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Review Article

# Significance of Diatoms in Diagnosis of Drowning Deaths: A Review

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## Abstract

Diatom test is an important tool in diagnosis death in drowning cases. As the typical features of ante-mortem drowning disappeared very rapidly with commencement of putrefaction and hence diatom test plays an important role for diagnosis and confirmation of drowning deaths. Diatoms have number of characteristic like their widespread presence in water, high diversity in species, habitat specificity and have a good potential for preservation and thus this group can be used in forensic geosciences for criminal investigations. Extraction and identification of diatoms present in the tissue samples can be used as supportive evidence in drowning and also for site specific diatoms. Temperature and pH variations also play important role in the distribution of diatoms in a particular location. Diatoms species detection in both water and tissue samples gave a reliability of the diatom test in solving cases pertaining to drowning deaths. Hence the possible means and methods must be practiced on a regular basis by the forensic examiner as well practitioners so that new possibilities must always be explored for extraction and identification of diatoms in near future. This paper reviews the recent year's progress on diatom test and its application in forensic science.

**Keywords:** Diatoms;Drowning; Postmortem; Tissues; Acid digestion; Microscopical examinations

## Introduction

Diatoms (Bacillariophyceae) are unicellular, photosynthetic, autotrophic organisms that have a characteristic structure-frustules, comprising two thecas i.e. silica cell walls. Each frustule is made up of two halves which are known as valves, one of which is slightly smaller than the other and fit together one inside the other. Depending on the shape of the frustule, the diatoms are subdivided into two major orders-Centrales and Pennales. The Centrales are radially symmetric and the Pennales have bilateral symmetry [1]. The diatoms are a highly diverse taxon, comprising more than 200000 species [2]. Diatoms generally range in size from 2-200µm and are composed of a cell wall comprising silica. This siliceous wall can be highly patterned with a variety of pores, ribs, minute spines, marginal ridges and elevations which can be utilized to delineate genera and species [3].

In drowning deaths there is a suspicion of ante-mortem or postmortem drowning. In these medico legal aspects diatom test plays a very important role to ascertain the cause of death. Therefore, in drowning related death a correlation has to be established between the diatoms extracted from the organ samples and the water samples collected from the putative sites of drowning for the successful determination of drowning deaths in Forensic laboratories [4-6].

Diagnosis of drowning deaths in forensic pathology is one of the most difficult tasks and number of tests were developed to confirm the cause of drowning deaths. Diatom test has emerged as one of the most important tests in forensic science by detection of diatoms in tissues samples [7]. Diatom analysis can further used in forensic science by identifying the individuals, clothing or belongings from the sites of investigation [8]. The two factors for the diatoms test are the concentration of diatoms in the lungs and the development of a river monitoring programme in the district of the study [9]. Continuous monitoring of fresh water sites and comprehensive species level inventories of diatom flora at these sites may be useful in the medico legal investigation of drowning deaths [10].

## Brief History of Diatom Test

Diatoms were first detected in lung fluid by Hofmann [11]. Incze [12] successfully detected diatoms in blood and parenchymal organs and demonstrated that diatoms could enter into the circulation through lungs. Diatoms were detected in bone marrow by Tamasaka [13]. Porawski [14] in his study showed that presence

of diatoms in organs and bone marrow is indication of the ante-mortem inhalation of water during drowning. In the 1960's and 1970's, Timperman [15] presented his research for the presence of diatoms in the bone marrow, lung, liver, spleen, kidney and brain tissue and thus developed a method for diatom test for direct screening drowning deaths, whereas the presence of diatom can be verified and analyzed both qualitatively and quantitatively. The method of its extraction was improved by acid digestion of the tissues by Pollanen [16]. In 90% drowning cases, diatom in bone marrow matched with the drowning medium. Pollanen [17] reported that the sensitivity of the diatom test has been one of its chief criticisms to date. "The medico legal utility of the diatom test for drowning could be significantly enhanced by increasing the sensitivity of the test". Cameron [18] reported that diatoms are diverse remains and can be identified with high taxonomic precision. These factors allow diatoms to be used in a range of application in forensic geosciences. Horton [19] in his study showed that diatom test act as a valuable tool in forensic science for the detection of drowning deaths.

## Extraction of Diatoms from Samples

Complete destruction of tissues samples is required for the extraction and detection of diatoms. Current techniques used for the detection of diatoms are acid digestion method. Acid digested organ tissues commonly bone marrow is removed by using spatula and placed in a flask. Approximately 50ml of nitric acid is added into the conical flask. The bone marrow acid suspension is boiled on hot plate in a fuming hood for 48hours. The suspension is then cooled and subjected to centrifugation and washing with double distilled water. The final sediment is placed on microscopic slide and examined on the phase contrast microscope for the detection of diatoms. Hurlimann et al. [20] used nitric acid for extraction of diatoms from bone marrow tissue and Bortolotti et al. [21] used nitric acid for the extraction of diatoms from lungs and sternum bone. Krstic et al. [5] used $H_2SO_4$ for extraction of diatoms from internal organs like kidney, liver, lung and brain. Auer and Mottonen [22] used a mixture of $HNO_3$ and $H_2O_2$ for the digestion of tissue samples of brain, lung, liver and kidney. Scott et al. [23] in their study showed that $H_2O_2$ extraction is the most efficient technique for the collection and analyzing of diatoms from clothing samples in order to aid in forensic investigation. Sidari et al. [24] tested the soluene-350 method to detect diatoms in three cases of sea water drowning.

There are limitations in the acid digestion methods as the structure of the diatoms may get destruct due to acid treatment. But newly developed method Lefort aqua regia (3:1 nitric acid to hydrochloric acid) by Huipin Wang et al. [25] has an improvement over the conventional acid digestion for recovery of diatoms from tissue samples. Conventional acid digestion methods of tissues for analyzing diatoms are time consuming, laborious and potentially dangerous. Enzymatic digestive method develops by Kakizaki and Yukawa [26] for solubilizing lung tissue by using Qiagen proteinase K, Qiagen Buffer ATL and 5N HCL can accelerate and simplify diatom extraction from suspected drowning cases. Molecular biology technique can be used for the detection of 16S rRNA subunits of ribosomal RNA for the detection of planktonic DNA from human tissues in drowned victims [27,28]. Shiwei et al. [29] advocated a new and rapid technique for drowning diagnosis of putrefactive corpse by using electric impedance spectroscopy. Fluorimetry can be used to locate and isolate diatoms in a sample of bone marrow or other tissue by luminescent properties. It can differentiate diatoms found at suspected site of drowning from other diatoms found in nature, by incorporating specific fluorescent tags [30].

With the development of more streamlined extraction methods with shorter processing times and higher sensitivity and specificity for successfully recovery of diatoms from various tissues and fluids and their comparison with drowning medium has been useful for the diagnosis of drowning deaths. Water from the drowning medium should also be examined for the presence of diatoms. It is important to take 1-2 liters of water sample from the surface of putative site and then add few drops of formalin. The solution is then left overnight. Then decant the solution and preserve the concentrate for microscopic examinations [20].

## Internal Organs used for Diatom Test

When a person gets drowned in water containing diatoms then due to aspiration of water they get enter into the lungs. Due to forceful inspiration and expiration microscopic tears got developed in alveolar wall, they get entered into the blood stream and get lodged into the internal organs of drowned victims. Several studies conducted throughout the world showed that different internal organs can be used for the detection of diatoms. Aghayev et al. [31] showed that left ventricular blood can be used for diatom test while Pachar and Cameron [32] showed that liver, kidney and brain could be used for the detection of diatoms. Matsumoto and Fukui [33] showed that lungs could be used for the detection of diatoms. In a study made by Nadia Fucci [34] Lung, liver, kidney and brain were used for the detection of diatoms in 10 cases of drowning deaths. Bone marrow is considered to be the best as it proves the hypothesis of antemortem drowning as well as it is least affected by contamination during post-mortem submersion [16,35]. Anand and Unmesh [36] utilized bone marrow, nasal sinus aspirate and lung bits for the detection of diatoms from 50 dead bodies in drowning cases and Pathak and Mangal [37] used sternum bone for the detection of diatoms in 86 cases related to drowning deaths.

## Qualitative and quantitative aspects of diatom test

Qualitative and quantitative analysis of diatoms in organ sample and water gives strong evidence that death of drowned victim had

occurred due to aspiration of water. Various Studies conducted throughout the world showed that validity of diatom test is based on the shape and number of valves recovered from tissue samples. Hurlimann et al. [20] suggested that 20-40 diatoms per 5 g for bone marrow are required for the diatom test. While Farrugia and Ludes [38] suggested that identification of more than 5 complete diatom from 100µl of sediment from the tissue sample of brain, kidney, liver and bone marrow is required for the positivity of diatom test. Krstic et al. [5] observed 37 diatoms valves in heart tissue, Giri et al. [39] observed 20 diatoms valves in the liver tissue and Auer and Mottonen [22] showed that 20 diatoms per microscopic slide from lung tissue is a sufficient concentration to exclude false positive results due to contamination. Various studies conducted throughout the world showed that by comparing the diatom species detected in organ sample with the drowning medium we can confirm the drowning site [40-43]. Systematic sampling of locations where submerged remains is frequently occurred allows for the creation of a predictive diatom database and such a database is suitable for comparison of diatoms from recovered tissues [44]. There are several factors which are responsible for the qualitative and quantitative distribution of diatoms in the body. According to Hurlimann et al. [20] diatoms density decreases by a factor between 10 and 100 when passing from drowning medium to lungs or to stomach. And their density further decreases from the lungs to blood, kidneys, liver and bone marrow to a factor of 100-1000.

The abundance of species composition varies in various sites of drowning according to their preferred conditions such as salinity, temperature, pH and impact of inter-species competition. Thus, the individual species or the species composition in forensic sample can provide important information about the habitat or location at a particular time of year [45]. Williams and Kociolek [46], Vanormelingen et al. [47] in their study showed that some species of diatoms have a restricted distribution as they are found only in particular area and thus are considered to be endemic. Site specific diatoms can be used as marker for site identification in cases of suspected drowning. The studies made by Krstic [5], Yadav et al. [48], Anu and Resmi [49] reported site specific diatoms in their studies. Comparison in number, nature and distribution of diatoms observed in the tissues samples with the submersion medium will indicate the death due to drowning.

## Importance of Diatom Test and its Limitations in Forensic Science

With the help of diatom test we can ascertain that whether the cause of death is drowning or not. Sometimes criminals dumped the bodies into the water after committing the crime to simulate the cause of death as drowning [50]. The macroscopical findings of autopsy in drowning deaths are plume of froth on the mouth and nostrils, lung emphysema, odema aquosum, Paltaufs spots, froth in trachea, elevated lung weights and pleural effusions [51]. Diatoms can resist putrefaction, so diatom test is more valuable in the cases where decomposition is advanced and post mortem symptoms of drowning had got diminished. Studies conducted on drowning deaths showed that in skeletonized bodies and the bodies with advanced stage of decomposition, only diatom test can tell antemortem drowning by detection of diatoms in organ sample and its comparison with control water sample [37,52-54]. From the historical perspective the study of drowning deaths required a sensitive, specific and easily applicable test. Diatoms test has emerged as the most important test used in forensic laboratories for the detection of drowning deaths.

The main criticism of the validity of diatom test is the potential of ante-mortem and post-mortem penetration of diatoms and detection of diatoms in non-drowned bodies. Presence of diatoms in high abundance is required in putative site of drowning for the positive findings. Hurlimann et al. [20] in their study showed that density of diatoms decreases many folds when the diatoms penetrate the dependent parts from the drowning medium. Sometimes rapid death in water body due to victim's heart conditions or weakness of pulmonary and circulatory system causes decrease in the length of time taken for drowning and thus reduces the quantity of water inhaled [16,55]. Various studies conducted throughout the world showed that only small diatoms or valve fragments can penetrate into the tissues of the drowning victims. Lunette et al. [56] in their study showed that maximum length of diatom that can penetrate the alveolo-capillary barrier is around 110µm and Pachar and Cameron [32] showed that the diatoms detected in internal organs were of size less than 30µm. All reagents and glass containers must have checked for diatoms presence before use and contamination of exogenous diatoms must be avoided by using diatom free water, protecting the organs from the clothes and skin during autopsy of the victims [38]. In a study made by Anand and Unmesh [36] the lung samples of 80% non-drowning cases showed diatoms and 1-2 frustules in the slides prepared from bone marrow. Acid digestion method used for detecting diatoms is not safe. Strong acids when heated eliminate harmful gases; such as nitrogen oxide and Sulphur dioxide which can cause health hazards. Detection of planktons other than diatoms by the acid digestion method is not possible [6]. From these studies we concluded that rapid death in drowning medium, low abundance of diatoms in drowning medium, inefficient methods for extraction and detection and inappropriate tissue sample can cause negative results or lesser number of diatoms in tissue samples.

## Conclusion and Summary

Qualitative and quantitative analysis of diatoms can be done by detection of diatoms in the samples and by counting the number of species. Results pertaining to such studies should also interpreted

in context to postmortem reports and the police investigations. The diatom test is significant even occasionally the diatoms may also have recovered from the internal organs of non-drowning bodies. Since diatoms vary on the basis of morphological and taxonomic characteristics on a particular habitat, so it is possible to find the location/site of the drowning. Water from the putative site of drowning should be examined and compared with the organ sample to establish the site of drowning in drowning deaths. Advanced technologies such as Nuclear Magnetic Resonance (NMR), Fluorimetry, Molecular biological techniques, Automatic Diatom Identification and Classification (ADIAC) can be used for the detection of diatoms in near future.

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