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Original Article

Diatoms Don't Lie: Forensic Insights into Drowning through Autopsy-Based Analysis

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ABSTRACT

Background: Drowning is a major cause of accidental death worldwide and poses a significant challenge in forensic investigations. Classical autopsy findings of drowning are often non-specific or may be obscured due to decomposition, making confirmation of drowning difficult. The diatom test has emerged as an important supplementary forensic tool to establish drowning as the cause of death by detecting microscopic algae in internal organs.
Aim: To evaluate the significance of the diatom test in establishing drowning as the cause of death and to compare diatom species found in organs with those present in water samples collected from the alleged site of recovery.
Materials and Methods: This observational study was conducted in the Department of Forensic Medicine and Toxicology at Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab over a period of 18 months. Fifty cases of suspected drowning brought for medico-legal autopsy were included. Samples of liver, brain, bone marrow, and water from the recovery site were collected. Diatoms were extracted using the acid digestion method followed by centrifugation and microscopic examination. The species of diatoms identified in organ samples were compared with those present in the water samples.
Results: Out of the 50 cases studied, 84% were males and the majority belonged to the age group of 20–49 years. Most bodies were recovered from canals (68%) and running water (86%). Diatoms were detected in 86% of cases, while 14% showed negative results. Mixed species of diatoms were the most commonly identified, followed by Synedra, Achnanthidium, Aulacoseira, Stephanocyclus, Pinnularia, and Navicula. In most cases, the diatom species detected in organ samples corresponded with those found in water samples from the recovery site, supporting the diagnosis of antemortem drowning.
Conclusion: The diatom test is a valuable adjunct in forensic investigations of drowning. Detection and comparison of diatom species in tissues and water samples can assist in confirming antemortem drowning and identifying the site of drowning, especially in decomposed bodies where classical autopsy findings may be inconclusive.

Keywords: *Drowning; Diatom test; Forensic pathology; Antemortem drowning; Diatom analysis; Medico-legal autopsy; Aquatic death investigation.*

INTRODUCTION

Drowning represents one of the most significant yet preventable causes of accidental death worldwide and remains a major public health concern across both developed and developing countries. According to the World Health Organization (WHO), drowning accounts for approximately 372,000 deaths annually, making it the third leading cause of death due to unintentional injury globally. The burden of drowning mortality is disproportionately higher in low- and middle-income countries, where factors such as easy access to open water bodies, inadequate supervision, lack of swimming skills, and limited rescue infrastructure significantly increase the risk of drowning incidents. In countries like India, where rivers, canals, ponds, wells, and irrigation systems are widely distributed, drowning deaths constitute a substantial proportion of medico-legal autopsies performed in forensic departments. [1]

The forensic investigation of drowning poses considerable challenges to pathologists and medico-legal experts. Unlike many other causes of death, drowning does not always produce pathognomonic autopsy findings. Although several classical signs such as fine froth at the nose and mouth, waterlogged lungs, and washerwoman's skin have traditionally been associated with drowning, these signs are not always present and may be absent in decomposed bodies or in atypical forms of drowning. Furthermore, bodies recovered from water may have died from causes other than drowning, including trauma, poisoning, or natural disease, and were subsequently disposed of in water to conceal the crime. Therefore, determining whether a person was alive at the time of immersion and whether drowning was the cause of death remains a crucial but difficult task in forensic pathology. [2]

Historically, forensic diagnosis of drowning relied primarily on external and internal autopsy findings. External signs such as fine white froth around the mouth and nostrils, washerwoman's changes in the skin, and cutis anserina were commonly used as indicators of drowning. Internally, the presence of over-distended lungs, pulmonary edema, water in the stomach, and debris such as mud, algae, or sand in the respiratory tract were considered suggestive of drowning. However, these findings are not entirely specific and may be altered or obscured by decomposition, postmortem immersion, or environmental factors. Consequently, reliance solely on gross autopsy findings often results in diagnostic uncertainty. [2]

To overcome these limitations, forensic scientists have explored various supplementary techniques to improve the accuracy of drowning diagnosis. Among these methods, the diatom test has emerged as one of the most widely studied and utilized laboratory investigations in suspected drowning cases. The diatom test is based on the detection of microscopic algae known as diatoms in the internal organs and tissues of drowning victims. Diatoms are unicellular algae belonging to the class Bacillariophyceae and are characterized by their unique siliceous cell walls called frustules. These frustules possess intricate and species-specific morphological patterns, which allow accurate identification under microscopic examination. Because of their silica composition, diatoms are highly resistant to decomposition and can persist in tissues long after death, making them valuable forensic indicators. [3]

The principle underlying the diatom test is that during antemortem drowning, water containing diatoms is aspirated into the lungs. From the lungs, these microscopic organisms may pass through the alveolar-capillary barrier into the bloodstream and subsequently circulate to distant organs such as the liver, kidneys, brain, and bone marrow. The detection of diatoms in these organs suggests that the victim inhaled water while alive, thereby supporting the diagnosis of drowning. In contrast, when a body is immersed in water after death, diatoms may enter the lungs passively but are unlikely to reach the systemic circulation due to the absence of blood circulation. Thus, the presence of diatoms in distant organs is considered supportive evidence of antemortem drowning. [4]

The importance of the diatom test becomes particularly evident in cases involving decomposed bodies or skeletonized remains. Decomposition often obliterates classical signs of drowning, making diagnosis extremely difficult. In such circumstances, the persistence of diatom frustules in tissues such as bone marrow and brain provides valuable evidence for forensic investigation. Consequently, diatom analysis has become an important adjunct tool in medico-legal autopsies involving suspected drowning cases. [4]

Apart from confirming drowning as the cause of death, diatom analysis may also provide additional forensic information. By comparing the species composition of diatoms found in the victim's tissues with those present in the water body where the body was recovered, forensic investigators may determine whether the drowning occurred at that particular location. This comparative analysis can assist in reconstructing the circumstances of death and may help identify the primary drowning site in cases where bodies are transported by water currents. Such applications highlight the broader forensic significance of diatom research beyond simple confirmation of drowning. [5]

Despite its advantages, the diatom test is not without limitations. Environmental variability, contamination during sample collection, and the presence of airborne diatoms may lead to false-positive results. Similarly, the absence of diatoms does not necessarily exclude drowning because certain water bodies contain very low diatom concentrations. Additionally, advanced decomposition may affect the recovery of diatoms from tissues. Therefore, the interpretation of diatom findings requires careful consideration of environmental factors, laboratory techniques, and the overall medico-legal context of the case. [6]

Another challenge in diatom analysis is the variation in diatom species across different aquatic environments. Rivers, lakes, ponds, and marine environments often contain distinct diatom communities influenced by factors such as water chemistry, temperature, and seasonal changes. Forensic scientists must therefore possess adequate knowledge of local diatom flora to accurately interpret the results of diatom testing. Establishing regional diatom databases is considered an important step in improving the reliability of forensic diatomology. [7]

In addition to technical considerations, the use of diatom analysis in forensic investigations raises important legal and ethical issues. The results of diatom testing may have significant implications in criminal trials, especially in cases involving suspected homicide disguised as drowning. Therefore, strict adherence to standardized protocols, proper documentation, and maintenance of chain-of-custody records are essential to ensure the admissibility of diatom evidence in courts of law. Ethical considerations regarding the respectful handling of human remains must also be observed during sample collection and laboratory analysis. [2]

Given these complexities, the diatom test should be considered as part of a comprehensive forensic approach rather than a standalone diagnostic tool. Integration of autopsy findings, histopathological examination, toxicological analysis, and scene investigation is necessary for accurate determination of cause and manner of death in drowning cases. When interpreted in conjunction with these factors, diatom analysis can significantly enhance the diagnostic accuracy of forensic investigations.

The present study aims to evaluate the significance of the diatom test as a reliable forensic tool in determining the cause of death in suspected drowning cases. By analyzing the presence and distribution of diatoms in various organs, the study seeks to assess the usefulness of diatom analysis in distinguishing antemortem drowning from postmortem immersion. The findings of this study may contribute to improving forensic diagnostic practices and strengthening medico-legal investigations involving drowning deaths.

REVIEW OF LITERATURE

Concept and Definition of Drowning

Drowning is defined as the process of experiencing respiratory impairment due to submersion or immersion in a liquid medium. This impairment occurs when the airway is obstructed by liquid, preventing the normal exchange of oxygen and carbon dioxide in the lungs. The modern definition emphasizes drowning as a process rather than an outcome, acknowledging that drowning incidents may result in fatal, non-fatal, or no morbidity outcomes.[8]

Drowning can occur in a variety of environments including oceans, rivers, lakes, wells, swimming pools, and bathtubs. Contrary to popular belief, drowning does not require complete submersion of the body; obstruction of the mouth and nose by water is sufficient to initiate the drowning process. Consequently, drowning can occur even in shallow water, particularly among children or incapacitated individuals.[8]

Globally, drowning remains a major cause of accidental death, particularly among children, adolescents, and young adults. Epidemiological studies have shown that males are significantly more affected than females due to greater exposure to aquatic environments and higher participation in risk-taking activities.[9]

Classification of Drowning

Drowning can be broadly categorized into typical and atypical forms. Typical drowning, also known as wet drowning, involves inhalation of water into the lungs and is the most common form encountered in forensic practice. In wet drowning, water entering the respiratory tract leads to asphyxia and progressive hypoxia. This type may occur in both freshwater and saltwater environments, each producing distinct physiological effects on the body.[8]

Freshwater drowning results in rapid absorption of hypotonic water through the alveoli into the bloodstream, leading to hemodilution, hemolysis, and electrolyte imbalance. These changes may precipitate ventricular fibrillation and sudden cardiac arrest. Saltwater drowning, on the other hand, involves aspiration of hypertonic saline water, which draws fluid from the bloodstream into the lungs, resulting in pulmonary edema and hypoxia.[8]

Atypical drowning includes conditions where classical water inhalation is absent or minimal. These include dry drowning, immersion syndrome, and submersion of unconscious individuals. Dry drowning occurs due to reflex laryngospasm triggered by water entering the upper airway, leading to asphyxia without significant water aspiration. Immersion syndrome, also known as vagal inhibition, involves sudden cardiac arrest caused by reflex vagal stimulation upon sudden contact with cold water. Submersion of unconscious individuals may occur due to pre-existing conditions such as epilepsy, cardiac disease, or head injury.[8]

Mechanism of Drowning

The physiological mechanism of drowning involves a sequence of events beginning with panic and struggle following submersion in water. The victim initially attempts to hold their breath while struggling to stay afloat. As oxygen levels decrease and carbon dioxide accumulates in the blood, the urge to breathe becomes overwhelming, leading to involuntary inhalation of water.[8]

The entry of water into the airways triggers laryngospasm, temporarily sealing the airway and preventing further water aspiration. However, this also prevents air from entering the lungs, leading to progressive hypoxia. As hypoxia worsens, the victim loses consciousness, after which the laryngospasm relaxes and water enters the lungs. This interferes with pulmonary gas exchange and further exacerbates hypoxia, ultimately resulting in cardiac arrest and death.[8]

Postmortem Findings in Drowning
Autopsy findings in drowning cases include both external and internal signs. External findings commonly observed include fine white froth around the nose and mouth, washerwoman's skin changes in the hands and feet, and cutis anserina caused by contraction of erector pili muscles due to cold exposure. Cadaveric spasm may also occur, with the victim grasping objects such as weeds or mud during the final struggle.[8]

Internal findings typically include heavy, waterlogged lungs exhibiting pulmonary edema, sometimes referred to as emphysema aquosum. On sectioning, the lungs exude blood-stained frothy fluid. Water and debris such as sand or algae may also be present in the stomach and intestines, indicating ingestion of water during drowning.[8]

However, these findings are not specific to drowning and may be absent in decomposed bodies or atypical drowning cases. Therefore, additional laboratory investigations are often required to support the diagnosis.

Forensic Significance of Diatoms
Diatoms are microscopic algae characterized by their siliceous cell walls, which make them highly resistant to environmental degradation and decomposition. They are widely distributed in aquatic environments including freshwater, marine, and even moist terrestrial habitats. Due to their durability and abundance, diatoms have become important biological indicators in forensic science.[10]

During antemortem drowning, diatoms present in water may enter the lungs and subsequently pass into the bloodstream. Circulating diatoms may become lodged in distant organs such as the liver, kidneys, brain, and bone marrow. Detection of these organisms in internal organs provides supportive evidence that the victim was alive at the time of submersion.[11]

Studies on Diatom Analysis in Forensic Investigations
Numerous researchers have investigated the reliability of diatom analysis in diagnosing drowning. Neidhart and Greendyke (1967) examined 43 drowning cases and found diatoms in the lungs of 83% of victims and in systemic organs in 42% of cases. Their study demonstrated that diatoms rarely enter systemic circulation in postmortem immersion, thereby supporting the diagnostic value of the test.[12]

Seshagiri et al. (1993) conducted a study on 60 drowning cases and reported the highest concentration of diatoms in the lungs, followed by the heart, kidneys, liver, and bone marrow. Their findings suggested that bone marrow and brain tissues are particularly reliable samples for diatom detection due to lower contamination risk.[13]

Anderson et al. (1996) analyzed 50 autopsy cases and reported a 90% detection rate of diatoms in drowning victims. Their study emphasized the importance of examining multiple organs to increase diagnostic accuracy.[14]

Peabody (1997) investigated the presence of diatoms in bone marrow and reported positive findings in 85% of drowning cases, highlighting the usefulness of bone marrow examination in decomposed bodies.[15]

Bierens et al. (2002) conducted a large-scale study involving 100 drowning cases and found that diatoms were detected in 95% of freshwater drowning cases compared to 80% of saltwater cases, suggesting environmental differences in diatom distribution.[16]

Lunetta et al. (2004) emphasized the importance of integrating diatom testing with other forensic investigations to improve diagnostic accuracy in cases where drowning is suspected but autopsy findings are inconclusive.[17]

Subsequent studies have also focused on improving laboratory techniques for diatom detection. Dussault et al. (1998) compared acid digestion with enzymatic digestion methods and concluded that enzymatic digestion produced higher diatom counts and better preservation of frustule morphology.[18]

More recently, molecular approaches such as polymerase chain reaction (PCR) and next-generation sequencing have been explored for identifying diatom DNA in tissue samples. Yang et al. (2016) reported a 98% success rate in identifying diatom species using PCR techniques, indicating the potential of molecular methods in forensic diatomology.[19]

Similarly, Li et al. (2023) demonstrated the effectiveness of next-generation sequencing in identifying diatom species with high accuracy, suggesting that advanced molecular technologies may enhance the reliability of diatom analysis in forensic investigations.[20]

Several epidemiological studies have also highlighted the demographic patterns associated with drowning deaths. For instance, Ambade et al. (2013) reported that drowning cases were more common among males aged 31–40 years and frequently occurred in wells and natural water bodies.[21]

Recent research in India has further emphasized the forensic value of diatom testing. Shrilakshmi et al. (2017) found diatoms in 94% of drowning cases examined in Andhra Pradesh and Telangana, supporting the reliability of the test in medico-legal investigations.[22]

Similarly, Pal et al. (2017) reported a positive diatom test in 93.93% of drowning cases in Himachal Pradesh, confirming its usefulness as a diagnostic tool in forensic pathology.[23]

Limitations of Diatom Analysis

Despite its advantages, diatom analysis has several limitations. False-positive results may occur due to contamination from environmental sources or laboratory procedures. Airborne diatoms may also contaminate samples during autopsy or laboratory processing. Additionally, certain water bodies may contain very low diatom concentrations, resulting in false-negative findings even in genuine drowning cases.[24]

Seasonal variations in diatom populations also influence the reliability of the test. Studies have shown that diatom diversity and abundance vary significantly across seasons, with higher concentrations during spring and autumn. These variations must be considered when interpreting forensic results.[25]

Furthermore, the detectability of diatoms decreases with increasing postmortem interval due to tissue decomposition and environmental degradation. Therefore, timely sample collection and proper preservation techniques are essential for obtaining accurate results.[26]

AIMANDOBJECTIVES

1. TodetectthediatomsinvariousorgansandtissueinDrowning cases.
2. Tocompareandcontrastthevarioustypesofdiatomsinthetissues with a sample of water.
3. ToascertainwhetherthedrowningisAnte-mortemorPost-mortem.

MATERIALSANDMETHODS

Study area: The study will be conducted in the Mortuary under theDepartment of Forensic Medicine and Toxicology, G.G.S. Medical Collegeand Hospital, Faridkot, Punjab.

Studyperiod:18months

Studydesign: ObservationalStudy

Studypopulation:

Inclusion criteria:

Only those cases were included in this study where dead bodies were foundin water. Specimen of Liver and Brain is to be collected in dried and clean plastic jars and sample of water from where the dead body was recovered at the time of postmortem examination. Later on, these specimens were subjected to acid digestion for conducting diatom test in Mortuary under the Department of Forensic Medicine and Toxicology, G.G.S. Medical Collegeand Hospital, Faridkot, Punjab.

Collection of Bone Marrow specimen: Specimen of sternum dissected fromthe body and sample of water from where the dead body was recovered, was send to the Chemical Examiner, Govt. of Punjab, Kharar.

Exclusioncriteria:

Cases other than drowning were not included as confirmed by history and police investigation.

Sampling technique: Keeping in view of the availability and feasibility of the participants, a nonrandom convenient sampling technique will be used. So, consecutively 50 subjects will be considered for the study.

Data collectiontools: Proformaisattachedinannexures.

Methodology:

After taking informed consent from relatives/guardians of the deceased, the autopsy was conducted on all the selected cases brought to the mortuary of Guru Gobind Singh Medical College, Faridkot. Demographic information regarding the deceased including age, sex, identification status of deceased, residence, siteof recoveryof deadbodiesand timeof incidence wascollected from the police and relatives. The duration and medium of submersion was duly documented as available from the history and police records. The external state of the body i.e., fresh or decomposed was emphasized in documentation prior to conducting the examination. A thorough external and internal examination of the body was done and documented.

Following basic experimental protocol were followed for extraction and analysis of diatoms from each of the reported cases: -

Acid digestion: Concentrated nitric acid was used to extract the diatoms from the exhibits. For this, internal organs in the form of exhibitsareputintoseparatejarsand50mlof nitricacid waspouredin each of the jars. Jars having the exhibits and acid were put overnight undisturbed and covered. Next day, the digested or semi-digested exhibits were boiled for half an hour or more till the whole of thecontent changed into yellow liquid and were allowed to cool.

Centrifugation: Fat layer was removed and the yellow liquid was subjected to centrifugation at 4000 RPM for about 15 minutes. Centrifugation was repeated three times and the supernatant was discarded every time. Pellets were washed with distilled water and again centrifuged.

Microscopic Examination: After discarding the supernatant, pellets were used to prepare the microscopic slides and were examined forthe presence of diatoms under the microscope. Slides were also prepared from the water samples from which the dead bodies are recovered. Diatom types found in the organ samples and water samples were compared and analysis was done.

Data analysis plan: ThecollecteddatawasenteredinMSExceland appropriate statistical tests was applied accordingly.
Ethicalconsiderations:Writteninformedconsentwastakenfromthe relatives/guardians of deceased for the study.

OBSERVATIONSANDRESULTS

Thesexdistributioninthisstudy,withmales constituting 42 (84%) and females 08 (16%), the highest frequency of drowning occurred among the 20-49 years age group (74%), followed by 10-19 years age group i.e 5 cases (10%) followed by 50-59 years age group i.e.4 cases (8%) followed by 60-69 years age group i.e. 3 cases (6%) followed by 0-9 years age group i.e 1 case (2%), majority of the deceased (84%) were identified, while 16% remained unidentified majority of drowning victims were from rural areas (64%), followed by urban areas (20%), with 16% residency unknown.

The recovery sites were majority of bodies were found in canals (68%), followed by rivers or streams (20%). Ponds and other locations had lower frequencies (4% and8%, respectively) and mostdrowningsoccurredinrunning water (86%), compared to stagnant water (14%). The probable time of death and postmortem examination of the dead bodies is majority bodies recovered is within 2 and more than 10 days i.e 33 cases (66%) with putrefied changes followedby12-24hoursi.e.14cases(28%)followedby24-48hoursi.e.3 cases(6%). The highest frequency of cases occurred during the and summers and rainy/monsoon season (64%), followed by winters (28%).Autumn had the lowest occurrence at 8%.86% of the cases tested positive for diatoms, while 14% tested negative.

Table1Incidenceofdiatomspeciesidentifiedinwatersamples collected from site of recovery of dead body

Speciesidentifiedinwatersamples collectedfromsiteofrecoveryof dead body	Number of drowning cases	Percentage (%)
1.Sp.Achnanthidium	5	10.0
2.Sp.Aulacosiera	5	10.0
3.Sp.Navicula	2	4.0
4.Sp.Pinnularia	4	8.0
5.Sp.Proschkinia	1	2.0
6.Sp.Stephanocyclus	5	10.0
7.Sp.Synedra	7	14.0
8.MixedSpecies	21	42.0
Total	50	100.0

Tableno.1indicatesthediatomspeciesidentified in water samples collected from the site of recovery of dead body. Mixed species were the most frequently identified (42%), followed by Sp. Synedra (14%)and Sp.Achnanthidium, Sp.Aulacosiera and Sp. Stephanocyclus (10% each respectively) followed by Sp. Pinnularia (8%) followed by Sp. Navicula (4%) and last Sp. Proschkinia (2%).

Table no. 2 Incidence of diatom species identified in liver samples of the dead body

Speciesidentifiedinliversamples of the dead body	Number of drowning cases	Percentage (%)
1.Sp.Achnanthidium	4	8.0
2.Sp.Aulacosiera	5	10.0
3.Sp.Navicula	2	4.0
4.Sp.Pinnularia	4	8.0
5.Sp.Proschkinia	1	2.0
6.Sp.Stephanocyclus	5	10.0
7.Sp.Synedra	7	14.0
8.MixedSpecies	15	30.0
9.NoDiatoms present	7	14.0
Total	50	100.0

Tableno. 2 presents the diatom species identified in liver samples. Mixed species were the most common (30%), followed bySp.Synedra(14%)followedbySp.StephanocyclusandSp.Aulacosiera(10% each respectively) followed by Sp. Achnanthidium and Sp. Pinnularia (8% each respectively) followed by Sp. Navicula (4%) followed by Sp. Proschkinia (2%). In 14% of cases, no diatoms were present.

Table 3. Incidence of diatom species identified in brain samples of the dead body

Speciesidentifiedinbrain sample of the dead body	Number of drowning cases	Percentage(%)
1.SpeciesAchnantidium	2	4.0
2.SpeciesAulacoseira	2	4.0
3.SpeciesNavicula	0	0.0
4.SpeciesPinnularia	2	4.0
5.SpeciesProschkinia	0	0.0
6.SpeciesStephanocyclus	1	2.0
7.SpeciesSynedra	2	4.0
8.Mixedspecies	6	12.0
9.Nodiatomspresent	7	14.0
10.Liqeufiedbrainmatter	28	56.0
Total	50	100.00

Tableno.3indicatesthediatom speciesidentified inbrainsamplesofthedeadbody.Themajorityofsampleshadliquefiedbrain matter (56%), in 14% of the sample, no diatoms were present followed by mixed species were found in 12% of cases followed by Sp. Achnanthidium, Sp. Aulacosiera, Sp. Pinnularia and Sp. Synedra (4% each respectively) followed by Sp. Stephanocyclus (2%).

Tableno.4Comparisonofstudycasesoftypesofspeciesfoundin liver with that of water sample

Speciesidentified	Liversampleofthe dead body		Water sample collected from site ofrecoveryof deadbody	
	Frequency	Percentage(%)	Frequency	Percentage(%)
1.SpeciesAchnantidium	4	8.0	5	10.0
2.SpeciesAulacoseira	5	10.0	5	10.0
3.SpeciesNavicula	2	4.0	2	4.0
4.SpeciesPinnularia	4	8.0	4	8.0
5.SpeciesProschkinia	1	2.0	1	2.0
6.SpeciesStephanocyclus	5	10.0	5	10.0
7.SpeciesSynedra	7	14.0	7	14.0
8.Mixedspecies	15	30.0	21	42.0
SubTotal	43	86.0	50	100.0
9.Nodiatomspresent	7	14.0		
GrandTotal	50	100.0	50	100.0

The table no. 4, presents a comparison of the diatom species found in the liver sample of the deceased individual and the water sample collected from the alleged incident site.The liver sample contained a total of 43 diatom specimens, accounting for 86% of the total sample, the remaining 14% of the liver sample showed no presence of diatoms.The water sample contained 50 diatom specimens, representing 100% of the sample.

Comparisonofdiatomsspecies:The most common species in both the liver and water samples was mixed species, comprising 30% and 42% of the respective samples.Synedra was the second most prevalent species, making up 14% of both the liver and water samples.Achnantidium, Aulacoseira, Pinnularia, and Stephanocyclus each accounted for around 8-10% of both samples.Navicula and Proschkinia were the least common species, representing 2-4% of both samples.

Tableno.5Comparisonofstudycasesoftypesofspeciesfoundin brain with that of water sample

Speciesidentified	Brainsampleof the dead body		Water sample collectedfromsite of recovery of dead body	
	Frequency	Percentage(%)	Frequency	Percentage(%)
1.SpeciesAchnantidium	2	4.0	5	10.0
2.SpeciesAulacoseira	2	4.0	5	10.0
3.SpeciesNavicula	0	0.0	2	4.0
4.SpeciesPinnularia	2	4.0	4	8.0
5.SpeciesProschkinia	0	0.0	1	2.0
6.SpeciesStephanocyclus	1	2.0	5	10.0
7.SpeciesSynedra	2	4.0	7	14.0

8.Mixedspecies	6	12.0	21	42.0
Subtotal	15	30.0	50	100.0
9.Nodiatomspresent	7	14.0		
10.Liquefiedbrainmatter	28	56.0		
Total	50	100.0	50	100.0

The table and graph no. 5, provides a comparison of the diatom species found in the brain sample of the deceased individual and the water sample collected from the site of recovery of dead body.Thebrainsamplecontainedatotalof15diatomspecimens,accounting for 30% of the total sample, the remaining 70% of the brain sample consisted of either liquefied brain matter (56%) or no diatoms (14%) indicating significant decomposition.The water sample contained 50 diatom specimens, representing 100% of the sample.

Comparisonofdiatomspecies:The most common species in both samples is mixed species, comprising 12% and 42% each respectively of the total.Sp. Achnantidium, Sp. Aulacoseira, Sp. Pinnularia and Sp. Synedra, each made up 4% and 8-14% each respectively of the total.Sp. Stephanocyclus was the least common in both samples, representing 2% and 10 % each respectively of the total.NaviculaandProschkiniawerenotdetectedinthebrainsample

DISCUSSION

In this study the data shows that out of the 50 total drowning cases, 42 (84%) were males, while only 8 (16%) were females. Howland et al (1996)9 reported 45% of males, Franklin et al. (2010)27 76.4% of males,Ambade VN etal(2013)21whoreported241outof353casesweremales,KaushikNetal (2017)28 15 males out of 17 drowning cases, Pal SK et al (2017)23 75.75% males, Chhikara P et al (2018)29 40 males out of 41 drowning cases, Kym Roberts et al (2024)30 reported similar incidences.

This sex based difference may be due to high risk taking behaviour, water- related activities (boating, swimming, scuba diving), alcohol use in aquatic setting, physiological factors, cultural and social norms by males and its surrounding gender roles and water safety practices may also play a role in the observed disparities.

In this study, the majority of cases belonged to age group of 20-49years of age i.e. 37 cases (74%) followed by 50-59 years i.e. 4 cases (8%) followedby60-69yearsofagegroupi.e.3cases(6%)andlastly0-9years i.e. 1 case (2%). This study is in accordance with Phad et al (2018)31 who reported majority of drowning deaths belongs to the age group of 21-30 years i.e.49outof176cases(27.84%).

The higher drowning death rates in young adults are more likely to engage in high-risk water activities such as swimming in open water, boating, or water sports. They may be more likely to engage in occupational activities that involvewater,suchasfishingorworkingonboats.Thisincreasedexposureto water-related activities can contribute to the higher number of drowning incidents in this age group.

Inthisstudy,majorityofthedeceasedwereidentifiedi.e. 42 cases (84%) and 16 cases (16%) were not identified.Theseresultsare in accordance with that ofAmbade VN et al (2013)21 who reported 333 out of 353 cases were identified (94%).

It isthe effortof investigating agencies and relatives to find the dead bodies in water. The unidentified dead bodies may be travelled in running water to long distances or bodies get putrefied beyond identification.

In this study, majority of the cases were from rural area i.e. 32 cases (64%) followed by urban areas i.e. 10 cases (20%). Pal SK et al (2017)23 reported 65.15%, TT Reddy et al (2019)32, reported that the cases were from rural area and there was rural predominance.

Since majority of drowning victims are from rural areas and these areas have more of these natural water bodies, such as rivers, lakes, and ponds, which can increase the exposure and accessibility to water-related activities and the risk of drowning. Rural areas may have fewer resources and infrastructure dedicated to water safety, such as lifeguard stations, swimming pools, and water safety education programs, compared to urban areas. Rural residents may be more likely to engage in water-related recreational activities, such as fishing, boating, orswimmingin naturalwaterbodies, which can increasetheir exposure to drowning hazards. Rural areas may have longer emergency response times and limited access to rescue resources, which can impact the ability to respond effectively to drowning incidents and reduce the chances of survival.

In this study, majority of dead bodies were recovered from canals i.e. 34 cases (68%), followed by rivers or streams i.e. 10 cases (20%) followed by other locations such as swimming pool, sewer, etc,i.e.4cases(8%)andleastfrom Pondsi.e.2cases(4%).Thisstudyisnot in accordance with Pal SK et al (2017)23 who reported majority of dead bodies were recovered from water of natural flowing streams (khuds) (31.81%).

Majority of dead bodies recovered from canals and rivers and streams is due to canalsand rivers/streamsareoftenmoreaccessible and integrated into the local landscape, increasing the exposure and likelihood of individuals engaging in water-related activities in these locations. Canals and rivers/streams may have characteristics that increase the risk of drowning, such as strong currents, deep water, or poor visibility, making them more hazardous than other water bodies like ponds.

In this study, the majority of the 50 drowning cases, 43 cases (86%) involved running water. In contrast, 14% or 7 cases involved stagnant water. Kumar Naveen et al (2023)33 reported similar incidence (72%) drowning death occurred in running water. The majority of drowning death occurred in running water as running water often has strong currents and flow patterns, which can make it difficult for individuals to swim or stay afloat, increasing the risk of drowning.

Running water can be deeper and have poorer visibility than stagnant water, making it more challenging for individuals to navigate and avoid hazards. Running water is often associated with recreational activities such as swimming, boating, or fishing, which can increase the risk of drowning ifsafety measures are not taken.

In this study, the majority of the 50 drowning cases had a probable time of death and postmortem examination from 2 days to more than 10 days i.e 33 cases(66%) followed by 12-24 hours i.e. 14 cases (28%). This study is not in accordance with Rubio et al, Malaga, Spain (2014)34 who reports majority of the dead bodies were recovered in the postmortem interval of less than 12 hours i.e. 58 out of 78 dead bodies.

It may take a longer time to discover and recover the body after a drowning incident can significantly impact the postmortem interval. Since, this geographical region has a good canal system for irrigation purpose, the dead bodies which are drowned in water bodies tend to remain submerged under the water and since the dead body are only visible after they float above the surface time between their death and autopsy becomes more and hence the postmortem interval increases.
In this study, the highest frequency of drownings occurred during the summers and rainy/monsoon season i.e. 32 cases (64%), followed by equal frequencies in winters (28%) and Autumn had the lowest occurrence at 4 cases (8%). This is in accordance with Ambade VN et al (2013)21 who reported majority of dead bodies retrieved from water were in rainy season i.e. 181 out of 353 cases. Chikara Pet al (2018)29 reported that majority of drowning deaths occurred in rainy season13 out of 41 cases. Phad et al(2018)31 reported majority of drowning deaths occurred in rainy season (43.75%).

The rainy/monsoon season is characterized by heavy rainfall, which can lead to increased water levels and stronger currents, making it more hazardous for individuals to engage in water-related activities.

In this study, the majority of drowning cases i.e. 43 cases (86%) tested positive for diatoms, while 7 cases (14%) tested negative. The results are in accordance with Neidhart and Greendyke (1967)12reported that 36 out of 43 drowning cases (83%) tested positive for diatom test. Shrilakshmi P et al (2017)22 who reported 94 out of 100 cases tested positive for diatoms test (94%), Kaushik N et al (2017)28 reported 12 out of17 cases (70%) tested positive for diatom test, Pal SK et al (2017)23 who reported diatoms test was positive in 62 out of 66 cases (93.93%). Chikara P et al (2018)29 who reported 28 cases out of 41(68%) tested positive. Reddy TT et al (2019)32 who reported 35 out of 40 cases (87%) tested true positive for diatoms.

The presence of diatoms in the victim's organs and tissues, particularly brain and liver, is a strong indicator that the individual drowned in the water body where the diatoms were present.The high percentage of positive diatom tests supports the use of this test as a reliable forensic tool in confirming drowning as the cause of death.

Inthisstudy,Mixedspecieswerethemost frequently identified (42%), followed by Sp. Synedra (14%) and Sp. Achnanthidium, Sp. Aulacosieraand Sp. Stephanocyclus (10% each respectively).Sharma P et al (2019)35 reportedthat 12 different species were identified from 10 different waterbodies of Punjab. Mall et al(2021)36 reported a total of 13 species were identified from different sites of Rapti river, Gorakhpur. Tandon et al (2023)37 who reported 12 different species were identified in rivers of different region of Chhattisgarh were multiple species or genera (34 genera).Naturalwaterresourceswhichmayhavemixedspeciesofdiatoms.

In this study, Mixed species were the most common (30%), followed by Sp. Synedra (14%). In 14% of cases, no diatoms were present. This study is in accordance with Levkov etal, Skopje, Macedonia (2017)24 reported that in 7 out of 10 cases liversample contained diatom species with the dominant diatoms being pennate diatoms. Kaushik N et al (2017)28 reported that majority of diatoms species found in liver sample were sp. Navicula, Sp. Synedra, etc.

Reason being the diatoms which are found in natural water resources, and they are susceptible to climate and biowaste/industrial waste dumped in the natural water resources.

In this study, the majority of samples had liquefied brain matter (56%), in 14% of the samples No diatoms were present while mixed species were found in 12% of cases. This study is not in accordance with BandlaSeshagiri et al (1993)13 reported that smaller diatoms like sp. Synedra, sp. Pinnularia, sp. Navicula were found in brain samples of the dead body.

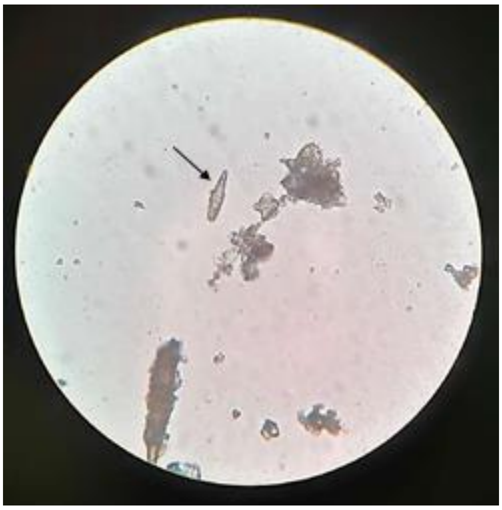
Practically in putrefied body diatom test on brain is difficult to perform. Therefore, except brain, other organs such as liver, bone marrow, etc should be preferred.

Inthiscomparisonstudybetweendiatomsspeciesidentifiedin liver with that of water sample. Liver sample contained a total of 43 diatom specimens (86%) of the total sample andWater sample contained 50 diatoms specimens(100%).Most commonspecies inboth the liverand watersamples was mixed species, comprising (15 cases) 30% and (21 cases) 42% of the respective samples. This study is in accordance with Levkov et al, Skopje, Macedonia (2017)24 reported that in 7 out of 10 cases liver sample contained diatom species while 10 out of 10 cases of water contained diatom species, the species found in water sample collected from alleged site of incident were comparable with that of liver with the dominantdiatoms being pennate diatoms. Kaushik N et al (2017)28 reported that 12 out 17 cases tested positive for diatom test and the diatom species identified in liver samples ofthe dead body recovered were comparable with that water collected from the alleged site of incident.

The diatom species found in liver and water are same in majority of cases as this gives a strong evidence of death has occurred after submersion in the water where the bodies are recovered. Person was live at the time of submersion in that water.

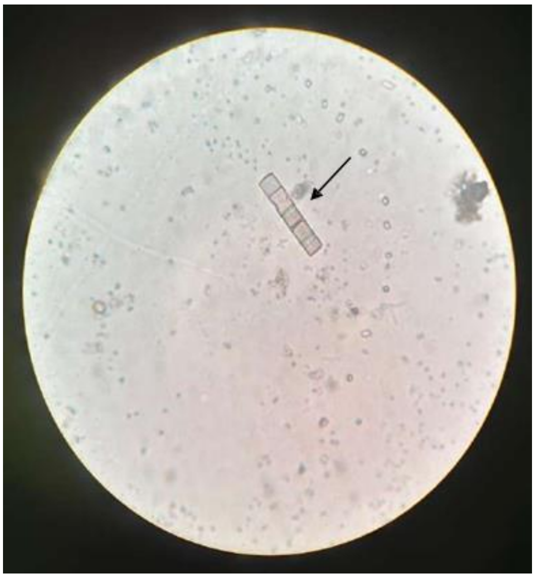
Inthiscomparisonstudybetweendiatomsspeciesidentifiedin liverwiththatofwatersample.Brainsamplecontainedatotalof15diatom specimens (30%) of the total sample and water sample contained 50 diatoms specimens(100%).Mostcommonspeciesinboththebrainandwater samples was mixed species, comprising (6 cases) 12% and (21 cases) 42%oftherespectivesamples.ThisstudyisnotinaccordancewithBandlaSeshagiri et al (1993)24 reported that smaller diatoms like sp. Synedra, sp.Pinnularia, sp. Navicula were found in brain samples of the dead body which were common with sample of water collected from the alleged site of incident. As the brain is far way organ and also it putrefies early. So, practically diatom test on brain is a difficult task to perform.

PHOTOGRAPHS

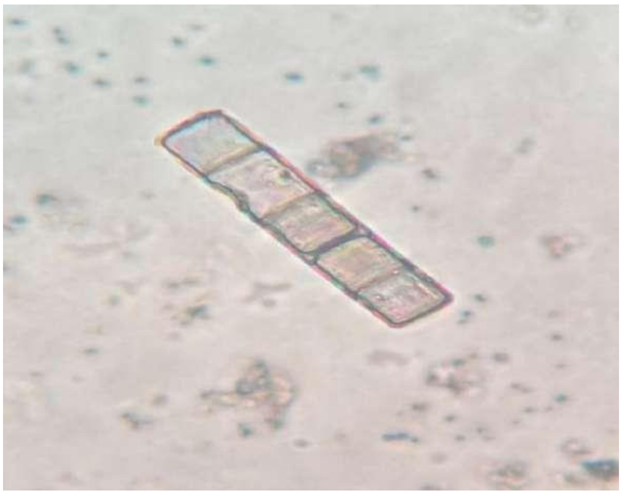


Photograph1 AShowingspeciesAchnanthidiumtakenat 40x magnification oil immersion

Photograph 1 B. Showinga close-up view of the above image of species Achnanthidium



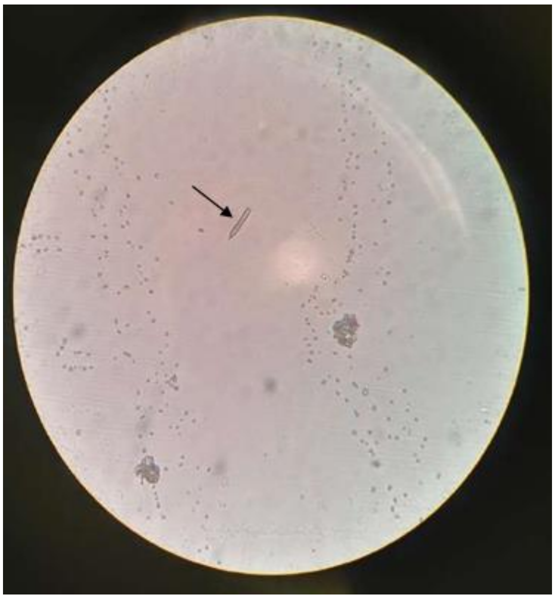
Photograph2A.ShowingspeciesAulacosieratakenat40x magnification oil immersion



Photograph 2 B. Showinga close-up view of the above image of species Aulacosiera

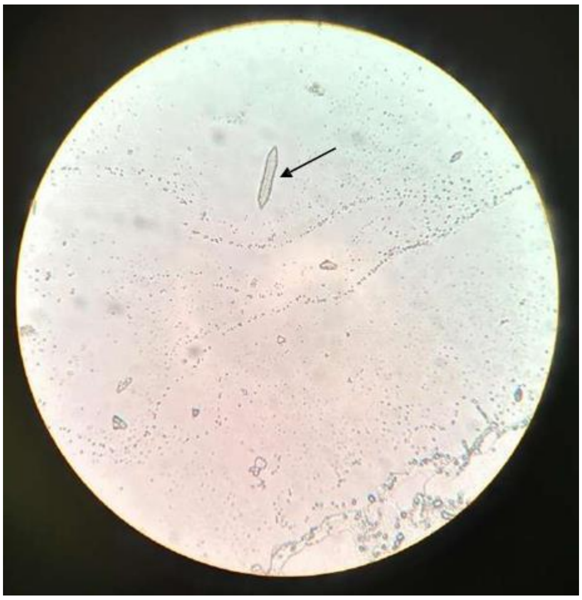
Photograph 3 A.Showing speciesNaviculataken at 40xmagnification oil immersion

Photograph 3 B. Showinga close-up view of the above image of species Navicula



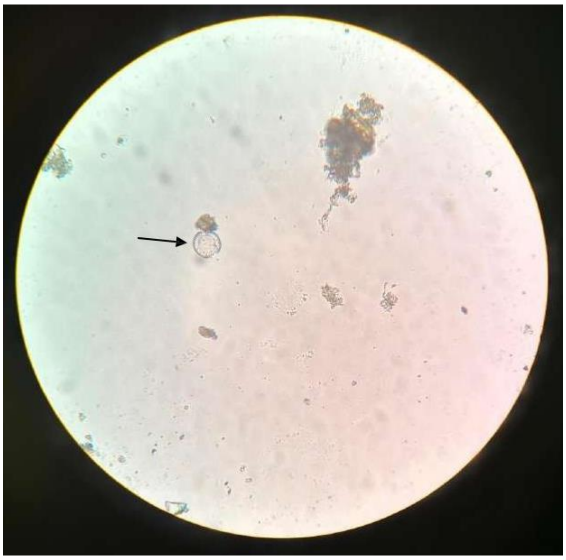
Photograph 4A.Showing species Pinnularia taken at 40x magnification oil immersion

Photograph 4 B. Showinga close-up view of the above image of species Pinnularia



Photograph5A ShowingspeciesProschkiniataken at40x magnification oil immersion

Photograph 5 B. Showinga close-up view of the above image of species Proschkinia

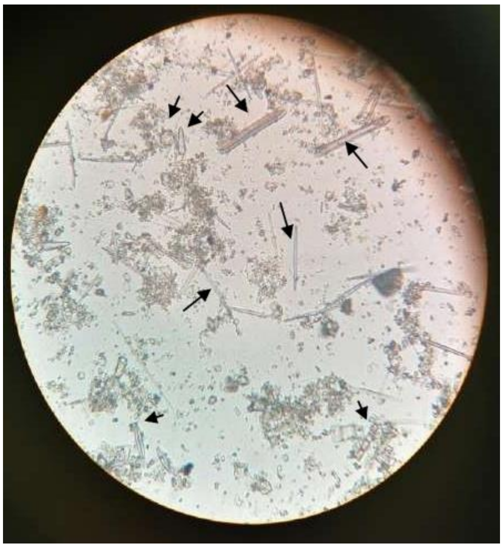


Photograph6AShowingspeciesStephanocyclustakenat40x magnification oil immersion

Photograph 6 B. Showinga close-up view of the above image of species Stephanocyclus

Photograph7A.ShowingspeciesSynedratakenat40xmagnificationoil immersion

Photograph 7 B. Showinga close-up view of the above image of species Synedra



Photograph8.ShowingMixedSpeciestakenat40xmagnificationoil immersion

Photograph9.ShowingMixedSpeciestakenat40xmagnificationoil immersion

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