*Review Article*

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# Diatoms and their usage in Forensics in different water Bodies: A Review

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**Abstract**

Diatoms are unique and single celled algae belonging to the family Bacillariophyceae. They are the rarest found algae in the water. Diatom plays important role in the prognosis of deathin drowning. Diatom present in tissue indicates the sign of drowning. The utility of diatom for the identification of death cases due to drowning was discussed soon after they were found inlungexudates. For diagnosis of diatom, hard bone and soft tissue of dead bodies found in water are often sent to Forensic Science Laboratories. Dissolution of evidence is not very difficult but the complete removal of these diatoms frustules from the evidence needs great care, protection, more attention, and expertise. During analyze of drowning cases a connection between the diatomextracted from the tissue sample and the sample obtained from the put at ivedrowning medium has to be confirmed for the successful resolution of the drowning site. As aresult a proper procedure for the extraction of diatom from the tissue and water evidence to keep away from even minor contamination. Diatom examination has been suggested to provide supportive evidence of drowning. The same type of diatoms is found in almost similarty pes of water bodies. Significant changes in diatom diversity were found with seasonal changes. The quantitative distribution of diatoms in water sources is strongly assisted by climatic conditions.

**Keywords:** Prognosis; Evidence; Putative; Contamination; Frustules; Drowning.

## INTRODUCTION

Diatoms are unique Photosynthesizing single celled algae that have a silica cell wall. They are mostly found in any wet atmosphere, including freshwater, marine water, soil, and also in the ocean. They are the world's largest biomass producer, contributing 25% of the world's total oxygen ($O_2$) production and being one of the most important contributors to the global carbonfixation. Diatoms are highly effective species evaluated by their versatility, range, productivity, and comparative antiquity.[1] Diatoms have a siliceous cell wall made up of two intricately sculpted halves, which makes them exceptional for a wide variety of applications for acknowledging and analyzing the degrees of species level complexity. The siliceous shell of death diatoms can sink to a depth of half a mile (0.5 miles) below the ocean floor. The taxonomy of diatoms is based on morphological features such as

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frustules or namentation shape and size. Diatoms are excellent because they provide nutrients to fish while increasing the $O_2$ (oxygen) level in the water. When diatoms populations are low, nitrogen (N) is no longer converted to dissolve oxygen ($O_2$) and is released as carbon dioxide ($CO_2$), which leads to the growth of toxic algae.[1]

Based on the structure of the frustules diatoms are divided in to 2 parts: Pennales and Centrales. Pennales also known as Bacillariales have bilateral symmetry and the apertures in each of their valves are slit along the raphes, and the length of their shells is normally parallel to these raphes.[2,3] They cause the cytoplasm to flow along the raphes, causing the cell to constantly move along the solid surfaces, while the centrales also known as Biddulphia lesare radially symmetric and they are made up of epitheca (upper valves) and hypotheca (lower valves). Incentral diatoms, the cytoplasm is located in the inner surface of the shell and allows the bigvacuole in the cell's center to be surrounded by a hollow lining.[2] A substance called "cellsap", is just like sea water but differs in particular ion content. Inside the cytoplasmic layer, there are other organelles present such as the chloroplasts and mitochondria. The nucleus of the centric diatoms is at the center of the valves and its moves toward the center of the cytoplasmic layer before the division is finished. Depending on the axis from which the shell extends and whether the spines are present, the central diatoms can be different shapes and sizes.[2,3]

Since the late 18th century diatoms have been studied, however the first actual advance in the first came in the early 19th century, when diatoms be come more favored among microscopist who took advantage of the new improvement in microscopic resolution. Regarding diatoms biolog is studied both their natural habitat and their culture, so we have great knowledge about their biology and ecology. Given sufficient nutrients and sunlight, a living group of diatoms doubles by a sexual multiple fissi on approximately every day (24 hours). A single diatom has a lifes pan of six days. About 20,000 species of diatoms are believed to exist. As nutrients in water diatoms keep out undesirable forms of algae by decreasing harmful filamentous algae and cyanobacteria a lgae. For all fresh water organisms diatoms are a major food source like amphibians, zooplankton, and crustaceans. Diatoms have the ability to increase the amount of dissolved oxygen in water bodies. It produces too much oxygen than it consumes during its life cycles.

## *Importance of Diatomsin Forensic*

Kapil Verma *et al.* (2016) role of diatoms in the world of forensic science. In India, drowning is one of the leading causes of death each year it can be accidental, suicidal, or homicidal. Drowning is when someone is submerged in liquid/water; they can drown because their breathing becomes impaired. Not every drowning results in death. The diagnosis of drowning for victims that have just been pulled from the water is mostly based on specific drowning indications, such as the presence of fine froth at the mouth or nose, petechial hemorrhages, ribimpressions on the lungs, and a few other histological findings. However, the identification of drowning in decomposed corpses and skeletonized bodies discovered in water is relatively challenging because such drowning indicators were obliterated.[4,5,8]

The best bio-indicators are diatoms. These characteristics make it easier to apply them in the evaluation of the ecological status of waters, as does the fact that they make up a dominant group of species in the phytobenthos. Every aquatic environment contains organisms unique to the local conditions because of the diversity of habitats and varying amounts of chemical compounds in water. This is significant because these species may be useful in the forensic field.[1,5] Diatoms can be used to findout whether an environment is comparable to the location of adrowning by performing quantitative analyses of the diatoms present in the drowning fluids that have accumulated in side the lungs, stomach, bone marrow, kidney, or liver and comparing the results with those from the environment from which the corpse was extracted.[5,7]

Manisha Nandan *et al.* (2016) in forensic identification of drowning death by the use of diatom analysis explained the diatoms in forensic help to get the information about:

Identify the cause of death the fundamental idea behind the diatom test in drowning is based on the assumption that diatom is present in a medium where the drowning occurred and that when water is inhaled, it enters the alveolar spaces of the lungs and penetrates from the alveoli into the blood circulation, where it is then transported to various body organ.[7,9,12] The diatoms discovered in side the drowning victim's body could provide additional native proof that the person died. Either the antemortem or post-mortem nature of drowning can be determined.[9,12]

**Timesince of death:** Using the diatoms testing, researchers can determine an approximate time of death by counting the number of diatoms in the organism.[9,12]

**Place of death**: If the body is dumped and ends up somewhere other than where it was supposed to be, diatom species can be matched and can find the original location of death.[8,9,12]

## *Over view of Collected Data from different Water Bodies*

C.S Singh, Bharti Rinku, *et al.* study on fresh water diatoms from the different habitats of Patna, Bihar, India. All the samples were collected from different water bodies in India (Bihar, Varanasi and Delhi). The below given table is the over view of data collected from different locations namely Kothwan Pondin Bihar, Yamuna River in Delhi, and River Ganga in Varanasi were compared on the basis of common species of diatoms like Melosira, Cymbella, Fragilaria, Brachysira, Synendra, and Cyclotella that were present or absent in these water bodies.[12,13,14]

**Table 1:** Over view of Collected Data from different Water Bodies

| Common Species | Water Bodies | Location | Present/Absent |
|---|---|---|---|
| | (a) Kothwan Pond | Bihar | Present |
| 1. Melosira | (b) Yamuna River | Delhi | Present |
| | (c) River Ganga | Varanasi | Present |
| | (a) Kothwan Pond | Bihar | Present |
| 2. Cymbella | (b) Yamuna River | Delhi | Present |
| | (c) River Ganga | Varanasi | Present |
| | (a) Kothwan Pond | Bihar | Absent |
| 3. Fragilaria | (b) Yamuna River | Delhi | Present |
| | (c) River Ganga | Varanasi | Present |
| | (a) Kothwan Pond | Bihar | Absent |
| 4. Brachysira | (b) Yamuna River | Delhi | Present |
| | (c) River Ganga | Varanasi | Absent |
| | (a) Kothwan Pond | Bihar | Absent |
| 5. Synendra | (b) Yamuna River | Delhi | Present |
| | (c) River Ganga | Varanasi | Present |
| | (a) Kothwan Pond | Bihar | Present |
| 6. Cyclotella | (b) Yamuna River | Delhi | Present |
| | (c) River Ganga | Varanasi | Present |
| | (a) Kothwan Pond | Bihar | Present |
| 7. Actino–phychus | (b) Yamuna River | Delhi | Absent |
| | (c) River Ganga | Varanasi | Absent |

## *Extraction Methods of Water Sample*

1. ***Extraction by Acid Digestion Methods:*** Rajvinder Singh and M.K. Thakar *et al.* (2006) Extraction methods of diatoms. Acid digestion methods are regular and most demanding for diatom extraction from the water sample and post-mortem sample. In this method, very strong chemicals are used such as $H_2SO_4$ (Sulfuric acid) and $HNO_3$ (Nitric acid). Sometimes continuous washing of samples removes the diatoms because of their corrosive nature. These acids are highly corrosive in nature and they are destructive to the bone marrow and lungs. These chemicals are used in water samples for diatom extraction in a modified way.[15,16] For the extraction of diatoms from the reference water sample by usingn it ricacid Rajvinder Singh and M.K. Thakar *et al.* used 50 ml to 60 ml of concentrated nitric acid ($HNO_3$) was added tothe sample in a beaker. The sample was then allowed to stand for three to four hours at room temperature. Then the sample was centrifuged for 15 to 20 minutes at 5000 rpm. The supernatant was discarded and the left over pellets were collected. 30 ml of distilled water was added and centrifuged.

The process was repeated three to four times. The left over pellets were then observed under the microscope[15] while GD Tyagi and TD Dogra *et al.* (1985) diatoms of Delhi. The samples were collected from the rock surface of the ponds and rivers in Delhi. The conc. HCL acid was used to treat the sample and the supernatant was removed. Then conc. $H_2SO_4$ (Sulfuric acid) was added which burned the present organic material and turned it blackish. The solid Sodium Nitrate ($NaNO_3$) was added to the cooled supernatant. The suspension was heated again and again on the hot plate until the solution changed its color to brown and lastly cleared. The resulting suspension of silica diatom cells was washed with distilled water, and any left over material was re-dissolved in acetone. M.S. Pollanen *et al.* the diatom test for drowning in Ontario also used acid digestion methods in the extraction of the diatom.[16,17]

2. ***Extraction by Enzymatic Methods:*** Ludes *et al.* assessed the validity and usefulness of qualitative and quantitative diatom analysis in 1994 using an enzymatic digestion technique for diatom extraction. It's believed that enzymatic digestion is preferable to the acid digestion method. It's an easy, quick, and non-corrosive method to extract diatom. Proteases such as proteinase K are used for enzymatic digestion and compared to the acid digestion method enzymatic methods are less damaging to post-mortem samples and reference water samples.[18] The waters ample was treated with hydrogen peroxide ($H_2O_2$). Then the sample was heated at 80°C for 12 to13 hours and after that, the sample was allowed to stand at room temperature for three to four hours. Then the sample was centrifuged at 2500rpm for 10 to 15 minutes. All the supernatant was discarded and finally, distilled water was centrifuged at 3000rpm too btaina pellet containing diatoms. The sediment was air dried after the removal of the supernatant again. The process was repeated four to five times. The left over pellets were then observed under the microscope.[18]

3. ***Preparation of Diatomslide for Amicroscope:*** N. Foged *et al.* used a small amount of left over material from the extraction and they made at least 10 to 15 slides. On the slide, the left over material was allowed to dry at room temperature, and let the diatoms settle down. To make it permanent Takatori, and Terazawa *et al* used DPX mountant (DPX is the combination of three chemicals *i.e.* distyrene, aplasticizer, and xylene) one achslide. All the slides were covered with acleanc over slip.[18,19]

## DISCUSSION

Aquatic are as contain awide variety and abundance of diatoms. They have forensic importance in drowning situations because of their singularity. Diatoms in the body may be detected through laboratory examinations. Diatoms are tiny algae that can be found in both fresh and salt water. Their skeletons are made up of silica, which resists degradation and can occasionally be found in severely degraded remains. When the person is already dead when they enter the water there will be no circulation, which will prevent the diatoms from entering the body and preventing the movements of diatoms cells to various organs. When a body is pulled from the water, it is commonly suspected that it was a victim of either an ante mortem or post-mortem drowning or drowning that occurs before or after death. The discovery of diatoms in the body tissue serves as highly valuable in these medicolegal cases. The successfully identify the drowning site in forensic laboratories, a correlation between the diatoms extracted from bone marrow and lung samples, and samples acquired from the drowning medium must be established in drowning related mortality cases. A predictive diatoms database can be created by carefully sampling areas where submerged remains are regularly found. Comparing such databases to recovered tissues is appropriate.

## CONCLUSION

Diatoms can be analyzed qualitatively and quantitatively by looking for them in samples and counting the number of species, in addition, post-mortem reports and police investigations should be taken into consideration when interpreting the finding of such studies. Even though occasionally the diatoms may have recovered from the internal organ of non-drowning bodies, the diatom test is still important. The location/site of the drowning can be identified because diatoms differ based on morphological and taxonomic traits in a specific habitat. To identify the place of drowning deaths,

water from the potential drowning area should be analyzed and compared with the organ sample.

In the upcoming future, the identification of diatoms may make use of advanced technologies like Nuclear Magnetic Resonance (NMR), Fluorimetry, Molecular Biological Method, and Automatic Diatom Identification and Classification (ADIAC).

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