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Diatoms As Forensic Bioindicators: Assessing Their Distribution In Ponds, Lakes, And Rivers Of Peninsular Malaysia

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Abstract

Diatoms are widely recognized as sensitive bioindicators of freshwater ecosystem conditions, with their diversity and distribution reflecting variations in environmental parameters. This study investigates diatom morphotype assemblages in ponds, lakes, and rivers across the Klang Valley, Selangor, and Negeri Sembilan, Peninsular Malaysia, to assess how nutrient regimes, light availability, substrate type, and water chemistry influence their occurrence. Centric diatoms were detected at only a limited number of sites, consistent with their dependence on stable and well-lit water columns, conditions that are uncommon in small ponds and fast-flowing rivers. In contrast, pennate diatoms, particularly motile raphid forms, were more frequently observed and appeared better adapted to turbulent, turbid, and benthic environments. Across the environmental gradient, diatom assemblages were often sparse or absent, suggesting constraints such as nutrient limitation, reduced light penetration, seasonal variability, or frustule dissolution under extreme pH conditions. These findings provide a preliminary morphotype-level baseline for freshwater systems in Malaysia that may support comparative aquatic provenance assessment, although limited taxonomic resolution and low abundance reduce discriminatory strength. Interpretation should follow evaluative reporting principles and acknowledge plausible alternative explanations, including contamination and methodological loss.

Introduction

According to the 2018 World Health Organization (WHO) estimates, drowning claims the lives of 360,000 people worldwide each year, making it a serious public health issue. While many bodies have been found in water, not all of them have drowned. Pathological evidence of drowning victims is frequently hard to come by, if not impossible. One of the main issues in forensic medicine is the autopsy diagnosis of drowning, particularly when the victim's recovery is delayed[1]. An eyewitness's testimony or a suicide note are examples of circumstantial evidence that can help determine if a crime, suicide, or accident has occurred[2]. The diagnosis of drowning is frequently made by ruling out alternative causes of death, because in 69.6% of the instances, there were no eyewitnesses due to the distance from residential areas[3]. Thus, because of these limitations, diatoms are used in forensic applications for further investigation.

Diatoms are single-celled organisms with complex skeletons that are found in practically every aquatic ecosystem, including rivers, lakes, ponds, and oceans. Two varieties are typically identified based on morphology: pinnate, with an elongated form and longitudinal symmetry, and centric, with radial symmetry[4]. Diatoms are unusual substrates that are very useful for forensic investigations of bodies found in water because of their peculiar siliceous cell walls, which offer them exceptional resilience to the external environment[5]. Assessing and interpreting diatoms found in various forms of evidence, such as lung tissues, internal organs, and bone marrow, is the primary focus of forensic diatomology, especially in cases involving drowning or bodies retrieved from aquatic environments. In these circumstances, the human may swallow or inhale diatoms before or during submersion in water. Their

presence and dispersion across different bodily tissues and fluids can offer important clues about the cause of death or the place where the deceased person was submerged[5].

There are no discernible macroscopic discoveries if it is a body in a more advanced stage of decomposition meaning acquiring data for old instances are equivocal because organ samples older than 11 months or even skeletal remains tended to show no diatoms[6,7], particularly in organs like the liver, which might not have diatoms when bodies are retrieved months later, decomposition delays may make detection less accurate[8]. Consequently, the exclusion criterion is still used far too frequently in drowning diagnoses. Given the morphological traits and distribution of diatoms in aquatic environments, a supplementary diatom test can be instrumental in this context[9,10]. Water floods the lungs after entering the respiratory system by inhalation in a drowning death. Diatoms in the surrounding environment may be unintentionally swallowed or inhaled by a person while submerged in water. After passing through the lung filter, the bloodstream distributes these diatoms throughout the body's fluids and tissues, including bone tissue. By identifying whether the submersion occurred before or after the death, the presence of these diatoms can provide information on the victim's immersion timeline[5]. However, because there is no circulatory activity in a dead person submerged in water, the diatoms can passively enter the respiratory system. Still, they cannot spread to the other organs. Therefore, the diagnosis of drowning can be strongly supported if diatoms are found in the interior viscera of an intact person[1,5].

For a long time, ecological mapping of water types and localities has also used diatom analysis. It has been reported in several studies as a sign of a drowning location[1,11–15]. The least common species will be the most helpful in identifying the drowning site when there is a high diversity of common species from several geographic areas[11,12,16]. Furthermore, if reference water samples are provided to compare the diatom species found in the viscera, diatom analysis can potentially reveal the location of drowning[7,10,17–21]. According to several studies, a local diatom database or diatomological map (D-map) that includes various water types over time and across seasons could be helpful for comparisons[13,22].

Nowadays, in Malaysia, diatom analysis is also used in forensic science, but it is more common in academic and research settings than in real case work. Based on research conducted in Malaysia, the diatom concentrations of cooked prawns and clams were around 8,360 and 29,054 diatom frustules per 50 g, respectively[23]. This study warns against false positives in diatom testing, as ingestion may introduce millions of diatoms per year, particularly when low thresholds (e.g., 5 frustules per 10 g bone marrow) are used. Apart from that, eight primary diatom morphologies were identified in research conducted in Selangor, including rivers such as Klang, Gombak, Ampang, Damansara, Kemensah, and Kanching. According to the study, these profiles can be utilized to determine which bodies of water the suspects or victims interacted with prior to their deaths[24]. Lastly, challenges in drowning cases in Malaysia include the effects of tropical climate conditions on decomposition rates, which can complicate diatom analysis[25].

The challenge is to localize diatom signatures to sites. Thus, the main objective of this study is to generate a reference profile that could be used for forensic comparison, in relation to cases of drowning or to water evidence more generally, by recognizing characteristic diatom assemblages in each victim and across different freshwaters. For sampling purposes, using standardized protocols and under sterile conditions, representative, uncontaminated freshwater samples will be collected from defined sites. To prevent cross-contamination and ensure that subsequent analysis is valid, these samples were kept intact. Identifying whether and what type of diatoms are present in each targeted region is another objective. The study will investigate the richness of diatoms through intensive morphological and taxonomic analysis. This will further the understanding of ecology and the applicability of species distribution patterns to forensic science. It concludes by emphasizing the importance of maintaining a strong chain of custody when collecting, transporting, and analyzing samples. Adherence to strict forensic protocols ensures that evidence remains admissible and can be used in court.

Results

Diatom morphotype presence/absence differed among lake, river, and pond samples (Table 1), with the pond represented by a single site (P1). Centric morphotypes were detected in lake sites L1, L2, L4–L6 and in river sites R1–R2 and pond site P1. This suggests that these morphotypes were relatively common but not consistently present across all habitats. In rivers, centric diatoms were detected in R1 and R2, whereas in the pond, they were observed only in P1. Residual plant fragments and organic detritus were present on some prepared slides despite digestion. In addition, some samples contained rotifers, further indicating the presence of other biological components in the environment (Fig. 1).

Pennate morphotypes included both araphid and raphid forms. Araphid morphotypes were detected at L1, L2, and R1 and were not observed in the remaining sampled sites. Raphid pennate morphotypes (monoraphid and biraphid) were detected in both lake and river samples, but their occurrence varied by site (Table 1). Symmetrical and asymmetrical biraphids were detected in multiple lake sites (L1, L2, L4, L5) and were primarily observed in river site R1 among the river samples. Descriptively, lakes contained a broader set of pennate morphotype categories than rivers in this dataset.

Within the biraphid group, specialized forms such as nitzschioid and eunotioid were sparsely distributed. Nitzschioid diatoms were absent in most sites but present in certain lakes (L1, L2, L4, L5) and occasionally in the pond (P1), while eunotioid diatoms were rare and only inconsistently detected. The pond sample (P1) contained both centric and multiple pennate morphotypes; given the single pond site, broader inference about ponds requires additional sampling. Overall, the results demonstrate that lakes supported the greatest diversity of morphotypes, rivers exhibited lower richness, and the single pond sample showed a mixed composition, with representation of several key groups.

<Insert Table 1>

<Insert Fig. 1>

Discussions

Diatom assemblages vary with water chemistry and physical habitat, which can make them informative markers of aquatic provenance. In forensic limnology, spatially variable diatom assemblages can support drowning-site or aquatic-provenance inference when compared between evidence and reference water. Centric morphotypes were detected in a subset of sites rather than across all samples[5]. One plausible explanation for site-to-site differences is habitat structure (e.g., water-column stability vs. benthic substrates), but this requires environmental measurements to test.

Many centric taxa are planktonic and favored by stable water columns, whereas many raphid pennates are benthic/attached; both groups occur in freshwaters, but relative prevalence depends on habitat conditions[26]. If river reaches were more turbulent or turbid at sampling, these conditions could reduce planktonic centric detection and favor benthic forms; measuring turbidity/flow/irradiance would enable a test of this mechanism. Raphid pennates can adhere and glide on substrates via the raphe system, supporting persistence in benthic microhabitats under flow[27]. Studies of river-lake transitions have shown that centric species dominate in slow-flowing or lacustrine zones, whereas pennate taxa increase with water velocity and disturbance[28–30].

Although diatoms appear scarce in our freshwater samples, their absence at several sites may be attributed to environmental factors. Diatom abundance and diversity are strongly influenced by nutrient availability, light penetration, substrate type, and water chemistry[31]. Unfavorable conditions, such as low nutrient concentrations, high turbidity, or competition with other phytoplankton groups, may have limited diatom growth during the sampling period. In general, freshwater diatom communities are relatively stable in terms of species diversity, supported by good water quality[32].

Nevertheless, seasonal variability also plays a role, as diatom populations often undergo pronounced fluctuations throughout the year. Such variability is typically driven by multiple surrounding parameters acting simultaneously. For instance, studies of Malaysian estuarine-mangrove ecosystems have shown that tidal mixing, nutrient availability, temperature, pH, and rainfall are the main drivers of seasonal diatom dynamics[33]. Additionally, methodological factors, such as sample preservation or frustule dissolution in highly acidic or alkaline waters, may have contributed to the lack of observable diatom frustules[34]. Further investigations across different seasons and with complementary techniques, such as pigment analysis and molecular approaches, are recommended to confirm whether diatoms are consistently absent or occur at undetectable levels at the sampling locations[35].

These data can be used as a morphotype-level baseline to support preliminary aquatic provenance comparisons when reference waters are available, but morphotype resolution alone is unlikely to maximize discrimination among candidate sites. Interpretation should align with evaluative reporting principles and modern interpretation standards by stating what the findings support, what they do not, and which alternative explanations remain plausible, including contamination and methodological loss.

This study relied exclusively on light microscopy (LM) for diatom detection and identification. Thus, taxa with very small or lightly silicified frustules may have been undetected or misidentified, and the absence of observed diatoms in our samples should be interpreted cautiously. Scanning electron microscopy (SEM) would provide the ultrastructural detail necessary for accurate diatom taxonomy. Molecular approaches (e.g., metabarcoding of 18S rRNA or RBCL markers and environmental DNA) can detect taxa present at low abundance or whose frustules are degraded. Pigment analysis using High-Performance Liquid Chromatography (HPLC) and chlorophyll fluorescence or epifluorescence microscopy can help confirm the presence of photosynthetic microalgae when frustules are absent. Increasing sample volumes, using size-fractionated filtration, and applying acid-cleaning or oxidation to concentrate and reveal frustules will also raise detection probability. Seasonal and repeated sampling are recommended to account for temporal variability.

Conclusions

This study demonstrates that diatom assemblages in Malaysian freshwater systems are strongly influenced by interacting physical and chemical factors. Centric diatoms occurred only in stable, well-lit environments, whereas pennate taxa, particularly motile forms, dominated habitats subject to turbulence, turbidity, and benthic substrates. The overall low abundance of diatoms across sites suggests that environmental constraints, including nutrient limitation, shading, and phytoplankton competition, may restrict their proliferation. The observed gradient between highly urbanised lakes and rivers and less disturbed sites underscores the impact of anthropogenic activity on diatom distribution and diversity. These findings highlight the importance of diatoms as bioindicators of ecosystem health, while also emphasising the need for improved methodological sensitivity.

Declarations

Declaration of competing interest

The authors have no conflicts of interest to declare for this study.

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CRediT authorship contribution statement

DR MUHAMAD NAIM BIN ABD MALEK: Results, formal analysis, discussion, limitations, recommendations, contributed contents, figure and table visualisations. **DR TERENCE RICKY CHIU**: Literature search, synthesised inputs from readings, and contributed initial contents and references. **DR EDISON EUKUN SAGE**: Methodology, restructured compiled manuscript, checked the English language, and contributed content in multiple drafts. **DR NURUL IZZATI MOHD ALI**: Geographical Information System (GiS) software, mapping the sampling area, and contributed content. **MADAM NURSYAHIDA BINTI BOHARI**: resource acquisition, reviewing and editing subsequent draft contents. **DR NUR-FADHILAH MAZLAN**: Conceptualisation/ conceived the study framework, data compilation, formal analysis, methodology, project administration, fund acquisition, added additional literature in multiple drafts, multiple rounds of tents.

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Tables

Table 1 is available in the Supplementary Files section.

Figures

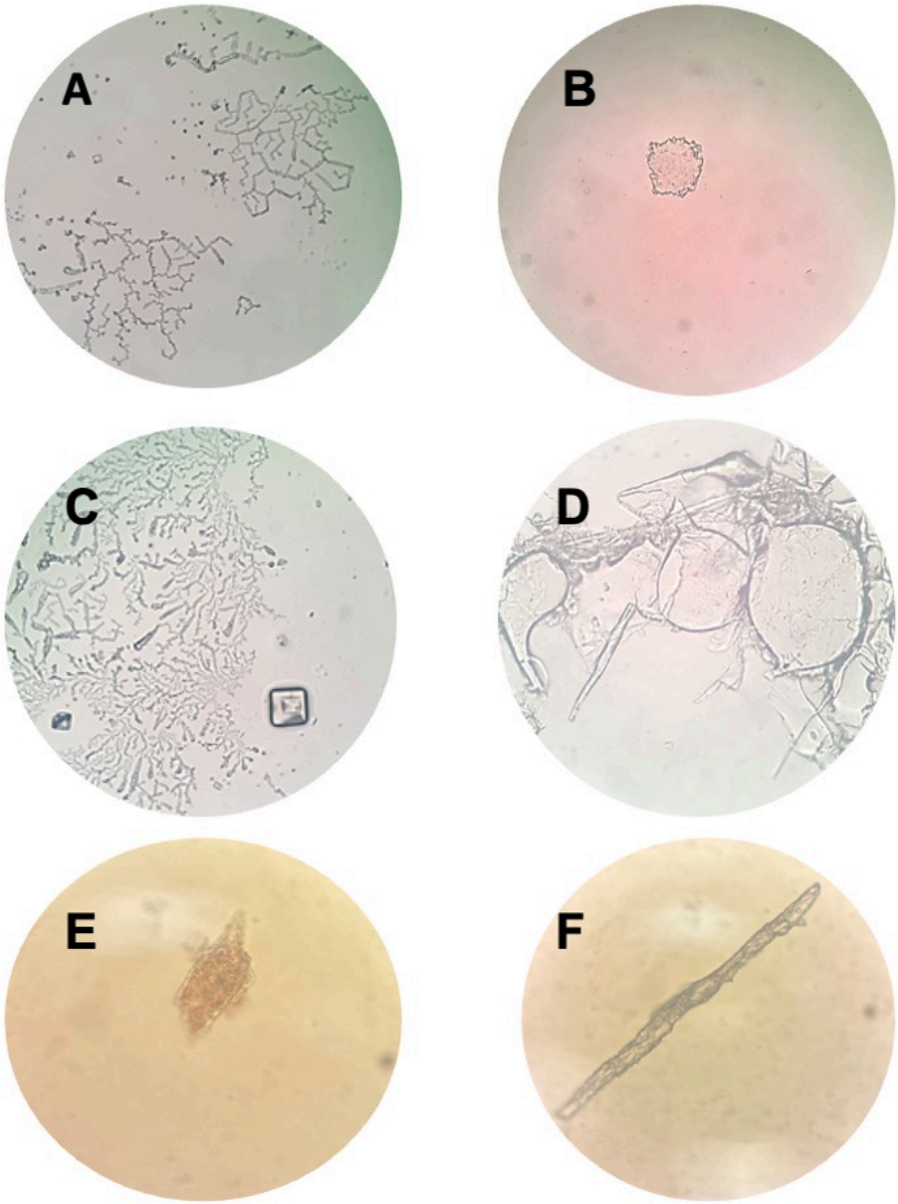


Figure 1

Light microscope images showing some of the diatom and other organic structures from water samples. A – **pennate** araphid diatom, B – organic detritus, C, D – mineral crystal, E – rotifer, and F – plant materials. Magnification: 40× – 100× (using 4× and 10× objectives with 10× eyepiece).

Supplementary Files

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