

■ ORIGINAL ARTICLE

## Forensic Database Management of Diatoms: A Tool for Comparison

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### ABSTRACT

**CONTEXT:** In forensic analysis diatoms plays an important role. Their structure and morphology is widely used to identify the location. Since their species are varied in different geographical locations. Therefore, can be used for diagnosing the suspected drowning and dumping cases.

**AIMS:** The study portrays the identification of different species of diatoms found in different geographical locations of Punjab.

**SETTINGS AND DESIGN:** Experimental study design was adopted to isolate and identify the different species of diatoms collected from different district of Punjab.

**MATERIALS & METHOD:** A total of 36 water samples from 18 different district in Punjab were collected. Each sample was of 500-1000 ml in quantity subjected to proper legend labeling of venue, time and date was procured followed by isolation using Acid digestion method for identification.

**RESULTS:** Different species of diatoms such as *Cylindrotheca Closterium*, *Triceratium*, *Haslestenopteroberia*, *Tabulariavariostriata*, *Tabellaria*, *Vulgariabory*, *Semiorbis*, *Fragalariforma*, *Distriionella*, *Diprorahaenaensis*, *Hantzschiaamphioxyz*, *Pinnularia*, *Peronia fibula*, *Plagiotropis*, *Pseudostaurosira*, *Cyclotella*, *Melosiraundulata*, *Aulacoseira*, *Stenopteroberia*, *Stauroneisaugustilanacea*, *Craspedastaurus*, *Achnanthes lanceolata* were isolated with the help of acid digestion method and were identified by keeping diatoms of North America as a reference standard.

**CONCLUSIONS:** Diatom species have been identified as indicators of drowning and dumping locations for the sake of criminal inquiry. Morphological analysis aids in the detection of the location where a body is discovered. Hence, site-specific diatoms from the Punjab region can help identify geographical locations.

**KEY MESSAGE:** In forensic science, diatom analysis is a useful tool for diagnosing suspected death by drowning cases. Diatoms at every few kilometers within a single water body changes thus making it easier as a shred of evidence in the identification of the exact location where the body drowned.

**KEYWORDS** | diatom, drowning, frustule, trace evidence

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### INTRODUCTION

**D**IATOMS ARE AN IMPORTANT BIOLOGICAL evidence, and gives information of forensic importance. Drowning deaths found to be the third most accidental death known so far. Due to their numerous species

they not only help in the direct conclusion of the cause of death, but it can also locate the location of a possible drowning site. The silical framework of diatoms do not decay readily, so they aid in diagnosing heavily putrefied bodies.

Specifically, in drowning cases the utilization of diatom testing from diatomaceous remnants seen on clothing and footwear of victim helps to identify the type of specie. To be more precise the molecular methods like DNA sequencing prompt forensic experts and law enforcement agencies to identify and study the species of diatom with the help of genetic markers.

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#### METHOD

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**Materials used:** Air tight containers, Microscopic slides, Coverslips, Lugol's iodine, Nitric acid, dropper, Immersion oil, Compound Microscope.

Different water bodies of Punjab region such as Patiala Beas, Gurdaspur Beas, Barnala Kotla canal, Nawanshahr Beas, Amrit Sarovar Lake, Ludhiana Sutlej river, Veyin Hoshiarpur, Gurdaspur Chakki, Mohali Sukhna lake, Pathankot Chakki, Muktsar Nangal Hydell, Mansa Manas river, Ropar Nawanshahr, Firozpur Harika Lake, Rupnagar Ropar Lake, Fatehgarh Sahib Sheesh Mahal pond, Kapurthala Kanjili were used for collection of water sample (1500-1000 ml approx.)

**Sample collection:** For the research of Diatom flora, two water samples were obtained from each selected site, totalling 36 samples from 18 districts in Punjab. Plastic bottles or containers were cleansed with distilled water at least 2-3 times before being collected. After cleaning the bottle, diatom-containing samples were collected in bottles containing 500-1000 ml from the designated sites, and the containers were tightly sealed with appropriate covers and labelled with the date and place of collection.<sup>35</sup>

**Extraction and isolation:** To extract and isolate diatoms from water samples, first apply 2, 3 drops of 2% Formalin solution to bottles containing water samples to limit diatom growth, then leave it overnight or for 4 hours for settlement. The next day, discard half of the water without shaking it, then vigorously shake it and pour it into a 500 mL beaker. 1-2

drops of Lugol's iodine solution are added, and the beaker is covered with aluminium foil and let to settle overnight. In the beaker containing the water sample, add 4-5 drops of strong Nitric acid (HNO<sub>3</sub>) to breakdown the organic materials found in the diatom cell wall, which is naturally resistant. These samples were kept undisturbed for two hours. It is then transferred to properly labeled tarson tubes and centrifuged for 10 minutes at 1500 rpm. The supernatant was pipette off leaving behind only a pellet at the bottom of the tube containing diatom frustules. To remove all traces of acid, pellet material was suspended in distilled water and centrifuged twice.<sup>35</sup>

**Microscopic Examination:** The pellets were placed on a microscopic slide and allowed to dry for a few minutes before being examined using a compound microscope at 10X, 45X, and 100X magnification. This process was repeated for all of the samples for Diatom examination and identification.<sup>35</sup>

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#### RESULTS

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After the isolation microscopic examination of diatoms following morphological features were identified. On the basis of identification features the species were named and marked.

##### **Cylindrothecaclosterium**

Belongs to Nitzschioid category, Frustules were narrow, elongated with drawn out ends, twisted, and very lightly silicified. Cells are elongated, solitary and are characteristically thin like needle. The valves including the keel and raphe canal wrap around each other forming a twisted frustule.

##### **Triceratium**

Cells are attached or free-living. Valve view is triangle in shape and girdle is tapered and oblong, corners are elevated and a projection at the center. Valves are shallow and ornamented with branched spines.

##### **Haslea Stenopterobia**

The valves of Stenopterobia are tapered and found to be sigmoid or straight in outline. The raphe is positioned inside a canal with the valve

Water Bodies	Location
Bathinda lake	Bathinda
Buddha nullah	Malwa
Harike lake	Firozpur
Kali Bein	Hoshiarpur
Mansa River	Mansa
Ropar lake	Ropar
Sirhind canal	Chandigarh
Sukhana lake	Chandigarh

Table 1- Different water bodies of the Punjab region were taken into account for sample collection.

margin. The canal is raised onto a keel above the valve.

#### **Tabularia**

Valves with lanceolate, acute apices and a central raphe system. Two or three thickened trans apical costae on either side of the valve center form a narrow fascia. The raphe sternum is thickened on one side of the axial area and is fused to the central ribs, give valves a fusiform appearance.

#### **Tabellaria**

The valves are elongated and capitate at the ends. The center of the valve is usually wider than the ends. In the center of the valve face, there is a rimoportula. Septae can be seen on copulae in abundance. Pseudosepta may be present as well. Mucilage pads connect zigzag colonies of cells.

#### **Vulgare Bory**

Frustules are heterovalvar, that is, raphe is present in one valve, while the other one lacks a raphe. Species of Vulgare are generally small in size, with narrow valves. The shape of the valve differ by species, but the ends may be rounded, capitate or rostrate. Striae usually uniseriate.

#### **Pinnularia**

Frustules of pinnularia are large, about 300um long and alveolar shaped striae. Internally, striae are confined in chambers. The elongated lines crossing the striae clearly shows the openings

of chambers. The raphe found to be straight or fused. Raphe is enlarged and bent externally.

#### **Fragalariforma**

Valve margins vary in shape, being lanceolate, elliptical, or linear. Frustules are rectangular in shape when viewed from the girdle. The center section of valves is either very narrow or missing. Striae are made up of separate uniseriate aerolae.

#### **Peronia fibula**

Peronia is heterovalvar and heteropolar. Valves have a tapered, wider headpole and a round footed pole. The headpole is round, while the footpole is narrow and rounded. Heterovalvar valves in which one valve has a raphe that elongates from each pole.

#### **Plagiotropis**

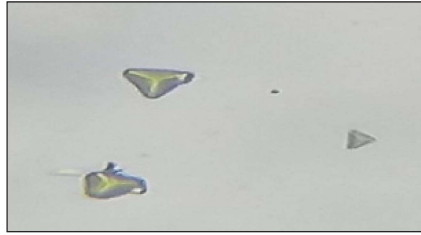
The valve margins are lanceolate in shape with narrow poles. Valve is folded on both sides. The raphe is positioned within a keel and itself raised above the valve face. The central area is variable in shape and axial area is narrow.

#### **Pseudostaurosi Rabraevistriata**

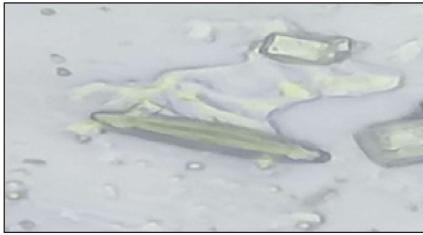
Valves are elliptical in smaller specimens and valve ends are round in shape. Face of the valve is flat and a gradual progression between the mantle and the valve face. The axial area is broadly lanceolate. Frustules are rectangular in shape connected by spines. Striae are small, visible, extending into the mantle, oval to round



Cylindrotheca Closterium  
Site 1: Patiala Beas



Triceratium  
Site 2: Gurdaspur Beas



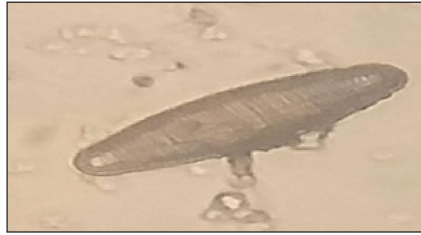
Haslea stenopteroibia  
Site 3: Barnala Kotla Canal



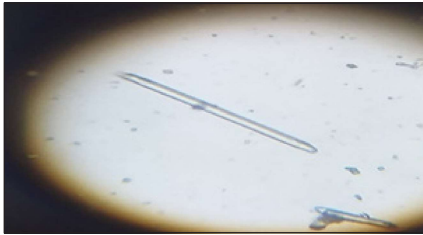
Tabularia variostrata  
Site 4: Nawanshahr Beas



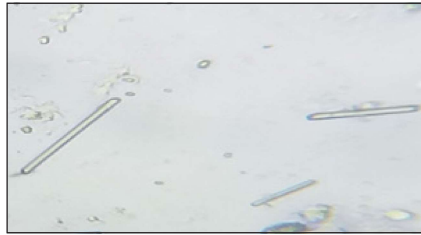
Tabellaria  
Site 5: Amrit Saravor Lake



Vulgare bory  
Site 6: Ludhiana Sutlej



Pinnularia  
Site 7: Veyn Hoshiarpur



Fragalariforma  
Site 7: Veyn Hoshiarpur

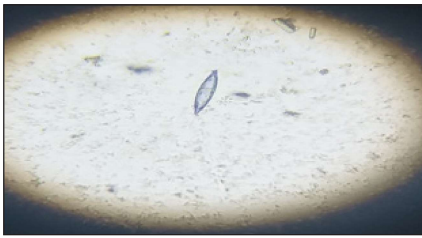


Cyclophora tenuis  
Site 8: Gurdaspur Chakki

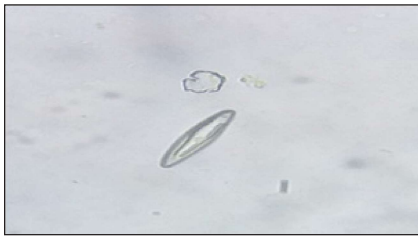


Peronia fibula  
Site 9: Bathinda Sirhind Canal

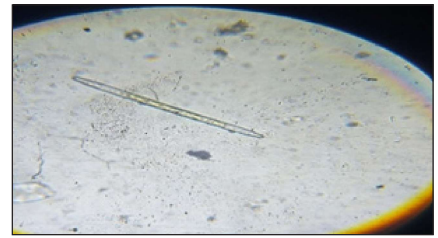




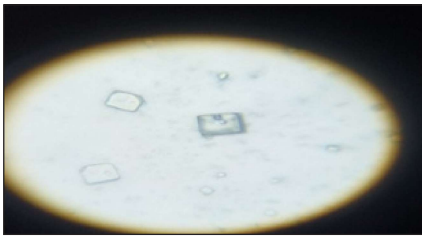
Plagiotropis  
Site 9: Bathinda Sirhind Canal



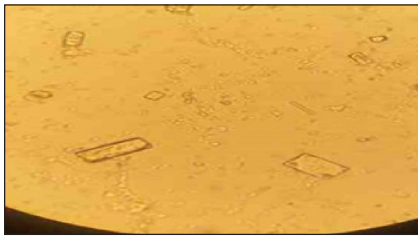
1) Pseudostaurosira  
Site 10: Mohali Sukhna lake



2) Craspedastauros  
Site 10: Mohali Sukhna Lake



1 Triceratium favis  
Site 11: Pathankot Chakki



2 Melosira undulate  
Site 11: Pathankot Chakki



Cyclotella  
Site 12: Mukstar Nangal Hydrel



Aulacoseira ambigua  
Site 13: Mansa Manas River



Achnanthes lanceolata  
Site 14: Ropar Nawan Shahr



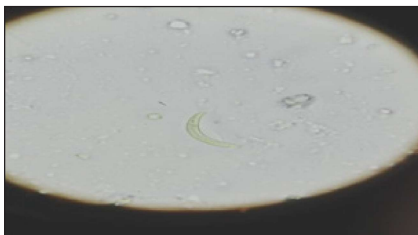
Stenopterobia  
Site 15: Firozpur Harika Lake



Stauroneis augustilancea  
Site 16: Rupnagar Ropar Lake



Diprora haenensis  
Site 17: Fategarh Sahib Sheesh Mahal pond



Semiorbis  
Site 18: Kapurthala Kanjili

aerolae, parallel to radiate near the valve's center to mildly radiate towards the valve's end Costae is a broad term. Except at the apices, spines can be seen along the valve face edge and are always found on the striae. The valve mantle has apical circular fields with round poroids close to the valve face. Copulae (girdle view) are open and lack perforations.

#### **Craspedastaurus**

Valves of Craspedastaurus are linear, with a slenderedstaurus or fascia. Frustules are narrow in girdle around the central staurus. Cells often present in girdle view, because of the extensive girdle. Numerous girdle bands are present.

#### **Distrionella**

Cells are attached or free-living. Valve view is triangle in shape and girdle is tapered and oblong, corners are elevated and a projection at the center. Valves are shallow and ornamented with branched spines.

#### **Melosira Undulate**

It has large, cylindrical shaped frustules linked in chains. Valves and mantles are distinctly enhanced with striae and rimoportulae. A ring of evenly positioned rimoportula encloses near the mantle valve. The mantle is thickened unevenly.

#### **Cyclotella**

Valves are large with a tangentially undulate valve face. Various central fuloportulae are present. Multiseriate striae are present.

#### **Aulacoseiraambigua**

Valves are 3-12um in diameter, and 5-15um mantle height. The ratio of the height of the mantle to diameter of the valve is greater than 1. The helical rows of the mantle aerolae are curved. Spines are positioned at the perivalvar costae ends. Linking spines are and short and triangular.

#### **Achnanthes lanceolata**

Frustules possess 2 valve in which raphe is present in one and other lacks raphe. Hence it is heterovalvar. It possess very small and tapered valves. Valve shape may differ depends on species. May be round or rostrate.

#### **Stenopterobia**

The valves of Stenopterobia are tapered and

found to be sigmoid or straight in outline. The raphe is positioned inside a canal with the valve margin. The canal is raised onto a keel above the valve.

#### **Stauroneis augustilanacea**

Stauroneis has solitary cells. It has two chloroplasts, on each side against the cingulum. Valves are lanceolate to linear to elliptic lanceolate. The central area is an eminent trans fascia known called a "staurus". The typical fascia elongates to the margin of the valves.

#### **Semiorbis**

Valves are thick and transverse. Costae is present externally between the striae. The costae often ends in spines at ventral and dorsal margins. Raphe, which is small is present adjacent to the poles. The proximal raphe ends are on ventral margin.

#### **Diprorahaenaensis**

Genus Diprora is monotypic (contain a single species). The taxon is somewhat similar to marine taxon, hyalinella. Frustules typically with a concave and convex valve. Valves are round with broad ends. The smallest valves are nearly circular. Presence of single row of pores along the valve margin. Frustules are joined into filaments. The valve filament is extended to form apical prows, a feature visible in girdle view.

#### **Hantzschia amphioxz**

The ratio of the height of the mantle to diameter of the valve is less than 1. The helical rows of the mantle aerolae are elongated.

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#### **CONCLUSION**

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In present study, a total of 22 species were identified from different locations of Punjab. The genera identified are *Cylindrothecaclosterium*, *Triceratium*, *Haslestenopterobia*, *Tabulariava-riostriata*, *Tabellaria*, *Vulgariabory*, *Semiorbis*, *Fragalariforma*, *Distrionella*, *Diprorahaenaensis*, *Hantzschiaamphioxz*, *Pinnularia*, *Peronia fibula*, *Plagiotropis*, *Pseudostaurosira*, *Cyclotella*, *Melosiraundulata*, *Aulacoseira*, *Stenopterobia*, *Stauroneis augustilanacea*, *Craspedastaurus*, *Achnanthes lanceolata*. These species have been identified morphologically

using Diatoms of North America as a reference. Diatom species have been identified as indicators of drowning and dumping locations for the sake of criminal inquiry. Morphological analysis aids in the detection of the location where a body has been discovered. It can also be useful to identifying the site based on the morphological characteristics of the Diatom Flora; site-specific diatoms from the Punjab region aid in the recognition and identification of the site in the event of drowning or dumping. This study is extremely useful for medico-legal applications, such as unclaimed bodies in cases of disputed drowning and dumping, as well as location correlation and identification in cases of uncertainty.<sup>8</sup>

The major job of forensics is to ascertain the cause of death, such as whether the deceased drowned or if the body was put into the water after death. However, because of variables such as chewing by fish or worms in the water, as well as corpse decomposition, dead bodies in water often lack the typical signs of drowning, making drowning diagnosis extremely difficult.

The diatom test is based on the fact that when a person drowns, diatom enters the lungs through inhalation of any liquid. They may penetrate the wall of lungs, and if the cardiovascular is efficient it will carry them to

other internal organs (heart, kidney, liver, etc.) where they will remain. Although some diatoms may still be present in the lungs if a person is already dead before entering the water, they will not be present in any other internal organ, and the cause of death may be something other than drowning. Examination of lungs and other internal organs for the presence of diatoms therefore yield supporting evidence for drowning, if diatom valves are found.<sup>28</sup>

Diatoms are naturally occurring, so its persistence and transfer of particulates to an evidential surface such as clothing or footwear, can impart valuable evidence to differentiate between suspect or victim and a crime scene. Diatom utility in anthropogenic by products such as pesticides, filters, paints and construction materials, mining of fossil diatom deposits, presents additional probability for presence and transfer of diatoms in Forensic samples.<sup>20</sup> **IJFMP**

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The authors declare that there is no commercial or financial links that could be construed as conflict of interests.

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