

Identification of diatom from the suspected cloth sample and their comparative study with the concord water body: Case Study

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ABSTRACT

Diatoms are regarded as valuable evidence to correlate the cause of death due to drowning or dumping and also for site characterization. In present study, there were six case studies suspected in which examination of diatoms from the cloth sample of dead body was used to correlate the actual site of drowning and dumping in different water resources in Allahabad. Samples were collected from the mortuary of SRN hospital Allahabad. For the purpose of obtaining concluded result, an experiment was performed on different varieties of cloth sample & biological sample for extraction & identification of diatoms, as well as for comparison with control sample. On the basis of these examinations, this can be proved that, diatoms can be extracted from different varieties of cloth samples which can be used as valuable evidence and can be used for site characterization. In these six cases, second, fourth and fifth cases shows site specification on the basis of extraction, identification and comparison of diatom, while in first and third case did not.

Key words: Drowning, Dumping, Microscopic, Unicellular, Organism.

INTRODUCTION

Diatoms are microscopic unicellular eukaryotic organisms ranging in size from approximately 5 microns to 1000 microns and of the most common types of phytoplankton. The word 'diatom' means 'cut in two' and is derived from the Greek: (*dia*) = 'through' + (*temnein*) = 'to cut', i.e. 'cut in half'. A characteristic feature of diatoms cell is that they are enclosed within a cell wall made of silica (hydrated silicon dioxide) called Frustules and is found in almost aquatic environment including fresh and marine waters, soils, in fact almost anywhere in moist environment. There are more than 200 genera of living diatoms and it is estimated that there are approximately 10,000 extant species **Hasle et al. (1997)**, either free-floating, planktonic forms or attached to substrate, Benthic forms that have 2µm to 1µm in size, (**Warner 1997**). They form the base of aquatic food webs in marine and fresh water habitats. Chloroplasts of diatoms are variable, but consistent within most taxa. Chloroplast may be many small centric diatoms and some pennates, or few large, plate-like chloroplasts are found in the majority of pinnate taxa.

Clothing was determined an appropriate recipient surface to examine diatom transfer due to its frequent presence at a range of crime scenes. The optimal

recovery and analysis of trace evidence from clothing is therefore imperative for the reliable comparison and exclusion of samples in forensic geoscience. In this study, two methods were tested on in contact with multiple water sites. The traditional method of rinsing with water was adopted and acidic treatment of water sample. The efficacy of each extraction method was assessed through consideration of the total diatom yielded in each experimental sample, the species richness of each experimental sample when compared to a control, and the similarity of sample composition as determined by correspondence analysis. Comparisons between diatoms in a piece of clothing and those in a water source depend on an extraction method that does not preferentially lose or damage specific diatom species. In addition, larger numbers of extracted and identified diatoms increase the reliability of the species composition analysis.

METHODOLOGY

1. COLLECTION OF SAMPLES

One important factor that should be taken into consideration before any sample processed is the cleanliness of glassware, especially if samples from several different sites are to be processed in the same laboratory. Cloth sample and biological sample of dead body were collected from Postmortem House of SRN hospital, Allahabad and also control sample taken from particular place from where the dead body was recovered.

2. EXTRACTION OF DIATOMS

2.1 FROM CONTROL SAMPLE

500ml to 1000ml water sample were collected from different places (Control sample from different water sources where dead body recovered). Water sample were collected in thoroughly washed bottle, then bottles were tightly fitted with cap & labeled with the location of sampling site, along with the date, time, and month. The water samples were collected from 6-different sites, Site1 (Under Yamuna bridge), Site2 (Mama-Bhanja Pond), Site3 & site4 (At the confluence of Ganga and Yamuna river), Site5 (Yamuna River), and Site6 (Well water) at Allahabad. 500ml water sample was taken into the beaker from each bottles and 10 ml of Lugol's iodine

(as preservative) was added in beaker and left it for overnight. According to **Ludes et al., (1996)**, 50ml of Conc. HNO_3 , KMnO_4 & H_2O_2 was added in 500ml water sample in 5:2:3 ratios. They oxidize the organic matter present in the water sample except diatom cell wall because cell wall is resistant to them. The next day samples were taken in Tarson tubes & centrifuged at 1500rpm for 5-7 minutes and the supernant were discarded. This step was repeated till full of the water sample contained in the beaker was centrifuged.

2.2 FROM CLOTH SAMPLE

Firstly we used different varieties of cloth sample such as siphon, cotton, georgette, and synthetic wearing cloth. These cloths were used as a sample one by one for extraction process. First of all, packed cloth sample was taken and transferred into a plastic tub for washing it with distilled water. This cloth sample was set aside overnight as such for appropriate exclusion of diatoms from cloth to distilled water and cover it from aluminum foil to prevent contamination from other substances. Next day cloth was removed from tub and the left distilled water sample transferred into beakers. After transference add 2-3 drops of 2% Formalin and left it whole night as such. After that these water sample was treated as described above. After completion of these processes, microscopic slides were prepared and observed under compound microscope. If possible picture of slide should also be taken with the help of microscope for comparison from standard sample's diatom as well as confirmation. This process would be repeated for all the cloth samples which taken from different water sources.

2.3 FROM BIOLOGICAL SAMPLE

For the extraction of diatoms from the biological materials acid digestion method is one of the best. In this method, One gram of biological sample (kidney, liver, and lung) was taken and placed in glass beaker. After that an equal amount of a mixture of strong sulphuric (conc.) and nitric acid (conc.) was added in it and placed in oven at 90°C overnight for dissolution of organic matter. Next day the beaker was taken out from the oven and washed with distilled water 2-3 times. Now the solution taken in centrifuge tube from the beaker with the help of

dropper and centrifuged at 4000 rpm for 2-3 times. After centrifugation the supernatant were discarded and residue was taken over a microscopic glass slide with the help of dropper. This microscopic glass slide was put over hot plate at 40⁰C for 3-5 min for drying. Microscopic slides prepared after following this procedure and observed under compound microscope at different magnifications.

3. PREPARATION OF MICROSCOPIC SLIDES

Slides for examination of diatoms were prepared by taking pellets from centrifuged tube with the help of dropper. After that putting a drop of diluted pellet on the microscopic slide and left it on hot plate at 30 – 40⁰ C to 2 to 3 minutes for drying. Finally mounted it with one drop of DPX (fixer) on the slides and cover slip is put over there for proper fixation. After fixation of pellets slide is kept in oven and left it for one hour at 60⁰ C. After applying this process the microscopic slide was observed under compound microscope at 10x, 45x, and 100x (oil emersion) magnifications. The same steps were repeated for all the samples for examination of diatoms.

4. EXAMINATION OF DIATOMS

Examination of diatoms was done according to standard international method in which the following parameters were considered (Metzeltin *et al.* 2005 and Vinayak 2012). They were as follows:

- Recognition of diatom frustules, if present.
- Awareness of the physical features of the diatoms such as diameter, raphe (centric, ex-centric).
- Compare no of rows of punctuate, strai diameter, valve length, breadth
- Confirm symmetry (bilateral, radial) after identification of diatoms.

CASE STUDIES

In first case, the dead body was found under the river bridge of Yamuna, Allahabad. It was a female aged near about 30-32 years. The body could not be identified. Police had sent the dead body for postmortem examination. Injury was found over the head. There was no wound, bruises etc. found on the body. Epidermis peeled of a place. In second case, an

unknown dead body of female was found in the pond of Naini, Allahabad. The age of the body was near about 19- 20 years. The police wanted to know about the cause of death whether she was drowned or dumped the body in the pond. After postmortem examination, there was no any mark of ligature found on the neck, no any wounds are present. According to his brother, she went to the pond for washing clothes and she slipped over there. In third case, there was a dead body found at the confluence of Ganga and Yamuna river (at Sangam), Allahabad. The dead body was floating in the river. It was a male and age was near about 30-35 year old. The body was started to putrefy. Injury was found over the head. Epidermis peeled off at places. In fourth case, an unknown dead body of a female was found at the confluence of Ganga and Yamuna river (at Sangam), Allahabad. The age was near about 40-45 years. Police had sent the body for postmortem examination. After postmortem, report did not show any mark of ligature on neck. Wall of thorax, ribs were healthy. Heart, liver, kidney, and spleen etc. was softened and started to decompose. No injury was found over the body. In fifth case, there was a dead body of male found in Yamuna River, Arail Ghat, Naini, Allahabad. The age was near about 24-26 years. The body emitted smell. According to postmortem examination eyes balls were putrefied and softened. Ears, eyes and lips not deformed. Scalp and skull were healthy. There was no ligature mark found on the neck. No apart external injury was over the body. In sixth case, an unknown female dead body, age nearly 26-27 years, was found in a well, Naini, Allahabad. The dead body was not completely putrefied. The police had sent the body into the hospital for postmortem examination. According to examination there was some injury found over the body. A ligature mark was not found on the neck. Kidney, spleen, and heart were started to putrefied.

RESULTS & DISCUSSION

The final results of these six case studies are given below:

Case no-1 After performing the acid digestion method, configuration and microscopic studies it that there was absence of diatoms from the biological sample while present in both water (control sample)

and also in cloth sample. In this case, on the basis of observed result it may conclude that, the death was due to dumping. And it may also concluded that if the body was recovered not in proper condition then we use the cloth sample as an evidence and used it for extraction of diatoms.

Case no-2. After analysis of diatoms it was observed that there was presence of distinctive spp. of diatom, which was matched with other two samples. In this case, some species are *Pinnularia acrospheria*, *Cyclotella operculata*, *Navicula caroline*, *Amphora pediculus*, *Boreozonacola olympica*, *Fragilariforma nitzschiodes* found common in all three samples which show similarity and connectivity to each other. After observation it may indicates that the death was due to drowning in that particular place. Also diatoms show site characterization.

Case no-3. After the performing acid digestion method, configuration and microscopic studies it may be concluded that there was absence of diatoms from the biological sample while present in both water (control sample) and also in cloth sample. In this case study, diatoms were extracted from both samples (Control sample & Cloth sample), but there were no diatoms found after extraction from biological sample of dead body. This indicates that death was due to dumping.

Case no-4. After examination, configuration and microscopic studies it was observed that there was presence of some spp. of diatom, which was matched with other two samples. In this case, *Cyclotella operculata*, *Cyclotella rossii*, *Achnantheidium druartii*, *Eunotia microcephala*, *Aulacoseira granunlate*, *Cymatopleura solea*, *Geissleria decussis*, *Nitzschia exilis* are common spp. of diatoms found in all three samples. There were no specific injuries found over the body but the diatoms extracted from the samples (Control sample, Cloth sample, and Biological sample) were generally same. This may indicates that the case was of drowning at the same site where dead body was recovered.

Case no-5. After the performing acid digestion method, configuration and microscopic studies it was observed that there were presence of distinctive spp. of diatom, which matched with other two samples. In this case, diatoms were extracted from all three

samples. Some common spp. found in between cloth and control sample were *Achnantheidium gracilimum*, *Cymbella affinis*, *Gomphonema mexicanum*, *Nitzschia biacrula*, *Pseudostaurosina elliptica*, *Navicula aitchelbee*. While common spp. found in between cloth sample and biological sample were *Navicula aitchelbee*, *Nitzschia paleavardeblis*, *Nitzschia acicularis*, *Cymbella affinis*. But there were no common spp. found in between control sample and biological sample. Therefore, different species were observed from different samples. It may indicate that the body had come from other place with the current of water. It may also indicate that the death due to drowning had occurred in other place.

Case no-6. After performing experiment diatoms were not found in water sample from which the body was recovered and also cloth sample of the dead body. In this case, there was no diatom extracted from any kind of sample. But on the basis of injury found on the body, it may conclude that the case was of dumping.

SUMMARY

Diatoms can be used in cases where a body is found at places far away from the actual site of drowning or dumping. Hence it can be specifically used to correlate the deceased with the actual scene of crime. Similarly clothes can also be used to link the presence of accused at scene of crime as many times these diatoms stuck to the cloth fibers. In present study, Six different suspected cloth sample and biological sample were collected from mortuary of Swaroop Rani Nehru hospital Allahabad, which were reported for drowning, dumping and as well as for site characterization of unclaimed body.

In present study, samples (biological sample, cloth sample and also control sample, taken from particular place from where the dead body recovered) were treated with acid digestion method used for extraction of diatom. After extraction, comparison and identification of diatoms, it was observed that, in first and third case study, some spp. of diatom found common in both cloth and control sample. But there were no diatom observed after extraction of diatoms from the biological sample of dead body. This shows that the body was recovered from different place where the crime was committed and also the case was

of dumping. In these two cases, the actual sites were not recognized. In second and fourth case study, some species are found common in all three samples which show similarity and linked to each other. After observation it may indicates that death was due to drowning in that particular site. In fifth case study, different species were observed from different samples. It may indicate that the body had come from other place with the current of water. It may also indicate that the death due to drowning had occurred in other place. In sixth case study, there were no diatoms observed from any kind of sample. This proves that the case was of dumping.

CONCLUSION

Diatom analysis is a biological test used to assess deaths due to drowning, dumping and also for site characterization of unclaimed body. This experiment proves that cloth sample should also conclude as valuable evidence for forensic purposes. In present study, six cases were reported which help to correlate the crime scene with the criminals and also cause and site of death. After performing experiments and examination it was concluded that cloth sample of dead body can also be used as an important evidence as well as biological sample to correlate the actual site of drowning with the help of comparison with control (water) sample.

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