

Forensic simulation examination of Diatoms from drowning White rats (*Rattus norvegicus*) in Wudil stream, Kano State, Nigeria

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ABSTRACT

This study examines the forensic utility of diatom analysis in drowning cases using white rats (*Rattus norvegicus*) in Wudil Stream, Nigeria. Diatoms, microscopic algae with siliceous cell walls, serve as biomarkers to distinguish drowning from post-mortem water submersion. While widely used in human forensics, experimental validation in mammals remains limited. We assessed diatom infiltration patterns in drowned rats to evaluate the reliability of this diagnostic method. Thirty adult white rats were used, 21 subjected to controlled drowning at varying depths (50, 70, 90 cm) in Wudil Stream, and 7 as non-drowned controls. Water and tissue samples (lungs, liver, and bone marrow) were analyzed for diatom content using acid digestion and microscopy. The findings showed that, *Navicula* spp. dominated stream water (28.5% at 50 cm, decreasing to 22.8% at 90 cm), while *Nitzschia* spp. remained stable (19–20%). Drowned rats showed significantly ($p < 0.001$) higher diatom counts in lungs (125–165 cells/g), liver (31–40 cells/g), and bone marrow (21–30 cells/g) compared to negligible counts in controls. *Navicula radiosa* was prevalent in lungs (30–35%), whereas *Nitzschia palea* and *Synedra ulna* were prominent in bone marrow, confirming systemic circulation post-drowning. The findings validate diatom analysis as a reliable forensic tool for drowning diagnosis, with tissue-specific infiltration patterns supporting ante-mortem inhalation. This study highlights its potential in regions with limited forensic resources.

Keywords: Forensic diatomology, drowning diagnosis, diatom test, *Rattus norvegicus*, Wudil Stream

INTRODUCTION

Diatoms are unicellular, photosynthetic algae with siliceous cell walls that are widely distributed in aquatic environment, Dhyani, S., & Bramhanwade, K. (2023). Their presence in tissues of drowning victims has been used as a forensic tool to determine the cause and location of death (Dahiya *et al.*, 2024). The "diatom test" is a well-established method in forensic pathology, where the presence of diatoms in bone marrow, lungs, and other organs indicates ante-mortem inhalation of water, confirming drowning as the cause of death (Ludes *et al.*, 1999; Gupta *et al.*, 2025). While human drowning cases have been extensively studied, limited research has been conducted on drowning in mammals, particularly in controlled experimental settings. White rats (*Rattus norvegicus*) are commonly used in forensic and biomedical research due to their physiological similarities to humans.

Drowning is one of the leading causes of accidental and homicidal deaths worldwide. However, determining whether a victim drowned or was disposed of post-mortem in water remains a challenge in forensic investigations. The diatom test has been used to resolve such cases, but its reliability depends on various factors, including diatom concentration in the water, the victim's inhalation mechanism, and post-mortem degradation (Auer & Mottonen, 1988; Hestiyani *et al.*, 2024). Despite its usefulness, the diatom test has limitations, including false negatives (due to low diatom concentrations in water) and false positives (due to environmental contamination). Additionally, most studies focus on human drowning cases, with limited experimental data on mammals. There is a need to validate the diatom test using controlled animal models to improve forensic accuracy (Cid-Rodríguez *et al.*, 2024).

Forensic diatomology plays a crucial role in medico legal investigations, especially in drowning cases where traditional autopsy findings are inconclusive. However, the technique's reliability depends on experimental validation under controlled conditions. Using white rats as mammalian models provides an ethical and practical approach to understanding diatom infiltration patterns, (Ren *et al.*, (2021).

Wudil Stream in Kano state is a significant freshwater source in Northern Nigeria, yet its diatom diversity remains understudied. This research will document diatom species in the stream while assessing their forensic applicability. The findings could aid Nigerian forensic pathologists in drowning investigations, particularly in regions where water-related deaths are common but forensic resources are limited. Moreover, this study may contribute to the broader scientific understanding of diatom-based drowning diagnostics. If successful, the methodology could be adapted for other mammalian species, including humans, improving drowning determination in forensic casework. In view of that, this study aimed at simulating drowning in white rats and examine diatom infiltration into their tissues to assess the reliability of diatom analysis in forensic investigations which could contribute to drowning diagnostics in both humans and animals, particularly in cases where the cause of death is ambiguous.

MATERIALS AND METHODS

Brief Description of the Study Area

Wudil Stream (Latitude: 11.7936° N, Longitude: 8.8436° E) was selected due to its ecological significance and accessibility. Water samples were collected from three different points along the stream for diatom diversity analysis.

Experimental Design

Thirty (30) adult white rats of average weight of 200 to 250 g were used during this study. Twenty-one (21) rats were used for simulation drowning and seven (7) of the rats as control group. The Seven (7) rats each were ethically submerged in Wudil Stream water with the average pH range of 7.2 to 7.8 at the depth of 50, 70 and 90 cm for 10 minutes to simulate drowning and the nine (9) rats (control) were euthanized without drowning (non-aqueous method, ie. CO₂ asphyxiation) after death for 20 minutes, then submerged in to the stream following same protocol of the drowned rats.

Sample Collection and Processing

Water Samples used during the study were collected from multiple points at the depth of 0.5 meter along Wudil stream in sterile bottles and, 100 mL of each water sample

were filtered through a 2- μ m cellulose nitrate membrane filter and rinsed filters with distilled water to remove organic debris and then preserved with Lugol's iodine (5%) to prevent diatom degradation and to easily detect the presence of diatoms, as it stains their silica cell walls, making them visible under microscopy. The experimental animals tissue Samples such as Lungs, liver, and femur bone marrow were extracted post-mortem for diatom extraction process.

Diatom Extraction, Digestion and Identification

The rat tissues (lungs, liver and bone marrow) of both those life rats submerged in water to death (drowned) and control (non- drowned) were excised. The two samples were placed in sterile containers and were then treated with 30% hydrogen peroxide (H₂O₂) and digested in Conc. nitric acid (HNO₃) in order to dissolve the organic matter of the samples while preserving diatom frustules, following the method described by Zhao *et al.*, (2023). The processed samples both from Wudil stream water samples and the recovery from drowned rat tissues were then centrifuged at 3000 rpm for 5 minutes at 60°C for 48 hrs and the supernatants were discarded.

Slide Preparation

The pellets were washed with distilled water (3 \times) to neutralize acid and the resuspend diatom pellets in distilled water were placed on glass slides allowed to dry. The diatom slides were prepared and examined under a light microscope (400x magnification). Diatom counts per 10 μ L of sediment (Relative abundance) were recorded, and species identification was performed using standard taxonomic keys.

Data Analysis

The data obtained during this study were subjected to Chi-square test (X^2) in order to compare diatom species distribution across tissues diatoms (the systemic) in drowning regions and Diatom Counts in Drowned and Non-Drowned Rats were also analyzed using Two-way ANOVA and One-way ANOVA to compare diatom abundance across depths respectively.

RESULTS

This study examines diatom distribution in Wudil stream water, drowned and non-drowned rats to assess forensic evidence for drowning. The result presented in Table 1 for relative abundance of the Diatom species at different depths showed that, *Navicula* spp was the most abundant species though its abundance declined slightly with increasing depth, (28.5% at the depth of 50 cm, which decreased to 22.8% at the depth of 90 cm). *Nitzschia* spp. remains relatively stable (19 to 20%) across depths, *Pinnularia* spp. and *Fragilaria* spp. In their relative abundance increased slightly with depth. Meanwhile, the relative abundance of *Gomphonema* spp decreased with depth from (12.2% at 50 cm to 9.5% at 90 cm). The pH of the stream remained near neutral (6.9 to 7.3), suggesting minimal pH influence on diatom distribution, since all diatom species identified fall within the tolerance range of freshwater pH (6.5–8.5), indicating no extreme acidity or alkalinity.

Table 1: Relative Abundance (%) of Diatom species Identified in Wudil stream at different Depths (cm) with pH

Diatom Species	50 cm	70 cm	90 cm	Freshwater pH
<i>Navicula spp.</i>	28.5	25.0	22.8	7.2
<i>Nitzschia spp.</i>	20.0	18.7	19.5	7.1
<i>Pinnularia spp.</i>	14.3	16.2	18.0	6.9
<i>Fragilaria spp.</i>	10.5	13.8	14.7	7.0
<i>Gomphonema spp.</i>	12.2	11.0	9.5	7.3
<i>Cyclotella spp.</i>	7.5	6.0	6.3	7.2
<i>Synedra spp.</i>	7.0	9.3	9.2	7.1
Total	100	100	100	—

F-statistic (2, 18) = 4.82, p = 0.021

The result presented in Table 2 showed the relative abundance (%) of diatom species which represented the proportion of each species among the total diatoms recovered per tissue in drowned rats, and also comparing diatom distribution across tissues (lungs, liver, and bone marrow). The Lungs recorded the highest diatom abundance species of *Navicula radiosa* (30 to 35%) which was consistently found in the lungs across all depths, reinforcing its diagnostic value in forensic drowning cases, followed by Bone marrow which showed (24 to 27%) of *Nitzschia palea* and *Synedra ulna* (22%). Meanwhile, the Liver had the lowest but detectable levels of *Pinnularia viridis*, *Gomphonema parvulum*, and *Fragilaria capucina*) also supporting systemic circulation post-drowning. The freshwater pH range (6.5–8.5) is ideal for diatom survival and is within typical values (7.2 → 7.8) for Wudil stream and the presence of identical diatom species (*Navicula radiosa*) across multiple depths supports ecological consistency and forensic reliability.

Table 2: Diatom species recovered from the tissues of drowned White rats in Wudil stream at varying water depths (cm)

Diatom Species	Relative Abundance (%)	Water Depth (cm)	Body Tissue	Freshwater pH
<i>Navicula radiosa</i>	32	50	Lungs	7.2
<i>Nitzschia palea</i>	27	50	Bone Marrow	7.2
<i>Pinnularia viridis</i>	18	50	Liver	7.2
<i>Cyclotella meneghiniana</i>	35	70	Lungs	7.5

Diatom Species	Relative Abundance (%)	Water Depth (cm)	Body Tissue	Freshwater pH
<i>Synedra ulna</i>	22	70	Bone Marrow	7.5
<i>Gomphonema parvulum</i>	14	70	Liver	7.5
<i>Navicula radiosa</i>	30	90	Lungs	7.8
<i>Nitzschia palea</i>	24	90	Bone Marrow	7.8
<i>Fragilaria capucina</i>	16	90	Liver	7.8

$$\chi^2 (6) = 18.7, p = 0.005$$

The result Diatom counts in drowned and non-drowned rats as presented in Table 3 showed that, drowned rats showed significantly ($p < 0.001$) higher diatom counts in all the tissues, especially in the lungs, with the highest counts (125–165 cells/g) followed by the Liver (31–40 cells/g) and, the least counts in Bone marrow (21–30 cells/g). Meanwhile, the non-drowned rats had recorded negligible diatoms counts (0 in bone marrow, < 20 cells/g in lungs/liver). The diatom counts recorded during the study increased with depth in drowned rats (165 cells/g at 90 cm vs. 125 cells/g at 50 cm).

Table 3: Diatom counts (cells/g) in organs of Drowned and Non-drowned White rats from Wudil stream at different depths (cm)

Depth (cm)	Group	Lungs (cells/g)	Liver (cells/g)	Bone Marrow (cells/g)
50	Drowned	125 ± 32	31 ± 22	21 ± 21
50	Non-Drowned	15 ± 12	10 ± 9.8	0.00
70	Drowned	142 ± 42	36 ± 24	26 ± 22
70	Non-Drowned	14 ± 12	12 ± 11	0.00
90	Drowned	165 ± 42	40 ± 28	30 ± 25
90	Non-Drowned	20 ± 14	15 ± 9.9	0.00

Main effect of drowning status $F(1, 24) = 68.3, p = 0.001$, Main effect of depth $F(2, 24) = 5.6, p = 0.010$, Interaction effect $F(2, 24) = 1.2, p = 0.32$

DISCUSSION

The findings of this study provide critical insights into the forensic application of diatom analysis in drowning investigations. The distribution of diatoms in Wudil stream water and their presence in drowned rat tissues support the utility of diatomology as a reliable forensic tool. The results align with previous research demonstrating that diatom

abundance and species composition can serve as indicators of drowning (Lunetta et al., 2015; Zhao et al., 2017; Đukic et al., 2021)

Diatom distribution in Wudil stream water

The relative abundance of diatom species in Wudil stream varied with depth, with *Navicula* spp. being the most dominant, though its prevalence decreased slightly with increasing depth. This trend may reflect light availability and nutrient distribution, as *Navicula* species are often reported to be benthic and thrive in well-lit shallow waters (Round et al., 1990; Cid-Rodríguez et al., 2024). The stability of *Nitzschia* spp. across depths suggests its adaptability to varying ecological conditions, while the increase in *Pinnularia* and *Fragilaria* spp. with depth may indicate their preference for lower light conditions (Battarbee et al., 2001; Xing et al., 2024). The near-neutral pH (6.9–7.3) of the stream further confirms that diatom distribution was not significantly influenced by extreme acidity or alkalinity, as most freshwater diatoms flourish within a pH range of 6.5–8.5 (Stevenson et al., 2010).

These findings are consistent with forensic diatomology studies that emphasize the importance of matching diatom profiles in water samples with those found in drowning victims (Hurlimann et al., 2000; Đukic et al., 2021). The presence of identical diatom species (*Navicula radiosa*) across multiple depths reinforces the ecological consistency of Wudil stream, enhancing the reliability of diatom-based drowning diagnoses.

Diatoms in the tissues of drowned rats

The recovery of diatoms from drowned rat tissues (lungs, liver, and bone marrow) supports the systemic circulation of diatoms following inhalation of water. The lungs exhibited the highest diatom abundance of *Navicula radiosa*, consistent with previous studies by Ludes et al., (1999) and Ren et al., (2021), reported that, *Navicula* species were found frequently dominant in pulmonary samples of drowning victims due to their abundance in aquatic environments and ease of aspiration during drowning. The presence of *Nitzschia palea* and *Synedra ulna* in bone marrow were particularly significant, as bone marrow diatom analysis was considered to be a gold standard in forensic drowning investigations due to its resistance to postmortem contamination (Auer & Mottonen, 1988; Dervišević et al., 2024). The detection of *Pinnularia viridis*, *Gomphonema parvulum*, and *Fragilaria capucina* in the liver further corroborates systemic distribution, as these species are less likely to be introduced postmortem (Piette & De Letter, 2006). The negligible diatom counts in non-drowned rats (< 20 cells/g in lungs/liver, 0 in bone marrow) strengthen the argument that diatom presence in tissues is a reliable marker of antemortem drowning, (Dervišević et al., 2024).

The findings of this study carry several forensic implications. First, the detection of site-specific diatom assemblages in both water and tissue samples provides strong circumstantial evidence linking a suspected drowning victim to a specific aquatic environment. This aligned with established forensic protocols, where matching diatom profiles from victims and suspected drowning sites can corroborate drowning diagnoses (Lunetta & Modell, 2005; San Ko et al., 2024). Second, the recovery of diatoms from closed organs such as bone marrow and liver confirms ante-mortem aspiration and systemic circulation, thereby strengthening conclusions about drowning as the cause of

death (Pollanen, 1998; Hestiyani *et al.*, 2024). The statistical differences between drowned and non-drowned groups in both species composition and diatom counts reinforce the reliability of diatom testing in distinguishing drowning from post-mortem immersion. Finally, the depth-dependent differences in diatom counts suggest that environmental profiling may assist in reconstructing the circumstances of drowning, such as estimating submersion depth or identifying the most likely drowning location within a body of water. This potential application, while promising, requires further validation in natural drowning cases with known immersion depths.

CONCLUSION

This study demonstrates that diatom analysis in both environmental and biological samples can yield valuable forensic information in drowning investigations. The significant differences in diatom assemblages and counts between drowned and non-drowned subjects, along with the ecological consistency of species between the Wudil stream and recovered tissues, support the inclusion of diatom testing as a complementary method in medico-legal drowning diagnosis. Future work should focus on expanding sample sizes, exploring seasonal variations in diatom communities, and integrating molecular diatom identification methods to improve specificity and reliability.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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