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CASE SERIES

Retrospective evaluation of diatom evidence in the forensic diagnosis of drowning

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ABSTRACT

Background and aims*: The diagnosis of drowning is a major challenge in forensic practice, particularly when bodies are recovered from aquatic environments in an advanced state of decomposition. Under such circumstances, classical autopsy findings are often obscured, necessitating the use of ancillary techniques. Diatom analysis has emerged as a valuable forensic tool for supporting the diagnosis of antemortem drowning and assisting in the identification of the probable site of drowning. The aim of this case series is to evaluate the forensic utility of diatom analysis in establishing the cause of death and correlating the probable site of drowning in decomposed bodies recovered from freshwater environments.* ***Methods:*** *A case series of six freshwater drowning cases was examined at the Central Forensic Science Laboratory, Chandigarh, India. Bone samples (sternum and femur) and corresponding reference water samples were subjected to acid digestion using concentrated nitric acid. Extracted samples were centrifuged, washed, and mounted on microscopic slides. Diatoms were identified using light microscopy at 100× magnification and compared morphologically between tissue and reference water samples.* ***Results:*** *Diatoms were detected in tissue and corresponding water samples in four cases, showing morphological similarity and taxonomic concordance. In two cases, diatoms were absent in bone samples but present in reference water samples, suggesting causes of death apart from drowning. The findings supported antemortem drowning in four cases and excluded drowning in two cases.* ***Conclusion:*** *Diatom analysis was a reliable adjunct in the forensic diagnosis of drowning, particularly in decomposed bodies. The presence of diatoms in bone samples strongly supported antemortem drowning, while negative tissue findings aided in excluding drowning. The correlation of diatom profiles between tissues and water sources strengthened medico-legal conclusions regarding both the cause and the site of death.*

Keywords*: Ante-mortem drowning; post-mortem drowning; sternum; asphyxia; acid digestion.*

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INTRODUCTION

Determining the cause of death of a body recovered from water is one of the most challenging tasks for investigating officers. Drowning is a form of asphyxia in which atmospheric air is prevented from entering the lungs of the victim.[1] In most drowning cases, the body is usually decomposed, due

to which the features suggesting antemortem and post-mortem drowning become indistinct. In such situations, diatoms in water bodies help resolve medico-legal uncertainties. The presence of diatoms in the body of the deceased, especially in the bone marrow of the sternum and long bones, is considered a positive indicator of drowning.[2] In antemortem drowning, a considerable amount of water, along with diatoms, reaches the lungs and other organs (bone marrow, spleen, kidney, and liver) through blood circulation.[3] In contrast, during post-mortem submersion, due to the absence of circulation, diatoms cannot reach distant organs.

Diatoms possess a hard silica cell wall, which makes them resistant to various tissue digestion methods, including acid, enzymatic, and Soluene-350 digestion. Therefore, diatoms can be used as a reliable tool not only for determining antemortem or post-mortem drowning but also for identifying the actual site of drowning. Different diatom species inhabit specific water bodies according to their nutritional requirements and the physicochemical parameters of those bodies. This ecological specificity helps in associating a body with a particular drowning site. For this reason, continuous assessment of water bodies for diatom profiles is recommended.[4] The diatom assemblage of a given aquatic environment can serve as a reference standard for comparison with diatoms recovered from human tissues for the determination of the probable site of drowning. In the present case series, decomposed bodies were recovered from multiple water bodies, and the post-mortem examinations were inconclusive with respect to the cause of death. As a result, samples of long bone and sternum obtained during the autopsy, along with corresponding reference water samples from the purported sites of occurrence, underwent diatom analysis at the Biology Division, Central Forensic Science Laboratory, Chandigarh. The primary forensic query was to determine whether the diatom profiles detected in tissue samples matched those recovered from the reference water sources. Diatoms were extracted from both bone and water samples using standard acid digestion techniques. Representative photomicrographs of the recovered diatom taxa were obtained using light microscopy and subjected to comparative morphological analysis for forensic correlation.

MATERIALS AND METHODS

All cases included in the present study involved freshwater drowning. Following post-mortem examination of the deceased individuals, relevant exhibits were submitted to the forensic laboratory. The study was based on cases received from various regions of India. Diatoms were extracted from biological exhibits (sternum and femur) and corresponding reference water samples using the acid digestion method, as described by Pollanen[5] and Pal et al.[6] Both biological and control water samples were processed and examined. A comparative chart was prepared to assist in the taxonomic identification of diatoms.

Examination of Diatoms in Bone Samples

The external surface of the bone samples was first cleaned by carefully removing adhering soft tissues and muscles using a sterile surgical blade, followed by thorough washing with distilled water. The cleaned bones were air-dried. A section of approximately 1 × 1 cm was cut from each bone using a bone cutter. The bone section was then placed in a 500 mL glass beaker, and 50 mL of concentrated nitric acid was added. The samples were kept in a fume hood until fume emission ceased.

After approximately 24 hours, 100 mL of molecular-grade water was added to the beaker. The suspension was strained, and undigested bone fragments and fatty materials were carefully removed. The filtrate and

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corresponding control water samples were transferred into separate, properly labelled 2 mL microcentrifuge tubes bearing the case and exhibit numbers. The samples were centrifuged at 4000 rpm for 10 minutes. After centrifugation, the supernatant was discarded, leaving the pellet intact, and the tubes were refilled with molecular-grade water. This washing step was repeated 4–5 times. After the final wash, traces of acid were removed by rinsing the pellet with a drop of molecular-grade water.

Microscopic slides were prepared from the pellet and mounted using a permanent mounting medium (DPX). The slides were examined under a light microscope at 100× magnification. Photomicrographs of each observed diatom species were taken and compared with the diatom species detected in the corresponding tissue and reference samples.

Examination of Diatoms in Reference Water Samples

Approximately 100 mL of each reference water sample was treated with 10 mL of concentrated nitric acid and a small amount (pinch) of potassium dichromate. The samples were incubated overnight. On the following day, the samples were centrifuged at 4000 rpm for 10 minutes. The supernatant was discarded, and the pellet was retained. The pellet was washed by refilling the tubes with molecular-grade water, and the centrifugation step was repeated 2–3 times to remove residual acids.

After the final wash, microscopic slides were prepared from the pellet and mounted using DPX. The slides were examined under a light microscope at 100× magnification. Photomicrographs of individual diatom species were captured and compared with those observed in the tissue (bone) samples for forensic correlation.

RESULTS

Case Study 1

The body of a 40-year-old male was recovered from a pond by police in Punch, Jammu and Kashmir. Police sent autopsy evidence samples, along with two reference water samples—one from where the body was recovered and another from the incident site—for diatom examination. Microscopic studies of slides prepared from the nitric acid extract of the sternum and trachea proved the presence of diatoms belonging to six genera (*Pinnularia, Cymbella, Achnanthidium, Encyonema, Synedra, and Gomphonema). They were identified from* both the case exhibit samples (sternum and trachea) and two reference water samples (*Achnanthidium, Pinnularia, Cymbella, Gomphonema, and Synedra)*. The diatom species recovered from the sternum and trachea were morphologically similar to those recovered from the reference water sample. Drowning was the most probable cause of death.

Case Study 2

The body of a 28-year-old male was recovered from a lake in Chandigarh. After post-mortem examination, the sternum bone and water reference samples were sent for diatom examination. Nitric acid extract of the sternum showed the presence of diatoms belonging to six genera *(Navicula, Cyclotella, Amphipleura, Pellucidaelliptica, Pinnularia, Melosira, and Cymbellaperfossilis).* The diatom species recovered from the piece of the sternum were morphologically similar to those recovered from the reference water sample. Drowning was the most probable cause of death.

Case Study 3

The body of a 38-year-old male was recovered from the water tank in Rangat, Middle Andamans, Andaman and Nicobar Islands,

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India. The body was sent to the civil hospital by the police. After the post-mortem, the sternum bone was sent for diatom examination along with a water reference sample from where the body was recovered for comparison. Microscopic studies of slides prepared from the nitric acid extract of the sternum showed the presence of diatoms belonging to six genera (Navicula, Hannaea arcus, Nitzschia longissima*, Pinnularia, and Amphipleura).* The diatom species recovered from the piece of sternum were morphologically similar to those recovered from the reference water sample. Drowning was the most probable cause of death.

Case Study 4

The putrefied body of a 34-year-old married female was found hanging with a rope in a well and was recovered by the police authorities of Kanke, Ranchi, Jharkhand. The father of the lady claimed that her daughter was murdered by her husband and then hung in the well to disguise it as a suicide hanging. No specific reason for the cause of death was observed at the time of autopsy. The sternum bone and reference water samples from the well were submitted to the lab for diatom examinations. No diatoms were detected in the bone nitric acid extract. However, four diatom species (Rhizosolenia imbricata, *Nitzschia longissima, Navicula, and* Surirella elegans) were detected in the water reference. The cause of death was attributed to other reasons than drowning. Later, her husband accepted that he had murdered his wife and then hung the body in the well with a rope, as mentioned in vernacular reports.

Case Study 5

Police authorities recovered the putrefied body of a 26-year-old lady from a well in Chainpur, Ranchi, Jharkhand. The body was taken to a civil hospital for autopsy examination. After post-mortem examination, the sternum bone is preserved for diatom examination. The sternum bone, along with the reference water sample, was sent to CFSL Chandigarh for diatom examination. After receiving the case, the case was opened with proper exhibit marking and photographs of all parcels and exhibits. Diatoms were found in both samples, and a comparative study of these diatoms was conducted. Diatoms of different species were detected from the sternum bone sample, and the same species of diatoms were recovered from the source of the reference water sample as well. Drowning was the most probable cause of death.

Case Study 6

Police authorities recovered the body of a 33-year-old lady from a well in Daltonganj, Jharkhand. The body was taken to a civil hospital for autopsy. After post-mortem examination, the sternum bone is preserved for diatom examination. The sternum bone, along with a reference water sample from the well, was sent to CFSL Chandigarh for diatom examination. After receiving the case, the case was opened with proper exhibit marking and photographs of all parcels and exhibits. Diatoms were found in both samples, and a comparative study of these diatoms was conducted. Diatoms of different species were detected from the sternum bone sample, and the same species of diatoms were recovered from the source of the reference water sample as well. Drowning was the most probable cause of death.

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COMPARATIVE ANALYSIS TABLE IN BRIEF

Sr. no.	Evidence sample	Types of diatoms Detected	Reference sample	Types of diatoms detected	Geographical location
1	Sternum and Trachea	*Achnanthidium, Pinnularia, Cymbella, Gomphonema, Synedra*	Pond Water	*Achnanthidium, Pinnularia, Cymbella, Gomphonema, Synedra*	Punch, J & K
2	Sternum	*Navicula, Cyclotella meneghiniana, Amphipleura pellucida, Pinnularia, Melosira*	Lake Water	*Navicula, Cyclotella meneghiniana, Pinnularia, Cymbellaperfossilis, Melosira species, Amphipleura pellucida*	Chandigarh
3	Sternum	*Navicula, Hannaeaarcus, Nitzschia longissimi, Pinnularia, Amphipleura,*	WaterTank	*Navicula, Hannaeaarcus, Nitzschia longissima, Pinnularia, Amphipleura*	Rangat (Andaman and Nicobar)
4	Sternum	-	Water Well	*Rhizosolenia imbricata, Nitzschia longissima, Navicula, and* Surirella elegans	Kanke, Ranchi, Jharkhand, India
5	Sternum	Navicula, Triceratium latus, Licmophora, Pinnularia viridis, Gyrosigma, Licmophora, Surirella elegans	Water Well	Navicula, Pseudotriceratiumcinanomeum, Triceratium latus, Licmophora, Pinnulariaviridis, Gyrosigma, Licmophora, Surirella elegans	Chainpur, Ranchi, Jharkhand, India
6	Sternum	Pinnularia viridis, Navicula, Surirella elegans, Pseudotriceratium cinanomeum, Triceratium latus, and Licmophora.	Water Well	Pinnularia viridis, Navicula, Surirella elegans, Gyrosigma, Pseudotriceratium cinanomeum, Triceratium latus, Licmophora, Naviculaelsoniana, Licmophora,	Daltonjganj, Jharkhand, India

The findings from the evidence examination in the above table indicate that the microscopic examination at 100X resolution revealed a morphologically similar structure.

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DISCUSSION

The forensic diagnosis of drowning remains inherently complex, particularly in cases involving advanced decomposition or limited circumstantial evidence. The findings discussed in this study reinforce the well-established role of diatoms as valuable adjunctive markers in the interpretation of drowning deaths. Diatoms, owing to their rigid siliceous frustules, demonstrate remarkable resistance to post-mortem degradation, allowing their detection even in severely decomposed remains. When conventional autopsy indicators become obscure, this property significantly enhances their forensic relevance.

The ecological specificity of diatom assemblages provides a scientific basis for correlating tissue-recovered diatoms with those from suspected drowning sites. Variations in nutrient composition, physicochemical characteristics, and environmental conditions contribute to water body–specific diatom profiles, enabling forensic investigators to associate a body with a probable site of drowning. The present observations are consistent with earlier studies emphasising the value of comparative diatom profiling for geographic source attribution in drowning cases.[7]

Previous research has demonstrated that the presence of diatoms in distant organs and bone marrow indicates antemortem aspiration and systemic dissemination via active circulation. Timperman (1962) was among the first to establish the diagnostic importance of detecting diatoms in bone marrow and vital organs.[8] Subsequent studies by Pollanen (1998)[2] and Singh et al. (2006)[9] further validated the reliability of acid digestion–based recovery methods. The current findings support these conclusions, particularly regarding the forensic utility of sternum and femur bones as optimal substrates for diatom detection.

Despite the diatom test's long-standing recognition, only specialised forensic laboratories routinely apply it. The limited availability of standardised reference databases, the lack of trained personnel, and the technically demanding nature of tissue digestion and microscopic identification have restricted its broader implementation, especially in resource-constrained settings. Within the Indian forensic context, only a limited number of laboratories routinely perform diatom analysis, although recent reports[10-12] demonstrate increasing acceptance of this technique in medico-legal investigations. An important limitation highlighted in both the literature and the present study is the possibility of false-negative results. Diatom absence in tissue samples suggests, but does not rule out, other causes of death besides drowning. Factors such as low diatom density in the drowning medium, rapid death due to neurogenic shock or vagal inhibition, and technical losses during sample processing can all contribute to negative findings. Therefore, diatom evidence should always be interpreted in conjunction with autopsy findings, toxicological results, and circumstantial evidence.

The generally accepted quantitative thresholds proposed by Ludes et al. (1996)[4] including the detection of ≥20 diatoms per 100 µL pellet from lung tissue and ≥5 diatoms from bone samples, provide useful guidance for interpretation; however, strict reliance on numerical cut-offs may not be universally applicable due to ecological and methodological variability. Future directions should focus on developing standardised protocols, expanding regional diatom reference databases, and incorporating molecular and automated imaging techniques to improve the specificity, sensitivity, and reproducibility of forensic diatom analyses.

In summary, this study reinforces the medico-legal significance of diatom testing as a supportive tool for differentiating antemortem drowning from post-mortem immersion and for assisting in the localisation of drowning sites when used alongside a comprehensive forensic investigation.

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