REVIEW

FORENSIC DIATOMOLOGY

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Abstract

Diatom frustules are examined routinely during autopsies of deaths due to drowning. Presence of same species of diatoms (in similar concentrations) as that of the putative drowning medium from the internal organs of drowning victims constitute a corroborative or even conclusive evidence to support the diagnosis of death. This paper highlights the relationship of diatoms with that of drowning victims and provides an overview as to the digestion techniques and microscopic examination of siliceous residues of diatoms. Role of diatoms in identification of a site of drowning has been emphasized with suitable case studies from literature.

Introduction

Death by drowning is the result of hampering of respiration by complete or partial submersion and subsequent entry of water into the air passages. Drowning can occur in any medium; a six inches puddle or deep sea.[1] Drowning in shallow waters is seen in alcoholics, epileptics or infants. Since the facts pertaining to the events in a drowning victim are primarily obtained from animal experiments and to lesser extent from observations of eye witnesses, our knowledge is by no means complete.

A diagnosis of death due to drowning is normally based on the presence of foreign particles like twigs / leaves or soil in the lobar bronchioles (deep air passages of lungs) on dissection of overinflated and waterlogged lungs during autopsy examination and further conclusively by exclusion of all other unnatural causes of death. It is difficult to furnish anopinion as to the cause of deathin cases of highly putrefied bodies (recovered from drowning medium and exhumations). The rate of decomposition of drowned bodies will be at a quicker pace due to imbibition of water.[2]It is not possible to identify classical autopsy findings (Paltauf sign, emphysema aquosum or pleural effusion) in a decomposing body. In such situations the forensic pathologist has to rely on somelaboratory investigations. Diatom, a unicellular alga with a siliceous cell wall is found in almost all water bodies. In wet drowning these diatoms along with water enter the lungs and reach various internal organs. Acid digested extracts of various internal organs demonstrates the presence of diatoms similar to those found in the drowning medium (Diatom Test).

The distribution of diatoms widely varies between different water bodies. It depends on the various parameters of the water body such as temperature, salinity, pH, other algal matter, impurities etc.[3]Such parameters can vary between different water bodies. Even within one water body the same parameters can vary from time to time. Thesevariations are the fundamental reasons behind the prevalence of different types of diatoms between water bodies and within a single source of water. Planktonologists (algal specialists) are the experts who study in depth about relationship of such parameters with the seasonal or temporo-spatial variations of diatoms.

Identification of the site of drowning is important in drowning investigations. This can be more efficiently located based on the above mentioned parameters. This is because such parameters (for a particular season) will be specific to a drowning medium. Quite often the site of discovery of a dead body will be far from the actual site of drowning, because water currents, tides etc. transfer the body for some distance. A diatom test must not only demonstrate the mere presence or absence of diatoms in the internal organs of a drowned victim, but must also demonstrate the same type of diatoms in similar concentrations in the drowning medium.

Biology of diatoms

Marine plant life consists largely of minute, free-floating forms collectively known as phytoplankton. The word 'plankton' is derived from Greek word *planktos*, which means a wanderer. This shows that planktonic organisms are drifters rather than powerful swimmers. Their horizontal distribution is mostly governed by currents in water than by the outcome of their own efforts. On land, plants of different species are present everywhere, but in sea and other water bodies majority of the plants are these phytoplankton. They are present in abundance everywhere, particularly in the sunlit water surfaces where the environmental conditions are favourable for them to photosynthesize and multiply. Phytoplankton produces half of the world's oxygen through photosynthesis. Since mostly autotrophic, they form the foundation of all the food webs in sea. Diatoms or *Bacillariophyceae* are conspicuous members of the phytoplankton. The other members include *Dinophyceae, Cyanobacteria, Chlorophyceae, Haptophyceae*etc.[4] Diatoms range in size from one-thousandth of a millimetre (1 micron) to nearly two millimetre. Some are single-celled while others exist in the shape of filaments, ribbons, or stellate colonies. There are more than 200 genera of living diatoms comprising approximately of 1,00,000 species.[5] They are found in oceans, freshwater bodies, soils etc. Their cell walls are hardened to a glassy consistency by siliceous material, intricately sculptured in miraculous patterns of pits and hollows. Some of the diatoms, especially marine forms are extremely beautiful. Therefore diatoms are better known as '*Ornaments of the Sea'* or '*Grasses of the Sea'*.

The cell wall of a diatom consists of two nearly equal, overlapping halves: hence diatom, meaning 'two atoms'. The box so formed may be circular (centric kinds) or oblong (pennatekinds) on the basis of their symmetry. Their cell wall is also called as frustule. The frustule consists of two parts called valves (thecae). The upper half (valve) is called epitheca and the lower half is called hypotheca. The joint between the two thecae is supported by bands of silica (girdle bands) that hold them together. This overlapping allows for some internal room expansion which is essential during reproduction. The epitheca closes on the hypotheca (smaller) like the lid of a pill box or*Petri Dish*.

The centrales are radially symmetrical, either circular or triangular whereas the pennates are bilaterally symmetrical. The linear (pennate) diatoms may be wedge-shaped, boat-shaped or keel-like in appearance. Most of the diatoms are non-motile while some move via flagellation. Many of them, have intracellular lipid vacuoles which help them to reduce the weight of cells and thereby their rate of sinking. Diatoms have a constant shape and size due to the presence of a siliceous wall. These walls are marked by minute pores or depressions that allow it to access to its environment. The frustule encloses a mass of living protoplasm which is demarcated into peripheral and central masses connected by strands.

Drowning and its medico-legal importance

The medico – legal system in our country is active to discover the real truth behind instances of a person being murdered and then disposed in water bodies to simulate drowning. In cases of death due to drowning, the Judiciary attributes as much importance to diatom test as *DNA profiling*. When a dead body is recovered from water, there is usually a suspicion as to whether death was due to drowning or if it was a case of post-mortem submersion. Detection of diatoms from internal organs of victims of wet drowning is considered as a positive proof of antemortem immersion by the forensic pathologists nationwide. The types of diatoms that are found in various body tissues / organs are compared with those present in the putative (suspected) drowning medium. This further helps in the reconstruction of events that had occurred prior to the death of an individual.

Drowning occurs when a person submerged in water attempts to breathe and instead aspirates water resulting in decreased oxygenation, hypoxemia and hypoxic brain damage finally leading to death. It is mostly accidental. In a relatively small number of cases it is suicidal. In some other cases death in the water is due to natural causes such as myocardial infarction (cardiac arrest due to absence of blood supply) or cerebral haemorrhage (bleeding from blood vessels of brain) during bathing or swimming. Disposal of a victim of homicide to simulate drowning is rare but due to such a possibility distinction between features of antemortem immersion (live entry) and postmortem submersion (dumping of a dead body) is of prime importance. Thus in the case of a body recovered from water, the crucial point is whether the person was alive or dead at the time of submersion.

Drowning is the final common pathway of different initiating causes of person's incapacitation in water. To arrive at an opinion as to the cause and manner of death in the case of a corpse recovered from water, collaborative investigations by forensic pathologists and police officers are required. Bodies recovered from water pose the most difficult medico legal problem especially when putrefied. The circumstances that lead to death are difficult to be established. Signs of antemortem immersion depend upon the type of drowning. The autopsy examination is an essential element, but it represents only a part of the investigations. Microscopic observations, diatom test, biochemical analysis, histological studies and the circumstances preceding death; all together are necessary to form a reliable and conclusive opinion. A complete autopsy examination along with toxicological screening is necessary since drowning is a *diagnosis of exclusion*. When a corpse is found in water, all other causes of death have to be excluded since even victims of intoxication (by drugs or alcohol) and those of heart attacks can accidently fall into water. Such persons can be even dumped into water bodies.

Diatom analysis – introduction

Diatom analysis is a biological test used to assess deaths due to drowning. Diatoms are ubiquitous in nature. Fossil diatoms can be found in substances such as board-chalk. But these fossil diatoms are different from the modern species prevalent in the water bodies around us. The fact is that when a person drowns in water containing diatoms, at least some of them will penetrate the alveolar capillaries and walls thereby entering the bloodstream. Penetration of diatoms into alveoli-capillary barrier has been proved using Transmission Electron Microscope and Scanning Electron Microscopy.[6] If a person is alive at this point of time with the heart beating, the diatoms will be transported and lodged in distant organs like kidneys, brain or even bone marrow before he dies. On the other hand in postmortem submersion though the diatoms may reach the lungs by passive percolation, it will not reach the distant organs due to circulatory failure. The presence or absence of diatoms in the internal organs depends on their ability to navigate the alveolar-capillary (lung - blood vessel) interface. The size of the diatoms is also important in order to eventually reach the bone marrow. Hence presence of large number of diatoms of same species as that of the putative drowning medium in the bone marrow of the deceased is used to confirm the cause of death as drowning.

It has been reported by Swann[7] that three minutes was only required during drowning for the original blood volume to be diluted by an equal volume of water. The transportation process of diatoms through respiratory system works since the siliceous frustules of diatoms are resistant to the mucus material of respiratory system. This enables diatoms to embolise from circulatory system into the internal organs. Bone represents a closed system, requiring circulation of blood to bring diatoms into bone marrow unlike other organs of the body where diatoms may be passively invaded (Figure 1).

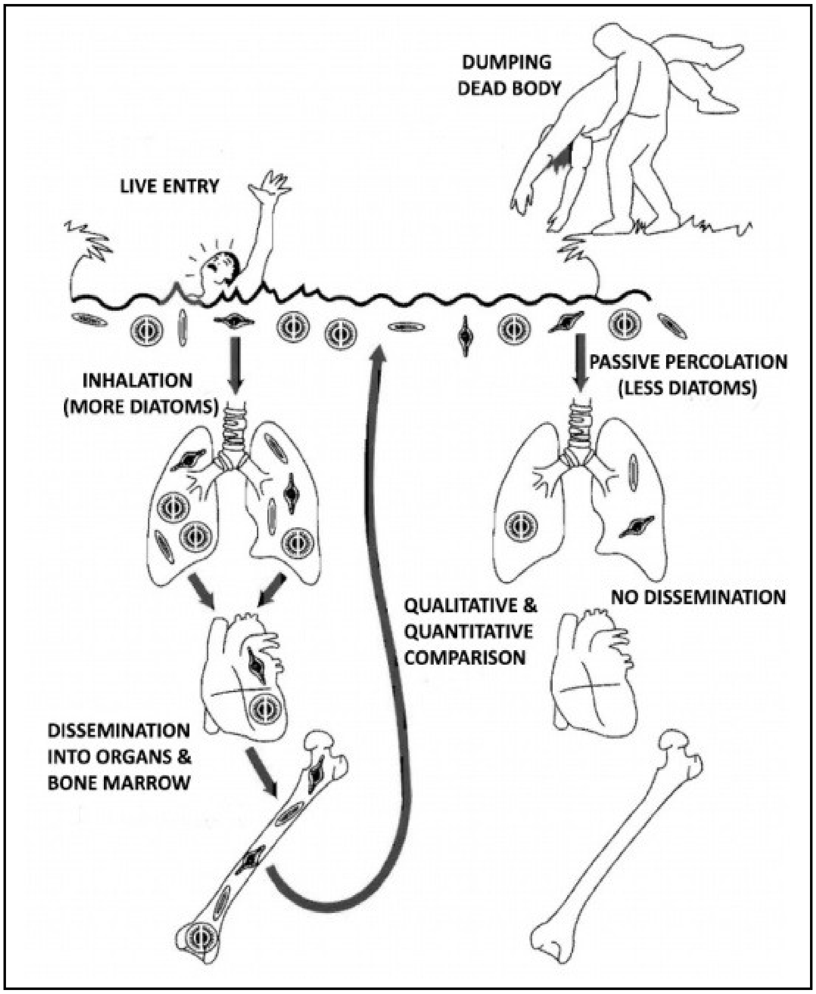

Figure 1. The essential differences in diatom entry and dissemination between live entry (antemortem immersion) and dumping of a dead body (postmortem submersion) into water

For this reason, bone marrow is preferred to other organs for diatom estimation, since the diatoms deposited will be mostly ante mortem in nature. Typically smaller diatoms will be found in the bone marrow ranging to less than 30 microns in size whereas larger diatoms may find their way to the other organs of body.[8] Due to such passive percolation, estimation of diatoms from other organs must be exercised with caution. The purpose of the digestion is to destroy other organic materials present in the water, thereby making the whole process of identification (of diatoms) as well as counting convenient. Due to the resistant siliceous cell walls, diatoms are spared from the fury of acid digestion. Though the organic components of diatoms are also lost along with other organic materials in the water or tissues, they can be easily identified from the siliceous framework (skeleton of diatoms) left over after acid digestion. Proper digestion method should be chosen, since the digestion techniques do disorganise the weaker diatoms to some extent. The classical strong acid digestion method is chemically hazardous for the operator and laboratory equipments. It also decreases the visibility of diatoms during microscopic examination.

Diatom analysis – evolution

Acid digestion method was brought to limelight by Timperman.[9] He dissolved bone marrow taken from sternum in Kjeldahl flask containing 50 ml of nitric acid. After half an hour the solution turned transparent. It was then cooled and centrifuged. The residue was put on a slide and examined under microscope. Peabody[9] also extracted diatoms using concentrated hydrochloric acid and concentrated sulphuric acid instead of using nitric acid alone. Boiling with sulphuric acid produced a black suspension. It was cooled and treated with solid sodium nitrate until colour was turned to brown and finally transparent. Resultant suspension was transferred to slides for reading under the microscope. Pollanenet al.[9] made a slight modification to the whole procedure by removing bone marrow (50gms) from femur bone. He used 50ml (millilitre) of concentrated nitric acid in boiling flask for the digestion process. The acid marrow suspension was simmered for about 48 hours on a hot plate placed under fume hood. Later it was cooled and centrifuged. The residue so obtained was again centrifuged after adding distilled water. The supernatant was discarded and the acid free pellet was spread over a glass slide for analysis.

'Acid Digestion' was the commonly used technique of diatom analysis.[8,9,10] This was because the method was cheap, easy, reliable and effective. But it had some demerits too. Firstly it was not safe due to the use of strong acids. Large amounts of acid were needed to digest few grams of tissues. Moreover the process of digestion was time consuming also. Lastly for some scientists (not Forensic Scientists) detection of plankton other than diatoms was not possible using acid digestion techniques. The use of hydrogen peroxide for oxidisation of the organic materials was criticised, since washing off the peroxide from samples was difficult.[9] Enzymatic digestion, a newer technique was safer and effective than acid digestion technique. It could be used to detect plankton other than diatoms. But it was costlier compared to acid digestion technique. Membrane filter method creates problem due to clogging of the pores with blood clots and other particles. Colloidal silica gradient centrifugation was not successful since during homogenisation many of the diatoms were destroyed. Ultrasonic irradiation and Dry Ash methods were less practicable and it is suspected to badly affect some diatom species. Therefore despite many limitations, acid digestion method is still frequently used for extraction of diatoms from water and tissues.

Diatom analysis – present scenario:

The combination method of nitric acid with hydrogen peroxide was developed by Auer and Mottonen[11] of the Department of Forensic Medicine, University of Helsinki, Finland. The authors have stressed on the qualitative estimation, which involves determination of diatoms up to the species level to understand the general environmental conditions of drowning medium. The basic concept is that diatoms recovered from tissues must be ecologically consistent with the environment of drowning.[8]This is because diatoms fairly reflect the water quality in terms of salinity, pH, water depth, bottom conditions, aquatic vegetation etc. On the other hand, quantitative assessment involves the comparison of ratio of different types of diatoms obtained from the drowned victims with that in the putative drowning medium.

Mei Ming and associates[12] from Department of Forensic Medicine, Wuhan University, China conducted a study with the objective of comparing the merits / demerits of four digestive methods so as to choose the best method for diagnosis of drowning. The four methods were (i) nitric acid plus hydrogen peroxide, (ii) proteinase K (enzymatic digestion), (iii) nitric acid in *disorganisation can* and (iv) soluene-350 (tissue solubiliser). The tests were compared on the basis of four indices. The indices compared were (i) the time demanded for complete digestion, (ii) the digestive capacity of method, (iii) the reclaiming ratio of diatoms and (iv) the degree of digestive destruction caused to diatoms. The time taken by nitric acid in *disorganization can* to complete digestion was shortest. This was followed by nitric acid plus hydrogen peroxide. Proteinase K was in third place and longest time for digestion was taken by Soluene-350. The digestive capacity was strongest for the nitric acid in *disorganization can* whereas nitric acid plus hydrogen peroxide took second place. Proteinase K was third in terms of digestive capacity and Soluene-350 was weakest. When analysing the reclaiming ratio, proteinase K method had the highest reclaiming ratio with nitric acid plus hydrogen peroxide at second place. Both the other methods had very low reclaiming ratio. To compare the destruction caused to diatoms, the structures were examined under Scanning electron microscope (SEM). The best results were given by Proteinase K. Here the other three methods showed destruction to different extents. The scientists had commented that with respect to time consumption; *disorganisation can* digestion technique was not very superior to the combined acid and oxidising method. The sea water diatoms were damaged to great extents by Soluene-350. Hence it could not be used for extraction of diatoms from sea water.

Finally the authors have concluded the best method to be enzymatic digestion using Proteinase K, with nitric acid plus hydrogen peroxide being its substitute.

Diatom analysis – identity of site of downing (Case studies)

The location of the site of drowning based on diatom investigations have been undertaken by various botanists including diatomologists. Auer and Mottonen[11] quote some case reports that favors identification of sites using diatom analysis. One case was that of a 52 year old man found dead in a brook. Police investigation raised suspicion that the body might have been drowned in a nearby small pond, not connected with the brook. Later someone could have carried the corpse to the brook. Analysis from tissues showed diatoms similar to those in the brook. These were the dominant species usually seen in flowing freshwater environment. The diatoms in the pond were totally different. In another case involving diatom analysis of a 48 year old woman found dead in a ditch, the outcome was of the same nature. The authors also have mentioned the relevance of absence of diatoms in some fresh water sources. Four cases were enumerated with respect to this aspect. Three cases (in Finland) had occurred in tap water source (i.e. drowning in a bath or swimming pool) and last one had occurred in a well. All the four cases had macroscopic findings suggestive of drowning but none of them yielded diatoms.

Ludes and his associates[13] had documented seasonal variations of diatom population (using river monitoring programs). They were also able to throw some light upon the relevance of diatom analysis in identification of the site of drowning. They studied two series of cases. The sites were ascertained through police investigations. They found a good correlation of 65% in the first series when the site was known. In these cases, the comparison of the relative abundance of the most frequent taxa (identified in the lung and water samples) was relatively a sensitive and specific test to indicate the site of drowning. They had concluded that the diatoms extracted from lungs may be an indicator of the site of drowning. But the results obtained must be interpreted on the basis of autopsy findings (with histology) and police investigations.

Peter, Wayne and Donald[14] bring an interesting case report in their published work. Peter was a Professor at Department of Botany, Connecticut College, New London. Wayne was a special agent at the FBI Academy, Quantico, Virginia. Donald was a lieutenant at Police Department, Waterford. During the July of 1991, two young boys were brutally attacked by multiple teenaged assailants while fishing at a suburban Connecticut pond. After being accosted at knife point, the victims were bound with duct tape, beaten with a baseball bat and dragged into the pond to be thrown. But one of the victims managed to free himself and rescue his colleague. They both summoned help from local residents. Subsequently police had apprehended the three suspects after an exhaustive investigation. In an effort to link the suspects to crime scene, sediment encrusted sneakers were seized from both assailants and the victims. They were analysed for diatoms and other algae. Reference samples were taken from the crime scene (pond) too. Marked similarities between the algal communities of the pond and those on sneaker residues supported the common origin of both the samples recovered. Besides, the location was proved to be the pond due to presence of same diatoms in similar ratios. Thereby the authors had proved the applicability of aquatic community ecology for forensic investigations.

Pollanen[8,15] has touched upon several cases in his work highlighting the precise localisation of site of drowning. In one of his cases, burnt remains of a teenage girl was found in a suitcase at a parking lot near a dumpster. An accelerant was also recovered from the scene. The autopsy did not reveal any injuries. No toxicological cause of death was also established. The lungs were mildly oedematous and congested. However five ml of watery fluid was found in the right maxillary sinus. Bone marrow extracts were taken from both the thigh bones. Nitric acid digestion revealed diatoms in the fluid from maxillary sinus (air spaces in skull). The bone marrow extracts also revealed diatoms, matching exactly with those in the sinus. Based on these findings the cause of death was opined as to be drowning that had occurred elsewhere. Her corpse was then burned using an accelerant to conceal the nature of crime. Lastly the charred remains were dumped at the parking lot. Such case scenarios highlight the importance of taking samples from unusual anatomical sites for analysis and further for conclusion (as drowning) in the absence of classical autopsy findings.

Pollanen[8] in his monograph had quoted the relevance of absence of diatoms in sources of domestic water. Spontaneous birth into the toilet is an important variant of domestic drowning. At times it could be infanticide by drowning of the newborn. There were practical difficulties in such situations to establish live birth. In spontaneous birth the mother would be mostly primiparous and denies pregnancy until the time of delivery. The positive test outcome of diatoms was usually less than 10% in such situations. This was due to lack of diatoms in domestic water.

The authors of this article had conducted a study on diatoms of six water sources in Kochi, Kerala.[16] It was found out that each source of water was having different types of diatoms.Domestic sources of water had lowest number of diatoms. The fresh and marine water sources showed specific genera of diatoms. At times the same genera of diatoms were found in both fresh and marine sources of water, but still they were having differences in size and morphology (Figure 2). This overlap of genera can be nullified if the diatoms are all classified up to their species level.

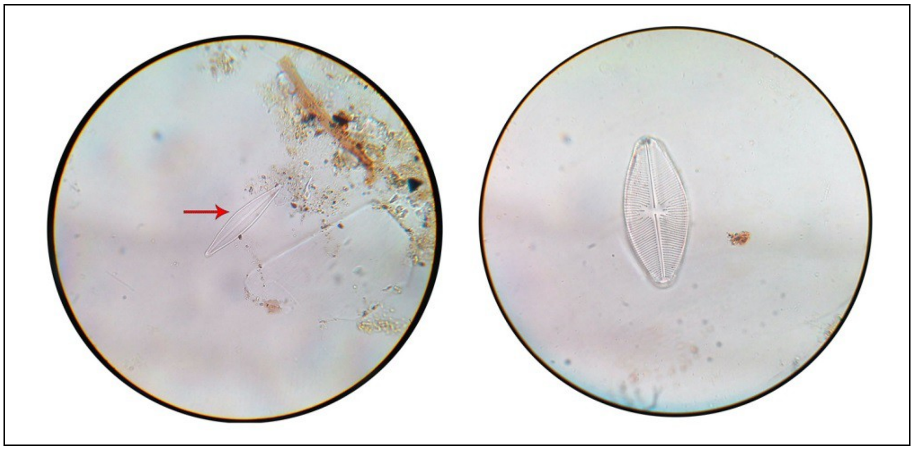

Figure 2. Photograph on Left: *Navicula* genera seen in well water (400x), Photograph on Right: *Navicula* genera seen in sea water (400x).

Diatom analysis – microscopic features of diatom frustules

Pollanen[8] was the only scientist to quote on the microscopic features of diatom frustules in his monograph. The frustules of diatoms have several characteristic microscopic features. The cell wall of most diatoms conforms to one of the two body plans. *Centric* shape is characterised by circular configuration with radial symmetry. *Pennate* shape is characterised by one or two axes of symmetry. The most common axis is the sagittal or longitudinal type whereas the other axis is transverse (orthogonal to the sagittal plane). Many pennate diatoms have a central line along the sagittal axis known as the raphe. Most of the frustules have periodic striae or pores on the surface. Such periodic striae or pores on the surface help the scientists to identify and delineate them into different genera. Each genus contains different species of diatoms. It requires specialised training as a diatomologist to identify the different species within one genus of diatoms.

Parallel rib like structures may radiate from the raphe of pennate diatoms. In centric diatoms radial lines emerge from a central zone. The frustules scatter light particularly at the edges. In water samples, diatoms may associate to form colonies. In addition to the basic features of identification, there are other subtle morphological characteristics that can be used to differentiate between taxa. In water samples diatom frustules are present in a wide range of sizes. Small diatoms are about 10 micrometre in size whereas larger species can exceed several 100 micrometres. However, diatoms extracted from bone marrow represent only a small subset of the total diatom population known as '*drowning associated diatom'* (Figure 3). This group has the required small size allowing them to embolise into the arterial system and eventually into the internal organs like bone marrow. They can be from 10 micrometres to 40 micrometres (few times the size of an erythrocyte) but seldom can be larger than 50 micrometres. In some cases, samples of putative drowning medium will not be obtained. Then a diagnosis of drowning only based on the frustules obtained from marrow would be difficult. In such situations, according to Pollanen[8,15] diatom frustules from unusual anatomical sites such as sinus fluid or stomach contents, if recovered should be compared with those obtained from bone marrow. Diatoms belonging to the same species if present in similar concentrations between the marrow and other anatomical sites can undoubtedly support the cause of death as drowning.

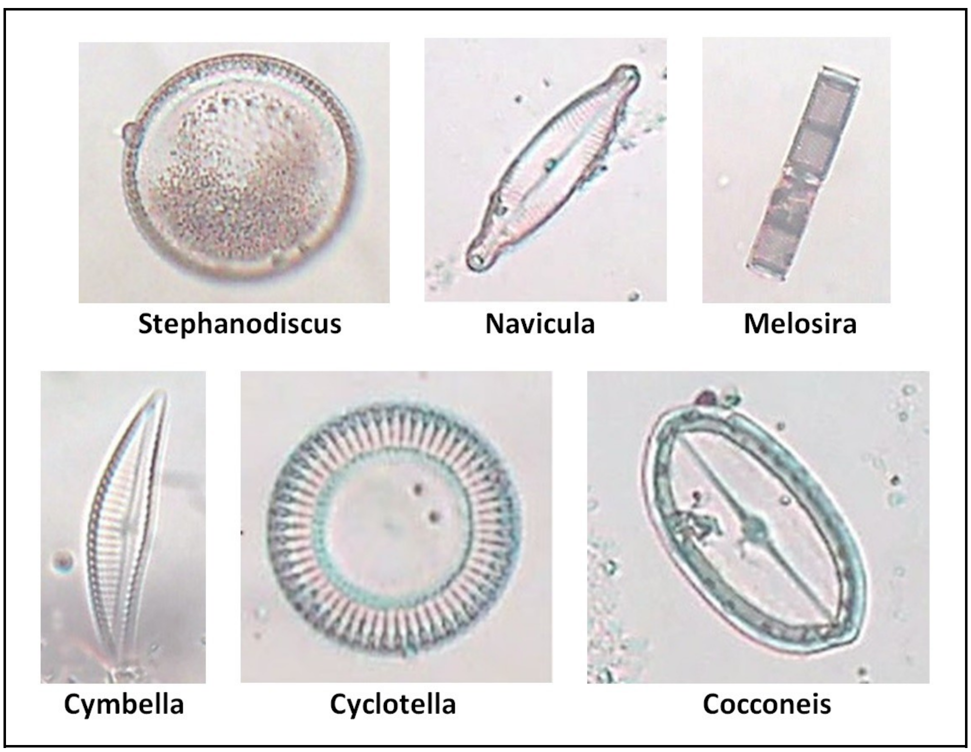

Figure 3. Different genera of *drowning associated diatoms*.

Many geometrically or regularly shaped microscopic particles can resemble a diatom frustule (Figure 4). These particles are crystals of inorganic silica. Since such crystals are composed of silica like the diatom frustules they can resist nitric acid digestion. These particles can be from human bone marrow extracts that co-purify with diatoms. They can also be acid insoluble crystals as a part of laboratory contamination. In some extracts the crystalline material may dominate and obscure the detection of diatoms. Although the silica crystals may have an overall configuration and size similar to a pennate diatom, several morphological features can be used to differentiate these crystalline materials from frustules. Each crystal has a regular geometric structure with a crystal face. The crystal has a continuous surface with no fine structure like raphe, pits or striae. Numerous crystals with identical morphological properties will be present in the extract. The crystals may aggregate into radiating arrays surrounding a nucleus of debris. The bone marrow burden of these siliceous materials may be related to occupational or environmental exposure during life.

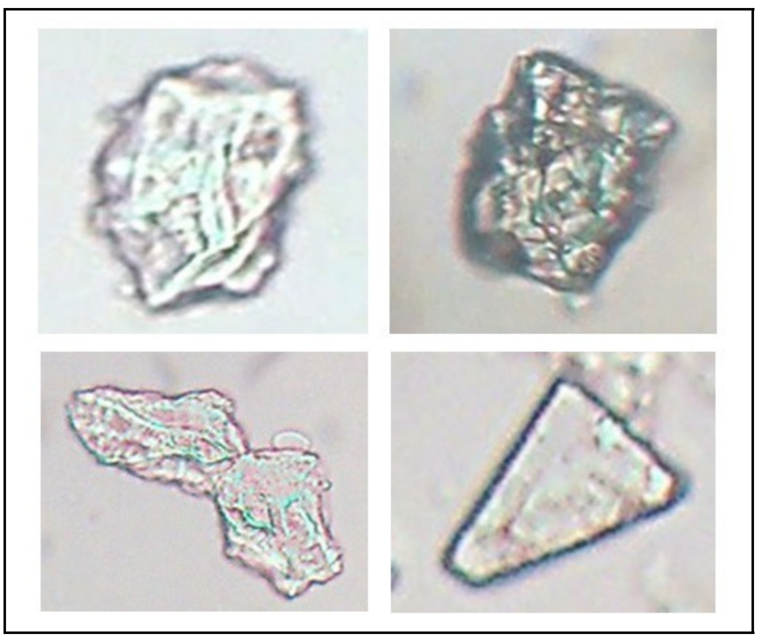

Figure 4. Various siliceous non diatomaceous crystalline structures that can be found during examination of diatom frustules.

There are some criteria to be fulfilled during analysis of diatom frustules. Frustules extracted from tissues must be whole and intact. In case of fragmented frustules, these must be cleaved along a line of symmetry thus allowing the observer to deduce the overall configuration. The frustules in the tissue extract should be concordant with diatoms in the drowning medium. Photomicrography can help in direct morphological comparison of diatom frustules. In most laboratories that perform the diatom test for drowning, acid digests are examined by light microscopy using phase contrast optics. However, some investigators advocate the use of scanning electron microscope for the detection of diatom frustules. Scanning electron microscope gives a higher resolution image of the surface features of the frustules. The greater magnification can be used to compare the individual morphological features of frustules extracted. However, for most of the forensic applications, degree of resolution provided by phase contrast light microscopy is satisfactory. Recently several new imaging techniques have been invented. One of the most promising methods developed recently are the scanning probe microscopes which include atomic force microscope. The atomic force microscope is a non-optical method of imaging the surface structure of microscopic and mesoscopic structures. This imaging method produces a computer generated topographical map of the sample surface. It has magnification and resolution at par with or beyond routine scanning electron microscopy. This can eventually be useful for routine forensic applications in future.

Summary

The medico-legal system in India requires revamping in diatom analysis. Currently diatom tests are not performed by Forensic Diatomologists. These tests are done either by biologists at the Forensic Science Laboratory or by chemists at the Chemical Examiner's Laboratory. Biologists or chemists are not having the sufficient knowledge and skills in examining diatoms of a medico-legal case. A planktonologist has to be trained in depth about forensics of drowning or vice versa. Such an expert in both fields of forensic medicine and planktonology can be entrusted with the examination and comparison of diatoms. He or she can only provide a foolproof opinion based on diatom tests in medico-legal cases. They will be able to do a proper qualitative and quantitative estimation of diatoms. Secondly a*diatom map or chart* can be prepared for different water bodies on the prevalence of different species of diatoms during different seasons. The map should also have information regarding variations of diatom population due to diurnal migration. When a case of drowning has occurred in such a common water body which has been mapped, then the information as to the ratio and type of diatoms prevalent during a particular time of day, month and year can be utilised for comparison with the diatoms found in the internal organs of the drowned victim.

Without such revamps diatom analysis which can be a good corroborative or sometimes a conclusive test, will disappear from the realm of Forensic Medicine.

References

1. DiMaio Vincent J, DiMaio Dominick. Death by Drowning. In: Forensic Pathology. 2nd ed. Boca Raton, FL: CRC Press 2001; p. 399-407.

2. Umadethan B. Principles and Practice of Forensic Medicine. Swamy Law House, Kochi, Kerala 2008.

3. Charles R, Alexander J. Phytoplankton. In: Limnology. McGraw-Hill International Book Company 1983.

4. Grahame John. The autotrophic plankton. In: Plankton and Fisheries. Edward Arnold Publishers Ltd. 1987; pages 38, 39, 49 & 50.

5. Round EF, Crawford RM, Mann DG. The Diatom Biology and Morphology of Genera. Cambridge University Press, Cambridge, England 1990; p. 7-20.

6. Lunetta P, Penttila A, Hallfors G. Scanning and transmission electron microscopical evidence of the capacity of diatoms to penetrate the alveoli-capillary barrier in drowning water. *Inter J Legal Med* 1998; **111**: 229-237.

7. Singh R, Singh R, Kumar S, Thakar MK. Forensic analysis of diatoms – A Review. Available from: *URL: http://www.anilaggrawal.com/ij/vol_007_no_002/papers/paper002.html*

8. Pollanen MS. Forensic diatomology and drowning. Elsevier Academic Press 1998.

9. Singh R, Singh R, Thakar MK. Extraction methods of diatoms – A Review. Indian Internet J Forensic Med Toxicol 2006;**4**: 1-9.

10. Thakar MK, Singh R. Diatoms – Wiley Encyclopedia of Forensic Science. Available from: *URL: http://onlinelibrary.wiley.com/doi/10.1002/9780470061589.fsa625b/full*

11. Auer A, Mottonen M. Diatoms and drowning. Z Rechtsmed 1988;**101**:87-98.

12. Ming Mei, Meng Xiangzhi, Wang Enyin. Evaluation of four digestive methods for extracting diatoms. *Forensic Sci Int* 2007;**170**:29-34.

13. Ludes B, Coste M, North N, Doray S, Tracqui A, Kintz P. Diatom analysis in victim's tissues as an indicator of the site of drowning. *Int J Legal Med* 1999; **112**:163-166.

14. Silver PA, Lord WD and McCarthy DJ. Forensic Limnology: The Use of freshwater algal community ecology to link suspects to an aquatic crime scene

in southern New England. *Journal of Forensic Sciences* 1994;**39**:847-853.

15. Pollanen MS. Diatoms and homicide. *Forensic Sci Int* 1998; **91**: 29-34.

16. Anu S, Umadethan B, Resmi S, Ajay B. Diatoms – a Study on its role in dentification of site of drowning. *International Journal of Medical Toxicology & Legal Medicine* 2012.