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Diatoms, the most common type of phytoplankton, are a major group of eukaryotic algae which are ubiquitous to a wide variety of aquatic habitats. These are useful in linking suspects and victims to crime scenes in and around water. When a person drowns, water is inhaled into the lungs and enters into the circulatory system, so any diatom present in the drowning medium can ultimately find their way into internal organs such as bone marrow, spleen and brain of a drowning victim. Any diatom found in these organs would thus provide an indication of the ante mortem inhalation of water, suggesting that either drowning was a cause or contributing factor to death. From a historical prospective, one of the most important issues in the study of drowning has been the search for a sensitive, specific, and easily applicable test for the cause of death. On this basis the diatom test has emerged as the foremost laboratory process for the detection of drowning. In the present study, five drowning cases were examined with the help of "Diatom Test" in the forensic science laboratory, Madhuban, Haryana (India). Out of these, four cases were found positive and one case was found negative for the drowning.

**Key Words:** Diatoms; Diatom Test; Drowning.**\*Corresponding Author:**

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**Introduction**

Death by drowning is defined as a death due to submersion in a liquid and the mechanism in acute drowning is hypoxemia and irreversible cerebral anoxia[1]. Although there are some typical signs of drowning known, it is still hard to determine a death by drowning when the post-mortem signs are impossible to find in case of deceased bodies[2]. The diagnosis of drowning is one of the most difficult diagnoses in forensic pathology and therefore a great number of tests have been proposed to allow a confirmation of death by drowning of a victim[3,4]. Diatom test is one of these test and works as an important tool in diagnosis of death due to drowning. According to Peabody[5,6] and Auer and Mottonen[7], diatom count can be used to discriminate between drowning and non-drowning cases. Detection of diatoms in tissues may contribute to diagnosis of drowning, therefore an efficient method of extraction and microscopic examination of diatoms from tissues is fundamental.

**Materials and Methods**

The study was conducted in Biology Division of Forensic Science Laboratory, Madhuban, Karnal (Haryana), India and all the cases reported here, are fresh water drowning cases. After post-mortem of dead bodies, internal organs (sternum, clavicle, femur and lungs) along with the water samples (From where the dead bodies were recovered) were sent as crime exhibits to the laboratory. Cases were opened and processed following standard methodology. In all cases exhibits were put into different jars. 50 ml of nitric acid was added in each jar containing the sternum, clavicle, femur and lungs. Samples were left undisturbed overnight and boiled for half an hour on next day. A clear yellow solution was obtained with a fat layer at the top. The fat layer was discarded and the remaining samples were centrifuged at 4000 rpm for 10 minutes. The process of centrifugation was repeated three times in the same way. Supernatant was discarded and the pellets were washed with distilled water and re-centrifuged. Microscopic slides were prepared from the pellets after washing, dried on hot plate and studied under the microscope after applying immersion oil. Slides were also prepared from the water sample sent to the laboratory in which possible drowning took place. Comparison of diatom species found in organ sample and water sample was done and correlations were drawn out accordingly.

**Results****Case 1**

The body of a 19 year old boy was found submerged in a water tank. No sign of injury were found at autopsy. Nitric acid extract of internal organs (sternum, clavicle, femur and lungs) revealed the presence of three types of diatom species (*Navicula lanceolata*, *Navicula oblonga* and *Gomphonema gracile*). The same three

types of diatom species were found in the water sample from which the body was recovered. So the cause of death was assigned due to drowning.

### Case 2

The body of a 26 year old lady was recovered from a canal. Signs of head and neck injury were traced during autopsy. Nitric acid extract of sternum and clavicle showed the absence of any diatom species. However, two diatom species (*Nitzschia subtilis* and *Navicula radiosa*) were detected in the water sample from where the body was found. The cause of death was attributed to the reasons other than drowning which was later accepted by her husband that he murdered her wife because of having extra-marital affair.

### Case 3

The body of a 23 year old lady was recovered from a village well. No sign of injury were found during autopsy. Father of the lady claimed that her daughter was being murdered and then thrown into the well to prove the case as suicide. Nitric acid extracts of sternum, clavicle and femur showed presence of three diatom species (*Nitzschia gracilis*, *Cymbella* and *Navicula radiosa*). Same three types of diatom species were detected from the water sample of well in which the body was found. Drowning was the cause of death.

### Case 4

The body of a 48 year old lady was recovered from a river. No specific reason of death was found at autopsy. Microscopic studies of slides prepared from nitric acid extracts of sternum, clavicle, femur and lungs proved the presence of three diatom species (*Cymbella cymbiformis*, *Gomphonema spheroporum* and *Nitzschia frustulum*) which were the same species as traced from the water sample of river (From which the body was recovered). Death was attributed to drowning. The family members also accepted that the lady was mentally disturbed and left the home a couple of days before the death.

### Case 5

A highly putrefied body of 30 year old male was recovered from a canal. No information about causes of death was obtained at autopsy. Studies from extracts of nitric acid of sternum, clavicle and femur proved the presence of two species of diatom (*Cymbella ventricosa* and *Coconeis placentula*). Water samples from multiple sites were sent to the laboratory to trace the original site of drowning. Same two types of diatom species were found in the water sample from a site distant from the site from where the body was recovered. The cause of death was drowning which was also proved by further police investigation.

## Discussions

The above mentioned cases describe the significance of diatom test in medico-legal investigation of drowning cases. But a little

controversy about the reliability of diatom test cannot be ruled out. Few workers have reported that diatom may be seen in tissues and bone marrow of those who have died by means other than drowning [8,9,10,11,12 and 13]. But it is suggested that if proper care is taken with all specification and analysis is performed without contamination, it can serve as significant supportive evidence in investigation of crime. As to contamination during sample preparation, it is generally accepted that instruments, gloves, papers, water supplies, and reagents represents potential contamination sources and tap water may contain diatoms [14,15]. However, in the present study, due care is taken to avoid contamination and use of tap water is avoided completely throughout the procedure of sample preparation. The medico-legal utility of diatom test for drowning could be significantly enhanced by increasing the sensitivity of the test and use of polymerase chain reaction (PCR) was suggested to amplify the minute quantities of diatom genome deposited in the bone marrow [16]. In conclusion, five cases reported in our study were solved by using diatom test for drowning and it was proved very significant by providing the actual cause of death.

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