

# Assessing Diatom Community Shifts with Salinity During Decomposition of Partially Submerged Buried Cadavers

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Diatoms are adaptable microbes that have numerous uses in areas including environmental monitoring and drowning investigations. Due to the ongoing exposure to moisture and changing conditions, traditional forensic techniques for detecting and estimating the postmortem interval (PMI) of partially submerged cadavers are frequently inefficient in aquatic situations. To ascertain the PMI and the location of partially submerged burial remains, this study sought to assess the efficacy of diatoms as biological indicators. This study was conducted in freshwater and brackish water ecosystems, where the fatty flesh of commercial *Sus scrofa* was buried in a shallow grave and allowed to decompose at several designated sampling points for 270 days of burial interval. Benthic diatoms were sampled from the associated soils to determine their abundances at each decomposition stage. Notably, several diatom species were identified as potential PMI indicators, including *Caloneis silicule* (freshwater) and *Chaetoceros coarctatum* (brackish water). Species potentially useful for locating submerged cadavers including *Coscinodiscus argus* for freshwater systems and *Fragilaria crotonensis* for brackish water. Additionally, strong correlations between certain diatoms (*Navicula cancellata*, *Fragilaria vaucheriae*, *Hantzchia* sp., *Nitzschia closterium*, and *Coscinodiscus gigas*) and lipid content further underscore their potential to be used in forensic investigations.

**Keywords:** Diatom diversity; partially submerge burial cadaver; forensic diatomology; freshwater ecosystems; brackish water ecosystems

## I. INTRODUCTION

Bacillariophyta, or diatoms, have been used in the green industry for a long time. Diatoms have been used as raw materials for industry, and the raw materials derived from diatoms are lipids and silica. Lipids derived from diatoms have been used as a more sustainable biofuel than petroleum fuels, while silica derived from diatom cell walls is used in biomedical applications such as drug delivery, analysis tools, nanoparticles and biosensors. In addition, diatoms have a foothold in the field of nutraceuticals and feed. The nutritional richness of diatoms has led to their use as food supplements for humans and as food supplements for cultured fish in the field of culture, benefiting the attractive

colour of ornamental fish due to their carotenoid content. Moreover, diatoms can be used to determine the quality of water (Sakset & Chankaew, 2013; Arumugham *et al.*, 2023; Jin *et al.*, 2024). Diatoms can react to any changes in physical and chemical parameters such as increased ammonia nitrogen, phosphorus, silicon, temperature, light intensity and pH (Juffermans *et al.*, 2025). The presence of a particular species or the dominance of a particular division can determine the health of the environment (Estuaries *et al.*, 2004). Diatoms have also been used as an indicator in investigations to determine the cause of death for bodies found in water bodies (Tambuzzi *et al.*, 2024).

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The diversity of benthic diatoms for each of the decomposition stages has been examined in this study. The fatty flesh of the commercial pig (*Sus scrofa*) has been used as a human model in a simulated burial experiment. The fatty flesh is buried in the soil system adjacent to a brackish water ecosystem at a depth of 15 cm from the topsoil, mimicking a burial in a shallow grave. The decomposition of fatty flesh was described into five distinct and well-documented stages of decomposition in terrestrial environments: initial decay, putrefaction, black putrefaction, butyric fermentation, and dry decay (Payne & King, 1972; Davis & Goff, 2000; Teo *et al.*, 2014; Ismail *et al.*, 2020). It has been postulated that the buried cadaver constantly under the influence of excessive moisture will demonstrate different trends. Even if it has been slightly altered, the decomposition of a partially submerged or buried body is still thought to proceed through these five stages, given that terrestrial bug species and other scavenger creatures can still access the body. The benthic diatoms were extracted from the topsoil and examined using a compound light microscope before subsequent statistical analyses to elucidate their potential to be used as bioindicators to locate partially submerged clandestine graves and to estimate the PMI of cadavers.

## II. MATERIALS AND METHOD

### A. Simulated Burial Experiment

#### 1. Burial sites

The burial sites for this study were freshwater (5°24.6429'N, 103°5.0692'E) and brackish water (5°24.6323'N, 103°5.4544'E) ecosystems, located on the Universiti Malaysia Terengganu (UMT) Campus (Figure 1). Both ecosystems have different soil types; the freshwater ecosystem has sandy loam soil, while the brackish water ecosystem has sandy soil type. Both environments have their particular physicochemical characteristics, slightly different in their physical and chemical properties (Table 1). The freshwater ecosystem has its uniqueness, where the source of water reservoirs is from groundwater and rain, with an average salinity of 0.294 ppt ± 0.117. Primary mangrove trees, which are plants from the genus *Bruguiera* that have an excellent tolerance for growing in freshwater conditions,

make up a large portion of the freshwater ecosystem. However, no macrobenthos was found in the freshwater ecosystems compared to that of the brackish water environments, which are rich in macrobenthos such as molluscs (gastropods), crustaceans (crabs), fish (Oxudercinae) and polychaetes. The water source for brackish water ecosystems is influenced by tides, with an average reading of 27.67 ppt ± 6.83.

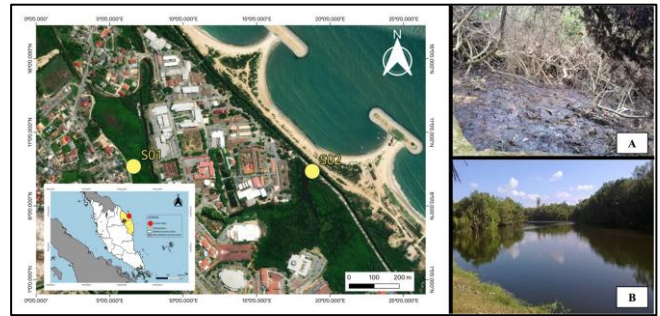


Figure 1. The location of burial sites for freshwater (SO1) and brackish water environments (SO2), in UMT Campus.

The weather data was obtained from the meteorological set-up, which was located in UMT campus. The mean temperature was 25.4 °C, with the maximum temperature being 25.9 °C (May) and the minimum being 24.9 °C (January). The average rainfall was 2498 mm, with the highest readings recorded in December and January, and the lowest in May.

Table 1. The physicochemical properties of soil (10 - 15 cm depth) for the freshwater and brackish water ecosystems.

Ecosystems	Freshwater	Brackish water
<b>Physical properties</b>		
<b>Sand (%)</b>	49.64	86.19
<b>Silt (%)</b>	46.20	12.85
<b>Clay (%)</b>	4.16	0.96
<b>Soil texture</b>	Sandy loam	Sand
<b>Chemical properties</b>		
<b>Total Carbons (%)</b>	7.322±1.681	8.433±1.986
<b>Organic Carbon (%)</b>	7.322±1.681	8.433±1.986
<b>pH</b>	6.75±0.41	7.09±0.30

## 2. Experimental design

The abdominal fatty flesh of the commercial pig (*Sus scrofa*) was used to replace human tissue due to ethical issues. A terra cotta pot with a diameter of 20 cm and a height of 15 cm was used as a burial vessel. Approximately 50 g of abdominal fatty flesh, containing skin, muscle, and fatty tissue, was weighed accurately. The fatty flesh was then placed into a clean pot which was initially filled with the interest soil at around 2 cm depth. After that, the soil was added to cover the flesh for a complete burial. The pots were labelled correctly and placed in the soil system adjacent to the brackish and freshwater ecosystems at 15 cm depth, mimicking a burial in a shallow clandestine grave. The fatty flesh was allowed to decompose at several designated sampling points over a 270-day burial period. These sampling points, which were replicated, represented the decomposition stages. The control burial vessels, with no fatty flesh, were prepared with similar procedures and replicated. The benthic diatoms and the associated soils were collected on specific days according to the sampling intervals for subsequent analysis.

## 3. Sampling

Sampling was carried out based on the sampling interval that was set at both study sites. Sampling was carried out during the lowest tide during the day. At each study site, the experimental pots and the specific control pots that were buried were taken out from the burial area. At the initial process of sampling, soil samples for extracting diatoms were taken from the control pots and experiment pots, along with replications of the experiment pots. Soil for extracting benthic diatoms was taken using a minicore. The amount of soil taken using a minicore was determined for all samples. A total of 4 ml of soil volume was taken using a minicore and transferred to a 15 ml centrifuge tube. Once the soil samples were placed in the centrifuge tube, the formalin solution was added as quickly as possible. The formalin solution was added until the centrifuge tube volume reading reached 11 ml to ensure that the soil samples for diatoms were correctly preserved. Formalin added to soil samples for diatom extraction was concentrated in the samples at 6% of formalin. Formalin solutions were used to preserve diatom samples and to kill the potential availability of zooplankton.

After formalin was added to the samples, the centrifuge tube was tightly closed to prevent formalin from evaporating into the air and decreasing its concentration. Then, the diatom samples were stored in styroform boxes to prevent exposure to direct sunlight and heat before being transported and stored in a chiller at the Trace Organics Laboratory 2, UMT. After completing the collection of soil samples for diatom extraction, the experimental pots with belly fatty flesh were removed and separated from the soil as soon as possible before being transported to the Marine Science Laboratory of UMT for further processing. The separation that was carried out was intended to inhibit the decomposition process. The remaining fatty flesh and the associated soil were wrapped separately in aluminium foil. At the same time, *in situ* measurements of physical and chemical parameters and locality were taken for future comparison and study. YSI Multiparameter (model: 556 MPS) was used to measure several parameters such as temperature (°C), conductivity (ms/cm), total dissolved solids (g/L), salinity (ppt), dissolved oxygen (% and mg/L), and acidity (pH). The locality of the study sites was taken using an iPhone 11 Pro Max (model: MWHK2MY/A).

## 4. Particle size analysis (PSA)

In analysing the size of the soil samples in both study environments, soil samples were taken before the study was conducted to determine the soil type. Soil samples were taken using a hand auger at a depth of 10 – 15 cm for each study environment. Then, the soils were taken to the Trace Organic Laboratory 1, UMT, and were dried using a universal oven for four days at a temperature of 60 °C. Then, the soil samples were put into a sieve and the results of 63 µm from the sieve were analysed using a particle size analyser (PSA 1190).

## 5. Diversity

The formula for the Shannon–Wiener Diversity index is as follows,

$$H' = - \sum_{j=1}^S p_i \ln p_i$$

where,  
 $H'$  = Shannon Diversity Index  
 $p_i$  = Fraction of the Entire Population from Species  $-i$   
 $S$  = Number of species

### 6. Statistical analysis

The raw data for this study were pre-treated, with the environmental data being tested using a normality test to determine the distribution of physicochemical data. The data was identified as non-normal data for the environmental parameters. Benthic diatom diversity was analysed in relation to environmental parameters, and the factors exerting significant influence were identified using the Kruskal–Wallis test. Principal component analysis (PCA) and the Spearman correlation test were also used to identify factors that influence the diversity of diatoms in the two different environmental areas. The analysis was carried out using XLSTAT.

## III. RESULT AND DISCUSSION

### A. Species Composition

In freshwater ecosystems, 23 species of diatoms were recorded and identified to the species classification level (Table 2). All 23 diatom species were found in the control soils; however, only 19 species of diatoms were recorded for the experimental soils with the decomposing fatty flesh. The four diatom species of *Cosinodiscus argus*, *Diatoma* sp., *Gyrosigma excentricum* and *Navicula glacialis* were not detected in the experimental soils. These four diatom species may have the potential to be used as bioindicators for the partially submerged buried bodies of the freshwater ecosystems, locating the clandestine graves and/or estimating the PMI. Furthermore, these diatoms were also seen to have high potential in identifying or estimating the PMI of a carcass based on the presence of species in the decomposition stage. The diatom species *Hantzchia* sp. was the only species found in the experimental soils during the early decomposition process, specifically in the initial decay and putrefaction stages. While *Cymbella lanceolata* sp. was only found during three decomposition stages of the initial decay, butyric fermentation and dry decay stages. *Fragilaria vaucheriae* sp. was found at four stages of the initial decay, putrefaction, butyric fermentation and dry decay stages. *Caloneis silicule* and *Gomphonema gracile* were detected during the putrefaction, black putrefaction, butyric fermentation and dry decay stages.

The absence of species at a particular decomposition stage can be attributed to the response of the benthic diatoms towards the environmental changes resulting from the presence of decomposing fatty flesh. The highest diatom species for relative abundance in the control soils were *Nitzschia sigma* (34.88%), *Navicula cancellate* (7.50%), *Fragilaria contruens* (7.21%), *Pinnularia tabellaria* (6.45%), and *Fragilaria vaucheriae* (5.75%). The same three species were present in the experimental soils, with the highest relative abundance groups of *Nitzschia sigma* (29.48%), *Eunotia lunaris* (7.94%), *Nitzschia closterium* (7.34%), *Pinnularia tabellaria* (6.97%), and *Fragilaria vaucheriae* (5.82%). For the control soils, there were five diatom species with low relative abundance, namely *Hantzchia* sp. (0.63%), *Stauroneis obtuse* (0.77%), *Navicula glacialis* (0.85%), *Eunotia major* (0.99%), and *Eunotia lunaris* (1.27%). The lowest reading for relative abundance for the study area in the freshwater environment is *Hantzchia* sp. (0.62%), *Caloneis silicule* (1.07%), *Eunotia major* (1.45%), *Cymbella lanceolata* (1.50%), and *Stauroneis obtuse* (1.50%). *Eunotia lunaris* sp. was among the species that showed a significant difference in the relative abundance between the control and experimental soils. This diatom species was found to be in the diatom group of low relative abundance for the control soils. However, it was in the diatom group with high relative abundance in the experimental soils.

A total of 27 diatom species were extracted from the soils of the brackish water ecosystem (Table 3), in which the experimental soils demonstrated lower numbers of diatom species than the control soils. Twenty-two species of diatoms were found in the experimental soils, while a total of 27 species of diatoms were found in the control soils. Both experimental and control soils shared a similar species composition, with five diatom species only found in the control soils. These five diatom species were *Fragilaria crotonensis*, *Melosira normanii*, *Pinnularia gigas*, *Surirella ovalis*, and *Triceratium favus*, which may have the potential to be used as indicators for locating dead bodies in brackish water environments. Besides, three diatom species have the potential to be used in determining the PMI of the bodies for brackish water environments; the diatom of *Chaetoceros coarctatum* was only found during the

putrefaction and dry decay stages, while *Rhizosolenia styliformis* was only found in the initial decay and dry decay stages. Unlike *Coscinodiscus gigas*, this species was not found only during the black putrefaction stage.

The five high-abundance species found in the control soils were *Fragilaria crotonensis* (13.83%), *Gyrosigma fasciolum* (11.65%), *Rhizosolenia hebetate* (7.17%), *Cyclotella litoralis* (6.97%), and *Guinardia delicatula* (6.05%). The experimental soils shared the same three high-abundance diatom species as the control soils; namely *Gyrosigma fasciolum* (21.53%), *Rhizosolenia hebetate* (7.96%), and *Cyclotella litoralis* (5.94%). Two diatom species that were only dominant in the experimental soils were *Navicula*

*rectum* (6.17%) and *Nitzschia sigmoidea* (5.84%). The lowest-composition diatom species extracted from the control soils were *Melosira normannii* (0.68%), *Navicula oblonga* (0.68%), *Pleurosigma angulatum* (0.74%), *Fragilaria striatula* (0.92%), and *Achnanthes longipes* (0.98%). Meanwhile, the lowest-composition diatom species extracted from the experimental soils were slightly different from the control soils, namely *Rhizosolenia styliformis* (1.02%) and *Chaetoceros coarctatum* (1.75%). However, three diatom species were present in both experimental and control soils, i.e. *Achnanthes longipes* (1.25%), *Pleurosigma angulatum* (1.73%), and *Fragilaria striatula* (1.75%).

Table 2. Occurrences, species composition and relative abundance of diatoms in freshwater environments. Note: Present = '+'; Absent = '-'.

No.	Species	Experiment Soil					Control Soil	Experiment Relative Abundance	Control Relative Abundance
		Initial Decay	Putrefaction	Black Putrefaction	Butyric Fermentation	Dry Decay			
1.	<i>Achnanthes coarctata</i>	+	+	+	+	+	+	3.05	3.10
2.	<i>Amphora libica</i>	+	+	+	+	+	+	5.07	2.45
3.	<i>Caloneis silicula</i>	-	+	+	+	+	+	1.07	2.66
4.	<i>Cocconeis placentula</i>	+	+	+	+	+	+	2.04	3.29
5.	<i>Coscinodiscus argus</i>	-	-	-	-	-	+	0.00	3.79
6.	<i>Cymbella lanceolata</i>	+	-	-	+	+	+	1.50	1.73
7.	<i>Diatoma sp.</i>	-	-	-	-	-	+	0.00	2.21
8.	<i>Eunotia lunaris</i>	+	+	+	+	+	+	7.94	1.27
9.	<i>Eunotia major</i>	+	+	+	+	+	+	1.45	0.99
10.	<i>Fragilaria contruens</i>	+	+	+	+	+	+	5.14	7.21
11.	<i>Fragilaria vaucheriae</i>	+	+	-	+	+	+	5.82	5.75
12.	<i>Frustulia javanica</i>	+	+	+	+	+	+	3.80	1.38
13.	<i>Gomphonema gracile</i>	-	+	+	+	+	+	2.07	2.12
14.	<i>Gyrosigma excentricum</i>	-	-	-	-	-	+	0.00	2.31

15.	<i>Hantzchia sp.</i>	+	+	-	-	-	+	0.62	0.63
16.	<i>Melosira granulata</i>	+	+	+	+	+	+	4.71	1.45
17.	<i>Navicula cancellata</i>	+	+	+	+	+	+	4.99	7.50
18.	<i>Navicula glacialis</i>	-	-	-	-	-	+	0.00	0.85
19.	<i>Nitzschia closterium</i>	+	+	+	+	+	+	7.34	4.53
20.	<i>Nitzschia sigma</i>	+	+	+	+	+	+	29.48	34.88
21.	<i>Pinnularia stricta</i>	+	+	+	+	+	+	5.45	2.67
22.	<i>Pinnularia tabellaria</i>	+	+	+	+	+	+	6.97	6.45
23.	<i>Stauroneis obtuse</i>	+	+	+	-	-	+	1.50	0.77

Table 3. Occurrences, species composition and relative abundance of diatoms in brackish water environments. Note: Present = '+'; Absent = '-'.

No.	Species	Experiment Soil					Control Soil	Experiment Relative Abundance	Control Relative Abundance
		Initial Decay	Putrefaction	Black Putrefaction	Butyric Fermentation	Dry Decay			
1.	<i>Achnanthes longipes</i>	+	+	+	+	+	+	1.25	0.98
2.	<i>Amphora lineata</i>	+	+	+	+	+	+	2.85	4.10
3.	<i>Chaetoceros coarctatum</i>	-	+	-	-	+	+	1.75	1.27
4.	<i>Coscinodiscus gigas</i>	+	+	-	+	+	+	3.80	2.49
5.	<i>Cyclotella litoralis</i>	+	+	+	+	+	+	5.94	6.97
6.	<i>Fragilaria crotonensis</i>	-	-	-	-	-	+	0.00	13.83
7.	<i>Fragilaria striatula</i>	+	+	+	+	+	+	1.75	0.92
8.	<i>Guinardia delicatula</i>	+	+	+	+	+	+	5.41	6.05
9.	<i>Guinardia flaccida</i>	+	+	+	+	+	+	5.49	4.08
10.	<i>Gyrosigma acuminatum</i>	+	+	+	+	+	+	2.04	2.05
11.	<i>Gyrosigma fasciolum</i>	+	+	+	+	+	+	21.53	11.65
12.	<i>Leptocylindrus minimus</i>	+	+	+	+	+	+	4.24	2.18

13.	<i>Manguinea fusiformis</i>	+	+	+	+	+	+	4.90	1.89
14.	<i>Melosira normannii</i>	-	-	-	-	-	+	0.00	0.68
15.	<i>Navicula oblonga</i>	+	+	+	+	+	+	3.03	0.68
16.	<i>Navicula pelagi</i>	+	+	+	+	+	+	4.91	3.58
17.	<i>Navicula rectum</i>	+	+	+	+	+	+	6.17	4.05
18.	<i>Nitzschia americana</i>	+	+	+	+	+	+	1.91	4.26
19.	<i>Nitzschia sigmoidea</i>	+	+	+	+	+	+	5.84	2.90
20.	<i>Pinnularia gigas</i>	-	-	-	-	-	+	0.00	1.79
21.	<i>Pleurosigma angulatum</i>	+	+	+	+	+	+	1.73	0.74
22.	<i>Pleurosigma naviculaecum</i>	+	+	+	+	+	+	2.31	1.75
23.	<i>Rhizosolenia hebetate</i>	+	+	+	+	+	+	7.96	7.17
24.	<i>Rhizosolenia styliformis</i>	+	-	-	-	+	+	1.02	1.46
25.	<i>Surirella ovalis</i>	-	-	-	-	-	+	0.00	3.31
26.	<i>Thalassiosira lineata</i>	+	+	+	+	+	+	4.15	4.28
27.	<i>Triceratium favus</i>	-	-	-	-	-	+	0.00	4.91

### B. Factors Affecting Benthic Diatom Diversity

Principal Component Analysis (PCA) was conducted to identify and evaluate the correlation between the diversity of benthic diatoms and the physicochemical factors that may influence their composition, particularly concerning the decomposition of partially submerged, buried cadavers in both freshwater and brackish water ecosystems. The PCA describes the distribution of samples from the burial ecosystems, consisting of two principal components, F1 and F2 (Figure 2). The variance explained by the principal components F1 and F2 is 55.26% and 17.44%. The samples were categorised into two groups based on their ecosystems, namely freshwater (green dots) and brackish water (blue dots). The ellipses surrounding the two groups for the environment are the confidence intervals with a value of 95%.

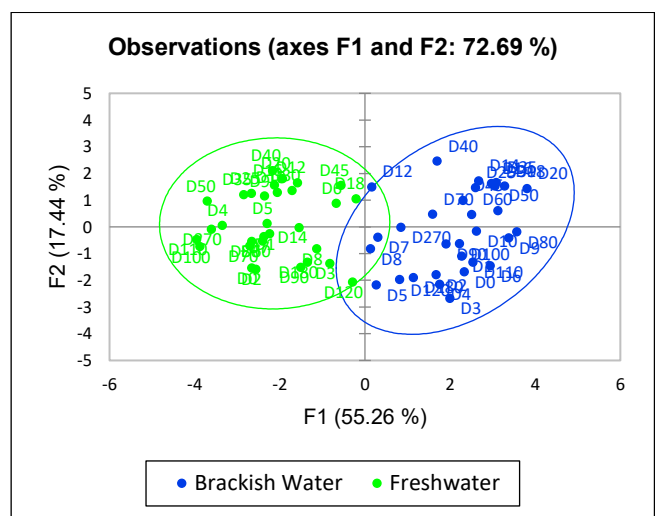


Figure 2. Principal component analysis (PCA) for the freshwater (green) and brackish water (blue) ecosystems, describing the relationship of physicochemical parameters and diversity of the benthic diatoms.

The results of PCA show the categorisation of the designated sampling points according to the physicochemical conditions of the burial sites of freshwater and brackish water environments. The resulting grouped scatter plots are from the diversity of characteristics and independent factors from each sampling location. This biplot observation is seen through the strength of the degree of scatter. The PCA analysis demonstrated that the burial in brackish water ecosystems had a greater degree of scatter than that of the freshwater ecosystems. Furthermore, there were sampling points that overlapped or shared the same cluster, i.e. day 120 for the freshwater ecosystem, and days 7, 8 and 12 for the brackish water ecosystem. This indicated that there were sampling points that may have the same diatom diversity, influenced by diverse environmental factors. However, most sampling points demonstrated differences in the diatom diversity as the two environments can be described as having different characteristics. Based on the observation, the data points for the freshwater ecosystem are clustered and dense, meaning that similar environmental physicochemical parameters influenced the diatom diversity of the cluster. Contrary to the freshwater ecosystem, the brackish water ecosystem's clustered structure was found to be scattered, suggesting that the diatom diversity in this ecosystem may be influenced by environmental physicochemical factors that vary significantly, with several levels within the dense cluster.

A Spearman correlation correlogram was performed to identify the correlation between environmental physicochemical parameters and diatom diversity for brackish water (I) and freshwater (II) ecosystems (Figure 3). Red and yellow colours describe positive and negative correlations for each comparison, while the numbers represent the strength and direction of the relative correlation for those characteristics. For the freshwater ecosystem, the environmental physicochemical parameters that demonstrated a significant correlation with diatom diversity are dissolved oxygen (DO) ( $r = 0.61, n = 31, p = 0.000$ ) and salinity ( $r = 0.49, n = 31, p = 0.006$ ). The presence of oxygen is crucial for diatoms to undergo cellular respiration (Gao *et al.*, 2021), which enables them to break down organic compounds and produce energy (Moreno *et al.*, 2018). The strength of the correlation between the

benthic diatoms and DO was moderate, as the diatoms have a variety of other alternatives to replace oxygen for metabolic pathways, such as the dissimilatory nitrate reduction to ammonium to produce energy without oxygen (Kamp *et al.*, 2011). Furthermore, salinity is one of the factors that affect the composition of diatoms, as diatom species have specific requirements or tolerances to certain changes in salinity (Zhang *et al.*, 2023). The salinity stress of an environment can affect the morphology of diatoms (Schmid, 1979; Stenger-Kovács *et al.*, 2023; Hu *et al.*, 2024).

Diatom diversity of the brackish water ecosystem was found to have a more significant correlation with the environmental physicochemical factors; DO ( $r = 0.29, n = 31, p = 0.00$ ), conductivity ( $r = -0.42, n = 31, p = 0.020$ ), water pH ( $r = -0.42, n = 31, p = 0.021$ ), total organic carbon (TOC) ( $r = -0.484, n = 31, p = 0.006$ ), and soil pH ( $r = -0.54, n = 31, p = 0.002$ ). Most studies show that many diatom species have interactions with environmental pH, where the water pH and soil pH demonstrated a negative correlation, i.e. the diatom diversity was lower with higher pH (Zepernick *et al.*, 2021). This is because diatoms require silica to build and form frustules; high pH values cause a decrease in silica solubility and inhibit frustule formation (Riedel *et al.*, 1987; Chauton *et al.*, 2015; Zepernick *et al.*, 2021).

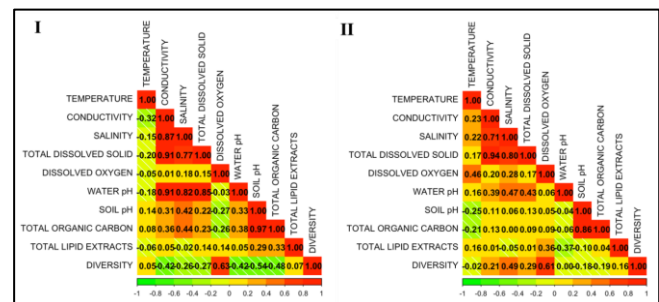


Figure 3. Illustration of correlation matrix for brackish water (I) and freshwater (II) environments with indication of positive (red) and negative (green) correlation.

The results of this study also discuss the correlation between environmental physicochemical factors of the partially submerged burial sites for both freshwater and brackish water ecosystems. In freshwater environments, there is a positive correlation between temperature and DO ( $r = 0.46, n = 31, p = 0.009$ ). This result does not agree with

the common interaction of temperature with DO, where the higher the water temperature, the lower the DO. However, this can be explained by the production of oxygen during photosynthesis, which is carried out by diatoms, for example, *Melosira* spp., *Hyalodiscus* sp., *Planothidium* sp. and *Nitzschia* spp., and other organisms (e.g. cyanobacteria) (Prelle & Karsten, 2022). In shallow water areas exposed to the sun, environmental conditions will be warm, increasing photosynthesis for algae (Wetzel, 2001). This condition may occur under specific conditions and at certain times only. For brackish water environments, there was no significant correlation with temperature. This may be due to the influence of water movement, which affects the abundance of periphyton in the area.

In the freshwater ecosystem, conductivity demonstrated a positive correlation with total dissolved solids (TDS) ( $r = 0.93$ ,  $n = 31$ ,  $p < 0.0001$ ), salinity ( $r = 0.71$ ,  $n = 31$ ,  $p < 0.0001$ ) and water pH ( $r = 0.39$ ,  $n = 31$ ,  $p = 0.030$ ). At the same time, conductivity for the brackish water ecosystem also had a positive correlation with the same factors, namely TDS ( $r = 0.91$ ,  $n = 31$ ,  $p < 0.0001$ ), salinity ( $r = 0.87$ ,  $n = 31$ ,  $p < 0.0001$ ), and water pH ( $r = 0.91$ ,  $n = 31$ ,  $p < 0.0001$ ). Besides, the conductivity of the brackish water environment was found to show a positive correlation with TOC ( $r = 0.36$ ,  $n = 31$ ,  $p < 0.049$ ). Most studies found that conductivity demonstrated a strong positive correlation with TDS, as conductivity is a direct measure of the concentration of ions in a liquid. Similar to TDS, salinity is a measure of the concentration of dissolved salts in water, which shows a very close relationship with conductivity and TDS (Thu *et al.*, 2019; Rebello *et al.*, 2020). All three environmental physicochemical parameters are closely related to the presence of ions in solution. The relationship between conductivity and pH depends on the water chemistry and the presence of buffering agents (Rebello *et al.*, 2020; McCleskey *et al.*, 2025).

Besides diatom diversity and conductivity, salinity had a positive correlation with TDS ( $r = 0.80$ ,  $n = 31$ ,  $p < 0.0001$ ) and water pH ( $r = 0.47$ ,  $n = 31$ ,  $p = 0.008$ ) for the freshwater ecosystem. In general, the results demonstrated a direct relationship between salinity and water pH. These ions raise the TDS, which affects the pH stability and buffering capabilities of the water. Higher pH levels potentially

resulted from the neutralisation of hydrogen ions ( $H^+$ ) by the dissolution of carbonate and bicarbonate salts. Besides diatom diversity and conductivity, salinity exhibited a positive correlation with both TDS and pH in the freshwater ecosystem, indicating that increased ionic strength contributes to enhanced alkalinity. In aquatic decomposition scenarios, the release of organic and inorganic compounds from decomposing tissues enriches the surrounding medium with various ions such as sodium, potassium, calcium, bicarbonate, and carbonate. These ions increase the TDS, thereby influencing the water's buffering capacity and pH stability. The dissolution of bicarbonate and carbonate salts neutralises hydrogen ions ( $H^+$ ), leading to higher pH values (Burcham *et al.*, 2024; Heudre *et al.*, 2021; Scott *et al.*, 2021). Elevated TDS and pH levels during decomposition can also modify the aquatic microenvironment, affecting nutrient availability and diatom community composition. Such physicochemical shifts are crucial in forensic interpretation, as they reflect the interplay between decomposition by-products, ionic enrichment, and microbial activity in determining post-mortem aquatic chemistry (Connor *et al.*, 2018). In the brackish water ecosystem, salinity had a positive correlation with four environmental factors, namely TDS ( $r = 0.77$ ,  $n = 31$ ,  $p < 0.0001$ ), water pH ( $r = 0.82$ ,  $n = 31$ ,  $p < 0.0001$ ), TOC ( $r = 0.44$ ,  $n = 31$ ,  $p = 0.015$ ) and soil pH ( $r = 0.42$ ,  $n = 31$ ,  $p = 0.018$ ). The difference between these two ecosystems can be distinguished by several significant correlations, where the salinity of the freshwater ecosystem affects benthic diatom diversity. However, the salinity of the brackish water ecosystem was influenced by soil pH and TOC.

TDS and water pH of both ecosystems had a significant positive correlation ( $r = 0.43$ ,  $n = 31$ ,  $p = 0.018$ ) for the freshwater, and ( $r = 0.85$ ,  $n = 31$ ,  $p < 0.0001$ ) for the brackish water ecosystems. This correlation for the brackish water ecosystem was greater than that of the freshwater ecosystem. Total Dissolved Solids (TDS) represent the sum of inorganic and organic matter dissolved in water and are closely related to salinity levels, as both reflect ionic strength and mineral content (Sá & Boyd, 2018). The observed positive correlation between TDS and pH in both ecosystems indicates that higher concentrations of dissolved ions, particularly bicarbonate and carbonate species, enhance the

buffering capacity of the water, thereby reducing hydrogen ion activity and increasing pH (Heudre *et al.*, 2021; Scott *et al.*, 2021). The DO demonstrated a positive correlation with the benthic diatom diversity for both ecosystems. This indicates that the benthic diatoms in the partially submerged burial sites require oxygen for the respiration process. In freshwater environments, DO had an interaction with total lipid extracts (TLEs) ( $r = 0.36$ ,  $n = 31$ ,  $p = 0.045$ ), however, the TLEs were found to have a weak negative correlation with water pH ( $r = -0.37$ ,  $n = 31$ ,  $p = 0.043$ ). TOC of the brackish water ecosystem demonstrated a positive correlation with water pH ( $r = 0.38$ ,  $n = 31$ ,  $p < 0.037$ ) and soil pH ( $r = 0.97$ ,  $n = 31$ ,  $p < 0.0001$ ). However, the TOC of the freshwater ecosystems only had a significant correlation with soil pH ( $r = 0.86$ ,  $n = 31$ ,  $p < 0.0001$ ), even though several studies revealed a negative correlation between TOC and pH (Xu *et al.*, 2019; Lu *et al.*, 2020).

### C. Interaction of Diatom Species with Submerged Buried Cadaver-Derived Material

A Spearman correlation test was performed to determine the relationship between benthic diatom composition and the concentration of TLEs, to confirm the potential of benthic diatoms as bioindicators to detect the partially submerged clandestine graves. In freshwater environments, four species have the potential to be used as bioindicators of the soil cadaveric-derived lipid concentrations, namely *Navicula cancellata* ( $r = -0.46$ ,  $n = 31$ ,  $p = 0.010$ ), *Fragilaria vaucheriae* ( $r = 0.36$ ,  $n = 31$ ,  $p = 0.049$ ), *Hantzschia* sp. ( $r = 0.47$ ,  $n = 31$ ,  $p = 0.009$ ), and *Nitzschia closterium* ( $r = 0.37$ ,  $n = 31$ ,  $p = 0.039$ ). For the brackish water ecosystems, only one species showed a significant correlation with the TLEs, namely *Coscinodiscus gigas* ( $r = -0.37$ ,  $n = 31$ ,  $p = 0.044$ ). Interactions between benthic diatoms may occur due to the need for nutrient sources by the diatoms themselves. The by-products of the decomposition process, such as fatty acids and other nutrients, can support the growth and proliferation of diatoms (Stonik & Stonik, 2015; Mukherjee, 2021). The correlation shows weak strength because diatoms do not rely entirely on the soil lipids for their growth. Diatoms themselves can produce lipids (structural lipids and storage lipids), especially polyunsaturated fatty acids, to serve various functions.

## IV. CONCLUSION

This study advances forensic ecological understanding by elucidating how physicochemical conditions at partially submerged burial sites influence benthic diatom community structure in freshwater and brackish water systems. The findings reveal significant insights into the physicochemical interactions governing aquatic ecosystems with partially submerged burial sites, highlighting the complex relationships between environmental factors, diatom communities, and decomposition processes. In freshwater environments, a positive correlation between temperature and dissolved oxygen suggests that diatom photosynthesis, driven by sunlight exposure, may override the typical inverse relationship, emphasising the role of biological activity in oxygen dynamics. The strong positive correlations between conductivity, TDS, salinity, and pH across both freshwater and brackish water ecosystems emphasise the influence of ionic content and mineral dissolution on water chemistry, which in turn affects diatom diversity and composition. Notably, the relationship between salinity and pH with soil and total organic carbon (TOC) illustrates how organic decomposition and ionic enrichment alter the microenvironment, impacting microbial and diatom communities.

Patterns of organic matter further reveal contrasting biogeochemical pathways across ecosystems; in freshwater systems, decomposition-derived lipids appear to modulate pH and oxygen availability, while, in brackish environments, high organic carbon and soil–water coupling amplify ion exchange and buffering processes. The observed relationships between specific diatom taxa (e.g., *Navicula cancellata*, *Fragilaria vaucheriae*, *Hantzschia* sp., *Nitzschia closterium*, and *Coscinodiscus gigas*) and cadaver-associated lipids provide preliminary evidence for the forensic potential of diatom–lipid interactions as indicators of decomposition process, albeit with modest effect sizes reflecting complex, multifactorial controls. The positive correlation between diatom diversity and dissolved oxygen further emphasises their potential as bioindicators for post-mortem aquatic conditions. Moreover, specific benthic diatom species demonstrate promising utility as indicators of cadaveric lipid presence, offering valuable forensic applications.

Overall, these findings underscore the dynamic interplay between physicochemical parameters and biological communities in submerged burial sites, advancing forensic interpretation and ecological understanding of decomposition in aquatic ecosystems. Collectively, these findings underscore the value of integrating diatom community profiling with detailed physicochemical characterisation in forensic aquatic investigations. Such integration can refine reconstructions of postmortem environmental conditions, assist in distinguishing freshwater versus brackish water depositional contexts, and contribute to the development of more reliable indicators for submerged body recovery sites. However, given the correlational nature and temporal constraints of this study, future work should employ experimental manipulations, extended temporal sampling, and multi-omics approaches to establish causal linkages and mechanistic insights. A controlled laboratory study of species-specific diatom culturing and lipid profiling shall also be conducted in the future to analyse their lipid biosynthesis under varying physicochemical conditions. This can help verify their correlation with cadaver-derived lipid compounds and refine their forensic reliability. Besides, extending sampling to multiple geographic locations can be done to determine whether the identified indicator species are consistently present and reliable in other regions, or if regional specificity limits their forensic application.

In the context forensic perspective, this study advances interpretive frameworks by demonstrating that diatom

community signatures, when contextualised within the surrounding physicochemical setting, can yield meaningful insights into the postmortem aquatic environment. Such integration enhances the ability to infer site-specific conditions of submerged cadavers, refine PMI estimations, and discriminate between freshwater and brackish water depositional circumstances. Ecologically, these findings extend the understanding of how decomposition-derived inputs, including lipids and other organic substrates, interact with aquatic microflora to drive localised biogeochemical gradients. By situating diatom ecology within a forensic framework, this research bridges aquatic biogeochemistry and forensic science, demonstrating that benthic diatoms, when interpreted through an ecosystem-specific lens, offer promising, context-sensitive markers for elucidating postmortem processes in aquatic burial environments, giving crucial information to determine the PMI and/or locate the partially submerged clandestine graves.

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