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Extraction and Identification of diatoms in relation to drowning, dumping & as well as for site characterization: a Review

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ABSTRACT

Diatoms in can be used to determine contact with surface water which can help link suspects to crime scenes. However, for the study of diatoms it is vital that they are first extracted from the samples and examined under investigation. 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. Extraction and identification of diatoms present in the body tissues like lungs, liver, spleen, blood, bone marrow, and Suspected cloth has been undertaken as supportive evidence in downing, dumping cases and also for site characterization. This paper presents a review on forensic analysis of diatoms from different variety of samples.

Key words: Diatom, Drowning, Dumping,

INTRODUCTION

Diatoms are unicellular having a cell wall made of silicon dioxide. The glass frustule is composed of two valves, which fit together with the help of a cingulum, or set of girdle bands. There are about 10,000 species and 174 genera of diatoms reported which are having different shapes and sizes varying from 1 to 500µm. Diatoms are traditionally divided into two orders: centric diatoms (Centrales), which are radially symmetric, and pennate diatoms, which are bilaterally symmetric (Pennales) but **Round *et al.* (1990),** classified diatoms into three classes: centric diatoms (Coscinodiscophyceae), pennate diatoms without a raphe (Fragilariophyceae), and pennate diatoms with a raphe (Bacillariophyceae). Most diatoms exist singly, although some join to form colonies. They are usually yellowish or brownish, and are found in fresh and salt water, in moist soil, and also on the moist surface of plants.

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. Linking suspects to a crime scene is a common forensic goal. In quite a lot of cases, diatoms were extracted from clothing to determine possible contact with surface water and which body of water was the most likely source of the diatoms. 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, **Rohn and Frade (2006) and Krstic *et al* (2002).**

Cleve (1894) reported that classification of diatom is predominantly based on the structure of the valve, its shape, intricate patterning, and ornamentation. In the late 1890's the Systematic and taxonomic investigations of modern and fossil diatoms began to be supported by studies of distributional ecology.

Living diatoms are distributed in almost all aquatic and damp terrestrial habitats. However, it was not until the 1920's that diatom analysis was recognized as a valuable tool in reconstructing ecology changes. However many interpretations are based on models developed in the 19th century, involving a simple classification of species into freshwater, brackish or marine forms, and provide only qualitative estimate of ecological conditions.

Timperman (1962) presented research into the presence of diatom in the bone marrow, lung, liver, spleen, kidney, and brain tissue has led to the development of the diatom test, a direct screening test for drowning, whereby the presence of diatom can be verified and analyzed both qualitatively and quantitatively. Considering the lengthy history of this method, it is no surprise that extensive literature exist on the topic. However, the methodology has remained largely unchanged since its refinement in the early 1960's.

Peabody (1977) extracted diatoms by removing organic matters using acid digestion method. But this time traces of $CaCO_3$ were removed by adding conc. HCI. It was again added with conc. H_2SO_4 and boiled until suspension turned black. Suspension was allowed to cool and solid $NaNO_3$ was added to it. Suspension was reheated until colour turned to brown and finally clear (HNO_3 produced oxidized the carbon to CO_2). Resultant suspension was washed thoroughly in diatom free water and re-suspended in acetone for the preparation of permanent slides.

Peabody (1980) demonstrated that Diatom analysis can be further use in forensic science through identifying the provenance of individuals, clothing or materials from sites of investigation.

Tyagi (1985) demonstrated the acid digestion method. He collected water samples from various water bodies like lakes, ponds, wells and drains.

Round *et al.* (1990) The Diatoms, Biology and Morphology of the Genera There are two types of diatom resting stages, resting spores and resting cells. Resting spores are heavily silicified stages that are morphologically distinct from the vegetative cells, whereas resting cells are similar to the vegetative cells with altered physiological and cytoplasm characteristics. Resting spore formation is more common in marine centric diatoms and is rare in pennates. But resting cell formation is observed more often in pennates and freshwater diatoms

Ludes *et al.* (1994) using an enzymatic digestion method for the diagnosis of putrefied drowned bodies. He also concluded that 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. He has proposed that 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. Until recently the diatom test for drowning has been based mostly on qualitative characteristics including the presence and concordance of diatoms in tissues. Performing the diatoms test in forensic laboratory, risk factor of contamination also increases because centrifuge process requires a great deal of fresh water, which sometimes is not free of diatoms.

Pollanen (1997) supported the validity and utility of the diatom tests for drowning through the analyses of 771 cases of drowning. In 90% of cases in which the sample of drowning medium was available, diatom in bone marrow matched assemblages in the drowning medium.

Pollanen (1998) extracted diatoms from putative water samples using acid digestion method. He 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".

Hurlimann *et al.* (1999) reported that one or more water samples from the site of drowning (from surface and bed) should be taken. Microscopicological analysis should record quantitative (diatom density), qualitative (species) and morphological (description of diatom valves) details for every samples. The algological conclusions were based primarily on the separation values of kater as well as on pair matching.

Yange *et al.* (1999) developed a new instrument called 'can' it was also based on acid digestion procedure. This instrument overcame the shortcomings of previous methods for the destruction of organic material so that their identification became easier. 'Can' consisted of three parts - a can body, an inner cover and an outer cover. For destruction procedure, the organic material was filled with Teflon, which made this instrument as corrosion resistance, heat resistance, pressure resistance, and leak proof etc. Under the fit of strong acid reaction and high temperature action, organic tissues were liquefied for the extraction of diatoms. 3 gm of tissue sample was added with 4 ml of strong nitric acid in this instrument. Can was then placed in a dry box at 102°C for 100 min. Then 'can' was cooled and post digestive liquid was filled in centrifugation tube. After centrifuging with distilled water, residue so obtained was put on slide for further analysis. In Japan many instruments were used for chemical digestion of tissue materials by making modifications as reported by Tomonaga (1954**).** Samples were digested with fuming nitric acid and sulphuric acid in a water bath (60°C - 180°C) or on a sand tray (80°C-300°C). Solution was centrifuged and added distilled water in it and this method was called 'Disorganization method'. In a highly modified method, after finishing the oxidation process, H_2SO_4 was added in amount necessary to complete the oxidation of organic matter. Saturated solution of potassium permanganate turned this solution violet and oxalic acid again discoloured it. After 48 hours of sedimentation, the probe was leveled to approximately 100 ml and centrifuged at 3000 rpm for 20 min, until the pH became neutral. Permanent diatom slides were prepared using the residue material.

Krstic *et al.* (2002) reported result obtained by examination of 22 human cases suspended for drowning ,one human cases of death other than drowning and several test on laboratory rats were used as a basis for evaluation of diatom method as supportive in forensic expertise of drowning .The recovery of diatom from various examined organs ,their qualitative and quantitative composition ,if properly treated without the possible of contamination ,can be a reliable proof of the time and place of drowning.

Cameron (2004) demonstrated the composition of diatoms is strongly related to water quality and aquatic habitats and because of their silica skeletons, diatom valves can be well preserved and provide a record of past and present environmental conditions. Diatom remains are often diverse and can be identified with high taxonomic precision. These factors allow diatoms to be used in a range of application in forensic geosciences. These include the matching of environmental samples with the identification of traces if diatomaceous materials used in the manufacture of materials or liquids.

Horton *et al.* (2006) examined the drowning cases on the bases of diatom test. In his study, a body was found face down in the pond and it was suggested that drowning took place in the pond. He collected samples for diatom analysis from four transects around the circumferences of the artificial pond, two samples of sediments from the center of pond to act as a control in the examination of diatom assemblages associated with three living tissue samples. After the diatom test, it was examined that diatom was detected in all body samples. Hence the result will be positive and death due to drowning.

Singh *et al.* (2006) Detected diatoms in tissues, and has been applied as an important sign of drowning since the beginning of 20^{th} century and utility of diatoms for the diagnosis of downing's cases was debated soon after they were first found in lung exudates. 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. Dissolution of these samples is not very difficult but complete extraction of diatoms frustules from these samples needs great care, attention and expertise. 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.

Horton (2007) suggested that the application of diatom analysis in determining whether drowning was the cause of death has proved to be valuable tool in forensic science. The basic principal of 'diatom test' in drowning is based on inference that diatom are present in the medium where the possible drowning took place and that the inhalation of water causes penetration of diatom into alveolar system and blood serum ,and thus their deposition into the brain ,kidney and other organs.

Stefan *et al.* (2010) tested three methods for extracting diatoms from cotton clothing: rinsing with water (RW), rinsing with ethanol (RE) and the dissolution of cotton with nitric and sulphuric acid (DI). The DI method produced the highest average yield and can be used to determine contact with water. The RE method extracted reproducible numbers of diatoms from two different T-shirts and the resulting species compositions were similar to their relevant reference water samples. Therefore, we present rinsing with ethanol as an effective extraction method for the qualitative and quantitative analysis of diatoms in (cotton) clothing.

Ajay *et al.* (2011) studied 7 drowning human cases are examined. Acid digestion test method on laboratory rats were used on the basic for evaluation of diatoms methods as supporting in forensic of drowning. Result revealed from examined of 7 human cases are suspected for drowning, four cases are positive (death due to drowning) while three cases are negative (death other than drowning).

Guangtao *et al.* (2011) established a model of drowning, and by investigating diatoms in lung, liver, kidney, and long bone marrow of rats at different time to discuss the cause of death. The organs of 35 rats were extracted 0.5 h, 1 h, 6 h, 12 h, 24 h and48 h after drowning and the organs of sham-drowning group killed by mechanical asphyxia were extracted 1 h after body immersed in water. The organs were digested by acid, and the diatoms were analyzed by statistics. Results shown the detection rate was 100% in lung, and the positive rate of all the extracted organs was 100% 6 hours after drowning except the sham-drowning group. No diatoms were detected in the liver, kidney and bone marrow of the sham-drowning group, just only one case was positive in the lung. So it is concluded that the detection rate of diatoms could be considered as important evidence in drowning determination.

Fucci (2012) has tested a minimal amount of H_2SO_4 diluted solution to detect diatoms in several tissues from human corpses under crime investigation, immersed in the sea and river water. The method was compared with a traditional method that includes digestion with a large amount of strong mixture of sulphuric and nitric acid (90%). The new procedure showed that all siliceous frustules of sea and river diatoms are more resistant to the H_2SO_4 diluted treatment and are still recognizable after digestion, and observation under the microscope is better than the other procedure.

Yashoda *et al.* (2012) presented an overview on extraction of diatoms from human tissues like lung; liver, kidney, brain and bone marrow, several methods like Acid Digestion Method, Soluene-350 Method, Enzymatic Method, Membrane Filter Method, Colloidal Silica Gradient Centrifugation Method, and Dry Ash Method etc. are used.

Yadav *et al* (2013) studied water sample from four sites of Yamuna River were collected for identification of diatom. Collected water sample were digested with acid digestion and examined under high power microscope after slide preparation. After examination it was found that total 41 diatom species was found at four sites of river. Out of 41 diatom species 10 diatom species were found as site specific. These site specific diatoms can be used as marker for site identification in cases of suspected drowning as well as for other forensic purposes.

Verma (2013) presented 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.

Anu and Resmi (2014) 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.

Kirstie (2014) Diatom analysis is currently an underused technique within the forensic geo-science approach, which has the potential to provide an independent ecological assessment of trace evidence. This study presents empirical data to provide a preliminary evidence base in order to be able to understand the nature of diatom transfers to items of clothing, and the collection of transferred diatom trace evidence from a range of environments under experimental conditions. Three diatom extraction methods were tested on clothing that had been in contact with soil and water sites: rinsing in water (RW), rinsing in ethanol (RE), and submersion in H_2O_2 solution (H). Scanning electron microscopy (S.E.M.) analysis was undertaken in order to examine the degree of diatom retention on treated clothing samples. The total diatom yield and species richness data was recorded from each experimental sample in order to compare the efficacy of each method in collecting a representative sample for analysis. Similarity was explored using correspondence analysis. The results highlight the efficiency of H_2O_2 submersion in consistently extracting high diatom counts with representative species from clothing exposed to both aquatic and terrestrial sites. This is corroborated by S.E.M. analysis. This paper provides an important empirical evidence base for both establishing that diatoms do indeed transfer to clothing under forensic conditions in a range of environments, and in identifying that H_2O_2 extraction is the most efficient technique for the optimal collection of comparative samples. There is therefore potentially great value in collecting and analyzing diatom components of geoforensic samples in order to aid in forensic investigation.

CONCLUSION

In present scenario there are a lot of cases have been reported in relation to drowning, dumping and as well as for site characterization of unclaimed body. Hence the possible means and methods must be practiced on a regular basis by the forensic examiner as well practitioners and new possibilities must always be explored for extraction and identification of diatoms in future. Therefore, it is very essential part of investigation that diatoms should be carefully analyzed in these types of cases and also considered as valuable evidence in forensic purposes.

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