

Identification of Diatom Genera at Different Ghats of Saryu River at Ayodhya Uttar Pradesh for Forensic Consideration

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ABSTRACT

Diatoms are unicellular, photosynthetic (autotrophic), eukaryotic algae. They belong to class Bacillariophyceae. In this work, 18 water samples were collected (3 from each site) from 6 different Ghats of Ayodhya i.e. Guptar Ghat, Raj Ghat, Ram Ghat, Lakshman Ghat, Janki Ghat, Naya Ghat. Water samples were collected from the surface of water. Following research, a total of 27 genera of diatoms were found. These diatom genera had significant ecological importance and were also crucial to criminal investigations involving post-mortem or anti-mortem drowning. Apart from 27 genera identification few diatom genera were found as site specific, these are *Cymbella*, *Gyrosigma*, *Surirella*, *Fragilaria virescens*, *Adalfia*, *Navicula*, *Brachysira*, *Anomoeoneis*, *Frauenfeldii*.

KEYWORDS: Diatoms, Saryu River, Ayodhya Drowning, Death, Acid Digestion, Postmortem, Anti Mortem, Forensic.

INTRODUCTION

The diatoms (algae) belonging to class bacillariophyceae, are unicellular algae present in aquatic environment (rivers, ponds, reservoirs, etc.) which play a pivotal role in forensic field. They are non-motile or show restricted movement through mucilaginous secretion from slits called raphe along the substrate they thrive on. Diatoms vary in sizes ranging from 20-200 μm to 2 millimeters. There are more than 20 genera and 100,000 species of diatom that are known till date. (Round *et al.*, 1990)[1]. Diatoms are important as supporting evidence in drowning cases, according to numerous studies (Horton *et al.*, 2006)[2].

The body of diatom is covered by cell wall made up of silica and is called frustule. This silicate cell wall consists of overlapping halves or two valves out of which the larger/older valve, called epitheca overlaps the smaller/younger valve called hypotheca. The girdle is made up of one or several connective bands of epicingulum and hypocingulum, frustules can have a wide range of symmetrical or asymmetrical shapes. The primary concept behind the "Diatom test" in drowning cases is centered on the association between the occurrence of diatoms in the area where a drowning may have occurred and the fact that diatoms

enter the bloodstream and alveoli when people breathe in water. These diatoms then get accumulated in various organs like brain, liver, among other organs. Hard bones from drowned individuals, such as the femur and sternum, and soft tissues, such as the organs like lungs, are usually sent to the laboratory for their forensic examination for diatom identification in drowning instances. Diatom communities are a well-liked tool for tracking both current and historical environmental conditions, and they were extensively used in water quality research. Due to the presence of silica cell-wall, they are resistant towards enzymes.

When drowning victims breathe in water, diatoms enter their respiratory system and bloodstream, which causes them to enter other body organs like bone marrow, liver brain, lungs, etc. (Gruspier and Pollanen, 2000)[3].

LOCATION

Saryu River is located at Ayodhya, Faizabad (U.P.) with coordinates 26.807096 latitude and 82.163520 longitude.

MATERIALS AND METHOD

The following method was used for the extraction and identification of diatom comparisons.

Collection of water sample

Total 18 water samples were collected from different ghats (Guptar Ghat, Raj Ghat, Ram Ghat, Lakshman Ghat, Janki Ghat and Naya Ghat). The water samples were collected from the surface water at a depth of one foot on both banks of the river and in the middle of the river. Before collecting samples in plastic bottles, they were thoroughly rinsed with the same water mixture two to five times. Following a thorough wash, 500ml of water samples was collected in each bottle.

Extraction of diatoms from water sample

The water samples collected from different ghats of Saryu River at Ayodhya were then brought to laboratory for extraction of diatoms. The process of extraction of diatoms from water involves the following. From each bottle 500 ml water was taken in a beaker and 4-5 drops of Lugol's iodine was added to it. Lugol's iodine was used as a preservative and the samples water was left overnight. (Ludes *et al.*, 1999)[4].

50ml conc. HNO_3 was then added in the water sample. Conc. HNO_3 oxidizes the organic matter present in the water sample except the diatoms because the diatoms cell wall is resistant to them. After that a few ml of digested water sample were taken from a beaker and transferred into tarson tubes. The water sample was centrifuged at 1000–1500 rpm for 10 minutes, then the pellets were obtained by discarding the supernatant. The procedure was then repeated 2-3 more times until the entire water sample contained in the beaker was centrifuge to obtain all of the pellets.

Preparation of Microscopic slide

For the examination of diatoms a slide was prepared to be studied under the microscope. For this purpose, the deponent pellets were taken out from the bottom of the centrifuge tube using a dropper and a drop was placed on a microscopic glass slide. Thereafter, the prepared slides were kept on hot plate maintained at 50-60°C for drying for 2-3 minutes. To fix the contents on the slide, a drop of the mounting medium, DPX, was added on the slide and a cover slip was gently placed over it to seal it.

After fixing the slides, they were examined under microscope at three different magnifications: 10X, 45X and 100X (oil immersion) (Taylor *et al.*, 2007)[5].

Identification of diatoms

For identifying the diatoms, images from the microscopic slide were collected, and their morphological structure was compared with the common diatoms database of North America.

RESULTS AND DISCUSSION

After collection, extraction and examination of diatom at 6 Ghats of Saryu River at Ayodhya. Total 27 diatoms genera were identified by comparing morphological features with diatom database of U.S (Diatoms of America).

Table 1: Total diatom found six Ghats of Saryu River

S.No	Genera
	<i>Brachysira</i>
	<i>Nitzschia</i>
	<i>Thalassiothrix</i>
	<i>Bacillaria</i>
	<i>Cyclotella</i>
	<i>Frauenfeldii</i>
	<i>Pseudotaursoir</i>
	<i>Melosera</i>
	<i>Craticulacitrus</i>
	<i>Pleurosigma</i>
	<i>Tabulaia</i>
	<i>Neofragilaria,</i>
	<i>Cymbella</i>
	<i>Gyrosigma</i>
	<i>Adlafia</i>
	<i>navicula</i>
	<i>Fragilariaspp</i>
	<i>Cyclostephanos</i>
	<i>Stephanocyclus</i>
	<i>Thalassionemanitzschioids</i>
	<i>Nitzschia</i>
	<i>Surirella</i>
	<i>Crapedostauros</i>
	<i>Anomoeoneis</i>
	<i>Thalassiosira</i>
	<i>Fragilariavirescens</i>
	<i>Eucoconeis</i>

Out of 27 genera of diatom, 17 genera of diatom were found at all sites.

Table 2: Common diatom genera were found at all site of Saryu River

S.No	Genera
1.	<i>Nitzschia</i>
2.	<i>Bacillaria</i>
3.	<i>Cyclotella</i>
4.	<i>Thalassiotherix</i>
5.	<i>Melosera</i>
6.	<i>Craticulacitrus,</i>
7.	<i>Tabularia,</i>
8.	<i>Neofragilaria</i>
9.	<i>Fragilariaspp</i>
10.	<i>Cyclostephano,</i>
11.	<i>Thalassionema nitzschioides</i>
12.	<i>Stephanocyclu</i>
13.	<i>Nitzschiaexilli</i>
14.	<i>Crapedostauros</i>
15.	<i>Thalassiosira</i>
16.	<i>Eucocconeis</i>
17.	<i>Pseudostaurosira</i>

Table 3: Site specific diatom genera found at six Ghats of river

S.No	Sites	Genera
	S1	<i>Cymbella, Gyrosigma</i>
	S2	<i>Pleurosigma, Surirella</i>
	S3	<i>Fragilaria virescens</i>
	S4	<i>Adlafia , Navicula</i>
	S5	<i>Brachysira, anomoeoneis</i>
	S6	<i>Frauenfeldii</i>

10 site specific diatom genera were found at different site of Saryu. Their characteristics identification features such as Raphe, diameter of cell wall and shape were observed and recorded for establishing their identity. They were then compared and matched with the standard online database of diatoms.

DISCUSSION

Diatoms are unicellular, microscopic, photosynthetic algae, which are found in all aquatic water bodies. The extra-cellular coat or frustules (made up of silica) of these single celled organisms is its most distinguishing feature. Diatoms may be collected from any water body which has running or stagnant water, salty or fresh water and even from some domestic water supplies, marine plants or barks of trees. The major objective of identifying diatoms in this study was to find out variation in diatom genera of

different Ghats or places of River. Skeletons of these diatoms are made of silica and hence do not easily disintegrate, and they can occasionally be found in severely degraded bodies. On the basis of their unique features like raphe, cell wall diameter and shape were examined for their identification (**Metzeltin et al., 2009**)[6]. Diatoms link crime scene locations, sites of accidental or suicide deaths, and enable a fully ecological review of forensic evidence in suspected dumping and drowning instances. The present work of research aimed to identify diatoms of Saryu River from water samples collected from Ghats of Ayodhya and to use them for site characterization. In this regard, diatoms were identified and classified in which 17 diatom types belonged to common genera and 10 were site specific. It's due to change in water quality, temperature salinity, pH, etc. maybe pollution level represented by **Ludes et al., (1999)**[7] continuous river monitoring. Diatoms extracted from the water sample by using the acid digestion method (**Pollanen et al., 1997**)[8] and examination under light microscope at different magnification power that is 45X and 100X. This study is in lined with the research work performed by **Mishra and Kumar (2017)**[9] where diatoms were used for site specification in Yamuna River at Delhi. The abundance of diatom genera varies in various sites of river and sites specific diatoms were used for locating of drowning or dumping by **Williams and Kociolek (2003)**[10] which can also help to justify the drowning sites. **Chepurnov et al., (2008)**[11] in their study showed that some species of diatoms have a restricted distribution as they are found only in particular area and thus are considered to be endemic. In situations when drowning is suspected, site-specific diatoms might be utilised as a marker to identify the scene. The studies made by **Scott et al., (2013)**[12] reported site specific diatoms in their studies in which they compared the number, nature and distribution of the diatoms they observed in the tissue samples.

SUMMARY

After the examining the diatoms in laboratory, total 27 genera were identified in the samples collected from Saryu River flowing through six Ghats of Ayodhya- Guptar ghat, Ram ghat, Naya ghat, Janki ghat, Lakshman ghat and Raj ghat. Out of all diatoms that were identified, 17 diatoms were common at all sites.

CONCLUSION

From this study it was concluded that after extraction and identification total 27 diatoms were identified. Diatom genera were identified from all 6 different Ghats of Saryu River at Ayodhya. 10 diatom genera were found as site specific at all 6 site that and is *Cybella*, *Gyrosigma*, *Pleurosigma*, *surirella*, *Fragilaraviresens*, *Adalfi*, *Navicula*, *Brachysirma Anonooneis* *Frauenfeldii*. These specific diatom genera can be used for characterization of site in cases of suspected drowning and dumping cases. Diatom diversity can be used for the preparation of diatom data bank as well as for medico legal purposes and for other area of research.

CONFLICT OF INTEREST: NIL

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ETHICAL CLEARANCE: Not required.

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