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Acid Digestion Method for Extraction of Diatoms in Drowning Cases: A Review

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ABSTRACT

Diatoms are single celled algae and is the most found phytoplankton in water sources like rivers, lakes, pools, ponds, etc. In cases of drowning, it becomes essential to prove that the person died due to drowning. In this aspect there are basically two conditions that need to be investigated, to prove it. The point of proving that it was an ante mortem drowning helps in determining the crime committed and plays a vital role in the conviction of the accused with the appropriate punishment. In ante mortem drowning the water is swallowed by the individual and since the person is still alive this water gets absorbed and enters the blood stream. Therefore, if a person was actually drowned which resulted in his or her death there is a possibility of finding some content of the water in which the drowning took place inside the individual and the region to look for this is the bone marrow. Further diatoms are the phytoplanktons that reach the bone marrow in cases of ante mortem drowning. Diatoms are present in all water bodies, but the species of diatoms present in each water body varies. Therefore, diatoms have been used in drowning cases to prove the person had drowned and the location where the drowning took place. The analysis of diatoms is still in the process of development. Since they have silica-based cell wall they are strong, and their analysis needs to be done in such a way that procedures employed should be accepted by the court as a valid protocol and subsequently admit the evidence in that particular case. Extraction of diatoms from bone samples is an important step to isolate diatoms from the human tissue. There are many ways to conduct this extraction, but the most efficient technique is not known. This study reviews the studies conducted on the acid digestion procedures for extracting diatoms and it is found that acid digestion method can be used for numerous types of samples. Some protocols were tweaked to get the best results but the basic utilization of acids like nitric acid, hydrochloric acid and sulphuric acid showed good results in the extraction of diatoms from samples of forensic significance.

KEYWORDS: Diatoms, Forensic investigation, Drowning

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1. INTRODUCTION

A Diatom is a photosynthetic, single celled micro-alga which is one of the most common forms in phytoplankton and lives in water sources such as rivers, oceans, lakes, seas, bogs, and damp rock surfaces. Diatoms are scientifically termed as Bacillariophycea. The striking feature of diatoms is their beautiful cell wall, which is of hydrated glass (SiO2.nH2O), and this cell wall split into two halves called Frustules. The cell wall is usually overlapping and interlocking as symmetrical sides with splits evident between them, hence the group name "Diatom" which comes from Greek (dia) means "through" and (temnein) means "to cut" and thus "cut in half". The silica frustules show wide diversity in form -, some quite intricate and ornate and the beauty of these organisms as observed through a microscope, has led to their being called "jewels of the sea" *(New World Encyclopedia, 2017).*

Diatoms are classified into two major groups - Centrales, the centric diatoms, and Pennales, the pennate diatoms. The centric diatoms are radially symmetrical, with parts radiating out from a central point and pennate diatoms exhibit bilateral symmetry, meaning the left and right halves are mirror images of each other *(S. Malviya, 2016).* A more recent classification by Round and Crawford (1990) divides the diatoms in to three classes - centric diatoms (Cosinidiscophyceae), pennate diatoms without a raphe (Fragilariophyceae) and pennate diatoms with a raphe (Bacillariophyceae). The raphe is a structure that allows diatom cells to move over surfaces. There are more than 200 genera of living diatoms, and it

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is estimated that there are approximately 100,000 existing species, although they can exist as colonies in the shape of filaments and ribbons. The Indian subcontinent accounts for approximately 7000 diatom taxa from fresh water, brackish water, marine environment, and fossil. They are commonly found between 20-200 microns in diameter or length, although sometimes they can be up to 2 millimeters long *(Vijayan D, 2016)*. Diatoms were first observed in 1703 by an unknown Englishman published by the Royal Society of London in the philosophical transactions. Tabellaria is the first written record of diatom in the scientific community. In the late 17th century, several European scientists began to use early simple or compound microscopes to look at natural material from aquatic habitats. In the latter half of the 18th century, many diatoms were observed and given classifications. The close of the 19th century left with a huge collection of diatom types collected on a world-wide basis. In the early twentieth century, fossil diatoms were first studied and most famously, Hustedt (1927- 66) produced a taxonomic and ecological study of diatoms which remains a key reference today. Diatoms were also the first specimen in which the details of cell division were examined. Each diatom species exhibits a specific environmental tolerance. Thus, diatoms form species assemblages corresponding to the habitat condition. Although several diatom species show a global distribution, species showing global distribution are mostly pollutant tolerant species *(Ajay Rana, 2018; David G. Mann, 2013).*

Diatoms play a vital role in Forensic Science even though this may be an unknown matter for most people. Forensic Limnology is a sub-field of Forensic Botany which makes use of living organisms in water in the investigation of forensic related cases. Currently analysis in forensic limnology is applied particularly for investigation of drowning cases. Guy (1861) observed that in drowned cases, which died due to inhalation of water, trachea, and larger bronchial tubes contain water which sometimes penetrates to the minute ramification and occasionally carries with it a portion of slime or mud and fragments of aquatic plants. Entering of water in blood circulation in case of submersion was described in a series of experiments of drowning dogs *(Brouardel & Vibert, 1880; Buri OS, 2015)*. Diatom was first detected in lung fluid by Hofmann in the year 1896. In the year 1904, Rovenstroff had successfully solved a case of a drowning mystery using the knowledge of diatoms.

The extraction method of diatom was improved by acid digestion of the tissues. Incze successfully detected diatoms in blood and parenchymal organs in 1942 and later Tamasaka detected diatom in bone marrow in 1949. In the 1960's and 1970's Timperman used large series of drowning cases and provided evidence for the validity of diatom test and he presented his research for the presence of diatom in bone marrow, lung, liver, spleen, kidney, and brain tissue, wherein he stated that presence of diatom can be verified and analysed qualitatively and quantitatively. Pollanen states that the sensitivity of the diatom test has been one of the 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". The study done by Horton shows that diatom tests act as a valuable tool in forensic science for the detection of drowning deaths (*Ajay Rana, 2018; Kumar A M, 2012)*.

2. ANALYSIS

In the study conducted by Ashiq Hussain Magrey and Mool Raj it was found that diatoms proved to be an important aspect in proving that the drowning in water was the manner in which the person died. They studied 31 cases of human drowning and conducted the analysis of diatoms in all these cases. The method used for the analysis of diatoms was acid digestion in the study that they conducted. They specifically used Nitric Acid Digestion as the method for analysis of diatoms. They used the sternum, clavicle, femur, and lungs of the bodies for the purpose of their analysis. The samples were put into nitric acid and left for about 12 hours and then boiled for about half an hour after that. Out of the thirty-one cases they found positive identification of diatoms in nine cases. A layer of fat was formed on the top. This layer was discarded, and the bottom layer was centrifuged at 5000 rpm for ten minutes. This process of centrifugation was done three times and every time the supernatant was discarded, and the pellet was washed with distilled water. After the final centrifugation, the pellet was dried on a hot plate and a microscopic slide was prepared. The slide was viewed under oil immersion. Simultaneously they prepared microscopic slides from the water samples collected from where the bodies were recovered *(Magrey & Raj, 2014)*.

Thomas et al. (1961), Timperman (1962) and Pollanen et al (1997) also used nitric acid digestion for the purpose of extraction of diatoms from the sternum and bone marrow of the femur bone. Pollanen et al. modified the normal procedure by simmering the suspension of the sample on a hot plate for approximately 48 hours *(Thomas F et al., 1961)*

Peabody (1977) extracted the diatoms using the acid digestion method but, in his procedure, he treated the samples with concentrated hydrochloric acid and concentrated sulphuric acid. The samples were also treated with solid sodium nitrate. This was done in addition to treating it with nitric acid *(Peabody, 1977)*.

The modification in this process helped to remove traces of calcium carbonate that may be present in the sample.

In a study conducted by Yange et al., the shortcomings of the acid digestion technique were overcome. This included the destruction of organic matter. Yange et al. developed an instrument for this purpose and name the instrument as 'Can". This instrument consisted of three parts. The first part is the body of the instrument, the second is the inner cover and the third is the outer cover. The instrument was filled with teflon which gave the properties of corrosion resistance, withstand high temperature, resistance to pressure, and prevention of leaking. It is in the "Can" that the acid digestion will take place. The can was placed in a dry box which was maintained at 102 ^{o}C for about one hour forty minutes. After which the can was cooled, and the digested solution was subjected to centrifugation *(Yange et al., 1999)*. Many modifications of the instrument were made by various scientists. Tomonaga (1954) modified this process by fuming the samples with concentrated nitric acid and sulphuric acid in a water bath kept at a range of 60-180 ° C. The experiment was also conducted by replacing the water bath with a sand tray kept at 80°-300 °C *(Tomonaga, 1954)*.

In another research conducted by Krstic et al. (2002), the samples were first treated with hydrogen peroxide for 24 hours for the purpose of oxidation. After the oxidation, the samples were then treated with concentrated sulphuric acid as per the quantity of the sample to be used. Further the resulting solution was treated with a saturated solution of $KMnO_4$. This resulted in the solution turning to violet *(Krstic et al., 2002)*.

This was then treated with oxalic acid which discoloured the solution. The solution was allowed to sediment for 48 hours and then subjected to centrifugation unit a neutral pH was achieved.

CONCLUSION

From the surveyed studies, it is evident that the acid digestion method is a good method for the extraction of diatoms from forensic samples. This method can be tweaked with the above-mentioned protocols to achieve better results depending on the type of sample obtained. Further it was observed in the studies conducted by Timperman and Krstic that the samples were left for twenty-four to forty-eight hours. Therefore, treating the sample properly with the proper acids and a time duration for the digestion to take place will help in the extraction of the diatoms from the sample. Since diatoms are present in the bone especially in the sternum, the extraction of the diatoms is important to ensure that ante mortem drowning can be proved or disproved. The above studies show a considerable detailed study on the acid digestion procedure of the extraction of the diatoms from various samples.

CONFLICT OF INTEREST

The author declares no conflict of interest in this research work

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