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Role of Diatoms in the World of Forensic Science

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Abstract

This article reviews the forensic aspects of Diatoms analysis and acid digestion method for diatoms extraction. A body recovered from the water does not necessarily imply that death was due to drowning. If the person is still alive when entering the water, diatoms will enter the lungs if the person inhales water and drowns. The diatoms are then carried to distant parts of the body such as the brain, kidneys, lungs and bone marrow by circulation. If the person is dead when entering the water, then there is no circulation and the transport of diatom cells to various organs is prevented because of a lack of circulation and diatoms cannot enter the body. When a body is recovered from water, there is usually a suspicion whether it was a case of ante-mortem or post-mortem drowning i.e. whether the body was drowned before or after death. In these medico legal cases, presence of diatoms in the body tissues is very useful evidence. In drowning related death cases, a correlation between the diatoms extracted from bone marrow and liver/lungs) samples and the samples obtained from drowning medium have to be established for the successful determination of drowning site in Forensic laboratories. Diatom analysis should be considered positive when number of diatoms is above a minimal established limit; 20 diatoms/ 100 µl of pellet obtained from 10 gm of lung samples and 50 diatoms from other organs and further matching of diatoms from bone marrow and drowning site can strengthen this supportive evidence and a positive conclusion can be drawn whether person was living or not when drowned. Detection of diatoms in the bone marrow is a proof that the individual was alive when entered the water.

Keywords: Diatom; Acid digestion methods; Organs; Lack of circulation; Drowning; Forensic laboratory

Introduction

Diatoms are photosynthesizing algae; they have a siliceous skeleton frustule and are found in almost every aquatic environment including fresh and marine waters, soils, in fact almost anywhere moist. They may be simple or branched, filamentous, and even enveloped in a gelatinous envelope or tube. All diatoms are enclosed by a frustule that is made up of two valves fitted together by a connective zone called a girdle. They are non-motile, or capable of only limited movement along a substrate by secretion of mucilaginous material along a slit-like groove or channel called a raphe (Table 1).

Diatoms are formally classified as belonging to the Division Chrysophyta; Class Bacillariophyceae Diatoms are divided into two Orders. The Centrales now called the Biddulphiales which have valve striae arranged basically in relation to a point, an annulus or a central areola and tend to appear radially symmetrical. The Pennales (Bacillariales), which have valve striae arranged in relation to a line and tend to appear bilaterally symmetrical; their silica-based skeletons do not readily decay and they can sometimes be detected even in heavily decomposed bodies. Diatoms do not occur naturally in the body. If laboratory tests show diatoms in the body that are of the same species found in the water where the body was recovered, then it may be good evidence of drowning as the cause of death.

Based on the study of drowning victims, where the diatoms are present in the medium, the penetration of diatoms into the alveolar system and blood stream has been caused by the breathing in of water by the drowning victims and then leads to the penetration of diatoms into other organs and parts of the body, such as bone marrow, the brain, kidneys and lungs [1,2]. Hard bones (sternum and femur) and soft tissues (lungs and liver etc) of drowned bodies are usually sent to the Forensic Science Laboratories for the detection of diatom. While solving drowning cases, a correlation between the diatoms extracted from these tissue samples and the samples obtained from putative drowning medium has to be established for the successful determination of drowning site. The occurrence of diatoms in the bone marrow is a proof that the individual was alive when entered the water. This means that the cause of death was due to the drowning.

More suggestions were given by [3] that diatom test would be of much importance in the diagnosis of drowning cases, origin of diatoms found in bone marrow is known i.e. matching of diatoms from both putative water medium and tissue of drowned body is must required for the success of this test. Some previous important reviews on diatoms and drowning have been published by Holden and Crosfill [4], Rushton [5], Timperman [6], Peabody [7]. Among the various works [8-15] have made good efforts to make this study live and hopeful one**.**

Examination of Diatoms

In 1942 Incze demonstrated that, during drowning, diatoms could enter the systemic circulation via the lungs. Their presence can be demonstrated in such tissues as liver, brain and bone marrow following acid digestion of the tissue. The use of diatoms as a diagnostic test for drowning is based upon the hypothesis that diatoms will not enter the systemic circulation and be deposited in such organs as the bone marrow unless the circulation is still functioning thus implying that the decedent was alive in the water. Before diatoms can be examined, they have to be cleaned. This involves the removal of cell contents, pigments, sand, mud or other material likely to interfere with microscope examination.

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Cell size	Diatoms are commonly between 20-200 microns in diameter or length, although sometimes they can be up to 2 millimeters long
Cell wall	SiO_2 or glass frustule
Chloroplasts	Many/cell, 3-5 thylakoid membranes/stack
Photo-pigments	Chlorophyll a & c, carotenoids
Reproduction:	Most often simple cell division, sexual reproduction
Ecological roles	Produce deposits (diatomaceous earth), toxic blooms
Common genera	Chaetoceros, Pseudonitzschia, Skeletonema

Table 1: Morphological characteristics of diatoms.

Extraction methods

Acid digestion method: The Acid digestion method for diatoms extraction accepted worldwide. It is easy to perform and gives good results; [2,11,16-44] are examples of workers who favoured this method for dissolving tissues samples.

Nitric acid method:

1. Samples are collected from the suspected drowning victim. Care should be taken as to not contaminate the sample with foreign diatoms during the process.
2. Intact femurs, for example, are removed at autopsy and washed in distilled water. Femurs are longitudinally sectioned using a clean band saw, and the bone marrow about 50g is removed using a clean spatula and placed into a boiling flask.
3. Approximately 50 mL of concentrated nitric acid is added to the flask, and the marrow-acid suspension is boiled on a hot plate for approximately 48 hours-under a fume hood.
4. The suspension is then cooled and centrifuged, in some instances two separate times, with the supernatant discarded and the resulting acid-resistant material dropped onto clean microscope slides and the sediment is examined under the microscope.

A remarkable collection of most delicate and beautiful skeletons of diatoms is seen in cases of death from drowning. In favorable circumstances, even the site of drowning can be determined since the fresh water and the sea water diatoms are different and the sea water plants even vary from place to place along the coast.

Sulphuric acid method:

1. This has the advantage of not causing violent foaming. Check that all calcareous compounds have been removed first; otherwise the sample will become totally useless because gypsum crystals will form.
2. When sample has settled completely, discard supernatant.
3. Add concentrated sulphuric acid until the volume is twice that of the original sample.
4. Add potassium bichromate. In contrast to the H_2O_2 method, no special care is necessary as no violent reaction occurs. Just add enough bichromate to make for a saturated solution.
5. Let stand for 24 hours or more, or speed up the reaction in a water-bath 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 settle completely, discard supernatant and rinse several times as described above.

The sulphurics acid method seems to remove resistant "dirt" somewhat better than the H_2O_2 method, mainly because the oxidation reaction is not as abrupt as with peroxide. But again, the principal point is patience, not the chemistry involved.

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 [45,46].

Transmission Electron Microscopy (TEM): This type of microscopy is best able to see the finer, delicate details of the diatom frustule (even if the frustule is not heavily silicified).

Scanning Electron Microscopy (SEM): 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).

The diatom test is considered positive when unequivocal diatoms are present on the slide. Incidences of positive diatom tests should be analyzed in relation to the season and month of drowning, if possible. As previously discussed, these factors can have an enormous effect on the number of diatoms discovered and the different genera represented. Discordances between diatoms found in the body and those in the putative drowning medium may suggest that either natural currents or human activity has moved the body, thus highlighting the importance of sampling the water source where the body is found [46]. By comparing the genera and species of diatoms found to regional samples, it may then be possible to localize the original site of a drowning in such cases [38].

The utility of the diatom test for drowning is directly linked to the incident of positive test results. With an occurrence of less than one third, the majority of drowning cases do not benefit from this method [45] have suggested three chief reasons for the low instances of positive test results.

The method of collection may not extract all the diatoms present in a sample and may miss diatoms altogether if only one or two individuals are present in the bone marrow--an issue that will be addressed later in a discussion of new methodologies increased test sensitivity.

Victims with heart conditions or other weaknesses of the circulatory or pulmonary systems who hyperventilate and pass out underwater or who experience a laryngeal spasm may die more quickly. This leads to a decreased volume of inhaled water and a lessened window of time where the circulatory system can embolize the diatom-containing medium [3,45,47].

Finally, there is a relatively low volume of blood circulating to and through the bone and bone marrow [45]. This fact makes the investigation of other major dosed organs all the more important. Relying on negative test results based on the bone marrow alone may lead an investigator to miss what is actually a positive indicator of drowning.

Again, however, care must be taken to investigate only dosed organs. The suggestion given here is a balanced approach where care is taken to avoid contamination or insecure sources of diatoms, but where every possible secure diatom source is investigated and tested. Further, the authors contend that the development and testing of new modalities may increase the sensitivity and reliability of the diatom test.

Many workers, Peabody [48] Mueller and Gorgs [49]; Timperman [50]; Fukui et al. [51] etc. made many successful attempts for the isolation and detection of diatoms from the organs of drowned bodies. Water samples from the putative drowning site have also been taken under analysis for the presence of various diatom species more or less associated with drowning. Tyagi [33] and Ludes et al. [47] conducted a water monitoring system and generated a data base of diatom species from various water bodies like pond, lakes and canals for diagnosis of suspected drowning cases. They observe , P. braunii, Mastoglia smithioi, N. graciloides, , N. bacillum, N. radiosa, N. simplex, N. pusilla, Pinnularia mesolepta, P. gibba, Cymbella cistula, N. cryptocephara, Camera lucida , Navicula pupula, N. meniscus and many more species of diatoms in those water bodies. The content of diatoms in 5 samples; lungs, kidney, liver and femur marrow from each of four drowned and non-drowned persons were investigated by Foged [31]. Centric diatoms were frequently found and cosmopolitan Navicula and Synedra ulna was occurring in all cases. Auer [3] made qualitative diatom analysis recovered from the drowned body organs in Finland. In different body parts various types of diatom species i.e. Campylodiscus noricus, C. echenels; Epithelia zebra, Melosira nummuloides and Navicula pregrina; Pinnularia subcapitata; Achnanthes taeniata and Navicula ; Eunotia; E. lunaris , Meridian; Fragilaria crotonensi, Asterionella Formosa; Cymbella cymbiformis; Pinnularia borealis; Pinnularia capsoleta, Tetracyclus lacustris and Cymbella were observed. During continuous river monitoring Ludes et al. [47] analyzed both water and tissue for the presence of diatoms and Navicula, Diatoma, Nitzschia, Stephanodicus, Fragilaria, Gomphonema, Gyrosigma, Melosira, Achnanthes, Amphora, Cocconeis, Cyclotella was commonly found diatoms.

Discussion

The main goal in this field is to differentiate a death by submersion from an immersion of a body. Laboratory tests may reveal the presence of diatoms in the body. Diatoms are microscopic algae found in both seawater and fresh water. Their silica-based skeletons do not readily decay and they can sometimes be detected even in heavily decomposed bodies.

If the person is dead when entering the water, then there is no circulation and the transport of diatom cells to various organs is prevented because of a lack of circulation and diatoms cannot enter the body. When a body is recovered from water, there is usually a suspicion whether it was a case of ante-mortem or post-mortem drowning i.e. whether the body was drowned before or after death. In these medico legal cases, presence of diatoms in the body tissues is very useful evidence. In drowning related death cases, a correlation between the diatoms extracted from bone marrow and liver/lungs) samples and the samples obtained from drowning medium have to be established for the successful determination of drowning site in Forensic laboratories

However, it is important to remember that the absence of diatoms does not immediately rule out drowning; the test does not prove the negative, and a thorough investigation is always required. Again, if specific types of diatoms recovered from the marrow can be matched with those found at the suspected drowning site, the results might implicate a particular locale of submersion and help identify the death scene, which in turn could lead to additional forensic evidence. The presence of diatoms can also corroborate investigative evidence in making accident determinations [37,45].

Diatom analysis can be of further use in forensic science through identifying the provenance of individuals, clothing or materials from sites of investigation [2,7,45,52-54]. Where materials have been submerged or there has been contact with littoral or riparian sediment or vegetation, diatom analysis of sediments or other diatomaceous traces present on clothing or footwear can be used to identify the type of habitat [54]. One or more water samples from the site of drowning should be taken. If there is any doubt about the drowning site, then water sample from the putative site of drowning can be collected and analyzed to determine the similarity of different species of diatoms in the water and the body.

Conclusion

Diatom analysis should be considered positive when number of diatoms is above a minimal established limit; 20 diatoms/ 100 μl of pellet (obtained from 10 gm of lung samples) and 50 diatoms from other organs [47] and further matching of diatoms from bone marrow and drowning site can strengthen this supportive evidence and a positive conclusion can be drawn whether person was living or not when drowned. The diatom test, while extremely specific, is of immense value considering the limited objective tests available for drowning diagnoses. A fresh outlook is necessary to rekindle use of this important application to medico legal investigations. It has been suggested that marrow of the sternum may be as good of a source of diatoms as femoral tissue. Death of a victim found in water should not always be related to drowning.

If the person is still alive when entering the water, diatoms will enter the lungs if the person inhales water and drowns. The diatoms are then carried to distant parts of the body such as the brain, kidneys, and bone marrow by circulation. If the person is dead when entering the water, then there is no circulation and diatoms cannot enter the body. Diatoms do not occur naturally in the body. If laboratory tests show diatoms in the corpse that are of the same species found in the water where the body was recovered, then it may be good evidence of drowning as the cause of death. Bone marrow is described as a sanctuary organ and if diatoms reach this tissue, the diagnostic of drowning could be assessed.

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