

Diatom Examination From Ganga River at Vindhyachal - A Forensic Approach

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ABSTRACT

Diatoms are unicellular, photosynthetic (autotrophic), eukaryotic organisms often classified as among the algae. They are scientifically known as Bacillariophyceae. Diatoms study can be used in medico-legal purpose to solve the cases of unclaimed drowned or dumped bodies. The detection of diatoms in the organs can contribute to a diagnosis of death by drowning, a process referred to as the 'diatom test'. This study was conducted in the department of Forensic Science, SHUATS, Prayagraj which included the extraction and identification of diatoms from the collected water samples from three different sites of Ganga River in Vindhyachal. The acid-digestion method was used for diatom extraction. 27 samples were collected (3 from each site) in the month of January, February, March 2022 from 3 different ghats of Vindhyachal, i.e. Babu Ghat, Pakka Ghat, Diwan Ghat. Samples were taken from surface, 1 feet and 2 feet deep inside river. After examination and analysis total 18 genera of diatoms were found. The identified diatoms were of great ecological importance and play an important role in criminal investigations related to pre-mortem or ante-mortem drowning deaths in Ganga river at Vindhyachal, district of U.P, India

Keywords: diatom, ganga river, vindhyachal, drowning death

INTRODUCTION

Diatoms are unicellular, eukaryotic, photosynthetic (autotrophic) algae that has more distinctive feature of crystalline extra cellular coat or frustules composed of silica and having unique patterns of symmetry and micro structure. They belong to kingdom Protista and class Bacillariophyceae. There are more than 200 genera and 100,000 species of diatoms that are known **Round et al. (1990)**¹

Diatoms are microscopic eukaryotic organisms ranging in size approx 5 micron to 1000 microns and of the most common types of phytoplankton. A special characteristic feature

of diatom cell is that they are enclosed within a cell wall made up of silica (hydrated silicon dioxide) called frustules found in almost aquatic environment including fresh and marine waters, soils in fact anywhere where moist environment present. **Warner (1997)**².

Numerous studies have shown that diatoms are valuable supportive evidence in cases of drowning **Horton et al. (2006)**³. The basic principle of "Diatom test" in drowning is based on correlation between diatoms which are present in the medium where the possible drowning took place and recovery of diatom from deceased body.

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When a dead body is recovered from water, there is usually a suspicion as to whether death was due to drowning or if it was a case of post-mortem submersion. In such cases detection of diatoms from internal organs of victim of wet drowning is considered as a positive proof of ante mortem immersion by the forensic pathologists nationwide.

The size of the diatoms is also important in order to eventually reach the bone marrow of dead victim when water is inhaled. Hence presence of large number of diatoms of same species as that of putative drowning medium in the bone marrow of the deceased is used to confirm the cause of death as drowning. **Peabody (1977)**⁴. Presence of diatoms in the biological sample serves as an indicator of drowning proving that such organisms are present in the submerging medium **Krystic (2002)**⁵, The diatom test is considered as a standard to confirm drowning as a cause of death and localize the site of drowning **Pollanen (1998)**⁶.

Ganga river is very well known for its drowning cases. With such high case of drowning there is very high possibility of post mortem submersion **Mishra (2014)**⁷. Diatom test and its study can also be useful in cases where body is found at places far away from the actual site of drowning or dumping. The objective of the study is to extract and identify diatoms from water samples of Ganga River at Vindhyachal from three different drowning prone sites.

MATERIALS AND METHODS

The present dissertation work entitled "Identification of Diatom Flora from Ganga River at Vindhyachal for Forensic

Consideration" was carried out in the Department of Forensic Science Laboratory, SHUATS, Prayagraj.

The materials required for conducting experimental work are, Beakers, Forceps, Tarson tubes,

Disposable pipettes or droppers, Measuring cylinder, Microscopic glass slides, Cover slips, Gloves all were taken new and fresh to avoid any contamination. Chemicals used are 2% Formalin solution, Conc. Nitric acid (HNO₃), Lugol's iodine solution and Emersion Oil were of analytical grade. Instruments used are Microscope, Hot plate and Centrifuge machine were cleaned well to avoid pre presence of any diatom of related material.

Collection of water samples

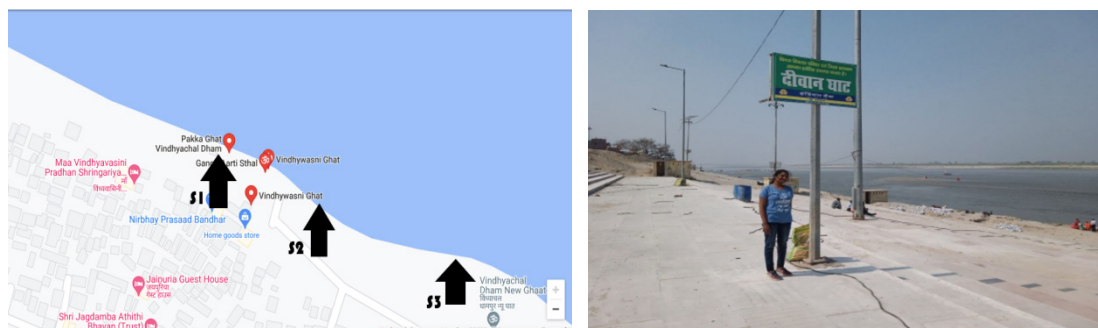
Total 27 samples were collected from Ganga river in the month of January, February, March 2022 from 3 different ghats of Vindhyachal. i.e. Babu Ghat, Pakka Ghat, Diwan Ghat. Samples were taken from surface, 1 feet and 2 feet deep inside river. Before collection in a plastic bottle, it was washed with plenty of the same water media 2-5 times. After thorough washing, water samples containing diatoms were collected (500 ml).

Sites of water sample collection of Ganga river at Vindhyachal were Pakka ghat (S1) Babu ghat (S2) and Diwan ghat (S3). The GPS locations of ghats were noted down.

Extraction of Diatoms from Water Samples

The collected water samples were brought to the laboratory for examination of diatoms. After that 2-3 drop of 2% formalin solution





(as preservative to prevent further growth of diatoms) was added with the help of dropper and was left as such for overnight. 500ml of water sample was taken into the beaker from each bottle and 4-5 drops of Lugol's iodine was added in the beaker and was covered with brown paper. Lugol's iodine is used as a preservative of water samples and was left overnight Ludes *et al.* (1999)⁸. 50ml of Conc. HNO_3 was added in the beaker the next day. They oxidize the organic matter present in the water sample except the diatoms because the diatoms cell wall is resistant to them. The following day samples were taken in tarson tubes & centrifuged at 1000-1500rpm for 10 minutes. After centrifugation pellet is obtained and supernatant was discarded. Again remaining sediments was taken in the centrifuged tube and this process was repeated till complete water sample contained in the beaker was centrifuged.

Preparation of Microscopic slide

For the examination of diatoms, the slide was prepared by taking one drop of deposited pellets from the bottom of centrifuge tube with the help of a dropper and put it on microscopic slide. After that the slides were kept on hot plate for 2-3 minutes at 50-60°C temperature and was allowed to dry. Now, one drop of DPX (mounting medium) was put on the slide and was covered gently with the help of cover slip to fix the slide. After fixation of slide they were observed under microscope at 10X, 40X and 100X (oil immersion) magnification respectively. These same process was repeated for all sample Taylor *et al.* (2007)⁹.

Identification of Diatoms

For identifying the diatoms, photographs were taken from the microscopic slide and then the structure was matched with the standard database Diatoms of North America ([https:// diatoms.org](https://diatoms.org)).

RESULTS

After collection, extraction and isolation of diatoms from water samples, various types diatom were identified by using standard online **Data base of diatom of United States**. They were examined on the basis of their characteristic features such as raphea, cell wall, diameter and shape for identification, these morphological parameters were compared and matched with the standard online database of diatoms.

As per result reported a total of 18 genera of diatoms were identified at different mentioned sites of Ganga river at Vindhyachal.¹⁸ diatoms genera were identified with following characteristics. Synedra, Achnanthisdium, Ulnaria, Nitzschia, Bacillaria, Cyclotella, Humidophila, Tryblionella, Aulacoseira, Surirella, Anomoeoneis, Fragilariforma, Pinnularia, Odontidium, Thalassiosira, Fragilaria, Cyclostephanos, Pseudostaurosira-

As per table no. 2 some common genera are identified at three different sites of Ganga river at Vindhyachal. A common type of diatom at three different sites has shown that there are little variations in diatom at nearby areas. This means that living diatoms often have specific salinity, temperature and other environmental tolerances. Some common genera among the sites are Synedra, Bacillaria, Fragilariforma and Pseudostaurosira.

Table 1: Identified Diatoms at all sites of Ganga river at Vindhyachal

Sr. No.	genera	Pakka ghat (S1)	Babu ghat (S2)	Diwan ghat (S3)
1.	Synedra	+ve	+ve	+ve
2.	Achnanthidium	+ve	+ve	-ve
3.	Ulnaria	-ve	+ve	+ve
4.	Nitzschia	+ve	-ve	-ve
5.	Bacillaria	+ve	+ve	+ve
6.	Cyclotella	+ve	-ve	+ve
7.	Humidiphilia	+ve	+ve	-ve
8.	Tryblionella	-ve	+ve	-ve
9.	Aulacoseira	+ve	-ve	-ve
10.	Surirella	+ve	-ve	-ve
11.	Anomoeoneis	+ve	-ve	-ve
12.	Fragilariforma	+ve	+ve	+ve
13.	Pinnularia	-ve	-ve	+ve
14.	Odontidium	+ve	-ve	-ve
15.	Thalassiosira	+ve	+ve	-ve
16.	Fragilaria	-ve	+ve	-ve
17.	Cyclostephanos	+ve	-ve	+ve
18.	Pseudostaurosira	+ve	+ve	+ve

Table 2. Common Diatoms genera at all sites of Ganga river at Vindhyachal.

Sr. No.	Genera	Pakka ghat (S1)	Babu ghat (S2)	Diwan ghat (S3)
1.	Synedra	+ve	+ve	+ve
2.	Bacillaria	+ve	+ve	+ve
3.	Fragilariforma	+ve	+ve	+ve
4.	Pseudostaurosira	+ve	+ve	+ve

As per table no. 3 some specific genera of diatoms were identified at three different sites of Ganga river at Vindhyachal.

In accordance with Table no. 1, it was observed that the pennales are found in abundant amounts as compared to centrales. The pennales which (bilaterally symmetric) are 84% of the total no. of diatoms found whereas the centrales which (radially symmetric) are only 16% of the total diatoms found. Total 18 genera of diatoms were found at Vindhyachal ghat out of which only 3 were centrales and rest 15 were pennales.

Table 3. Specific Diatoms genera at sites of Ganga river at Vindhyachal.

Sr. No.	Sites	Genera
1.	Pakka ghat (S1)	<ul style="list-style-type: none"> • Nitzschia • Cyclotella • Aulacoseira • Surirella • Anomoeoneis • Odontidium
2.	Babu ghat (S2)	Fragilaria Tryblionella
3.	Diwan ghat (S3)	Pinnularia

DISCUSSION

Diatoms are highly unicellular, microscopic, photosynthetic algae. They are found in water bodies. The most distinctive features of this unicellular organism are its extracellular coat or frustules, which is composed of silica. Diatoms can be picked up from anywhere, where there is a water body including seas, lakes, some domestic water supplies, even from moist surfaces or some marine plants and tree bark. The main goal of identification

of diatoms in this field is to differentiate a death by submersion from an immersion of a body. Silica based skeletons do not readily decay and they can sometimes be detected even in heavily decomposed bodies. On the basis of their unique features like that raphe, cell wall diameter and shape were examined for their identification Metzeltinet *al.* (2009)¹⁰. In the present study diatoms of Ganga River from Vindhyachal Ghat were identified to be used as indicators of drowning and dumping sites. Diatoms provide a freely ecological assessment of forensic evidence in suspected in dumping and drowning cases, linking crime scene locations, sites of accidental or suicide. Only a short-term (e.g. the perpetrator leaving a crime scene) contact between clothing and water body can cause significant transfers of diatoms, which could be collected for analysis in a criminal investigation Scott *et al.* (2014)¹¹. In this study a total 18 diatom genera were identified. The work performed in this study was discussed in accordance with the work performed earlier by Ludes *et al.* (1999)¹² for continuous river monitoring of diatoms in the diagnosis of drowning for diatom extraction. With the help of this work, we can also identify the site-specific diatoms which are present at some particular sites which can also help to justify the drowning sites. Pollanen *et al.* (1997)¹³ It is seen that common diatoms were found at all the sites. It shows that there are few variations in diatom at nearby areas. This study is in lined with the research work performed by Mishra and Kumar (2017)¹⁴ where diatoms were used for site specification in Yamuna River at Delhi. The individual species or the species composition in forensic sample can provide important information about the habitat or location at a particular time of a year by Williams and Kociolek (2003)¹⁵.

SUMMARY

In this study entitled "Identification of Diatom Flora from Ganga River at Vindhyachal for Forensic Consideration" was carried out in the Department of Forensic Science Laboratory, SHUATS, Prayagraj, with objective "To extract

and identify diatoms from water samples of Ganga River at Vindhyachal from three different sites."

Water samples were collected from 3 different site of Ganga River at Vindhyachal , water samples were collected from each selected site from January to March 2022. Collected water sample were treated for the digestion by using acid-digestion method.

4-5 drops of Lugol Iodine solution was added to the water samples, after that it was left overnight so that the impurities get digested. Diatoms are the unique genera which is undissolved because of their outer layer of silica Ludes *et al.* (1996)¹⁶. Next day, water sample which was mixed by formalin solution would be stunned strongly and relocated into a clean 500ml beaker and then 50ml of Conc. HNO₃ was added to 500ml water sample in the beaker. Now the sample was centrifuged at 1000rpm for 10 minutes to get pellets. Then the microscopic slides were prepared by spreading the thin layer of pellets on slide and then heated on hot plate. The slide is now ready for further observation. After that, diatoms were observed at 10X, 45X and 100X magnification. All observed diatom were identified and matched by "Diatom Data Bank of North America".

After the laboratory analysis total 18 genera were identified in Ganga River at Vindhyachal from 3 sites that is Pakka ghat, Babu ghat, Diwan ghat. Out of which 4 diatoms were common at all 3 sites. They are Synedra, Bacillaria, Fragilariforma, Pseudostaurosira. Pakka ghat had specific diatoms: Nitzschia, Cyclotella, Aulacoseira, Surirella, Anomoeoneis and Odontidium.

Babu ghat had specific diatoms: Fragilaria and Tryblionella. Diwan ghat had unique diatom: Pinnularia.

CONCLUSION

This study concluded that after extraction and identification total 18 diatoms genera were identified from all 3 different sites of Ganga River at Vindhyachal, acid digestion method

is suitable for the extraction and isolation of diatom flora. Out of which 9 diatoms genera are site-specific and 4 common diatom genera were identified from all 3 different sites, which can be utilized for examination, evaluation and identification of drowning death cases,

This work confirmed that the specific diatoms population is useful in determining anti- mortem or post-mortem submersion of the decomposing body and its decomposing phase if aquatic medium or any crime which is related to submersion in water in Ganga river at Vindhyaalocccoured.

Diatom study can be useful in medico legal purposes to solve the cases of unclaimed drowned or dumped body. Diatom finding from the water as well as biological sample provide a lot of information about the cause of death, locality and probable season also.

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ETHICAL CLEARANCE: Not Required.

REFERENCES

1. Round, F.E., Crawford, R.M. and Mann, D.G., *The Diatoms: Biology and Morphology of the Genera*. Cambridge University Press, Cambridge 1990; 747.
2. Ruhland, Kathleen, John P. Smol, JP Paul Jasinski, and Barry G. Warner. Response of diatoms and other siliceous indicators to the developmental history of a peatland in the Tiksi Forest, Siberia, Russia. *Arctic, Antarctic, and Alpine Research* 2000; 32 (2): 167-178.
3. Horton, B.P., and Boreham, S., and Hiller, C., *The development and application of diatoms based quantitative reconstruction technique in Forensic Science*. *Journal of Forensic Science* 2006; 51: 643- 650.
4. Peabody, A.J., *Diatom in Forensic Science*. *Journal of Forensic Science Society* 1977; 17-81
5. Krstic, S., Duma, A., Janevski, B., Levkov, Z. and Nikolova, K., *Diatoms in Forensic expertise of drowning-a Macedonian Experience*. *Forensic Science International* 2002; 127: 198-203.
6. Pollanen, M.S., Cheung, L. and Chiasson, D.A., *The diagnostic value of the diatom test for drowning: I. Utility: a retrospective analysis of 771 cases of drowning in Ontario, Canada*. *Journal of Forensic Sciences* 1997; 42: 281- 285.
7. Mishra, V. and Goyal, M.K., *Diatom Fingerprinting to Ascertain Death in Drowning Cases*. *Journal of Forensic Research* 2014; 4: 207
8. Ludes, B., Coste, M., North, Doray, S., Tracqui, A. and Kiatz, P., *Diatom analysis in victim's tissues as an indicator of the site of drowning*. *International Journal of Legal Medicine* 1999; 112: 163-166.
9. Taylor, J.C., Janse, M.S. and Pieterse, A.J.H., *The application and testing of diatom based indices in the Vaal and Wilge Rivers, South Africa* 2007; (1): 51-60.
10. Metzeltin, D., Lange-Bertalot, H. and Soninkhishig, N., *Diatoms in Mongolia*. *Iconographia Diatomologica* 2009; 20: 1-686.
11. Scott, K.R., Morgan, R.M., Jones, V.J., and Cameron, N.G., *The transferability of diatoms to clothing and the methods appropriate for their collection and analysis in forensic geosciences*. *Forensic Science International* 2014; 241: 127-137.
12. Ludes, B., Coste, M., North, Doray, S., Tracqui, A. and Kiatz, P., *Diatom analysis in victim's tissues as an indicator of the site of drowning*. *International Journal of Legal Medicine*, 1999; 112: 163-166.
13. Pollanen, M.S., Cheung, L. and Chiasson, D.A., *The diagnostic value of the diatom test for drowning: I. Utility: a retrospective analysis of 771 cases of drowning in Ontario, Canada*. *Journal of Forensic Sciences* 1997; 42: 281- 285.
14. Mishra, V. and Goyal, M.K., *Diatom Fingerprinting to Ascertain Death in Drowning Cases*. *Journal of Forensic Research* 2014; 4: 207
15. Levkov, Z., Williams, D.M., Nikolovska, D., Tofilovska, S. and Cakar, Z., *The use of diatoms in forensic science: advantages and limitations of the diatom test in cases of drowning*. *Geological Society of London* 2017; 261-277.
16. Ludes, B., Coste, M., North, Doray, S., Tracqui, A. and Kiatz, P., *Diatom analysis in victim's tissues as an indicator of the site of drowning*. *International Journal of Legal Medicine* 1999; 112: 163-166.