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Review Article

Nature helps to solve the crime – Diatoms study in case of drowning death

Pragnesh Parmar[1*], Gunvanti B. Rathod[2], Sangita Rathod[3], Ashish Parikh[4]

[1]Associate Professor, Forensic Medicine Department, SBKS MI & RC, Vadodara, Gujarat, India
[2]Assistant Professor, Pathology Department, SBKS MI & RC, Vadodara, Gujarat, India
[3]Assistant Professor, Department of Medicine, AMC MET Medical College, Ahmedabad, India
[4]Consultant Physician, Gayatri Hospital, Gandhinagar, Gujarat, India
*Corresponding author email: prag84@yahoo.co.in

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Abstract

Nature is everywhere surrounding us in different form. It includes single cell structure to extremely complex, living or non living, substance or creature. One of the important part of nature is diatoms – unicellular algae which are usually present in water sources like lake, pond etc. Death due to submersion of body into water is known since ages. Diatoms study in case of drowning death is very useful for forensic investigators. It is one of the best example in which nature helps to solve the crime. In present article, we discussed about diatoms, diagnosis of drowning death and length of submersion of body in water with help of diatoms, collection, identification and preservation of diatoms for forensic analysis.

Key words

Nature, Drowning, Diatoms, Crime, Submersion.

Introduction

Nature includes single cell structure to extremely complex, living or non living, substance or creature. One of the important part of nature is diatoms – unicellular algae which are usually present in water sources like lake, pond etc. Death due to submersion of body into water is known since ages. Diatoms study in case of drowning death is very useful for forensic investigators. It is one of the best example in which nature helps to solve the crime. As typical features of ante-mortem

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drowning disappear very rapidly with commencement of putrefaction, diatom test had gained the importance as gold standard for diagnosis and confirmation of drowning deaths.

Diatoms and other algae

The term 'algae' includes a wide range of organisms varying from single celled protists to colonial forms to huge multi cellular seaweeds such as kelp. Almost all algae contain chloroplasts. Although algae are ubiquitous in terrestrial and aquatic ecosystems, only the diatoms are useful in forensic analysis. Diatoms are unicellular algae belonging to the phylum Stramenopila (phylum Bacillariophyta) and are characterized by silica containing cell walls called as 'frustules'. They can be present in fresh water and salt water, as well as the surface of moist terrestrial habitats. Each frustule is made up of two halves, which are called as valves, one of which is slightly smaller than the other so that fit together one inside the other. Many species of diatom have a characteristic frustule design which helps in their identification. There are numerous species of diatoms and more than 5600 have been described and probably more than 1,00,000 in total. Their abundance and species composition vary between locations owing to different diatoms preferring different conditions such as temperature, salinity and pH and the consequences of inter - species competition. Therefore, the presence of individual species or the species composition in a forensic sample can provide an identifying feature of a habitat or location at a particular time of year.

Historical aspects of diatoms in relation to drowning

Diatom was first detected in lung fluid by Hofmann E [1]. However, Rovenstorff had successfully utilized it to solve a case of a drowning mystery in 1904. The method of its extraction was improved by acid digestion of the tissues. Incze successfully detected diatoms in blood and parenchymal organs in 1942 [2]. Tamasaka detected diatoms in bone marrow in 1949 [3]. In the 1960's and 1970's, Timperman using large series of drowning cases, provided evidence for the validity of diatom test [4].

Otto (1961) reported on workers in Kieselguhr industry who had contracted silicosis through continuous inhalation of diatoms in the lungs, without of course, having drowned [5]. Spitz (1963) and Peterson (1963) showed diatoms to be present in the major organs of non-drowned subjects, and came to conclusion that the diatom method was worthless [6, 7]. Porawski (1966) also found diatoms in the lungs, kidneys and bone marrow of non-drowned subjects [8]. Koseki (1968) reported their occasional presence in the lungs (and rarely in the liver and kidney) of non-drowned bodies [9]. Schellmann and Sperl (1979) had found diatoms in the bone marrow of 15 out of 16 non-drowned subjects [10].

Tamaska (1949) found no diatoms in the bone marrow of seven subjects who were shot before entering the water [3]. Muller (1963) did not find diatoms in the liver of 30 non-drowned bodies [11]. Neidhart and Greendyke (1966) failed to detect diatoms in the organs of 15 non-drowned bodies [12]. Peabody AJ (1977), Ludes B et al., (1994) and Pollanen MS (1997) strongly supported diatom test in diagnosis of drowning [13, 14, 22, 23]. Pollanen MS claimed that diatoms found in non-drowning cases could have caused by contamination during various process of autopsy and diatom test [22, 23]. Bortolotti F, et al., (2004) advocated the use of Environmental Scanning Electron Microscope (ESEM) over light microscope for better diagnosis of diatom [15]. Wang Lei, et al., (2004)

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advocated maximum value of diatom aspirated into the lung (number of diatoms per tissue in gram) to differentiate drowning and non-drowning deaths [16].

Diagnosis of drowning by help of diatoms

Drowning is the mechanical asphyxia death caused by aspiration of fluid into air passages due to submersion of body (nose and mouth) into water or fluid. Death due to drowning is very complex and mechanism of death is asphyxia. Autopsy findigs of drowning very difficult because signs of immersion only indicate that a body was underwater (or some other fluid) for certain time and not that the person died of drowning. Because diatoms can be found in many water sources, their presence in the lungs and other tissues has been used as an indication that the victim may have drowned [17]. Diatoms can be recovered from the stomach (indicating that water was swallowed), from the lungs (indicating that it was aspirated) and also from the blood, major organs and the bone marrow (indicating the water was aspirated whilst the victim was still alive). Owing to their small size and the damage caused to the lungs during drowning, diatoms may pass through the alveoli and be swept around the body in the blood stream [18].

However, once the blood circulation stop after death, any diatoms entering the lungs with water would not be transported elsewhere. By comparing the abundance and species profile of the diatoms found in the body with that of diatoms found in the river – or wherever the victim was recovered – it is possible to provide corroborating evidence with results from the autopsy to determine whether or not they drowned and if so, whether it was at that location [19]. For example, the presence of freshwater diatoms in a body recovered at sea would suggest that the victim may have died in a river and subsequently been swept out. The possibility that a person may have died in a water body other than the one in which they were found needs to be borne in mind when deciding on extraction techniques. For example, the frustules of marine diatoms are dissolved by Soluene - 350 (a solubilizing agent) whilst the frustules of freshwater diatom species are not [20]. Like all biological evidence, the results of diatom analysis need to be considered in context. For example, some workers question the sensitivity of the diatom test and the absence of diatoms does not mean drowning did not occur, nor does the presence of diatoms in the body tissues mean that it did. Although diatoms are extremely common, they are not found in all water sources and even if they were present they may not find their way into the body organs or be recovered if they did. Similarly, the abundance of diatoms means that contamination at the time the body was retrieved, during the autopsy or during laboratory analysis is always a possibility unless extreme care is taken. Furthermore, if a person repeatedly swims in a lake or the sea, it is possible that they may accumulate diatoms within their tissues over time so finding them does not mean that the person drowned there [21].

Diatoms may also be recovered from the tissues of persons who do not die of drowning or swim regularly. Diatoms are found in numerous man made products ranging from building materials to the powder used in rubber gloves. There are therefore many opportunities in which diatoms may be breathed in and there is also a possibility that they might be absorbed through the gastrointestinal tract when consumed with foodstuffs although the extent to which this occurs needs to be confirmed by further research. Consequently, diatoms can be found in the tissues of persons who died of causes other than drowning. However, it is generally accepted

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that finding diatoms within the bone marrow provides good corroborative evidence of drowning and identification to species level can exclude those that are contaminants [22, 23].

Determination of length of submersion and exposure by growth of algae

Length of submersion can be estimated by rate of colonization of submerged body by diatoms and other algae but there are fewer studies in this field [24]. Depth, substrate, local circumstances and time of year cumulatively affect the amount of growth of algae and therefore experimentation will help for any prediction. For example, in nutritionally rich pond, growth would be rapid in summer than during winter and compare to in nutrient poor mountain stream. Haefner, et al. (2004) described use of algal growth to determine the length of time a dead body remained in water [25]. Instead of identification of the species of algae and their relative large quantity, they determined total algal density by measuring the amount of the chlorophyll pigment in a sample collected.

Exposed objects and animal remains in terrestrial environments can be colonized by algae and their growth could be assessed. So called 'blue - green algae' are not algae at all but they are prokaryotic organisms because they lack an enclosed nucleus and membrane - bound organelles and are more accurately known as cyanobacteria. In spite of being tremendously common in both global and aquatic ecosystems their forensic potential has received little notice, although signs of colonization of exposed bones is said to become clear to the naked eye after 2 – 3 weeks under suitable situation [26].

Collection, identification and preservation of diatoms and algae for forensic analysis

Numerous types of algae, including diatoms, display seasonal growth quality and some are infamous for forming short lived toxic blooms. Therefore, if an effort is to be done at comparing the species composition recovered from body and specific area of lake or pond, it may be essential to take sequential samples through the year and, in the case of open water, samples should be taken from both the surface waters and the bed. In the case of a river or stream, samples should also be taken both above and below the site at which the body was found because there may be differences in the diatom flora and the body may have been moved by the water currents. Due to the small size of diatoms and their widespread distribution, every try should be done to evade the possibility of sample contamination occurring in both the field and the laboratory. All collecting equipments must be cautiously cleaned before use, and the samples should be processed in clean glassware within the laboratory and laboratory procedures must be adopted to lessen the chances of contamination. Samples of distilled water should be processed at the same time to check contamination.

Diatoms and other algae can be collected from open water source by using plankton nets which consist of a long funnel shaped net bag mounted on a circular frame and with a collecting vial attached to the narrower trailing end. The mesh size of the net will determine the size of the plankton which is usually small and require a fine net size of 0.1 – 0.3 mm. Algae and other microscopic organisms attached to underwater substrates are referred to as 'periphyton' and require specialized collection techniques [25]. After collection, samples may need further concentration, for example by centrifugation, although the method needs to be chosen with concern if the more delicate species of algae are to be preserved undamaged. The samples can be examined directly using an ordinary stage

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microscope although phase contrast illumination improves the detail examination. If one is only interested in the diatoms and there is a lot of contaminating organic matter present, the samples may be air dried and subjected to acid digestion although the majority of ordinary algae do not have siliceous cell walls and would be destroyed.

Diatoms can be extracted from body tissues, soil and other solid substances, by heating with concentrated nitric acid for up to 48 hours [17, 27]. Then after digest is centrifuged, the supernatant material is discarded, and the pellet is washed by one or two cycles of suspension in distilled water and centrifugation after which the final pellet is microscopically examined. This method depends upon the silicaceous frustules which remains after all organic matter is dissolved away but some authors like to use less dangerous reagents and have adopted DNA extraction technique [28] and ultrasonic digestion [29].

Morphological features of diatoms and algae help in their identification [30] but it is a time consuming and often very difficult process. Alternately, number of other processes has been discovered. Complex three dimensional shapes of diatom frustules can be detected by computer pattern recognition software and there have been future development of automatic devices that can simultaneously identify and count different diatom species [31]. However, even if automatic devices can be perfected, molecular evidence indicates that morphology on its own is a poor species indicator for diatoms and the existence of 'cryptic' and 'pseudo species' is widespread. This is an important consideration in a forensic context in which the identification of species composition is to form part of the evidence. Evans, et al. (2007) tested the suitability of a range of genes for phylogenetic analysis of diatom species and found that the CO1 (cytochrome oxidase subunit 1) gene offered considerable potential [32]. This is somewhat surprising because although this gene is commonly used in phylogenetic studies of animals it is considered to have limited applicability for plants. Abe, et al. (2003) attempted to identify the presence of diatoms in forensic samples using molecular techniques but although they found the approach to be sensitive and it could not be used to identify sufficient species of diatoms to distinguish the locality at which a person drowned [33]. It should be remembered that molecular techniques could not be used on diatom specimens isolated from soil or tissues using standard extraction techniques since the strong acids would destroy their DNA. Another alternative identification technique is to use Fourier transform infrared (FT - IR) spectroscopy to distinguish between species on the basis of their chemical composition. FT - IR has proved capable of distinguishing species that are difficult to tell apart on the basis of their morphology [34] and useful for population studies [35] but it does not appear to be in widespread use for identifying diatoms. Every life has an end on one day by natural or unnatural means. Death due to accident or drowning leaves a bad taste in society [36, 37, 38]. Study of nature in form of diatoms to solve drowning death is very useful.

Summary

Nature is everywhere around us and helps us in many way, one of it is to solve crime. Diatoms study in case of drowning death is one of the best examples of it. Invention in the fields of physics, chemistry, biology and science helps human to use nature for different purposes. Effective use of nature without deteriorating it is the best way to serve humankind.

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