USING AQUATIC MICROBIAL COMMUNITIES FOR FORENSIC SITE IDENTIFICATION: INSIGHTS FROM PHYTOPLANKTON AND DIATOM STUDIES

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Abstract. Aquatic microbial communities—particularly phytoplankton and diatoms—have emerged as precise and reliable bioindicators for forensic site identification. This review offers a comprehensive synthesis of advancements in traditional microscopy, molecular techniques (such as environmental DNA and metabarcoding), artificial intelligence (AI)-driven analysis, presenting an integrative framework for the field of forensic hydroecology. By examining the habitat specificity, structural resilience, and taxonomic richness of these microbial groups, we evaluate their applicability in both drowning diagnosis and the analysis of aquatic trace evidence. The review also highlights recent methodological progress, representative case studies, and persistent challenges, including cadaver relocation, ecological variability, and the lack of standardized forensic protocols. Looking forward, we emphasize the need for global reference databases, AI-assisted species identification with improved model interpretability, and GIS-integrated spatial forensics. By bridging ecological complexity with forensic requirements, this review proposes a unified, interdisciplinary approach to advance microbial applications in legal investigations.
Keywords: *forensic hydroecology, aquatic microbial communities, diatom analysis, environmental DNA (eDNA), molecular techniques. site identification*

Introduction

Forensic investigations involving bodies recovered from water present unique and complex challenges, primarily revolving around three critical legal questions: distinguishing between ante-mortem drowning and post-mortem disposal, identifying the precise drowning site—especially when bodies have drifted—and estimating the Post-Mortem Submersion Interval (PMSI). Traditional autopsy findings are often inconclusive due to decomposition or the lack of specific macroscopic signs (Andresen and Edlund, 2001; Dahiya et al., 2024). Consequently, investigators increasingly rely on independent, objective biological evidence to reconstruct these aquatic events.

Aquatic environments maintain diverse microbial communities that fluctuate according to specific environmental factors, including temperature, pH, nutrient load, and geography (Pollanen, 1997; Tambuzzi et al., 2024). This ecological specificity generates distinctive microbial signatures that act as natural "fingerprints" for forensic site identification (Timperman, 1972). Among these biological markers, phytoplankton—specifically diatoms—are particularly valuable due to their ubiquity, habitat specificity, resistance to decay, and high taxonomic diversity (Smol and Stoermer, 2010; Verma, 2013).

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Diatoms (Bacillariophyceae) are unicellular, photosynthetic algae characterized by siliceous cell walls (frustules) that display intricate, species-specific patterns (Kojić et al., 2021). The forensic application of these organisms began with Hofmann's observation of diatoms in the lungs of drowning victims in the 19th century (Porawski, 1966). Since then, the "diatom test" has become a standard tool for drowning diagnosis. More recently, its scope has expanded to site attribution, achieved by comparing species assemblages found in victim tissues with those from suspected drowning locations (Díaz-Palma et al., 2009; Thakar and Singh, 2010a; Thakar et al., 2018; Zhou et al., 2020).

Beyond diatoms, other phytoplankton groups, including green algae, dinoflagellates, and cyanobacteria, have demonstrated forensic utility (Chardez and Lambert, 1985; Yoshimura et al., 1995; Bailet et al., 2020; Allwood et al., 2020). Concurrently, the integration of molecular techniques, such as environmental DNA (eDNA) metabarcoding and next-generation sequencing (NGS), has significantly improved sensitivity and taxonomic resolution. These molecular approaches facilitate the detection of trace and degraded samples that might be overlooked by traditional microscopy (Lunetta et al., 2013; Adserias-Garriga et al., 2017; Deiner et al., 2017; Young and Linacre, 2021).

However, the field still faces significant hurdles, such as sample degradation, contamination risks, ecological variability, and a lack of standardized protocols (Medley and Clements, 1998; Gogoi et al., 2019; Sijen and Harbison, 2021). To address these limitations, this paper evaluates the role of aquatic microbial communities in forensic site identification. We assess current distribution patterns and analytical methods while addressing practical challenges like cadaver displacement and legal admissibility. Unlike previous surveys, we propose an integrated framework combining microscopy, molecular tools, artificial intelligence, and GIS-based analysis to bridge the gap between ecological complexity and the strict requirements of legal investigations.

Figure 1 illustrates the integrative framework proposed in this review, connecting aquatic microbial communities (phytoplankton, diatoms, other microalgae) with forensic applications (drowning diagnosis, aquatic trace evidence), key analytical tools (microscopy, eDNA/NGS, AI methods), and challenges (ecological variability, cadaver relocation, lack of protocols). This schematic highlights the interdisciplinary and methodological innovations discussed in the review to advance forensic hydroecology.

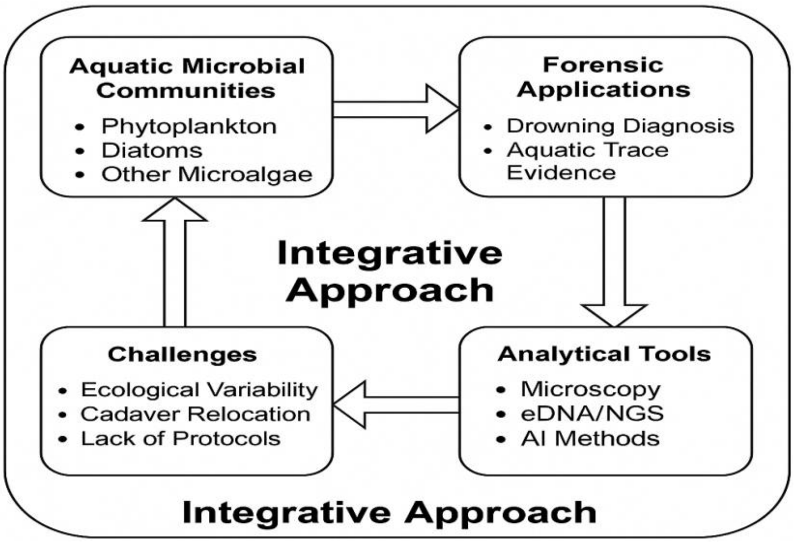

Figure 1. *Conceptual framework and key innovations of this review*

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Aquatic microbial communities in different water bodies

Aquatic ecosystems harbor diverse microbial communities that vary significantly based on water body type, geographical location, and environmental conditions. Understanding these variations is crucial for forensic applications, as they provide the foundation for site-specific identification. This section examines the distribution, composition, and ecological dynamics of microbial communities across different aquatic environments, with particular emphasis on phytoplankton and diatoms.

Freshwater ecosystems

Freshwater bodies, including lakes, ponds, rivers, and streams, support distinct microbial communities shaped by factors such as water flow, nutrient availability, light penetration, and anthropogenic influences (Smol and Stoermer, 2010; Gogoi et al., 2019). In lentic systems (standing waters), phytoplankton communities typically exhibit greater diversity and abundance compared to lotic systems (flowing waters), where physical disturbance from water movement can limit microbial establishment (Medley and Clements, 1998).

Lakes and ponds represent important freshwater habitats with characteristic microbial assemblages. Baba and Pandit (2014) documented significant variations in phytoplankton composition and diversity in Wular Lake, Kashmir, identifying 107 species belonging to *Bacillariophyceae*, *Chlorophyceae*, *Cyanophyceae*, *Euglenophyceae*, and *Dinophyceae*. Their study revealed that diatoms dominated during winter months, while green algae and cyanobacteria prevailed during summer, demonstrating clear seasonal succession patterns. Similarly, Singh et al. observed seasonal variations in diatom communities in Mawatha pond, Himachal Pradesh, India, with distinct species assemblages corresponding to different seasons (Gamier et al., 1995).

Rivers and streams harbor microbial communities adapted to flowing water conditions. Pajunen et al. (2017) investigated microbial stream communities and found that hierarchical factors, including water chemistry, substrate characteristics, and flow regimes, significantly influenced community composition. Their research demonstrated that certain diatom species serve as reliable indicators of specific stream conditions, making them potentially valuable for forensic site identification. Medley and Clements (1998) further highlighted the influence of longitudinal variation on diatom communities in streams, noting that species composition changed predictably along the stream continuum in response to environmental gradients.

Thakar et al. (2018) conducted forensic studies of phytoplankton ecology in two water bodies in Kurukshetra, India, developing "Phytoplankton maps" (P-maps) for forensic applications. Their research identified 138 species of phytoplankton belonging to five major groups, with certain species being site-specific to each water body. These findings demonstrate the potential for using phytoplankton assemblages as natural markers for specific freshwater locations in forensic investigations.

Forensic Implication: From an investigative standpoint, the distinct community structures of lentic (still water) versus lotic (flowing water) systems serve as critical exclusionary evidence. For instance, the recovery of a victim exhibiting a high abundance of pond-specific taxa (e.g., specific cyanobacteria or chlorophytes) while being found in a fast-flowing river strongly suggests post-mortem relocation. Therefore, establishing the specific "hydrological fingerprint" of a water body is a prerequisite for accurately reconstructing the crime scene timeline.

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Marine and estuarine environments

Marine and estuarine ecosystems host distinctive microbial communities that are shaped by salinity gradients, tidal regimes, and dynamic physicochemical conditions. These habitats differ markedly from freshwater systems, offering unique opportunities for forensic applications (Crump et al., 2004; Cloern et al., 2017). Phytoplankton communities in coastal and estuarine waters are influenced by a complex interplay of terrestrial runoff, nutrient fluxes, temperature, and seasonal monsoon cycles, which generate highly structured and site-specific assemblages (Canini et al., 2013; Naik et al., 2020).

Estuarine environments, such as mangrove-lined river mouths, are particularly sensitive to seasonal hydrological changes. Canini et al. (2013) documented monsoon-driven shifts in phytoplankton composition in a Philippine mangrove estuary, highlighting their potential as temporal and spatial bioindicators. Similarly, diatom assemblages from lagoonal sediments have proven to be effective markers of anthropogenic pollution and ecological change in coastal forensic investigations (Egres et al., 2019; Farqan et al., 2025).

In marine forensic science, the robust siliceous frustules, habitat specificity, and high diversity of marine diatoms offer considerable value in drowning site determination (Nakanishi et al., 2024). Sushanth and Rajashekhar (2012) recorded 46 diatom taxa along the Karnataka coastline and demonstrated that community composition fluctuated predictably with changes in salinity, nutrient concentrations, and water temperature. Moreover, nutrient uptake kinetics in marine diatoms such as *Chaetoceros calcitrans* provide insights into environmental conditions of suspected drowning locations (Tantanasarit et al., 2013; Bastos et al., 2022).

The advent of environmental DNA (eDNA) and high-throughput sequencing has greatly enhanced the taxonomic resolution and geographic attribution capabilities of microbial forensic tools (Reid et al., 1995; Allwood et al., 2020). These molecular approaches allow for the reconstruction of complex microbial signatures that can be spatially mapped using Geographic Information Systems (GIS) and artificial intelligence-based classifiers (He et al., 2022; Oliveira et al., 2024).

Future forensic hydroecology will increasingly rely on integrative technologies, including drone-assisted water sampling and satellite-based remote sensing. These approaches enable targeted and non-invasive microbial sampling from inaccessible aquatic sites, potentially increasing the spatial precision and forensic reliability of site identification efforts (Grimes et al., 2014; Lally et al., 2019).

Forensic Implication: In coastal investigations, the presence of specific halophilic (salt-loving) taxa on clothing or remains can definitively distinguish saltwater drowning from freshwater incidents. Furthermore, because estuarine communities react rapidly to salinity gradients and tidal cycles, they can act as high-resolution spatial markers. This allows investigators to potentially pinpoint the exact entry location within a complex tidal zone, significantly narrowing the search radius for the primary crime scene.

Phytoplankton and diatoms as forensic indicators

The application of phytoplankton and diatoms in forensic science has evolved significantly since their initial use in drowning investigations. This section examines their characteristics as forensic indicators, the principles underlying their forensic applications, and the methodologies developed for their use in site identification.

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Biological characteristics relevant to forensic applications

Several biological characteristics make phytoplankton, particularly diatoms, valuable forensic indicators. Their siliceous cell walls, habitat specificity, resistance to degradation, and taxonomic diversity contribute to their utility in forensic investigations (Auer and Möttönen, 1988; Verma, 2013; Dahiya et al., 2024).

Diatoms possess siliceous cell walls (frustules) composed of silicon dioxide, which resist decomposition and remain identifiable even under harsh conditions (Seo et al., 2014; Dahiya et al., 2022). This structural durability allows diatoms to persist in tissues and environmental samples long after death, making them valuable for forensic analysis in cases with extended postmortem intervals. Auer and Möttönen (1988)demonstrated that diatoms could be identified in drowning victims even after significant decomposition had occurred, highlighting their resistance to degradation.

Habitat specificity represents another crucial characteristic for forensic applications (Fløjgaard et al., 2019; Iannella et al., 2021). Different phytoplankton species exhibit preferences for particular environmental conditions, creating distinctive assemblages associated with specific water bodies (Burdis et al., 2025). Thakar et al. (2018) identified site-specific phytoplankton species in two water bodies in India, including *Cylindrospermum* sp., *Epithemia turgida*, and *Eunotia rhomboidea* in one location, and *Leptolynbya granulifera*, *Arthospira jenerii*, and *Cymbella cymbiformis* in another. These site-specific phytoplankton assemblages act as natural forensic signatures, enabling the attribution of samples to specific aquatic environments.

Taxonomic diversity provides another advantage, with over 200,000 diatom species estimated to exist globally (Tambuzzi et al., 2024). This remarkable diversity creates highly specific community profiles for different water bodies, enhancing the discriminatory power of phytoplankton analysis for forensic site identification. Verma et al. (2013) utilized this diversity to determine drowning sites by comparing diatom assemblages from victim tissues with those from suspected locations.

Seasonal stability within predictable patterns also contributes to the forensic utility of phytoplankton (Gayoso, 1999). While communities change seasonally, these changes follow predictable succession patterns that can be accounted for in forensic analyses (Dmitrijs et al., 2022). Singh et al. (Saini and Rohilla, 2020) documented these seasonal patterns, providing a foundation for developing temporally appropriate reference collections.

The diatom test in drowning investigations

The diatom test represents one of the earliest and most established forensic applications of aquatic microorganisms. Based on the principle that diatoms enter the bloodstream during drowning and are transported to various organs, this test has been used to diagnose drowning and identify drowning sites (Piegari et al., 2019).

When drowning occurs in diatom-rich water, these microorganisms enter the lungs with inhaled water and can penetrate the alveolar-capillary barrier to enter the bloodstream (Zhang et al., 2020). Blood circulation then distributes them to various organs, including the brain, liver, kidney, and bone marrow. Ludes et al. (1999) established quantitative criteria for positive diatom tests, suggesting minimum thresholds of 20 diatoms per 100 μL of pellet from lung samples and 5 diatoms per 100 μL from other organs to confirm drowning.

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The presence of diatoms in closed circulatory organs, particularly bone marrow, is considered strong evidence of ante-mortem drowning, as these locations are protected from postmortem contamination (Bogusz et al., 2024). Pollanen (1997) evaluated the diagnostic value of diatoms in bone marrow, concluding that their presence provided reliable evidence of drowning when appropriate controls and quantitative thresholds were applied.

However, the diatom test has faced criticism due to potential false positives and false negatives (Shen et al., 2019). Lunetta et al. (2013) investigated false-positive diatom tests using standardized protocols, finding that diatoms could occasionally be detected in non-drowned victims due to environmental contamination or dietary sources. Yen and Jayaprakash (2007) documented the prevalence of diatom frustules in non-vegetarian foodstuffs, highlighting a potential source of false positives. Conversely, false negatives may occur in water bodies with low diatom concentrations or when drowning occurs in winter months when diatom populations naturally decline (Girela-Lopez et al., 2020).

Despite these limitations, the diatom test remains valuable when properly implemented with appropriate controls and interpretation guidelines. Marshall et al. (2023) reviewed diatom tests in drowning cases, concluding that they provide valuable diagnostic information when combined with other evidence and interpreted within the context of case-specific circumstances.

Beyond drowning: Site identification applications

The forensic application of phytoplankton and diatoms has expanded beyond drowning diagnosis to include site identification in various contexts. By comparing microbial assemblages from evidence with those from potential crime scenes, investigators can establish links between suspects, victims, or objects and specific aquatic environments (Metcalf et al., 2016; Schmedes et al., 2018; Speruda et al., 2022).

Siver et al. (Geradts et al., 1994) pioneered the use of freshwater algal community ecology to link suspects to an aquatic crime scene in southern New England. By analyzing diatoms and scaled chrysophytes from sediment encrusted on the suspects' sneakers and comparing them with samples from the crime scene, they established a connection that contributed to the conviction (Siver, 2012). This case demonstrated the potential for using phytoplankton communities as natural trace evidence in forensic investigations.

Thakar and Singh (2010b) developed the concept of diatom-based forensic mapping to support drowning site identification. By creating detailed inventories of diatom species in various water bodies and comparing them with diatoms recovered from victim tissues, they established methods for determining the specific location where drowning occurred (Fucci et al., 2017; Rana and Manhas, 2018). Their approach has been particularly valuable in cases where bodies have been moved from the original drowning site.

The forensic application of phytoplankton has extended to clothing and objects associated with aquatic environments. Scott et al. (2014) investigated the transferability of diatoms to clothing and developed methods for their collection and analysis in forensic geoscience. Their research demonstrated that distinctive diatom assemblages could be recovered from clothing exposed to different water bodies, potentially linking suspects or victims to specific locations.

Uitdehaag et al. (2010) specifically examined the extraction of diatoms from cotton clothing for forensic comparisons, developing optimized protocols for recovering these microorganisms from fabric. Their methods enabled the detection of site-specific diatom

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assemblages on clothing, providing another avenue for establishing connections between individuals and aquatic crime scenes.

Beyond diatoms, other phytoplankton groups have shown forensic potential. Yoshimura et al. (1995) detected green algae (Chlorophyceae) in tissues of drowning victims, demonstrating that these organisms could complement diatoms in forensic analyses. Similarly, Díaz-Palma et al. (2009) developed standardized methods for microalgae testing in drowning cases, incorporating multiple phytoplankton groups to enhance diagnostic accuracy and site identification capabilities.

Recent research has explored the use of bacterial communities for drowning site identification. Studies have shown that bacterial composition analysis combined with machine learning algorithms can accurately infer drowning sites, particularly in flowing rivers where traditional methods face limitations (Su et al., 2025). This expanding toolkit of microbial indicators enhances the capability of forensic investigators to establish connections between cases and specific aquatic environments.

Analytical tools and emerging techniques

This integrative framework illustrates how aquatic microbial communities—including phytoplankton, diatoms, and other taxa—can support forensic site identification. It links key analytical methods (traditional microscopy, eDNA/metabarcoding, and AI-based classification) to specific forensic applications such as drowning diagnosis, aquatic trace evidence analysis, and spatial attribution.

As shown in *Figure 2*, an integrative conceptual framework brings together traditional, molecular, and AI-assisted methods for analyzing aquatic microbial communities and applying them to various forensic scenarios.

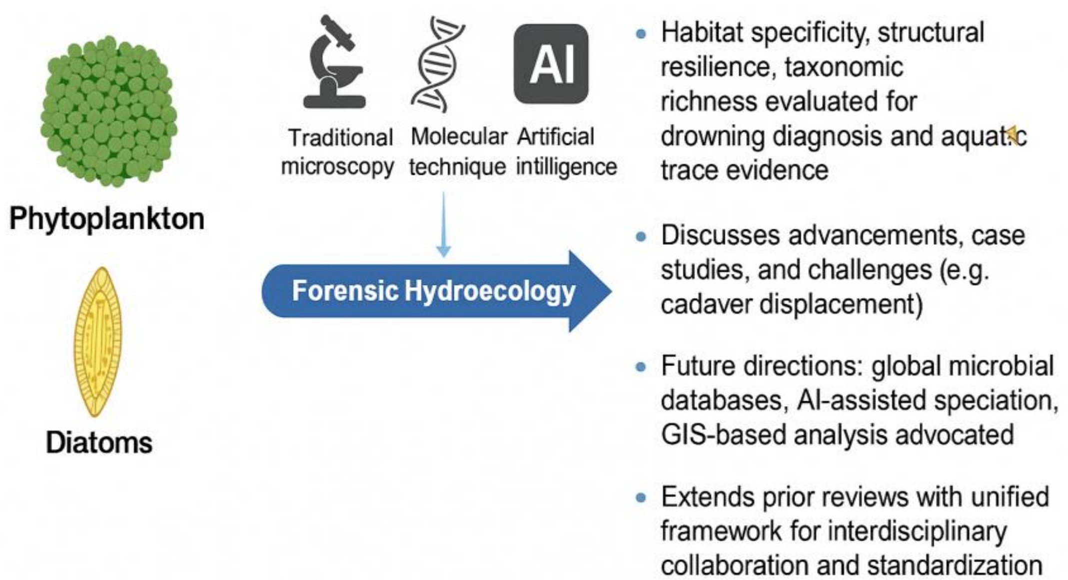

Figure 2. *Conceptual framework for forensic hydroecology. Source: Created by the authors*

The detection, identification, and analysis of aquatic microbial communities for forensic purposes rely on a diverse array of methodologies, ranging from traditional microscopic examination to cutting-edge molecular techniques. This section explores the

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analytical tools and emerging technologies that enable the forensic application of phytoplankton and diatoms.

Sample collection and preparation

Effective sample collection and preparation represent critical first steps in the forensic analysis of aquatic microorganisms (Benson et al., 2019). Different approaches have been developed for environmental samples and biological tissues, each addressing specific challenges related to sample type and investigation context (Rivera et al., 2020).

For environmental reference samples, standardized collection protocols ensure representative sampling of the water body in question. Rivera et al. (2020) optimized sampling strategies for diatom metabarcoding in monitoring networks, recommending multiple samples from different locations and depths to capture spatial heterogeneity. Water samples are typically collected in sterile containers and preserved with formalin or ethanol if immediate analysis is not possible (Nayebi et al., 2023).

Biological samples from drowning victims require specialized collection and preparation techniques. Lunetta et al. (2013) outlined protocols for collecting samples during autopsy, emphasizing the importance of avoiding contamination by using sterile instruments and collecting control samples. Bone marrow, typically extracted from the sternum or femur, is considered particularly valuable due to its protection from postmortem contamination (Cartiser et al., 2011; Vandenbosch et al., 2020).

Sample preparation methods vary depending on the analytical approach. For microscopic examination, samples typically undergo digestion to remove organic material while preserving diatom frustules. Hu et al. (2013) developed a combination of microwave digestion and vacuum filtration for processing water and tissue samples, achieving efficient recovery of diatoms while minimizing damage to their structures.

For clothing and other physical evidence, specialized extraction methods have been developed. Scott et al. (2014) evaluated techniques for recovering diatoms from clothing, finding that hydrogen peroxide digestion followed by centrifugation effectively isolated diatoms while preserving their identifying features. Uitdehaag et al. (2010) further refined these methods for cotton clothing, optimizing protocols for forensic comparisons.

Traditional microscopic methods

Light microscopy remains a fundamental tool for identifying and analyzing phytoplankton in forensic contexts. This approach allows direct visualization of morphological features critical for species identification, particularly for diatoms with their distinctive siliceous frustules (Crawford et al., 2001).

Brightfield microscopy represents the most basic approach, allowing examination of general morphological features. For more detailed analysis, phase contrast and differential interference contrast microscopy enhance visualization of fine structures without staining (Siver and Hinsch, 2010). These techniques are particularly valuable for examining the intricate patterns on diatom frustules that serve as taxonomic identifiers.

Scanning electron microscopy (SEM) provides significantly higher resolution and depth of field compared to light microscopy, enabling detailed examination of surface structures. Garcia et al. (Vijayan et al., 2020) comprehensively reviewed the forensic relevance of diatoms, underscoring the critical contribution of scanning electron microscopy to improving the precision of diatom identification. Zgłobicka et al. (2021) used advanced microscopic imaging to study diatom cell wall formation and

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morphogenesis, demonstrating the power of SEM for revealing taxonomically significant ultrastructural details.

Quantitative analysis of diatoms typically involves counting and identifying specimens on prepared slides using standardized protocols. Ludes et al. (1999) established quantitative criteria for the diatom test, recommending counts of diatoms per unit volume of sample to determine significance. These approaches require considerable taxonomic expertise but provide reliable results when performed by experienced analysts.

Molecular and DNA-based techniques

Molecular methods have revolutionized the analysis of aquatic microbial communities, offering increased sensitivity, specificity, and throughput compared to traditional approaches. DNA-based techniques enable the detection and identification of a broader range of organisms, including those difficult to distinguish morphologically (Hebert et al., 2003; Packer et al., 2009).

DNA metabarcoding represents a powerful approach for characterizing entire microbial communities from environmental or forensic samples. This technique involves amplifying and sequencing specific genetic markers (barcodes) from mixed DNA samples, followed by bioinformatic analysis to identify the organisms present (Taberlet et al., 2012). Bailet et al. (2020) compared diatom DNA metabarcoding protocols used in six European countries, highlighting the need for standardization while demonstrating the technique's potential for ecological assessment and forensic applications.

Environmental DNA (eDNA) analysis has emerged as a particularly promising technique for forensic hydroecology (Harrison et al., 2019). This approach detects DNA shed by organisms into their environment, allowing identification of species without direct observation or capture (Thomsen and Willerslev, 2015). Allwood et al. (2020) reviewed the future of environmental DNA in forensic science, highlighting its potential for linking samples to specific locations based on their microbial signatures.

Next-generation sequencing (NGS) technologies have dramatically increased the throughput and decreased the cost of DNA analysis, enabling comprehensive characterization of microbial communities from forensic samples (Valentini et al., 2016; Young and Linacre, 2021). Giampaoli et al. (2020) developed a semi-automated protocol for NGS metabarcoding and fungal analysis in forensic contexts, demonstrating its applicability to various sample types including those from aquatic environments.

Quantitative PCR (qPCR) offers another molecular approach, allowing quantification of specific microbial taxa in forensic samples (Reid et al., 1995; Kuiper, 2016). This technique can provide information about the abundance of particular species, potentially enhancing the discriminatory power of microbial analysis for site identification.

Spectroscopic and chemical approaches

Spectroscopic and chemical methods complement morphological and molecular approaches by providing information about the biochemical composition of phytoplankton communities, offering additional discriminatory power for forensic applications (Kim et al., 2020; Novikova et al., 2022).

Fluorescence spectroscopy exploits the natural fluorescence of photosynthetic pigments to detect and characterize phytoplankton. Garrido et al. (2013) evaluated the effects of sample conservation on photosynthetic efficiency measurements using pulse amplitude modulation (PAM) fluorometry, providing insights relevant to forensic sample handling and analysis.

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Raman spectroscopy offers another non-destructive approach for analyzing diatoms and other phytoplankton (Edwards et al., 2005). This technique provides information about the molecular composition of cell walls and other structures, potentially enhancing species identification and discrimination between populations from different water bodies.

Elemental analysis of diatom frustules can reveal information about the chemical environment in which they formed. Diatoms incorporate various elements from their surroundings during frustule formation, creating chemical signatures that may reflect specific water bodies (De Tommasi et al., 2017; Lobus et al., 2021). This approach offers potential for distinguishing between diatoms from different locations even when they belong to the same species.

Artificial intelligence applications in forensic hydroecology

Artificial intelligence and automated identification

Artificial intelligence and machine learning approaches are increasingly applied to the analysis of aquatic microbial communities, offering potential solutions to the challenges of taxonomic expertise requirements and processing large datasets (Cordier et al., 2018).

Automated image analysis systems have been developed for diatom identification, using computer vision algorithms to recognize and classify diatom species based on their morphological features (Kloster et al., 2020). These systems can process large numbers of images more rapidly than human experts, potentially increasing the throughput of forensic diatom analysis.

Machine learning algorithms have shown promise for inferring environmental conditions and geographical origins based on microbial community compositions. Recent research demonstrated that bacterial composition analysis combined with random forest algorithms could accurately identify drowning sites in flowing rivers (Su et al., 2025), highlighting the potential of AI-assisted approaches for forensic site identification.

Deep learning approaches, particularly convolutional neural networks, have demonstrated impressive performance in classifying diatom species from microscopic images (Gunduz and Gunal, 2024). These techniques continue to improve as training datasets expand, potentially reducing the reliance on specialized taxonomic expertise for forensic diatom analysis.

Integration of multiple data types through machine learning offers particularly promising avenues for forensic applications. By combining morphological, molecular, and environmental data, these approaches can potentially provide more accurate and specific site identifications than any single method alone (Huang et al., 2020).

Despite its potential for automating microbial identification and advancing forensic workflows, the legal application of artificial intelligence remains constrained by several critical challenges. These limitations, including issues of model bias, overfitting, lack of interpretability, and concerns regarding legal admissibility, are discussed in the following section.

Despite these promising developments, several unresolved issues continue to hinder the forensic adoption of AI-based tools. These challenges are discussed in the following section.

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Challenges and limitations of artificial intelligence in forensic hydroecology

Although artificial intelligence (AI) has shown significant promise in automating the identification of aquatic microbial communities, particularly through deep learning and image recognition technologies (Cordier et al., 2018; Kloster et al., 2020), several critical limitations must be addressed before widespread forensic adoption.

One major concern is training data bias. Models developed using geographically or taxonomically limited datasets may perform well on internal test sets but fail when applied to novel or ecologically diverse forensic samples (Kloster et al., 2020). This issue raises serious concerns about generalizability and the risk of misclassification in real-world forensic investigations.

A related issue is overfitting, wherein AI models learn noise or specific patterns in the training data that do not generalize to new input (Kloster et al., 2020; Gunduz and Gunal, 2024). To mitigate this, robust cross-validation, the use of independent external datasets, and data augmentation techniques must be incorporated into model development workflows (Cordier et al., 2018).

The lack of interpretability in many deep learning models—commonly referred to as the “black box” problem—poses a substantial obstacle to legal admissibility. In forensic contexts, expert testimony must be transparent and defensible; opaque algorithmic decisions that cannot be explained or audited risk being challenged in court (Cordier et al., 2018; Young and Linacre, 2021). Thus, the integration of explainable AI (XAI) frameworks is increasingly viewed as essential to ensure that AI-generated findings meet forensic evidentiary standards (Gunduz and Gunal, 2024).

Finally, legal and regulatory acceptance of AI-assisted forensic tools remains limited. Courts generally require that forensic methods be reproducible, validated, and peer-reviewed. Without standardized validation protocols and transparent reporting mechanisms, AI-generated microbial evidence may not be deemed admissible (Young and Linacre, 2021).

Addressing these limitations will require interdisciplinary collaboration between forensic scientists, microbiologists, data scientists, and legal professionals to ensure that AI tools are not only accurate, but also trustworthy and legally defensible.

To better illustrate the relative strengths, limitations, and optimal applications of different analytical approaches, a detailed comparative overview of traditional microscopy, eDNA/metabarcoding techniques, and AI-assisted identification is provided in *Table 1*.

Comparative overview of analytical methods

Given the diversity of analytical approaches discussed, it is crucial to systematically compare the traditional microscopy, eDNA/NGS methods, and AI-assisted identification techniques in terms of their forensic applicability. *Table 1* summarizes the key features, advantages, and limitations of each method, highlighting their respective roles and optimal use scenarios in forensic hydroecology.

As shown in *Table 1*, while traditional microscopy remains critical for morphological confirmation, eDNA/NGS methods offer enhanced sensitivity for degraded or complex samples. AI-assisted techniques, although currently experimental, present promising opportunities for high-throughput, objective, and scalable forensic analyses, pending further validation and regulatory acceptance. A visual comparison of these analytical approaches across core evaluation criteria is provided in *Figure 3*.

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Table 1. *Comparative overview of traditional microscopy, eDNA-based Methods, and AI-assisted approaches in forensic hydroecology*

Criteria	Traditional Microscopy	eDNA/Metabarcoding	AI-Assisted Identification
Cost	Low equipment cost; high labor cost (Ludes et al., 1999)	Moderate to high (sequencing & bioinformatics) (Allwood et al., 2020, Bailet et al., 2020, Young and Linacre, 2021)	High initial; low per-sample post-deployment (Cordier et al., 2018)
Detection Speed	Slow (manual identification; hours to days) (Ludes et al., 1999)	Moderate (1–3 days) (Allwood et al., 2020, Young and Linacre, 2021)	Fast (real-time once trained) (Kloster et al., 2020, Cordier et al., 2018)
Sensitivity	Moderate (requires frustule preservation) (Auer and Möttönen, 1988, Dahiya et al., 2022)	High (detects trace & degraded DNA)(Allwood et al., 2020, Harrison et al., 2019)	Very high (with adequate training data) (Kloster et al., 2020, Gunduz and Gunal, 2024)
Specificity	High for distinct morphologies(Crawford et al., 2001, Vijayan et al., 2020)	High if reference databases are complete (Bailet et al., 2020, Harrison et al., 2019)	Depends on model quality and dataset (Kloster et al., 2020, Gunduz and Gunal, 2024)
Best Use Case	Intact biological material (tissues, clothing, water samples)(Ludes et al., 1999)	Complex or degraded samples, trace evidence (Allwood et al., 2020, Harrison et al., 2019)	Large-scale, rapid screening(Cordier et al., 2018)
Main Limitation	Observer bias, labor-intensive, expertise dependency(Ludes et al., 1999)	Contamination risks, incomplete reference databases (Allwood et al., 2020, Harrison et al., 2019)	Training bias, explainability issues, legal hurdl (Cordier et al., 2018, Gunduz and Gunal, 2024)

Note: "Typical Sources of Error" summarize the main causes of inaccuracies for each method. For traditional microscopy, human error and morphological degradation are major concerns (Ludes et al., 1999). For eDNA/metabarcoding techniques, contamination during sampling or laboratory processing poses a significant risk (Allwood et al., 2020). For AI-assisted identification, biases in training data and lack of model explainability represent key challenges (Kloster et al., 2020)

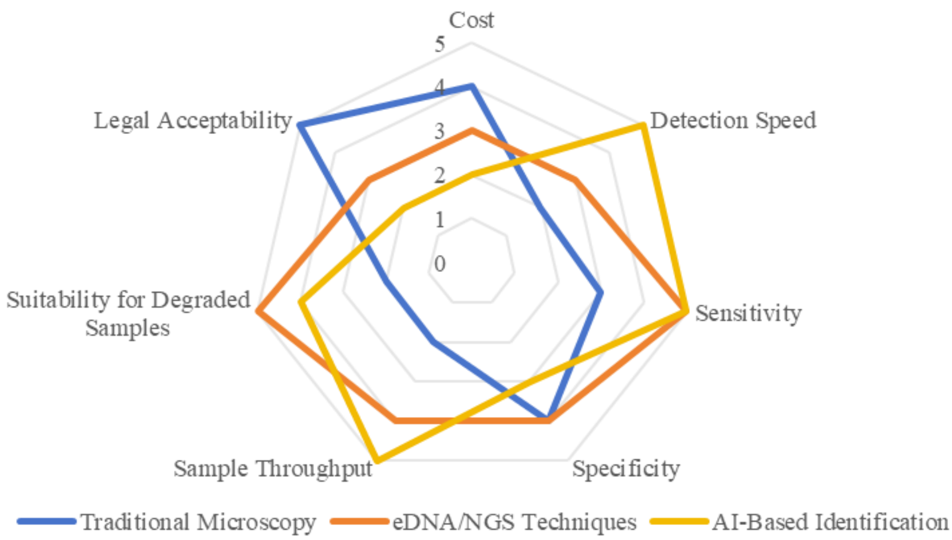

Figure 3. *Comparative radar chart evaluating three analytical approaches—traditional microscopy, eDNA/metabarcoding, and AI-assisted identification—across seven forensic-relevant criteria*

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To provide a systematic assessment of the main analytical approaches in forensic hydroecology, we compared traditional light microscopy, environmental DNA (eDNA) and metabarcoding techniques, and artificial intelligence (AI)-assisted identification across multiple criteria, including cost, detection speed, sensitivity, specificity, forensic applicability, and legal acceptability (*Table 1*).

Given these diverse profiles, no single method universally outperforms the others; rather, an integrative approach leveraging multiple techniques is increasingly advocated (Young and Linacre, 2021). Additionally, a SWOT analysis was conducted to further summarize the strengths, weaknesses, opportunities, and threats associated with each method (*Table 1*).

These findings emphasize that while traditional microscopy remains a cornerstone of forensic hydroecology, molecular and AI-based technologies offer transformative potential when appropriately validated and standardized (Cordier et al., 2018; Allwood et al., 2020; Bailet et al., 2020). The future of aquatic forensic science likely lies in multi-modal strategies that integrate morphological, molecular, and computational analyses to maximize evidentiary reliability and forensic robustness (Cordier et al., 2018; Young and Linacre, 2021).

Case studies and real-world applications

The practical application of aquatic microbial communities in forensic investigations has been demonstrated through numerous case studies. These real-world examples illustrate both the potential and limitations of phytoplankton and diatoms as forensic tools, providing valuable insights for future applications.

Drowning site identification cases

Several documented cases have successfully employed diatom and phytoplankton analysis to identify specific drowning locations, particularly in situations where bodies were moved from the original site of submersion (Geradts et al., 1994; Thakar and Singh, 2010b).

A landmark case described by Siver et al. (2012) involved the murder of a victim whose body was recovered from a pond in southern New England. Investigators analyzed diatoms and scaled chrysophytes from sediment on the suspects' sneakers, comparing them with samples from the crime scene. The distinctive assemblage of algae provided compelling evidence linking the suspects to the specific pond, contributing significantly to their conviction. This case demonstrated the potential for using freshwater algal communities as natural trace evidence in forensic investigations.

Thakar and Singh (2010b) documented a case where a body was recovered from a river, but diatom analysis of the victim's tissues revealed a species assemblage inconsistent with the recovery site. Comparison with samples from other water bodies in the region identified a pond with a matching diatom profile, leading investigators to the actual drowning location. This case highlighted the value of regional "diatomological mapping" for forensic applications.

Verma (2013) conducted diatom tests on lung and bone marrow samples from drowning victims, alongside environmental water samples, to determine drowning site. Their study revealed a mismatch between the diatom species found in the victim's tissues and those at the reported recovery site, suggesting postmortem body movement. This case highlighted the forensic value of comparing internal diatom assemblages with

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environmental references to infer original drowning locations, while also emphasizing challenges such as low diatom abundance in cold seasons.

Su et al. applied bacterial community profiling combined with machine learning algorithms to identify original drowning sites in a flowing river environment (Su et al., 2025). The study demonstrated that even under conditions of microbial drift and environmental variability, machine learning models could accurately attribute drowned individuals to specific river segments based on microbial fingerprints. However, the research also pointed out the complexities introduced by hydrodynamic factors and temporal shifts, underscoring the need for robust sampling and model training strategies in forensic hydroecology.

To provide a clearer overview, the key forensic cases discussed are summarized in *Table 2*, highlighting the analytical methods used, main findings, and challenges encountered.

Table 2. *Summary of key forensic cases involving aquatic microbial community analyses*

Case	Method Used	Main Findings	Challenges Noted
Siver et al. (New England murder case) (Siver, 2012)	Diatom and scaled chrysophyte analysis on sediment	Matched suspect's sneakers to specific pond; critical forensic linkage	Difficulty in capturing full environmental variability
Ma et al. (Yellow Sea river contamination study) (Thakar and Singh, 2010b)	Microbial source tracking using community profiling (MST)	Identified specific sources of microbial contamination entering a river flowing into the Yellow Sea	Ecological variability and multiple-source interference complicating attribution
Verma et al. (Site attribution case) (Verma, 2013)	Diatom test on lungs and bone marrow vs environmental samples	Proved body was moved postmortem from original drowning site	Low diatom concentration in winter samples
Su et al. (Flowing river study) (Su et al., 2025)	Bacterial community profiling and machine learning	Accurate identification of original drowning site in flowing river	Complex microbial shifts in high-flow environments
Scott et al. (Clothing transfer study) (Scott et al., 2014)	Diatom recovery from clothing fibers	Demonstrated diatom transferability from water bodies to garments	Variability in transfer efficiency across fabric types
Uitdehaag et al. (Clothing forensic comparison) (Uitdehaag et al., 2010)	Optimized diatom extraction from cotton fabrics	Enabled forensic site attribution from suspect clothing	Risk of post-exposure contamination

These cases collectively illustrate the strengths and limitations of applying aquatic microbial analyses in forensic investigations, reinforcing the need for method standardization and ecological contextualization.

Environmental forensics and pollution monitoring

Aquatic microbial communities have proven valuable in environmental forensics, helping to identify sources of pollution and establish timelines for environmental contamination events (Wu et al., 2021; Ma et al., 2023).

Arumugham et al. (2023) applied diatom analysis to identify pollution sources in freshwater ponds, demonstrating that specific diatom assemblages reliably indicated particular types of contamination. Their approach enabled investigators to trace pollutants

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to their sources and establish responsibility for environmental damage, highlighting the forensic utility of diatoms beyond drowning cases.

Desianti et al. (2017) used sediment diatoms as environmental indicators in coastal lagoons, developing methods to assess historical changes in water quality and identify sources of environmental degradation. Their research demonstrated how diatom assemblages in sediment cores could reveal temporal patterns of pollution, providing forensic evidence for environmental litigation.

The application of diatoms in environmental forensics extends to industrial settings. Tiwari and Marella (2019) reviewed the potential of diatoms for industry-specific wastewater treatment and monitoring, noting that certain species thrive in waters containing specific industrial pollutants. These specialized adaptations create distinctive community signatures that can potentially identify the sources of industrial contamination in environmental investigations.

Linking suspects to aquatic crime scenes

Several cases have demonstrated the potential for using aquatic microbial communities to link suspects to specific water bodies, providing valuable evidence in criminal investigations (Siver et al., 1994; Speruda et al., 2022).

Scott et al. (2014) investigated the transferability of diatoms to clothing, demonstrating that distinctive assemblages could be recovered from garments exposed to different water bodies. Their research provided a foundation for forensic comparisons between diatoms found on suspects' clothing and those from crime scenes, enabling investigators to establish or refute connections between individuals and specific aquatic locations.

Uitdehaag et al. (2010) further refined methods for extracting diatoms from cotton clothing, developing optimized protocols for forensic comparisons. Their techniques have been applied in cases where suspects denied presence at aquatic crime scenes, with diatom evidence contradicting their claims and placing them at the specific water bodies in question.

Temporal aspects and cold cases

Diatom frustules are highly resistant to degradation. This durability allows their application in cold cases and in cases where remains are discovered long after death (Cumming and Smol, 1993; Horton et al., 2006).

Horton et al. (2006) developed and applied a diatom-based quantitative reconstruction technique in forensic science, demonstrating its utility for establishing timelines in cases involving aquatic environments. Their approach enabled investigators to determine when bodies or objects had entered water bodies based on the succession patterns of diatom communities, providing crucial temporal information for cold cases.

The analysis of diatoms in sediment cores has proven valuable for establishing historical environmental conditions relevant to cold cases. Cumming and Smol (1993) developed diatom-based models for paleoenvironmental reconstruction, providing methods that can potentially determine whether particular water bodies existed or had specific characteristics at the time of historical crimes.

In a remarkable application, researchers analyzed diatoms from the clothing of historical drowning victims, successfully identifying the specific water bodies where the drownings occurred despite the passage of many decades (Scott et al., 2014). This demonstration of the long-term persistence of diatom evidence highlights its potential value for cold case investigations involving aquatic environments.

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Challenges and current limitations

Sample degradation, contamination, and preservation issues

Sample integrity represents a fundamental challenge in forensic microbial analysis, with degradation, contamination, and preservation issues potentially compromising results (Yen and Jayaprakash, 2007; Lunetta et al., 2013).

Postmortem degradation of tissues can affect the recovery and identification of microorganisms from bodies recovered from water. Lunetta et al. (2013) investigated the impact of decomposition on diatom testing, finding that advanced decomposition complicated analysis but did not necessarily preclude successful testing when appropriate methods were employed. Nevertheless, the interpretation of results becomes increasingly challenging as decomposition progresses.

Contamination presents another significant concern, particularly for the diatom test in drowning investigations. Yen and Jayaprakash (2007) documented the prevalence of diatom frustules in non-vegetarian foodstuffs, highlighting a potential source of false positives. Similarly, Otto (1961) reported the presence of diatoms in the lungs of non-drowned individuals due to inhalation from air, further complicating interpretation.

Sample preservation methods can significantly impact the recovery and identification of aquatic microorganisms. Garrido et al. (2013) evaluated the effects of different preservation approaches on phytoplankton analysis, finding that some common methods altered community composition or hindered species identification. These findings underscore the importance of standardized preservation protocols for forensic samples.

Żarczyńska et al. (2023) reviewed nucleic acids persistence in forensic genetics, highlighting both benefits and limitations relevant to DNA-based analysis of aquatic microbial communities. Their research emphasized the importance of appropriate sample handling and storage to maximize DNA preservation, particularly for environmental samples that may contain inhibitory substances.

Time delay and cadaver migration interference

Temporal factors and physical movement of bodies in water create additional challenges for forensic applications of aquatic microbial communities (Leth and Madsen, 2017; Armstrong and Erskine, 2018).

Time delays between death and recovery can significantly impact the interpretation of microbial evidence. Armstrong and Erskine (2018) reviewed practical aspects of drowning death investigations, noting that extended postmortem intervals allowed for changes in microbial communities both within the body and in the surrounding environment. These temporal changes complicate comparisons between victim samples and environmental reference samples collected after recovery.

Cadaver migration in flowing water bodies presents a particular challenge for drowning site identification. Bodies may travel considerable distances from the original drowning location, especially in rivers with strong currents. Leth and Madsen (2017) investigated drowning cases using post-mortem computed tomography and autopsy, documenting instances where bodies had moved significant distances from the drowning site, complicating the interpretation of microbial evidence.

Seasonal changes in aquatic microbial communities further complicate temporal aspects of forensic investigations. Singh et al. (2010) documented distinct seasonal assemblages of diatoms in water bodies, highlighting the importance of considering seasonal succession patterns when comparing samples collected at different times. This

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temporal dimension necessitates the development of season-specific reference databases for accurate forensic comparisons.

Lack of unified databases and standard procedures

The absence of standardized protocols and comprehensive reference databases represents a significant limitation for the forensic application of aquatic microbial communities (Ludes et al., 1999; Bailet et al., 2020).

Methodological variations complicate comparisons between different laboratories and investigations. Bailet et al. (2020) compared diatom DNA metabarcoding protocols used in six European countries, finding significant differences in results despite analyzing identical samples. Their research highlighted the urgent need for standardization to ensure reliability and comparability of forensic microbial analyses.

Reference databases for aquatic microorganisms remain incomplete and fragmented, particularly for less-studied geographical regions. Ludes et al. (1999) emphasized the importance of regional reference collections for accurate drowning site identification, noting that diatom assemblages vary significantly between different geographical areas. The lack of comprehensive databases limits the applicability of microbial approaches in regions without established reference collections.

Taxonomic inconsistencies further complicate the development of unified databases. Ongoing revisions to phytoplankton taxonomy, particularly for diatoms, create challenges for maintaining consistent identification standards across different studies and time periods (Trabert, 2017). These taxonomic issues necessitate regular updates to reference databases and careful attention to nomenclatural changes.

Tambuzzi et al. (2024) reviewed the current state of forensic diatom analysis, highlighting the lack of standardized protocols as a significant limitation. They called for international collaboration to develop consensus guidelines for sample collection, processing, analysis, and interpretation, emphasizing that standardization would enhance the credibility and admissibility of microbial evidence in legal proceedings.

Ecological complexity vs. forensic interpretability

The ecological complexity of aquatic microbial communities creates challenges for forensic interpretation, requiring careful consideration of multiple environmental factors and their interactions (Cumming and Smol, 1993; Lobo et al., 2015).

Spatial heterogeneity within water bodies complicates site-specific identification. Even within a single lake or river, microbial communities can vary significantly between different locations, depths, and microhabitats (Rivera et al., 2020). This spatial complexity necessitates comprehensive sampling strategies to characterize the full range of community variations within potential crime scenes.

Temporal dynamics, including daily migrations, seasonal succession, and long-term ecological changes, add another layer of complexity to forensic interpretations. Cumming and Smol (1993) highlighted how diatom communities in lakes change over time in response to environmental variations, creating challenges for historical comparisons in cold cases.

Multiple environmental factors simultaneously influence aquatic microbial communities, creating complex ecological relationships that can be difficult to disentangle. Lobo et al. (2015) developed the Trophic Water Quality Index based on diatom communities, demonstrating how multiple environmental parameters interact to

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shape community composition. This multifactorial influence complicates the interpretation of microbial evidence in forensic contexts.

Yang et al. (2021)investigated how spatial variation in bacterial community assembly processes affects the accuracy of source tracking in urban environments. Their research revealed that different ecological mechanisms dominated in different cities, highlighting the importance of understanding local ecological dynamics for accurate forensic interpretations.

Balancing ecological complexity with forensic interpretability requires sophisticated analytical approaches and careful consideration of limitations. Statistical methods, including multivariate analyses and machine learning algorithms, can help identify patterns in complex ecological data, but their application must acknowledge the inherent uncertainties and limitations of ecological inference in forensic contexts (Su et al., 2023).

Given the significant challenges posed by sample degradation, contamination, and ecological variability, the establishment of standardized sampling protocols is crucial to ensure consistency, reliability, and forensic applicability of aquatic microbial evidence. The following section outlines key recommendations for sampling standardization and management of ecological variability.

Standardization of sampling and management of ecological variability

The forensic application of aquatic microbial communities critically depends on robust and standardized sampling protocols to minimize ecological variability and improve reproducibility. However, current studies show significant heterogeneity in sampling approaches, leading to difficulties in cross-study comparisons and case interpretations. To address this issue, we propose the following key standardization strategies:

Minimum Sample Volume and Replication: For environmental sampling (e.g., water from suspected drowning sites), a minimum of 1 liter per site should be collected. To adequately capture microhabitat variability, at least three replicate samples from spatially distinct points within the same site are recommended (Rivera et al., 2020).

Simultaneous Recording of Environmental Parameters: Critical abiotic factors, including water temperature, pH, conductivity, dissolved oxygen, turbidity, and GPS coordinates, should be measured during sample collection. Recording these metadata enables ecological interpretation of microbial communities and helps control for temporal and spatial variability (Pajunen et al., 2017; Gogoi et al., 2019).

Sample Preservation and Storage: For microscopy-based analyses, immediate fixation using neutral formalin at a final concentration of 2–4% is recommended to preserve cellular integrity. For molecular analyses (eDNA or NGS), filtration of water samples on-site followed by immediate freezing at –20°C or preservation in ethanol is advised to prevent DNA degradation (Rivera et al., 2020; Allwood et al., 2020; Nayebi et al., 2023).

Temporal Considerations: Sampling protocols should account for the seasonal succession patterns of aquatic microbial communities. Reference databases must incorporate temporal variability by repeatedly sampling the same sites across different seasons (Gamier et al., 1995; Dmitrijs et al., 2022).

Contamination Control Measures: All sampling equipment must be sterilized before use to avoid exogenous contamination. Negative control samples (e.g., blanks) should be collected alongside actual samples, particularly for workflows involving environmental DNA, to monitor and control for potential contamination sources (Yen and Jayaprakash, 2007; Lunetta et al., 2013).

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Implementing standardized protocols as outlined above will enhance the reliability, comparability, and forensic admissibility of aquatic microbial evidence. Future efforts should aim at developing international guidelines to formalize these procedures across forensic laboratories.

Future perspectives in forensic hydroecology

Forensic hydroecology lies at the promising intersection of ecology, forensic science, and technological innovation. This section explores emerging directions and future possibilities for the application of aquatic microbial communities in forensic science.

Building global or regional "Water body microbial databases"

Comprehensive reference databases represent a critical foundation for advancing the forensic application of aquatic microbial communities (Deiner et al., 2017; Allwood et al., 2020).

Allwood et al. (2020) outlined the future of environmental DNA in forensic science, emphasizing the importance of developing standardized reference databases for different geographical regions and water body types. They proposed collaborative efforts to systematically sample and characterize microbial communities across diverse aquatic environments, creating resources that would enhance the specificity and reliability of forensic comparisons.

Deiner et al. (2017) reviewed environmental DNA metabarcoding applications for surveying animal and plant communities, highlighting approaches that could be adapted for forensic database development. Their work emphasized the importance of standardized sampling and analysis protocols to ensure database consistency and compatibility across different regions and research groups.

Ruppert et al. (2019) conducted a systematic review of environmental DNA methods, monitoring approaches, and applications, providing a framework for developing forensic-specific databases. They identified best practices for sample collection, preservation, and analysis that could be implemented in large-scale database development efforts.

International collaboration will be essential for building truly comprehensive microbial databases. Taberlet et al. (2018) discussed approaches for environmental DNA monitoring, emphasizing the value of coordinated international efforts to develop shared resources and standardized methodologies. Similar collaborative approaches could significantly advance the development of forensic hydroecology databases.

eDNA + Artificial intelligence + GIS combined technology prospects

The integration of environmental DNA analysis with artificial intelligence and geographic information systems offers particularly promising avenues for forensic applications (Bell et al., 2016; Young and Linacre, 2021).

Environmental DNA (eDNA) analysis continues to advance rapidly, with increasing sensitivity and specificity for detecting and identifying aquatic organisms. Thomsen and Willerslev (2015) reviewed the application of eDNA for monitoring biodiversity, highlighting techniques that could be adapted for forensic purposes. These approaches enable the detection of a broader range of organisms from water samples, potentially enhancing the discriminatory power of microbial analysis for site identification.

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Artificial intelligence applications in microbial forensics are expanding beyond automated identification to include predictive modeling and source attribution. Bell et al. (2016) reviewed future prospects for DNA barcoding in forensic palynology, discussing how machine learning approaches could enhance the interpretation of complex biological data. Similar approaches could be applied to aquatic microbial communities, potentially enabling more accurate site identification from limited sample material.

Geographic information systems (GIS) provide powerful tools for integrating spatial data with microbial community information. By mapping the distribution of microbial taxa across landscapes and correlating these patterns with environmental variables, GIS approaches can potentially predict the geographical origin of unknown samples based on their microbial signatures (Yang et al., 2021).

Young and Linacre (2021) discussed how massively parallel sequencing is unlocking the potential of environmental trace evidence, highlighting the integration of multiple data types through computational approaches. They envisioned systems that could combine DNA sequence data with geographical information and environmental parameters to provide highly specific source attributions for forensic samples. These multi-modal approaches are conceptually summarized in *Figure 4*, which visualizes the integration of AI, molecular tools, and GIS-based forensics using aquatic microbial communities.

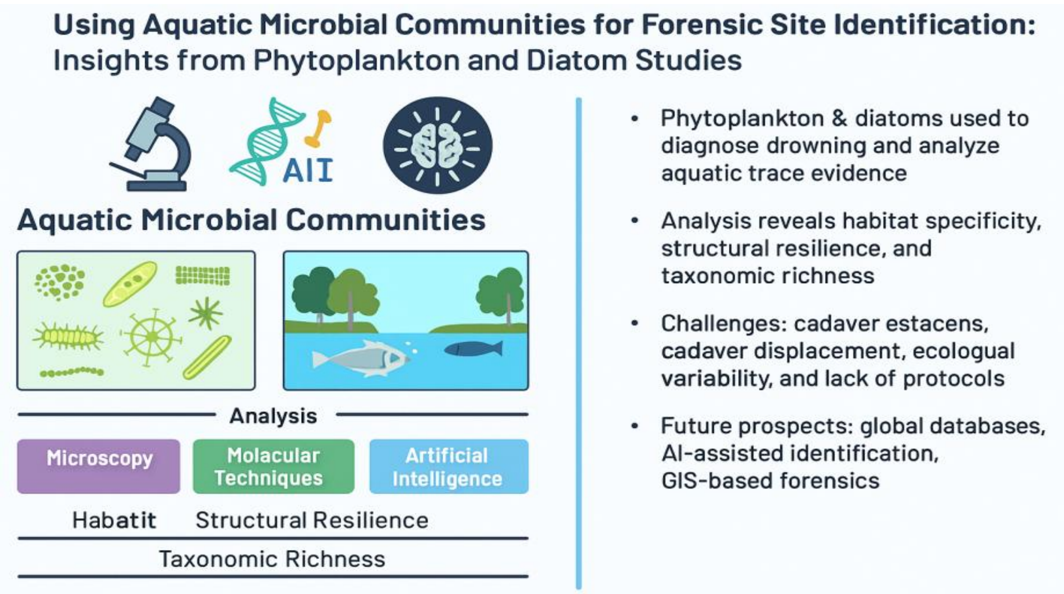

Figure 4. *Integrative future applications of microbial forensics. Source: Created by the authors*

Environmental tracing with remote sensing and drones

The combination of microbial analysis with remote sensing technologies and unmanned aerial vehicles (drones) offers innovative approaches for environmental forensics and site identification (Sijen and Harbison, 2021).

Remote sensing technologies can provide valuable contextual information for interpreting microbial evidence. Satellite imagery and aerial photography can document environmental conditions, water body characteristics, and landscape features relevant to forensic investigations. When combined with microbial data, this information can

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enhance the specificity of site identification and help establish temporal contexts for forensic events.

Drones enable targeted sampling and monitoring of aquatic environments, particularly in remote or difficult-to-access locations. Equipped with water sampling devices, these platforms can collect reference samples from specific coordinates, creating precise spatial records of microbial distributions in water bodies of forensic interest (Foster et al., 2023).

Sijen and Harbison (2021) discussed emerging approaches for identifying body fluids and tissues in forensic investigations, highlighting the potential for integrating multiple analytical techniques. Similar integrative approaches could combine microbial analysis with remote sensing data and geographical information to enhance the specificity and reliability of forensic site identification.

Wiltshire (2016) explored applications of mycology in palaeoecology and forensic science, emphasizing the value of integrating multiple lines of evidence. This integrative approach, combining microbial analysis with remote sensing and geographical data, represents a promising direction for future developments in forensic hydroecology.

Introduction of microecology in forensic education and interdisciplinary collaboration

Advancing forensic hydroecology will require significant educational developments and enhanced collaboration across disciplinary boundaries (d'Ippolito et al., 2015; Trabert, 2017).

Forensic science education programs increasingly need to incorporate ecological principles and microbial taxonomy to prepare practitioners for applying aquatic microbial approaches. Training in sampling techniques, identification methods, and ecological interpretation will be essential for the effective implementation of these approaches in forensic investigations (Tambuzzi et al., 2024).

Interdisciplinary collaboration between forensic scientists, ecologists, microbiologists, and data scientists represents a critical foundation for advancing the field. d'Ippolito et al. (2015) demonstrated the value of cross-disciplinary approaches in their work on lipid metabolism in marine diatoms, combining expertise from multiple fields to develop novel analytical methods. Similar collaborative approaches could significantly enhance the development and application of microbial techniques in forensic contexts.

International networks and collaborative research initiatives offer promising mechanisms for advancing forensic hydroecology. Martin et al. (2014) demonstrated the value of collaborative approaches in their work on lipid profiles in microalgae, bringing together expertise from multiple institutions to address complex research questions. Similar collaborative networks could accelerate the development of forensic applications for aquatic microbial communities.

This diagram summarizes current and emerging uses of aquatic microbial communities in forensic site identification. It integrates traditional and molecular techniques (e.g., microscopy, eDNA, metabarcoding) with AI-assisted classification and GIS-based spatial analysis. Key applications include drowning diagnosis, trace evidence linkage, and environmental attribution, framed within a multi-omics and explainable AI context.

Conclusions

This review highlights the significant potential of aquatic microbial communities, particularly phytoplankton and diatoms, in forensic site identification. By integrating traditional microscopy, molecular techniques, artificial intelligence, and GIS-based

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spatial analysis, forensic hydroecology can overcome limitations in drowning diagnosis and environmental trace evidence analysis. The advancement of standardized protocols, global reference databases, and explainable AI frameworks will be critical in transitioning these methods into routine forensic practice, enhancing legal reliability and operational applicability.

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REFERENCES

[1] Adserias-Garriga, J., Hernández, M., Quijada, N. M., Lázaro, D. R., Steadman, D., Garcia-Gil, J. (2017): Daily thanatomicrobiome changes in soil as an approach of postmortem interval estimation: an ecological perspective. – Forensic Science International 278: 388-395. https://doi.org/10.1016/j.forsciint.2017.07.017

[2] Allwood, J. S., Fierer, N., Dunn, R. R. (2020): The future of environmental DNA in forensic science. – Applied and Environmental Microbiology 86: e01504-19. https://doi.org/10.1128/AEM.01504-19

[3] Andresen, N. A., Edlund, M. B. (2001): Index to Diatom Research: Volumes 1-14, 1986–1999. – Diatom Research 16: 125-246. https://doi.org/10.1080/0269249X.2001.9705516

[4] Armstrong, E. J., Erskine, K. L. (2018): Investigation of drowning deaths: a practical review. – Academic Forensic Pathology 8: 8-43. https://doi.org/10.23907/2018.002

[5] Arumugham, S., Joseph, S. J. P., Nooruddin, T., 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

[6] Auer, A., Möttönen, M. (1988): Diatoms and drowning. – Zeitschrift für Rechtsmedizin 101: 87-98. https://doi.org/10.1007/BF00200290

[7] Baba, A., Pandit, A. K. (2014): Species composition, diversity and population Dyanamics of phytoplankton at Saderkot in Wular Lake, Kashmir. – J Eco Ecogr 4: 142-50. https://doi.org/10.4172/2157-7625.1000142

[8] Bailet, B., Apothéloz-Perret-Gentil, L., Baričević, A., Chonova, T., Franc, A., Frigerio, J.-M., Kelly, M., Mora, D., Pfannkuchen, M., Proft, S. (2020): Diatom DNA metabarcoding for ecological assessment: Comparison among bioinformatics pipelines used in six European countries reveals the need for standardization. – Science of the Total Environment 745: 140948. https://doi.org/10.1016/j.scitotenv.2020.140948

[9] Bastos, C. R., Maia, I. B., Pereira, H., Navalho, J., Varela, J. C. (2022): Optimisation of biomass production and nutritional value of two marine diatoms (Bacillariophyceae), *Skeletonema costatum* and *Chaetoceros calcitrans*. – Biology 11: 594. https://doi.org/10.3390/biology11040594

[10] Bell, K. L., Burgess, K. S., Okamoto, K. C., Aranda, R., Brosi, B. J. (2016): Review and future prospects for DNA barcoding methods in forensic palynology. – Forensic Science International: Genetics 21: 110-116. https://doi.org/10.1016/j.fsigen.2015.12.010

[11] Benson, J., Hanlon, R., Seifried, T. M., Baloh, P., Powers, C. W., Grothe, H., Schmale III, D. G. (2019): Microorganisms collected from the surface of freshwater lakes using a drone water sampling system (DOWSE). – Water 11: 157. https://doi.org/10.3390/w11010157

[12] Bogusz, I., Bogusz, M., Borowska, B., Szleszkowski, Ł., Żelazna-Wieczorek, J. (2024): A critical assessment of the diatom test of rib bone marrow as a supporting procedure in the case of drowning. – Journal of Forensic and Legal Medicine 107: 102753. https://doi.org/10.1016/j.jflm.2024.102753

[13] Burdis, R. M., Ward, N. K., Manier, J. T. (2025): Phytoplankton assemblage dynamics in relation to environmental conditions in a riverine lake. – Aquatic Ecology 59: 467-485. https://doi.org/10.1007/s10452-025-10174-1
[14] Canini, N. D., Metillo, E. B., Azanza, R. V. (2013): Monsoon-influenced phytoplankton community structure in a Philippine mangrove estuary. – Tropical Ecology 54: 331-343. https://doi.org/10.1016/j.jaridenv.2013.04.006
[15] Cartiser, N., Bévalot, F., Fanton, L., Gaillard, Y., Guitton, J. (2011): State-of-the-art of bone marrow analysis in forensic toxicology: a review. – International Journal of Legal Medicine 125: 181-198. https://doi.org/10.1007/s00414-010-0525-6
[16] Chardez, D., Lambert, J. (1985): Protozoaires ciliés et thanatologie. – Forensic Science International 28: 83-101. https://doi.org/10.1016/0379-0738(85)90065-9
[17] Cloern, J. E., Jassby, A. D., Schraga, T. S., Nejad, E., Martin, C. (2017): Ecosystem variability along the estuarine salinity gradient: Examples from long-term study of San Francisco Bay. – Limnology and Oceanography 62: S272-S291. https://doi.org/10.1002/lno.10537
[18] Cordier, T., Forster, D., Dufresne, Y., Martins, C. I., Stoeck, T., Pawlowski, J. (2018): Supervised machine learning outperforms taxonomy-based environmental DNA metabarcoding applied to biomonitoring. – Molecular Ecology Resources 18: 1381-1391. https://doi.org/10.1111/1755-0998.12926
[19] Crawford, S. A., Higgins, M. J., Mulvaney, P., Wetherbee, R. (2001): Nanostructure of the diatom frustule as revealed by atomic force and scanning electron microscopy. – Journal of Phycology 37: 543-554. https://doi.org/10.1046/j.1529-8817.2001.037004543.x
[20] Crump, B. C., Hopkinson, C. S., Sogin, M. L., Hobbie, J. E. (2004): Microbial biogeography along an estuarine salinity gradient: combined influences of bacterial growth and residence time. – Applied and Environmental Microbiology 70: 1494-1505. https://doi.org/10.1128/AEM.70.3.1494-1505.2004
[21] Cumming, B. F., Smol, J. P. (1993): Development of diatom-based salinity models for paleoclimatic research from lakes in British Columbia (Canada). – Hydrobiologia 269: 179-196. https://doi.org/10.1007/BF00028017
[22] d'Ippolito, G., Sardo, A., Paris, D., Vella, F. M., Adelfi, M. G., Botte, P., Gallo, C., Fontana, A. (2015): Potential of lipid metabolism in marine diatoms for biofuel production. – Biotechnology for Biofuels 8: 1-10. https://doi.org/10.1186/s13068-015-0212-4
[23] Dahiya, P., Kumar, S., Shukla, M. A., Yadav, C. S. (2022): Forensic limnology: Diversity of diatom population in relation to environmental factors in Gujarat region, India. – Materials Today: Proceedings 69: A14-A24. https://doi.org/10.1016/j.matpr.2022.11.421
[24] Dahiya, P., Makwana, M. D., Chaniyara, P., Bhatia, A. (2024): A comprehensive review of forensic diatomology: contemporary developments and future trajectories. – Egyptian Journal of Forensic Sciences 14: 2. https://doi.org/10.1186/s41935-023-00378-7
[25] De Tommasi, E., Gielis, J., Rogato, A. (2017): Diatom frustule morphogenesis and function: a multidisciplinary survey. – Marine Genomics 35: 1-18. https://doi.org/10.1016/j.margen.2017.07.001
[26] Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursiére - Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., De Vere, N. (2017): Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. – Molecular Ecology 26: 5872-5895. https://doi.org/10.1111/mec.14350
[27] Desianti, N., Potapova, M., Enache, M., Belton, T. J., Velinsky, D. J., Thomas, R., Mead, J. (2017): Sediment diatoms as environmental indicators in New Jersey coastal lagoons. – Journal of Coastal Research 78: 127-140. https://doi.org/10.2112/SI78-011.1
[28] Díaz-Palma, P., Alucema, A., Hayashida, G., Maidana, N. (2009): Development and standardization of a microalgae test for determining deaths by drowning. – Forensic Science International 184: 37-41. https://doi.org/10.1016/j.forsciint.2008.11.015

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_29392968

[29] Dmitrijs, F., Guo, J., Huang, Y., Liu, Y., Fang, X., Jiang, K., Zha, L., Cai, J., Fu, X. (2022): Bacterial succession in microbial biofilm as a potential indicator for postmortem submersion interval estimation. – Frontiers in Microbiology 13: 951707. https://doi.org/10.3389/fmicb.2022.951707

[30] Edwards, H. G., Villar, S. E. J., Parnell, J., Cockell, C. S., Lee, P. (2005): Raman spectroscopic analysis of cyanobacterial gypsum halotrophs and relevance for sulfate deposits on Mars. – Analyst 130: 917-923. https://doi.org/10.1039/b503533c

[31] Egres, A. G., Hatje, V., Miranda, D. A., Gallucci, F., Barros, F. (2019): Functional response of tropical estuarine benthic assemblages to perturbation by Polycyclic Aromatic Hydrocarbons. – Ecological Indicators 96: 229-240. https://doi.org/10.1016/j.ecolind.2018.08.062

[32] Farqan, M., Xiang, L., Jiang, L., Wang, W., Zheng, M., Mu, X., Liu, X., Song, X., Zhu, Z., Ayyamperumal, R. (2025): Diatom assemblages indicated ecological shifts over the past two centuries in Bosten Lake, northwest China: Natural and anthropogenic forcings. – Quaternary International 716: 109629. https://doi.org/10.1016/j.quaint.2024.109629

[33] Fløjgaard, C., Frøslev, T. G., Brunbjerg, A. K., Bruun, H. H., Moeslund, J., Hansen, A. J., Ejrnæs, R. (2019): Predicting provenance of forensic soil samples: Linking soil to ecological habitats by metabarcoding and supervised classification. – PLoS One 14: e0202844. https://doi.org/10.1371/journal.pone.0202844

[34] Foster, N. R., Martin, B., Hoogewerff, J., Aberle, M. G., De Caritat, P., Roffey, P., Edwards, R., Malik, A., Thwaites, P., Waycott, M. (2023): The utility of dust for forensic intelligence: Exploring collection methods and detection limits for environmental DNA, elemental and mineralogical analyses of dust samples. – Forensic Science International 344: 111599. https://doi.org/10.1016/j.forsciint.2023.111599

[35] Fucci, N., Campobasso, C. P., Mastrogiuseppe, L., Puccinelli, C., Marcheggiani, S., Mancini, L., Marino, L., Pascali, V. L. (2017): Diatoms in drowning cases in forensic veterinary context: a preliminary study. – International Journal of Legal Medicine 131: 1573-1580. https://doi.org/10.1007/s00414-017-1565-y

[36] Gamier, J., Billen, G., Coste, M. (1995): Seasonal succession of diatoms and Chlorophyceae in the drainage network of the Seine River: Observation and modeling. – Limnology and Oceanography 40: 750-765. https://doi.org/10.4319/lo.1995.40.4.0750

[37] Garrido, M., Cecchi, P., Vaquer, A., Pasqualini, V. (2013): Effects of sample conservation on assessments of the photosynthetic efficiency of phytoplankton using PAM fluorometry. – Deep Sea Research Part I: Oceanographic Research Papers 71: 38-48. https://doi.org/10.1016/j.dsr.2012.09.004

[38] Gayoso, A. (1999): Seasonal succession patterns of phytoplankton in the Bahía Blanca Estuary (Argentina). – Botanica Marina 42(4). https://doi.org/10.1515/BOT.1999.042

[39] Geradts, Z., Keijzer, J., Keereweer, I. (1994): A new approach to automatic comparison of striation marks. – Journal of Forensic Sciences 39: 974-980. https://doi.org/10.1520/JFS13676J

[40] Giampaoli, S., De Vittori, E., Frajese, G., Paytuví, A., Sanseverino, W., Anselmo, A., Barni, F., Berti, A. (2020): A semi-automated protocol for NGS metabarcoding and fungal analysis in forensic. – Forensic Science International 306: 110052. https://doi.org/10.1016/j.forsciint.2019.110052

[41] Girela-Lopez, E., Beltran-Aroca, C. M., García-Mozo, H. (2020): Diatoms in forensic analysis. – In: Cristóbal, G., Blanco, S., Bueno, G. (eds.) Modern Trends in Diatom Identification. Developments in Applied Phycology, vol 10. Springer, Cham. https://doi.org/10.1007/978-3-030-39212-3_14

[42] Gogoi, P., Sinha, A., Das Sarkar, S., Chanu, T. N., Yadav, A. K., Koushlesh, S. K., Borah, S., Das, S. K., Das, B. K. (2019): Seasonal influence of physicochemical parameters on phytoplankton diversity and assemblage pattern in Kailash Khal, a tropical wetland, Sundarbans, India. – Applied Water Science 9: 1-13. https://doi.org/10.1007/s13201-019-1034-5

[43] Grimes, D. J., Ford, T. E., Colwell, R. R., Baker-Austin, C., Martinez-Urtaza, J., Subramaniam, A., Capone, D. G. (2014): Viewing marine bacteria, their activity and response to environmental drivers from orbit: satellite remote sensing of bacteria. – Microbial Ecology 67: 489-500. https://doi.org/10.1007/s00248-013-0363-4
[44] Gunduz, H., Gunal, S. (2024): A lightweight convolutional neural network (CNN) model for diatom classification: DiatomNet. – PeerJ Computer Science 10: e1970. https://doi.org/10.7717/peerj-cs.1970
[45] Harrison, J. B., Sunday, J. M., Rogers, S. M. (2019): Predicting the fate of eDNA in the environment and implications for studying biodiversity. – Proceedings of the Royal Society B 286: 20191409. https://doi.org/10.1098/rspb.2019.1409
[46] He, Q., Niu, X., Qi, R.-Q., Liu, M. (2022): Advances in microbial metagenomics and artificial intelligence analysis in forensic identification. – Frontiers in Microbiology 13: 1046733. https://doi.org/10.3389/fmicb.2022.1046733
[47] Hebert, P. D., Cywinska, A., Ball, S. L., Dewaard, J. R. (2003): Biological identifications through DNA barcodes. – Proceedings of the Royal Society of London. Series B: Biological Sciences 270: 313-321. https://doi.org/10.1098/rspb.2002.2218
[48] Horton, B. P., Boreham, S., Hillier, C. (2006): The development and application of a diatom-based quantitative reconstruction technique in forensic science. – Journal of Forensic Sciences 51: 643-650. https://doi.org/10.1111/j.1556-4029.2006.00120.x
[49] 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 Science International 226: e48-e51. https://doi.org/10.1016/j.forsciint.2013.01.010
[50] Huang, L., Xu, C., Yang, W., Yu, R. (2020): A machine learning framework to determine geolocations from metagenomic profiling. – Biology Direct 15: 1-12. https://doi.org/10.1186/s13062-020-00278-z
[51] Iannella, M., D'Alessandro, P., De Simone, W., Biondi, M. (2021): Habitat specificity, host plants and areas of endemism for the genera-group Blepharida sl in the afrotropical region (Coleoptera, Chrysomelidae, Galerucinae, Alticini). – Insects 12: 299. https://doi.org/10.3390/insects12040299
[52] Kim, B. K., Jung, J., Lee, Y., Cho, K.-H., Gal, J.-K., Kang, S.-H., Ha, S.-Y. (2020): Characteristics of the biochemical composition and bioavailability of phytoplankton-derived particulate organic matter in the Chukchi Sea, Arctic. – Water 12: 2355. https://doi.org/10.3390/w12092355
[53] Kloster, M., Langenkämper, D., Zurowietz, M., Beszteri, B., Nattkemper, T. W. (2020): Deep learning-based diatom taxonomy on virtual slides. – Scientific Reports 10: 14416. https://doi.org/10.1038/s41598-020-71165-w
[54] Kojić, F., Mandić, D., Ilić, V. (2021): Resistance training induces similar adaptations of upper and lower-body muscles between sexes. – Scientific Reports 11: 23449. https://doi.org/10.1038/s41598-021-02867-y
[55] Kuiper, I. (2016): Microbial forensics: next-generation sequencing as catalyst: The use of new sequencing technologies to analyze whole microbial communities could become a powerful tool for forensic and criminal investigations. – EMBO Reports 17: 1085-1087. https://doi.org/10.15252/embr.201642794
[56] Lally, H., O'Connor, I., Jensen, O., Graham, C. (2019): Can drones be used to conduct water sampling in aquatic environments? A review. – Science of the total environment 670: 569-575. https://doi.org/10.1016/j.scitotenv.2019.03.252
[57] Leth, P. M., Madsen, B. H. (2017): Drowning investigated by post mortem computed tomography and autopsy. – Journal of Forensic Radiology and Imaging 9: 28-30. https://doi.org/10.1016/j.jofri.2017.02.002
[58] Lobo, E. A., Schuch, M., Heinrich, C. G., Da Costa, A. B., Düpont, A., Wetzel, C. E., Ector, L. (2015): Development of the Trophic Water Quality Index (TWQI) for subtropical

temperate Brazilian lotic systems. – Environmental Monitoring and Assessment 187: 1-13. https://doi.org/10.1007/s10661-015-4586-3
[59] Lobus, N. V., Kulikovskiy, M. S., Maltsev, Y. I. (2021): Multi-element composition of diatom Chaetoceros spp. from natural phytoplankton assemblages of the Russian Arctic Seas. – Biology 10: 1009. https://doi.org/10.3390/biology10101009
[60] Ludes, B., Coste, M., North, N., Doray, S., Tracqui, A., Kintz, P. (1999): Diatom analysis in victim’s tissues as an indicator of the site of drowning. – International Journal of Legal Medicine, 112, 163-166. https://doi.org/10.1007/s004140050224
[61] Lunetta, P., Miettinen, A., Spilling, K., Sajantila, A. (2013): False-positive diatom test: a real challenge? A post-mortem study using standardized protocols. – Legal Medicine 15: 229-234. https://doi.org/10.1016/j.legalmed.2013.03.002
[62] Ma, J., Lai, Q., He, F., Zhang, X., Shui, J., Yu, M., Wei, G., Li, W. (2023): Microbial source tracking identifies sources of contamination for a river flowing into the Yellow Sea. – Frontiers in Microbiology 14: 1111297. https://doi.org/10.3389/fmicb.2023.1111297
[63] Marshall, M., Chraïbi, V., Morgan, R. (2023): A method for bone marrow extraction of diatoms for forensic science applications. – Journal of Forensic Sciences 68: 1343-1351. https://doi.org/10.1111/1556-4029.15297
[64] Martin, G. J., Hill, D. R., Olmstead, I. L., Bergamin, A., Shears, M. J., Dias, D. A., Kentish, S. E., Scales, P. J., Botté, C. Y., Callahan, D. L. (2014): Lipid profile remodeling in response to nitrogen deprivation in the microalgae Chlorella sp. (Trebouxiophyceae) and Nannochloropsis sp. (Eustigmatophyceae). – PLoS One 9: e103389. https://doi.org/10.1371/journal.pone.0103389
[65] Medley, C. N., Clements, W. H. (1998): Responses of diatom communities to heavy metals in streams: the influence of longitudinal variation. – Ecological Applications 8: 631-644. https://doi.org/10.1890/1051-0761(1998)008[0631:RODCTH]2.0.CO;2
[66] Metcalf, J. L., Xu, Z. Z., Weiss, S., Lax, S., van Treuren, W., Hyde, E. R., Song, S. J., Amir, A., Larsen, P., Sangwan, N. (2016): Microbial community assembly and metabolic function during mammalian corpse decomposition. – Science 351: 158-162. https://doi.org/10.1126/science.aad2646
[67] Naik, S., Mishra, R. K., Sahu, K., Lotliker, A. A., Panda, U., Mishra, P. (2020): Monsoonal influence and variability of water quality, phytoplankton biomass in the tropical coastal waters–a multivariate statistical approach. – Frontiers in Marine Science 7: 648. https://doi.org/10.3389/fmars.2020.00648
[68] Nakanishi, H., Takada, A., Yoneyama, K., Kodama, S., Sakai, K., Saito, K. (2024): The utility of drowning site inference through metagenomic diatom analysis. – Legal Medicine 71: 102548. https://doi.org/10.1016/j.legalmed.2024.102548
[69] Nayebi, B., Khurana, P., Pulicharla, R., Karimpour, S., Brar, S. K. (2023): Preservation, storage, and sample preparation methods for freshwater microplastics–a comprehensive review. – Environmental Science: Advances 2: 1060-1081. https://doi.org/10.1039/D3VA00043E
[70] Novikova, N. I., Matthews, H., Williams, I., Sewell, M. A., Nieuwoudt, M. K., Simpson, M. C., Broderick, N. G. (2022): Detecting phytoplankton cell viability using NIR Raman spectroscopy and PCA. – ACS Omega 7: 5962-5971. https://doi.org/10.1021/acsomega.1c06262
[71] Oliveira, M., Marszałek, K., Kowalski, M., Frolova, A., Łabaj, P. P., Branicki, W., Madureira-Carvalho, Á., Da Silva, D. D., Dinis-Oliveira, R. J. (2024): Sequencing Technologies in Forensic Microbiology: Current Trends and Advancements. – Forensic Sciences 4: 523-545. https://doi.org/10.3390/forensicsci4040035
[72] Otto, H. (1961): Über den Nachweis von Diatomeen in menschlichen Lungenstauben. – Frankfurt. Z. Path. 71: 176.
[73] Packer, L., Gibbs, J., Sheffield, C., Hanner, R. (2009): DNA barcoding and the mediocrity of morphology. – Molecular Ecology Resources 9: 42-50. https://doi.org/10.1111/j.1755-0998.2009.02631.x

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_29392968

[74] Pajunen, V., Luoto, M., Soininen, J. (2017): Unravelling direct and indirect effects of hierarchical factors driving microbial stream communities. – Journal of Biogeography 44: 2376-2385. https://doi.org/10.1111/jbi.13046

[75] Piegari, G., De Biase, D., D'aquino, I., Prisco, F., Fico, R., Ilsami, R., Pozzato, N., Genovese, A., Paciello, O. (2019): Diagnosis of drowning and the value of the diatom test in veterinary forensic pathology. – Frontiers in Veterinary Science 6: 404. https://doi.org/10.3389/fvets.2019.00404

[76] Pollanen, M. (1997): The diagnostic value of the diatom test for drowning, II. Validity: analysis of diatoms in bone marrow and drowning medium. – Journal of Forensic Sciences 42: 286-290. https://doi.org/10.1520/JFS14112J

[77] Porawski, R. (1966): Investigations on the occurrence of diatoms in organs in death from various causes. – Journal of Forensic Medicine 13: 134-137. https://doi.org/10.1006/jsre.1994.1125

[78] Rana, A., Manhas, S. (2018): Significance of diatoms in diagnosis of drowning deaths: A review. – Journal of Forensic & Genetic Sciences 5: 77-81. https://doi.org/10.32474/PRJFGS.2018.01.000121

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

[80] Rivera, S. F., Vasselon, V., Bouchez, A., Rimet, F. (2020): Diatom metabarcoding applied to large scale monitoring networks: Optimization of bioinformatics strategies using Mothur software. – Ecological indicators 109: 105775. https://doi.org/10.1016/j.ecolind.2019.105775

[81] Ruppert, K. M., Kline, R. J., Rahman, M. S. (2019): Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. – Global Ecology and Conservation 17: e00547. https://doi.org/10.1016/j.gecco.2019.e00547

[82] Saini, E., Rohilla, P. (2020): Forensic diatomological mapping: a data base for diatom profiling to solve drowning cases. – Int J Cur Res Rev. 12: 23. https://doi.org/10.31782/IJCRR.2020.122405

[83] Schmedes, S. E., Woerner, A. E., Novroski, N. M., Wendt, F. R., King, J. L., Stephens, K. M., Budowle, B. (2018): Targeted sequencing of clade-specific markers from skin microbiomes for forensic human identification. – Forensic Science International: Genetics 32: 50-61. https://doi.org/10.1016/j.fsigen.2017.10.004

[84] Scott, K. R., Morgan, R. M., Jones, V. J., Cameron, N. G. (2014): The transferability of diatoms to clothing and the methods appropriate for their collection and analysis in forensic geoscience. – Forensic science international 241: 127-137. https://doi.org/10.1016/j.forsciint.2014.05.011

[85] Seo, Y., Ichida, D., Sato, S., Kuroki, K., Kishida, T. (2014): An improved method for the diatom test utilizing DNA binding ability of silica. – Journal of Forensic Sciences 59: 779-784. https://doi.org/10.1111/1556-4029.12390

[86] Shen, X., Liu, Y., Xiao, C., Zheng, C., Huang, J., Shi, H., Xu, Q., Cheng, J., Liu, C., Zhao, J. (2019): Analysis of false-positive results of diatom test in the diagnosis of drowning—would not be an impediment. – International Journal of Legal Medicine 133: 1819-1824. https://doi.org/10.1007/s00414-019-02021-4

[87] Sijen, T., Harbison, S. (2021): On the identification of body fluids and tissues: a crucial link in the investigation and solution of crime. – Genes 12: 1728. https://doi.org/10.3390/genes12111728

[88] Singh, M., Lodha, P., Singh, G. P. (2010): Seasonal diatom variations with reference to physico-chemical properties of water of Mansagar Lake of Jaipur, Rajasthan. – Res J Agric Sci 1: 451-457.

[89] Siver, P. A., Lord, W. D., Mccarthy, D. J. (1994): Forensic limnology: the use of freshwater algal community ecology to link suspects to an aquatic crime scene in southern New England. – Journal of Forensic Sciences 39: 847-853. https://doi.org/10.1007/BF01225421

[90] Siver, P. A., Hinsch, J. (2010): The use of interference reflection contrast in the examination of diatom valves. – Journal of Phycology 36: 616-620. https://doi.org/10.1046/j.1529-8817.2000.99141.x

[91] Siver, P. A. (2012): The biology of Mallomonas: morphology, taxonomy and ecology. – Springer Science & Business Media.

[92] Smol, J. P., Stoermer, E. F. (2010): The diatoms: applications for the environmental and earth sciences. – Cambridge University Press. https://doi.org/10.1017/cbo9780511763175.004

[93] Speruda, M., Piecuch, A., Borzęcka, J., Kadej, M., Ogórek, R. (2022): Microbial traces and their role in forensic science. – Journal of Applied Microbiology 132: 2547-2557. https://doi.org/10.1111/jam.15426

[94] Su, Q., Yang, C., Chen, L., She, Y., Xu, Q., Zhao, J., Liu, C., Sun, H. (2023): Inference of drowning sites using bacterial composition and random forest algorithm. – Frontiers in Microbiology 14: 1213271. https://doi.org/10.3389/fmicb.2023.1213271

[95] Su, Q., Zhang, X., Chen, X., Yu, Z., Wu, W., Xiang, Q., Yang, C., Zhao, J., Chen, L., Xu, Q. (2025): Integrating microbial profiling and machine learning for inference of drowning sites: a forensic investigation in the Northwest River. – Microbiology Spectrum 13: e01321-24. https://doi.org/10.1128/spectrum.01321-24

[96] Sushanth, V., Rajashekhar, M. (2012): Seasonal variation in diatoms in response to physico-chemical characteristics of coastal waters of Uttara Kannada district, West Coast of India. – International Journal of Environmental Sciences 2: 1543-1552. https://doi.org/10.6088/ijes.002020300039

[97] Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., Willerslev, E. (2012): Towards next-generation biodiversity assessment using DNA metabarcoding. – Molecular Ecology 21: 2045-2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

[98] Taberlet, P., Bonin, A., Zinger, L., Coissac, E. (2018): Environmental DNA: For biodiversity research and monitoring, Oxford University Press. – https://doi.org/10.1093/oso/9780198767220.001.0001

[99] Tambuzzi, S., Gentile, G., Zoia, R. (2024): Forensic Diatom Analysis: Where Do We Stand and What Are the Latest Diagnostic Advances? – Diagnostics 14: 2302. https://doi.org/10.1158/1538-7445.AM2024-2302

[100] Tantanasarit, C., Englande, A. J., Babel, S. (2013) Nitrogen, phosphorus and silicon uptake kinetics by marine diatom *Chaetoceros calcitrans* under high nutrient concentrations. – Journal of Experimental Marine Biology and Ecology 446: 67-75. https://doi.org/10.1016/j.jembe.2013.05.004

[101] Thakar, M. K., Singh, R. (2010a): Diatomological mapping of water bodies for the diagnosis of drowning cases. – Journal of Forensic and Legal Medicine 17: 18-25. https://doi.org/10.1016/j.jflm.2009.07.016

[102] Thakar, M. K., Singh, R. (2010b): Diatomological mapping of water bodies for the diagnosis of drowning cases. – Journal of Forensic & Legal Medicine 17: 18-25. https://doi.org/10.1016/j.jflm.2009.07.016

[103] Thakar, M. K., Luthra, D., Khattar, J. S. (2018): Forensic studies of phytoplankton ecology of two water bodies of Kurukshetra area of Haryana, State in India. – Egyptian Journal of Forensic Sciences 8: 1-15. https://doi.org/10.1186/s41935-018-0068-4

[104] Thomsen, P. F., Willerslev, E. (2015): Environmental DNA–An emerging tool in conservation for monitoring past and present biodiversity. – Biological Conservation 183: 4-18. https://doi.org/10.1016/j.biocon.2014.11.019

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

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2402_29392968

[106] Tiwari, A., Marella, T. K. (2019): Potential and application of diatoms for industry-specific wastewater treatment. – Application of Microalgae in Wastewater Treatment: Volume 1: Domestic and Industrial Wastewater Treatment, pp. 321-339. https://doi.org/10.1007/978-3-030-13913-1_15
[107] Trabert, Z. (2017): The diatom genus Gomphonema from the Republic of Macedonia. – Acta Botanica Hungarica 59: 276-277.
[108] Uitdehaag, S., Dragutinovic, A., Kuiper, I. (2010): Extraction of diatoms from (cotton) clothing for forensic comparisons. – Forensic Science International 200: 112-116. https://doi.org/10.1016/j.forsciint.2010.03.039
[109] Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F. (2016): Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. – Molecular Ecology 25: 929-942. https://doi.org/10.1111/mec.13428
[110] Vandenbosch, M., Rooseleers, L., Bogaert, W. V. D., Wuestenbergs, J., Cuypers, E. (2020): Skeletal tissue, a viable option in forensic toxicology? A view into post mortem cases. – Forensic Science International 309: 110225. https://doi.org/10.1016/j.forsciint.2020.110225
[111] Verma, K. (2013): Role of diatoms in the world of forensic science. – J Forensic Res 4: 181-84. https://doi.org/10.4172/2157-7145.1000181
[112] Vijayan, A., Kallumpurat, A., Christal, L. G. (2020): Diatoms: a review on its forensic significance. – Journal of Forensic Dental Sciences 12: 197-200. https://doi.org/10.18311/jfds/12/3/2020.566
[113] Wiltshire, P. E. (2016): Mycology in palaeoecology and forensic science. – Fungal Biology 120: 1272-1290. https://doi.org/10.1016/j.funbio.2016.07.005
[114] Wu, J., Song, C., Dubinsky, E. A., Stewart, J. R. (2021): Tracking major sources of water contamination using machine learning. – Frontiers in Microbiology 11: 616692. https://doi.org/10.3389/fmicb.2020.616692
[115] Yang, T., Shi, Y., Zhu, J., Zhao, C., Wang, J., Liu, Z., Fu, X., Liu, X., Yan, J., Yuan, M. (2021): The spatial variation of soil bacterial community assembly processes affects the accuracy of source tracking in ten major Chinese cities. – Science China Life Sciences 64(9): 1546-1559. https://doi.org/10.1007/s11427-020-1843-6
[116] Yen, L. Y., Jayaprakash, P. (2007): Prevalence of diatom frustules in non-vegetarian foodstuffs and its implications in interpreting identification of diatom frustules in drowning cases. – Forensic Science International 170: 1-7. https://doi.org/10.1016/j.forsciint.2006.08.020
[117] Yoshimura, S., Yoshida, M., Okii, Y., Tokiyasu, T., Watabiki, T., Akane, A. (1995): Detection of green algae (Chlorophyceae) for the diagnosis of drowning. – International Journal of Legal Medicine 108: 39-42. https://doi.org/10.1007/BF01845616
[118] Young, J., Linacre, A. (2021): Massively parallel sequencing is unlocking the potential of environmental trace evidence. – Forensic Science International: Genetics 50: 102393. https://doi.org/10.1016/j.fsigen.2020.102393
[119] Żarczyńska, M., Żarczyński, P., Tomsia, M. (2023): Nucleic acids persistence—benefits and limitations in forensic genetics. – Genes 14: 1643. https://doi.org/10.3390/genes14081643
[120] Zgłobicka, I., Gluch, J., Liao, Z., Werner, S., Guttmann, P., Li, Q., Bazarnik, P., Plocinski, T., Witkowski, A., Kurzydlowski, K. J. (2021): Insight into diatom frustule structures using various imaging techniques. – Scientific Reports 11: 14555. https://doi.org/10.1038/s41598-021-94069-9
[121] Zhang, P., Kang, X., Zhang, S., Xiao, C., Ma, Y., Shi, H., Xu, Q., Zhao, J., Chen, L., Liu, C. (2020): The length and width of diatoms in drowning cases as the evidence of diatoms penetrating the alveoli-capillary barrier. – International Journal of Legal Medicine 134: 1037-1042. https://doi.org/10.1007/s00414-019-02164-4

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DOI: http://dx.doi.org/10.15666/aeer/2402_29392968

[122] 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 sciences research 5: 98-105. https://doi.org/10.1080/20961790.2020.1718901

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