidence present in different body tissues or fluids, forensic investigators can determine if a person was alive or dead at the time of water immersion and potentially identify the location of immersion as well (Figs. 1 and 2, Table 1).

Methods for sample collection and analysis

To identify the accurate reason for death and the crime location, it is necessary to perform appropriate sampling and analysis with minimum human errors.

Sample collection

The first step for diatom testing is sample collection (Harding et al. 2007). 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 (Zhou et al. 2020), and even footwear (Bailet et al. 2020) of the victim. Hence, it is very much necessary to thoroughly examine the site and collect the samples appropriately to avoid contamination. The reference water samples from or around the crime scene must also be collected from multiple sites to account for any possible spatial or temporal variation errors.

Filtration

For washing diatoms from the water samples, a membrane filter of pore size 0.45 μm or 1.0 μm is used commonly. This filter has the property of retaining diatoms and allowing only non-diatomaceous substances and other debris to pass through.

Fig. 1 Pennates

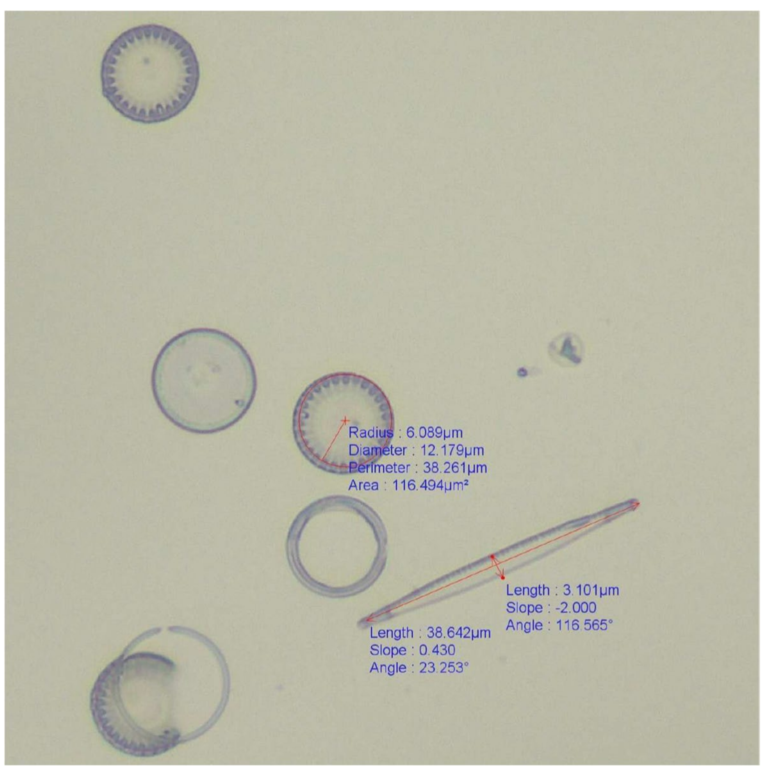

Fig. 2 Centrales

Table 1 Characteristic properties to distinguish between ante-mortem (true) drowning and post-mortem (false) drowning (Pérez-Burillo et al. 2021; Hamsher et al. 2011; Bailet et al. 2020)

Characteristic properties	Ante-mortem drowning	Post-mortem drowning
Definition	*Drowning while conscious	*Drowning after death
Cause of deaths	Suicidal or accidental	Homicidal
Incidents from all drowning cases*	80%	20%
Lungs	Tears in alveoli may be observed	No tears in the alveoli
Diatom test	Positive, except in the case of dry drowning**	Negative, except if water enters the lungs by passive absorption
Diatom presence in the lungs	20 diatom frustules per 100 ml of the residue obtained by enzymatic digestion from 10 g of lung tissue more than 5 diatom frustules	Absent or fewer
Diatom presence in another internal organ	Per 10 g of sediment from other internal organs	Absent

* Data might vary according to different regions

** Other types of ante mortem drowning where diatoms may not enter even lung cavities, due to laryngeal spasm and glottis spasm which prevent the flooding of the lungs with water. Here, the victims die due to fatal cerebral hypoxia caused by 'suffocation'

Extraction methods

For samples containing solid particles or sediments, several extraction methods can be used to extract and obtain diatoms. Extraction and analysis of diatom colonies from body tissues like the heart, liver, bone marrow, and lungs can be taken as evidence while investigation. As mentioned earlier, there is a significant difference in the amount of diatom in post-mortem and antemortem drowning. The diatoms retrieved from body tissues, along with analyzed diatom colonies in the water bodies, can be compared to uncover the accurate place of drowning. Many extraction techniques are being employed which include acid digestion method, enzymatic digestion, ultrasonic radiation method, Soluene-350 method, and physical methods like simple centrifugation or gradient centrifugation methods are also used (Hamsher et al. 2011; Dahiya et al. 2022).

- *Acid digestion method:* This 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 can then be isolated through filtration and centrifugation.
- *Ashing method:* Dry ashing refers to the use of a muffle furnace capable of maintaining temperatures of 500–600 °C for several hours. Inorganic matter, water and volatiles are vaporized, and organic substances are burned in the presence of oxygen and converted to CO_2 and oxides of nitrogen.
- *Enzyme digestion method:* For this method, a buffer solution containing Tris–HCl (pH 8.0), EDTA, and sodium chloride is prepared and added to the sample for maintaining ionic strength and pH. Thereafter, proteinase-K enzyme is added (0.1–1 mg/ml) and the sample is incubated for a few hours or overnight. A protease inhibitor is added to stop the activity of the enzyme and then the processed sample is centrifuged to isolate the diatoms.
- *Oxidation methods:* Here, the sample is treated with strong oxidizing agents such as hydrogen peroxide (H_2O_2) or a mixture of hydrogen peroxide and sulfuric acid (H_2SO_4). The strong acidic medium helps in breaking down the organic matter while diatoms remain unaffected due to their siliceous coat. Diatom then can be separated by filtration or centrifugation.
- *Density separation method:* This extraction technique utilizes the different density properties of diatoms and other particles in the sample by using a density gradient medium like sodium poly-tungstate or ludox. Diatoms can also be separated from other materials based on their buoyancy or sedimentation rate.
- *Microscopy and micromanipulation:* For some applications, microscopic manipulation and manual picking of diatoms using fine tools under a microscope might be needed. This method allows for precise selection and identification of diatom species. This method is not only time-consuming but also requires expertise for collecting diatoms.

Among all these methods, acid digestion with nitric acid (HNO3) is prominently used for extracting diatoms from body tissues as it completely digests non-diatomaceous matter (Levkov et al. 2017b; Bailet et al. 2020; De Carvalho et al. 2020; Mushtaq et al. 2022).

Leaving diatom frustules remaining unaffected. Heated hydrogen peroxide (H_2O_2) is found to be more efficient in terms of reduction of sediment, extraction of the material, and preservation of diatoms, proving to be a feasible alternative to conventional approaches with acids in terms of costs and operator safety. Sometimes, even simpler methods like sieving might be used where the pore size of the sieves may range between 20 and 100 µm as per the size of interested diatoms. In cases of periphyton samples, where diatoms are present in submerged rocks and vegetation, surface scraping using a brush, blade, or scapula is useful. If the vast majority of diatoms found in the recently gathered material are identified as lifeless cells (frustules without chloroplasts), it is advisable to discard the sample since conducting additional analysis will not provide an accurate representation of the current water quality at the specific sampling location (Yange et al. 1999).

Decalcification is solely required when samples are to be treated with nitric or sulfuric acid, as these acids react with calcium, resulting in the formation of an insoluble precipitate. This step can be skipped if it is known that the sample does not originate from a location containing calcareous rock in the catchment area or while employing the hot HCl and $KMnO_4$ technique. Each of these processes involves specific combinations of chemical reagents and variable temperatures to facilitate the efficient extraction of diatoms from the samples (Taylor et al. 2007; Levin et al. 2017).

Challenges and limitations

Diatoms are typically collected from lungs, but the detection and preservation of diatoms can depend upon factors like the duration between death and sample collection, the condition of the body and quality, and quantity of the sample itself as diatoms might tend to degenerate over time due to enzymatic activities, autolysis of cells, bacterial growth, and exposure to body fluids (Levkov et al. 2017c; Zhou et al. 2020).

Diatom identification and interpretation completely rely upon comparing the obtained samples with reference databases. These reference databases are formed using forensic limnology techniques but due to limited resources and databases, many regions do not have a diatom archive at all or are bound to specific localities. Also, factors like pollution (Farrugia and Ludes 2011), microorganism grazing, turbulence, light availability, and the chemical constitution of water may alter the diatom species richness (Martín, De Los Reyes Fernández 2012). Variations due to the abovementioned factors lead to difficulty in finding the accurate association between the obtained sample and the location of the crime scene. Diatoms can be transferred to secondary locations and can persist on objects. This makes it even more difficult to find whether the diatoms collected from the body or belongings of the victim are from the actual crime scene or any other environment.

Contamination is one of the major problems while using biological markers and systems for any purpose, as it could mislead the researchers and the law. False positives refer to a case where the testing incorrectly identifies the presence of diatoms when they are absent. Samples could be contaminated via multiple sources, like sample collection site itself, laboratory contamination, cross-sample contamination, airborne contamination, sample storage unit contamination, and reagent contamination. False positives are generally a result of contamination, but cross-reactivity (between multiple diatom samples) and human errors (methodological or interpretation errors) can also lead to inaccurate results (Horton 2007; Verma 2013; Scott et al. 2014).

Advancements in diatom testing through emerging technology

Molecular approaches for species identification

As species identification and interpretation through traditional methods (i.e., direct observation of morphology) is relatively difficult and requires a lot of expertise as well as time, molecular methods are more useful and efficient in diatom testing. These methods involve the use of DNA-based methodologies to identify and differentiate between diatom species.

Some commonly used molecular methods in diatom testing:

Species DNA barcoding This method involves sequencing a short section of DNA from a standardized region of the genome to identify and distinguish species. The commonly used DNA barcode marker for diatoms is in the ribosomal DNA (rDNA) region, specifically the small subunit (SSU) or the 18S rDNA gene. This gene region is highly conserved across diatoms but contains variable regions that can be used to distinguish between different species. To conduct this method, DNA is extracted from diatom samples and amplified using polymerase chain reaction (PCR) techniques. The amplified DNA is then sequenced and compared to existing reference databases to identify the species. Some diatom species have similar or identical DNA codes, making it difficult to identify species using this technique alone. Also, DNA barcoding may not capture the full extent of diatom diversity since it relies on specific gene markers that might not represent the entire genome.

Next Generation Sequencing (NGS) NGS platforms allow for high throughput sequencing of DNA samples, as it can generate millions to billions of short DNA sequence reads in a single sequencing run. Most NGS platforms produce relatively short DNA sequence reads, typically ranging from 50 to 600 base pairs in length. These short reads are then aligned to a reference genome or assembled de novo to reconstruct the original genetic sequences. Moreover, whole genome synthesis (WGS), RNA sequencing, exome sequencing, nucleotide sequencing, and epigenetic analysis can also be performed through NGS platforms.

Fluorescence in situ hybridization (FISH) This is a molecular technique that allows the detection and visualization of specific nucleic acid sequences in diatom samples. Special oligonucleotide probes (tags) are designed to target a specific nucleic acid sequence that is to be visualized. This probe is complementary to the specific sequence and is labeled with a fluorescent dye for visualization. The probes are added to the diatom samples and incubated under controlled conditions for the probes to bind to their complementary sequences. The excess and unbound probes can be washed off and the prepared sample is ready to be visualized under a fluorescence microscope.

Metabarcoding or environmental DNA (e-DNA) barcoding Metabarcoding is a special molecular technique, used to identify and interpret multiple species present in an environment by evaluating complex mixtures like samples from soil, water, or communities of insects or bacteria. Metabarcoding uses massively parallel sequencing of complex bulk samples at once, through high-throughput sequencing. While species DNA barcoding is specific and limited to a single organism, metabarcoding allows for the simultaneous identification of many taxa within the same sample. Hence, metabarcoding is proven to be quick and efficient to study the biodiversity of an ecosystem or to interpret the composition of a microbial system. Moreover, it also provides valuable information related to species interactions, ecological patterns, and overall health of the ecosystem· (Timperman 1972; Smol and Stoermer 2010; Lobo et al. 2016).

Automated diatom identification software

The traditional diatom identification techniques are based on manual microscopic examination, which is very a tedious task (Venkataramanan et al. 2023). Advancements in computer vision and image analysis have led to the development of automated methods that use computer algorithms and machine learning to identify and classify diatoms.

The simplest protocol to use this method is described below:

- A database is prepared by acquiring images of diatoms from various views and angles using a microscope, equipped with a digital camera or other image-capturing systems.
- These images are then processed to enhance their quality. Operations like denoising, image resizing, and contrast enhancement are done for the same.
- Various parameters like morphological and textural features are examined and extracted from these images for the characterization of diatoms.
- For classification, machine learning algorithms are applied, which are based on the extracted features.

Hence, the advancement of an artificial intelligence-based system capable of automatically identifying and classifying diatoms would significantly enhance the scientific rigor and objectivity of forensic diatom testing (Vinayak, Gautam 2019; Dahiya et al. 2022).

Future prospects

There are many areas and prospects in which the true potential of diatoms is yet to be explored. Due to their distinct morphological characteristics, immense diversity and abundance, environmental sensitivity, photosynthetic efficiency, and higher genetic manipulability, diatoms have immense applications in several fields like environmental monitoring, climate change research, paleolimnology, paleoenvironmental studies, bio-monitoring, biofuel production, nanotechnology, drug delivery systems, wastewater treatment are all yet to be developed using diatoms.

To ensure that the true potential of diatoms is completely exploited and the accuracy is enhanced in diatom testing, the following steps could be taken:

➢ Establishing standardized protocols in diatom testing, factors like quality control, comparability, and consistency could be ensured while collecting and interpreting data. Furthermore, in the long run, methodological advancements, trend analysis, collaboration, and long-term monitoring would be much easier and ultimately, it would enhance our knowledge of aquatic ecosystems such that we would be able to make informed decisions related to environmental protection and conservation.

➢ Collaboration between different fields of research in diatomology would lead to tremendous development by incorporating diatom analysis into fields like forensic anthropology, forensic botany, forensic entomology, and forensic limnology, additional evidence is provided, establishing vital connections between crime scenes, victims, suspects, and the surrounding environment.

This collaborative utilization of diatom analysis across multiple forensic disciplines strengthens the overall forensic investigation process, fostering a holistic comprehension of intricate forensic scenarios.

Discussion

Forensic diatomology is a specialized field dealing with the study of diatoms. Their application is observed in many fields apart from forensics. Methods currently used are time-consuming, sometimes resulting in damage, variability in efficiency, and lack of standardization. To counter these problems, new methods are being developed around the world. Forensic diatomology continues to evolve as new techniques and technologies emerge.

The risk of contamination is a major problem faced in diatom testing leading to false results. Some steps could be taken to minimize false testing results, like implementing rigorous quality control and validation procedures and identifying and preventing potential sources of contamination during sample handling. Also, participation in inter-laboratory proficiency testing for comparison can bring improvement. Providing continuous education and training to analysts enhances their skills and encourages peer review and collaboration for knowledge exchange. Continuous conduct of internal and external checks assures quality and helps identify weaknesses.

Molecular methods for identifying diatom species are much more efficient and accurate than existing methods which are completely dependent on direct observation and comparison. Among several other methods, DNA barcoding appears to be the future of molecular diatom identification as multiple extensive DNA databases for diatoms already exist and the overall protocol for this method is standardized. This method also has a more targeted approach towards specific gene regions and hence, making the comparison process much easier. However, DNA degradation and contamination are major drawbacks of molecular methods. Automation can decrease the chance of contamination and increase the efficiency of molecular methods.

Further, artificial intelligence in diatom analysis can also revolutionize the field of forensic diatomology. AI can be trained to identify diatom species from microscopic images with high accuracy and speed. Deep learning algorithms can recognize complex diatom features and patterns, streamlining the identification process. It can efficiently process and analyze large datasets of diatom samples, enabling researchers to identify trends, patterns, and correlations that might not be easily detectable through manual methods, and it can also aid in detecting and flagging potential contamination in diatom samples. AI can further be applied to predict diatom distributions and changes in aquatic ecosystems based on environmental factors, aiding in environmental impact assessments and pollution monitoring. But proper training datasets, algorithm validation, and interpretability of AI-driven results are critical factors that need to be carefully addressed to ensure the reliability and ethical use of AI in forensic diatomology and environmental studies.

However, integrating molecular techniques with traditional morphological identification methods can surely make great advancements in the future. The combination of diatom analysis with various forensic methodologies allows for a multidisciplinary approach that strengthens investigative capabilities and enables more thorough forensic evaluations. As extraction techniques and analytical methods continue to improve, the role of diatoms as valuable forensic evidence is likely to expand further, contributing to the resolution of criminal cases, and advancing our understanding of ecosystems and their interactions with human activities.

Conclusions

Forensic diatomology has emerged as a powerful and reliable tool in cases involving bodies found in aquatic environments or suspected drownings. The present review highlights the main principles, methodologies, and applications used nowadays by forensic practitioners, researchers, and scientists to have an easy understanding of the potential and significance of diatoms in the field of forensic science. These diatoms have proven to be extremely useful as supporting evidence in case of drowning-related deaths because of their diverse ecological distribution. The methods employed to extract, identify, and characterize these diatom species from collected forensic samples are elaborately mentioned in this review. The recent advancements made in this domain include developments in imaging and analytical techniques. Further, new approaches are introduced which are based on automated identification algorithms and machine learning. In conclusion, this study not only combines the current state of forensic diatomology but also underlines its immense potential as a valuable forensic tool. By promoting the use of diatoms as reliable and objective evidence, the field is contributing to the advancement of forensic science as a whole. With its positive trajectory, forensic diatomology is poised to drive advancements in forensic science, ultimately serving justice and upholding the principles of truth for society as a whole.

Abbreviations

pH	Potential of hydrogen
TDS	Total dissolved solids
DNA	Deoxyribonucleic acid
AI	Artificial intelligence
FISH	Fluorescence in situ hybridization
RNA	Ribonucleic acid
SEM	Scanning electron microscope
NGS	Next generation sequencing
e-DNA	Environmental DNA
WGS	Whole genome synthesis
SSU	Small subunit

EDTA Ethylenediaminetetraacetic acid
CT Computed tomography
MRI Magnetic resonance imaging
EDX Energy dispersive X-ray

Acknowledgements
Not applicable.

Authors' contributions
Study conception and design: PD, MM, PC, and AB. Drafting of the manuscript: MM, PC, and AB. Critical revision: PD and AB. All authors have read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Given by all authors.

Competing interests
The authors declare that they have no competing interests.

Received: 25 September 2023 Accepted: 19 December 2023
Published online: 08 January 2024

References

Andresen NA, Edlund MB (2001) Index to diatom research: volumes 1–14, 1986–1999. Diatom Res 16(1):125–246. https://doi.org/10.1080/0269249x.2001.9705516

Armstrong EJ, Erskine KL (2018) Investigation of drowning deaths: a practical review. Acad Forensic Pathol 8(1):8–43. https://doi.org/10.23907/2018.002

Arumugham S, Joseph SJP, Gopinath PM, Thajuddin N, Subramani N (2023) Diversity and ecology of freshwater diatoms as pollution indicators from the freshwater Ponds of Kanyakumari district, Tamilnadu. Energy Nexus 9:100164. https://doi.org/10.1016/j.nexus.2022.100164

Auer A, Möttönen M (1988) Diatoms and drowning. Zeitschrift Für Rechtsmed 101(2):87–98. https://doi.org/10.1007/bf00200290

Bailet B, Apothéloz-Perret-Gentil L, Baričević A, Chonova T, Franc A, Frigerio J, Kelly M, Mora D, Pfannkuchen M, Proft S, Ramon M, Vasselon V, Zimmermann J, Kahlert M (2020) Diatom DNA metabarcoding for ecological assessment: comparison among bioinformatics pipelines used in six European countries reveals the need for standardization. Sci Total Environ 745:140948. https://doi.org/10.1016/j.scitotenv.2020.140948

D'Ippolito G, Sardo A, Paris D, Vella FM, Adelfi MG, Botte P, Gallo C, Fontana A (2015) Potential of lipid metabolism in marine diatoms for biofuel production. Biotechnol Biofuels 8(1):1. https://doi.org/10.1186/s13068-015-0212-4

Dahiya P, Kumar S, Shukla MA, Yadav CS (2022) Forensic limnology: diversity of diatom population in relation to environmental factors in Gujarat region, India. Mater Today 69:A14–A24. https://doi.org/10.1016/j.matpr.2022.11.421

De Carvalho JC, Magalhães AI, De Melo Pereira GV, Medeiros ABP, Sydney EB, Rodrigues C, Aulestia DTM, De Souza Vandenberghe LP, Soccol VT, Soccol CR (2020) Microalgal biomass pretreatment for integrated processing into biofuels, food, and feed. Bioresource Technology 300:122719. https://doi.org/10.1016/j.biortech.2019.122719

Desianti N, Enache M, Griffiths ML, Biskup KJ, Degen A, DaSilva M, Millemann DR, Lippincott L, Watson EB, Gray AB, Nikitina D, Potapova M (2019) The potential and limitations of diatoms as environmental indicators in Mid-Atlantic coastal wetlands. Estuaries Coasts 42(6):1440–1458. https://doi.org/10.1007/s12237-019-00603-4

Farrugia A, Ludes B (2011) Diagnostic of drowning in forensic medicine. In: InTech eBooks. https://doi.org/10.5772/19234

Fields MW, Hise AM, Lohman EJ, Bell TAS, Gardner RD, Corredor L, Moll KM, Peyton BM, Characklis GW, Gerlach R (2014) Sources and resources: importance of nutrients, resource allocation, and ecology in microalgal cultivation for lipid accumulation. Appl Microbiol Biotechnol 98(11):4805–4816. https://doi.org/10.1007/s00253-014-5694-7

Garrido N, Costa AM, Stallman RK (2016) Drowning: a leading killer! Motricidade 12(2):2. https://doi.org/10.6063/motricidade.9482

Gogoi P, Sinha A, Sarkar SD, Chanu TN, Yadav AK, Koushlesh SK, Borah S, Das S, Das BK (2019) Seasonal influence of physicochemical parameters on phytoplankton diversity and assemblage pattern in Kailash Khal, a tropical wetland, Sundarbans, India. Appl Water Sci 9(7):1–3. https://doi.org/10.1007/s13201-019-1034-5

Hamsher SE, Evans K, Mann DG, Poulíčková A, Saunders GW (2011) Barcoding diatoms: exploring alternatives to COI-5P. Protist 162(3):405–422. https://doi.org/10.1016/j.protis.2010.09.005

Harding B, Archibald CGM, Taylor J (2007). A methods manual for the collection, preparation and analysis of diatom samples

Hoffman E (1878) Lehrbuch der GerichtlichenMedicin ("Textbook of Legal Medicine"), p 629

Horton BP (2007) Diatoms and forensic science. Paleontol Soc Papers 13:181–190. https://doi.org/10.1017/s1089332600001534

Hu S, Liu C, Wen J, Dai W, Wang S, Su H, Zhao J (2013) Detection of diatoms in water and tissues by combination of microwave digestion, vacuum filtration and scanning electron microscopy. Forensic Sci Int 226(1–3):e48–e51. https://doi.org/10.1016/j.forsciint.2013.01.010

Karkola K, Neittaanmäki H (1981) Diagnosis of drowning by investigation of left heart blood. Forensic Sci Int 18(2):149–153. https://doi.org/10.1016/0379-0738(81)90152-3

Leth PM, Madsen BH (2017) Drowning investigated by post mortem computed tomography and autopsy. J Forensic Radiol Imaging 9:28–30. https://doi.org/10.1016/j.jofri.2017.02.002

Levin E, Morgan R, Scott K, Jones V (2017) The transfer of diatoms from freshwater to footwear materials: an experimental study assessing transfer, persistence, and extraction methods for forensic reconstruction. Sci Justice 57(5):349–360. https://doi.org/10.1016/j.scijus.2017.05.005

Levkov Z, Williams DM, Nikolovska D, Tofilovska S, Cakar Z (2017a) The use of diatoms in forensic science: advantages and limitations of the diatom test in cases of drowning. In: The Geological Society of London on behalf of The Micropalaeontological Society eBooks, p 261–277. https://doi.org/10.1144/tms7.14

Levkov Z, Williams DM, Nikolovska D, Tofilovska S, Cakar Z. (2017b) The use of diatoms in forensic science: advantages and limitations of the diatom test in cases of drowning. In: The Geological Society of London on behalf of The Micropalaeontological Society eBooks, p 261–277. https://doi.org/10.1144/tms7.14

Levkov Z, Williams DM, Nikolovska D, Tofilovska S, Cakar Z (2017c) The use of diatoms in forensic science: advantages and limitations of the diatom test in cases of drowning. In: The Geological Society of London on behalf of The Micropalaeontological Society eBooks, p 261–277. https://doi.org/10.1144/tms7.14

Lobo EA, Heinrich CG, Schuch M, Wetzel CE, Ector L (2016) Diatoms as bioindicators in rivers. In: Springer eBooks, p 245–271. https://doi.org/10.1007/978-3-319-31984-1_11

Ludes B, Quantin S, Coste M, Mangin P (1994) Application of a simple enzymatic digestion method for diatom detection in the diagnosis of drowning in putrified corpses by diatom analysis. Int J Legal Med 107(1):37–41. https://doi.org/10.1007/bf01247273

Lunetta P (2016) Autopsy findings: drowning and submersion deaths. In: Encyclopedia of Forensic and Legal Medicine, 2nd ed. Amsterdam; Elsevier, p 315–322. https://doi.org/10.1016/b978-0-12-800034-2.00048-3

Marshall M, Chraïbi VLS, Morgan R (2023) A method for bone marrow extraction of diatoms for forensic science applications. J Forensic Sci 68(4):1343–1351. https://doi.org/10.1111/1556-4029.15297

Martín G, De Los Reyes Fernández M (2012) Diatoms as indicators of water quality and ecological status: sampling, analysis and some ecological remarks. In: InTech eBooks. https://doi.org/10.5772/33831

Martin P, Lampitt RS, Perry MJ, Sanders R, Lee C, D'Asaro EA (2011) Export and mesopelagic particle flux during a North Atlantic spring diatom bloom. Deep Sea Research Part I: Oceanographic Research Papers 58(4):338–349. https://doi.org/10.1016/j.dsr.2011.01.006

Medley CN, Clements WH (1998) Responses of diatom communities to heavy metals in streams: the influence of longitudinal variation. Ecol Appl 8(3):631. https://doi.org/10.2307/2641255

Mushtaq SO, Dahiya P, Awasthi A, Yadav CS (2022) Depletion in basic soil components and increased heavy metal load is associated with declined saffron growth in Kashmir valley region. Mater Today 69:1581–1586. https://doi.org/10.1016/j.matpr.2022.08.246

Pajunen VI, Kahlert M, Soininen J (2020) Stream diatom assemblages as environmental indicators – a cross-regional assessment. Ecol Ind 113:106183. https://doi.org/10.1016/j.ecolind.2020.106183

Pérez-Burillo J, Trobajo R, Leira M, Keck F, Rimet F, Sigró J, Mann DG (2021) DNA metabarcoding reveals differences in distribution patterns and ecological preferences among genetic variants within some key freshwater diatom species. Sci Total Environ 798:149029. https://doi.org/10.1016/j.scitotenv.2021.149029

Piette M, De Letter EA (2006) Drowning: still a difficult autopsy diagnosis. Forensic Sci Int 163(1–2):1–9. https://doi.org/10.1016/j.forsciint.2004.10.027

Pollanen MS (1998) Diatoms and homicide. Forensic Sci Int 91(1):29–34. https://doi.org/10.1016/s0379-0738(97)00162-x

Porawski R (1966) Investigations on the occurrence of diatoms in organs in death from various causes. J Forensic Med 13(4):134–7. PMID: 5972674

Rabiee N, Khatami M, Soufi GJ, Fatahi Y, Iravani S, Varma RS (2021) Diatoms with invaluable applications in nanotechnology, biotechnology, and biomedicine: recent advances. ACS Biomater Sci Eng 7(7):3053–3068. https://doi.org/10.1021/acsbiomaterials.1c00475

Rácz E, Könczöl F, Tóth D, Patonai Z, Porpáczy Z, Kozma Z, Poór VS, Sipos K (2016) PCR-based identification of drowning: four case reports. Int J Legal Med 130(5):1303–1307. https://doi.org/10.1007/s00414-016-1359-7

Rai A, Khan MJ, Ahirwar AK, Deka R, Singh N, Schoefs B, Marchand J, Varjani S, Vinayak V (2022) Hydrogen economy and storage by nanoporous microalgae diatom: special emphasis on designing photobioreactors. Int J Hydrogen Energy 47(100):42099–42121. https://doi.org/10.1016/j.ijhydene.2022.01.057

Reid M, Tibby J, Penny D, Gell P (1995) The use of diatoms to assess past and present water quality. Austral Ecol 20(1):57–64. https://doi.org/10.1111/j.1442-9993.1995.tb00522.x

Rivera MJ, Luís AT, Grande JA, Sarmiento AM, Dávila JM, Garrido JCF, Córdoba F, Díaz-Curiel J, Santisteban M (2019) Physico-Chemical influence of surface water contaminated by acid mine drainage on the populations of diatoms in dams (Iberian Pyrite Belt, SW Spain). Int J Environ Res Public Health 16(22):4516. https://doi.org/10.3390/ijerph16224516

Scott KR, Morgan RM, Jones VJ, Cameron N (2014) The transferability of diatoms to clothing and the methods appropriate for their collection and analysis in forensic geoscience. Forensic Sci Int 241:127–137. https://doi.org/10.1016/j.forsciint.2014.05.011

Singh R, Singh R, Thakar M (2006) Extraction method of diatoms-a review. Indian Internet J Forensic Med Toxicol 4:1–9

Smol JP, Stoermer EF (2010) The diatoms: applications for the environmental and earth sciences. Cambridge University Press

Tantanasarit C, Englande AJ, Babel S (2013) Nitrogen, phosphorus and silicon uptake kinetics by marine diatom Chaetoceros calcitrans under high nutrient concentrations. J Exp Mar Biol Ecol 446:67–75. https://doi.org/10.1016/j.jembe.2013.05.004

Taylor JC, Harding WR, Archibald CGM (2007) An illustrated guide to some common diatom species from South Africa (pp. 1-225). Pretoria: Water Research Commission

Timperman J (1972) The diagnosis of drowning. A review. Forensic Sci 1(4):397–409. https://doi.org/10.1016/0300-9432(72)90015-5

Tiwari A, Marella TK (2019) Potential and application of diatoms for Industry-Specific Wastewater Treatment. In: Springer eBooks, p 321–339. https://doi.org/10.1007/978-3-030-13913-1_15

Uitdehaag S, Dragutinovic A, Kuiper I (2010) Extraction of diatoms from (cotton) clothing for forensic comparisons. Forensic Sci Int 200(1–3):112–116. https://doi.org/10.1016/j.forsciint.2010.03.039

Venkataramanan A, Faure-Giovagnoli P, Regan C, Heudre D, Figus C, Usseglio-Polatera P, Pradalier C, Laviale M (2023) Usefulness of synthetic datasets for diatom automatic detection using a deep-learning approach. Eng Appl Artif Intell 117:105594. https://doi.org/10.1016/j.engappai.2022.105594

Verma K (2013) Role of diatoms in the world of forensic science. J Forensic Res 4(2):181. https://doi.org/10.4172/2157-7145.1000181

Verma P (2020) Diatoms analysis of well water sample of different districts of Punjab region. International Journal of Forensic Sciences 5(3). https://doi.org/10.23880/ijfsc-16000202

Vinayak V, Gautam S (2019) Diatoms in forensics: a molecular approach to diatom testing in forensic science. Diatoms 9781119370741:435–470. https://doi.org/10.1002/9781119370741.ch18

Wagner M, Hendy IL, Lai B (2022) Characterizing Ag uptake and storage in the marine diatom Thalassiosira pseudonana: implications for Ag biogeochemical cycling. Mar Chem 247:104175. https://doi.org/10.1016/j.marchem.2022.104175

Yange L, Chuanying H, Wang C, Wang X (1999) Development of can for destruction of organic material in use for forensic diatom examination. Forensic Sci Int 101(3):163–166. https://doi.org/10.1016/s0379-0738(98)00196-0

Zgłobicka I, Gluch J, Liao Z, Werner S, Guttmann P, Li Q, Bazarnik P, Płociński T, Witkowski A, Kurzydłowski KJ (2021) Insight into diatom frustule structures using various imaging techniques. Sci Rep 11(1):14555. https://doi.org/10.1038/s41598-021-94069-9

Zhou Y, Cao Y, Huang J, Deng K, Ma K, Zhang T, Chen L, Zhang J, Huang P (2020) Research advances in forensic diatom testing. Forensic Sci Res 5(2):98–105. https://doi.org/10.1080/20961790.2020.1718901

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