

## ROLE OF DIATOMS IN DETERMINATION OF TIME SINCE DEATH IN FORENSIC SCIENCE

### Forensic Biology

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### ABSTRACT

The most common application of diatoms in forensic science is the determination of drowning death. A body discovered from the water does not always mean that the death was caused by drowning. If the individual is still alive when they reach the water, diatoms will enter their lungs and cause them to drown. Circulation then transports the diatoms to distant regions of the body such as the brain, kidneys, lungs, and bone marrow. If the individual is dead when they enter the water, there is no circulation, and the transport of diatom cells to various organs is hampered by the absence of circulation, and diatoms cannot enter the body. It is possible to determine whether the drowning occurred ante-mortem or post-mortem. By detecting diatoms in tissue samples, the diatom test has emerged as one of the most essential tests in forensic science. Drowned bodies and water samples from potential drownings are typically sent to Forensic Science Laboratories for diatom detection. The acid digestion test has been determined to be one of the most effective ways for assessing cases of forensic drowning.

### KEYWORDS

Diatoms, Drowning, Acid digestion test, Forensic Science Laboratory, Organs.

### INTRODUCTION

Diatoms are truly a boon to mankind and nature. These naturally found creatures in our environment come under the biological classification of Algae. These neither come under the plant or the animal category, probably the most unique creature on planet earth. They share the prime biological features of both the plants as well as the animals.

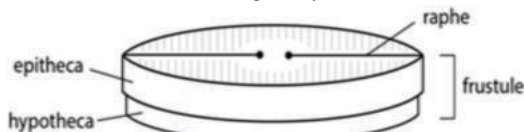
Scientific name of diatom is Bacillariophyceae. Bacillariophyta is the taxonomic phylum that includes diatoms. A diatom is a unicellular eukaryotic alga with a siliceous coating and symmetrical body. Diatoms are mostly aquatic, occurring in fresh, brackish, and saltwater. Some can be found in damp environments, such as dirt, but it can also be found in places such as soil and basically any place in our surroundings where there is a good amount of moisture for them to reside, them, being autotrophic in nature. Best part of these Diatoms is that, they prepare their own food by the process of photosynthesis.

They can live alone or in groups. They develop numerous forms in colonies, including as ribbons, zigzags, stars, and fans. Talking about its structure, nearly all diatoms are microscopic whereas the biggest ones are almost in length of a human hair. They are about 10- 100 microns in diameter. They can be broadly classified into two different orders which are Biddulphiales and the Bacillariales. The former tends to appear in a radially symmetric structure whereas the latter are bilaterally symmetrical in structure.

### Here is a brief description about the life-cycle of a Diatom:

Reproduction of these Diatoms is generally through cell division where the overlapping shells made of silica, split apart into two equal halves and new diatoms are formed from the bottom half of the shell. The life cycle of a Diatom includes both sexual and asexual phases. During sexual reproduction, two parent's cells align and one produces two active gametes while the second produces two passive gametes. The active gametes migrate and fuse towards the passive gametes which results in a structure called Auxospore.

Here are some features pertaining to what diatoms do. They tell about the environmental factors of an aquatic system.



**Figure 1 :** Structure of frustules

**Source:** <https://www.forensicscienceindia.com/post/significance-of-diatoms-in-ascertaining-the-cause-of-death>

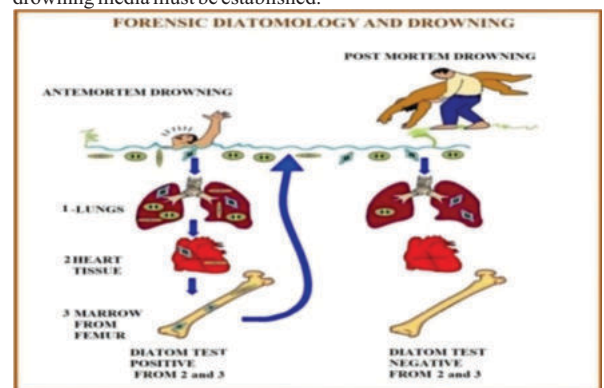
The application of scientific methods, knowledge, and principles to the resolution of legal matters, whether criminal or civil, is referred to as forensic science. A drowning death is described as one caused by submersion in a liquid, with the mechanism in acute drowning being hypoxemia and permanent cerebral anoxia. Although there are certain

common indicators of drowning, it is difficult to identify a drowning death when postmortem signs are impossible to locate in the case of a deceased corpse.

There is a possibility of antemortem or postmortem drowning in drowning incidents. In these medicolegal aspects, the diatom test is particularly essential in determining the cause of death. For the effective diagnosis of drowning fatalities in Forensic laboratories, a link between the diatoms recovered from organ samples and the water samples acquired from the suspected sites of drowning must be established.

Drowning death diagnosis is one of the most challenging tasks in forensic pathology, and several tests have been developed to determine the cause of drowning deaths. By identifying diatoms in tissue samples, the diatom test has emerged as one of the most essential tests in forensic science. Diatom analysis may also be used in forensic science to identify persons, clothes, or possessions from crime scenes. The concentration of diatoms in the lungs and the formation of a river tracking system in the study district are the two parameters for the diatoms test. Ongoing monitoring of fresh water locations, as well as complete species-level inventories of diatom flora at these sites, might be beneficial in the medicolegal examination of drowning deaths.

According to the study of drowning victims where diatoms are present in the medium, the breathing in of water by the drowning victims causes the penetration of diatoms into the alveolar system and blood stream, which then leads to the penetration of diatoms into other organs and parts of the body, such as bone marrow, the brain, kidneys, and lungs. Hard bones (sternum and femur) and tissues (lungs and liver, for example) from drowned victims are often transported to Forensic Science Labs for diatom detection. In order to successfully determine the drowning site, a connection between the diatoms recovered from these tissue samples and the samples acquired from suspected drowning media must be established.



**Figure 2 :** Diatom test for drowning using femoral bone marrow as the source to extract diatoms

**Source:** <https://vandanavinayak.com/wp-content/gallery/research-projects/image3.jpeg>

### Examination Of Diatoms

After acid digestion of the tissue, their existence may be proven in tissues such as the liver, brain, and bone marrow. The use of diatoms as a drowning diagnostic test is based on the premise that diatoms will not penetrate the systemic circulation and be deposited in organs such as bone marrow unless the circulation is still operating, suggesting that the deceased was alive in the water. Diatoms must be cleaned before they can be inspected. It entails the removal of cell contents, colors, sand, dirt, or other anything that might interfere with microscope inspection.

The forensic pathologists around the world have accepted that the detection of diatoms from the internal organs of the victim's body is considered as a conclusive positive proof for ante-mortem drowning cases.

Drowning cases may be accidental. It can be suicidal, can be due to cardiac arrest. Thus it becomes important to know whether the person was alive or dead before entering the water body.

The important aspect we need to understand here is that Diatoms do not occur naturally in our body. So, if there were presence of diatoms in internal organs, then it gives us a clue that the person was alive before entering the water which tells us that the cause of death might be due to drowning. Although the opposite might not be true.

### Extraction Of Diatoms From Water Samples Using Acid Digestion Method

About 50 ml of water sample was transferred into a sterilized 100 ml glass beaker and 10-12 ml of concentrated nitric acid (HNO<sub>3</sub>) was added to the bottles were shaken thoroughly before analyzing. The sample were analyzed with the help of 'Acid Digestion Method'. These samples were kept as such for two hours. Then the samples were centrifuged at 5000 rpm for 10 minutes in properly labeled plastic centrifuge tubes. The supernatant was pipette off leaving behind only a pellet containing diatom frustules at the bottom of tubes. Pellet material was suspended in distilled water and centrifuged twice to remove all traces of acid.

### Preparation Of Slides

After the final centrifugation except for 1 ml, the whole supernatant was discarded by using a sterilized dropper. The pellet along with 1 ml of supernatant was stirred well and entire volume of the pellet suspension (1 ml) was aspirated using a Pasteur pipette and droplets of the aspirate was poured microscopic slides for each sample. Permanent slides were prepared in order to avoid the skipping of any diatom species. This material was completely dried and mounted with the help of DPX. Diatoms were examined with an optical compound microscope fitted with light source at magnification of 1500X oil immersion. Slides were fully scanned with the help of microscope and representative images of the diatom were captured using a computerized photo- capturing device/camera fitted on microscope objected lens.

### Sulfuric Acid Method

1. This has the advantage of not causing violent foaming. Check that all calcareous compounds have been removed first or the sample will become totally useless because Gypsum crystals will be formed.
2. When the sample has settled completely, discard the supernatant.
3. Add concentrated sulfuric acid until the volume is twice that of the original sample.
4. Add potassium dichromate. In contrast to the H<sub>2</sub>O<sub>2</sub> method, no special care is necessary as no violent reaction occurs. Just ensure to add enough dichromate to make for a saturated solution.
5. Let it stand for 24 hours or more, or speed up the reaction in a water-bath around 60 degrees. Even so, it may take several hours before the sample is clean. The sediment should look grayish and no plant fragments etc. should remain.
6. Let it settle completely, discard supernatant and rinse several times as described above. The sulfuric acid method seems to remove resistant "dirt" somewhat better than the H<sub>2</sub>O<sub>2</sub> method, mainly because the oxidation reaction is not as abrupt as with peroxide. Since diatoms resist putrefaction, the diatom test is particularly valuable, where decomposition is advanced. Diatom

test is negative in dead bodies thrown in water and in dry drowning. In diatom examination invariably the control water samples must be used for comparison purpose. Standard diatom samples can be preserved on slides and can be used as standards for comparison purpose.

### Electron Microscopy

In order to examine the morphology of diatoms, both transmission and scanning electron microscopes are able to provide a much more detailed image than light microscopes. These microscopes were necessary for taxonomical purposes, with the distinctions between species being so minute at times. Electron or dark phase microscopy is currently the main methods used for analysis. These allow for more detailed imaging than simple light microscopy.

### Transmission Electron Microscopy

This type of microscopy is best able to see the inner, delicate details of the diatom frustule (even if the frustule is not heavily silicified).

### Scanning Electron Microscopy

SEM is best suited for visualizing the entire diatom frustule. It is a tool that can aid in viewing the gross morphology of a diatom (both internal and external parts).

### Diatoms Tell Us About The Health Of Aquatic System

Diatoms are particular about the quality of water in which they live. They have a different values pH in each of the aquatic environments where they grow.

The pH of Diatoms in freshwater ranges from 5.0 to 8.0. The pH of Diatoms in pond water ranges from 6.5 to 8.5.

### Case Studies

#### Case 1

The body of a 19 year old boy was recovered from Dal lake three kms from Lalchowk, Srinagar. No signs of injury were found during the autopsy. Nitric acid extract of internal organs (sternum, clavicle, femur and lungs) revealed the presence of three types of diatom species (Navicula lanceolata, Navicula oblonga and Gomphonema gracile). The same three types of diatom species were found in the water sample from which the body was recovered. So the cause of death was assigned due to drowning.

#### Case 2

The body of a 26 year old lady was recovered near the bank of Wular lake, the largest lake in Asia. Signs of head and neck injury were traced during autopsy. Nitric acid extract of sternum and clavicle showed the absence of any diatom species. However, two diatom species (Nitzschia subtilis and Navicula radiosa) were detected in the water sample from where the body was found. The cause of death was attributed to the reasons other than drowning which was later accepted by her husband that he murdered her wife because of having extra-marital affair, thus it was proved that it was not a case of drowning.

### RESULT

To conclude, we have seen how diatoms play a vital role in forensic science in diagnosing the death due to drowning. The test for diatoms proved very important in solving drowning cases and also helps in medico legal cases which were in the advanced stage of decomposition and serve a great help to medico-legal cases during the process of investigation.

### CONCLUSION

The diagnosis of drowning is difficult in human forensic pathology. Our findings indicate that the diatom test is a reliable method for assisting in the identification of drowning in forensic pathology. Histological and anatomopathological findings in drowning patients, on the other hand, are less specific since they can be detected in a wide variety of reasons other than drowning. Diatom density and position were found to differ between drowned and non-drowned cadavers in our study.

The most important use of diatoms in forensic science is the identification of drowning deaths.

The high specificity of the diatom test shows to be very useful in advanced stages of decomposition and in skeletonized remains, when autopsy results do not reveal drowning as the cause of death, therefore

providing as convincing proof in such circumstances. A number of diatom test investigations have revealed that the most common choice for diatom extraction from the body is bone marrow. When bone marrow is collected from a closed organ where diatoms cannot reach from an external source, the potential of contamination is greatly reduced or eliminated. If diatoms are detected in bone marrow, it means that drowning was a role in the individual's death and that they were still breathing when they entered the water. When a dead person is placed into water, diatoms passively trickle into the alveoli; however, because the heart is not beating, the diatoms do not spread to peripheral tissues and organs. As a result, the cause of death by drowning can be determined.

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