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Identification of diatom flora of Garra River at Shahjahanpur for forensic consideration

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Abstract
Diatom is a unicellular, photosynthetic (autotrophic) alga, that has most distinctive features of crystalline extra cellular coat or frustules composed of silica and having unique patterns of symmetry and micro structure draws the attention of researchers worldwide. In this paper, identification of diatom flora of Garra River at Shahjahanpur, a small district in Uttar Pradesh India has been made from collected water samples at prone areas of drowning cases. For this purpose, 12 water samples were collected (4 at each site) in the month of January, February and March 2020 from different sites i.e. garra, hanumat dham, ram Ganga. Total 18 species of diatom were identified, in which 5 species were site specific. The diverse, asexually-reproducing organisms occur in a great variety of colors, sizes and shapes, most being unicellular but some forming simple colonies allows for their study to be of great use in certain legal investigations.

Keywords: diatom, forensic science, morphological analysis, drowning, legal investigation

Introduction
Forensic Science is a multidisciplinary field; it includes various fields of science such as chemistry, biology, physics, geology, psychology, social science, engineering, etc. Among them, among them, the widely growing field is Forensic limnology, a sub-field of forensic botany, which is the study of freshwater ecology in a legal scenario, basically the presence of diatoms in crime scene samples and victims. Diatoms are unicellular, eukaryotic oxygen- synthesizer, photosynthetic algae, bilaterally symmetrical, having siliceous (hydrated silicon oxide, a layer of organic material called *Frustules*,), (epitheca + hypotheca = frustules) walls causes' significant variety of distinguished color, shape, and sizes (approx. 2-200 micrometer/ 0.5 mm) Warner (1997), evolved some 180 million years ago. There are about more than 11,000 species of diatoms identified till now. Occurrence can be in aquatic environments, but also in soils and a range of other moist environments. Death by drowning is defined as a death due to submersion in a liquid. The mechanism in acute drowning is hypoxemia and irreversible cerebral anoxia. There are several criteria that have been used till now for the confirmation of drowning related cases. Diatom identification test is one of them. The basic principle is based on the fact that diatoms are present in that area where drowning took place. During inhalation, it get enters into the alveoli of the victim and then via circulatory system transports to different part of the body. Microscopic analysis of diatom can be used to ascertain the anti-mortem or post mortem of drowning death cases.

Methodology
Collection of Water Samples
To avoid contamination, glassware and bottles were thoroughly cleaned. 1000 ml of water samples were collected (4 at each site i.e. A1, A2 and A3), tightly closed and labelled with sampling site name, date, time and month respectively. Water sample were collected in the month of January, February and March year 2020 continuously. Water sample were collected at the bank, mid & across the river. The samples were collected from three prone sites of drowning at Garra River of Shahjahanpur.

Extraction Procedure
Collected water samples were brought to the laboratory for the extraction and isolation of the diatom. Before extraction and isolation of diatom from water sample add, 2-3 drop of 2% formalin solution (as preservative to prevent further growth of diatoms) was added and left overnight. Next day the upper half amount is discarded and after shaking the sample thoroughly and vigorously, transferred into a clean glass beaker.

Further, 2-5 drops of Lugol's iodine solution (settlement of diatoms in beaker) in water sample is added. Then, the beaker was covered with the aluminium foil and left overnight. After that, with the help, of dropper, water samples were transferred in a centrifuge tube, and then centrifuge it at 1500 rpm for 6-8 minutes. After centrifugation, pellet is obtained and supernatant was discarded.

Again, this process is repeated 3-4 times for obtaining the pellets in more amounts. After complete centrifugation, the obtained pellet were mixed with the conc. Nitric acid 2-3 drops and distilled water was added in centrifuge tube kept it 8-9 hrs without disturbance. After the completion of the above steps, the pellets were washed with the help of distilled water. Tyagi *et al.*, (1985) [10], Pollanen (1998) [6].

Slide preparation

The microscopic slides were prepared by taken the pellets which were deposited at the bottom of centrifuge tube with the help of dropper on microscopic slide. Slide was kept on hot plate at 25-35 ^{0}C for 2-3 min. for drying. Then, one drop of DPX is dropped on a slide, covered with cover slip and fixed. After that slide was placed under the microscope and observed at different magnification such as 10X, 40X and 100X (oil immersion) magnification respectively. Same procedure was repeated for all sample. Taylor *et al*., (2007) [7], Metzeltin *et al*., (2005) [4].

Results and Discussion

After microscopic examination at different magnification various species of diatom were identified i.e. mentioned in table 3.1.

Table 1: The Diatom species identified at all three sites (A1, A2, A3) of Garra River Shahjahanpur among months of January to March 2020.

S.No.	Name of Diatom genera	S.No.	Name of Diatom genera
1.	*Nitzschia paleavardeblis*	10.	*Cymbella affinis*
2.	*Achnanthidium reimeri*	11.	*Rhopalodia brebissonii*
3.	*Cyclotella rossii*	12.	*Melosera granulate*
4.	*Fragilariforma nitzschiodes*	13.	*Nupela impexiformis*
5.	*Fragilaria intermedia*	14.	*Fragilariforma nitzschiodes*
6.	*Aulacoseira granunlate*	15.	*Diploneis*
7.	*Synedra affinis*	16.	*Surirella amphioxys*
8.	*Nymphaea*	17.	*Triblionella calida*
9.	*Amphora pediculus*	18.	*Actinocyclus normanii*

Table 2: Peculiar species of diatom representing to Hanumat dham (A1), Shahjahanpur Region.

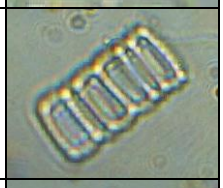
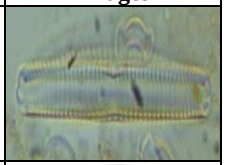
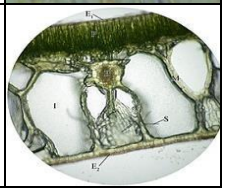
S.No.	Name of Genera	Images
1.	*Amphora veneta*	
2.	*Melosira varians*	
3.	*Triblionella calida*	

Table 3: Peculiar species of diatom representing to Ramganga (A3), Shahjahanpur.

S. No.	Name of genera	Images
1.	*Fragilariforma nitzschiodes*	
2.	*Nymphaea*	

In the present study, 18 diatom species were identified in all sites of Garra River at Shahjahanpur were identified in which species three was site.

Specific at Hanumat dham (A1) and two species was specific in Ramganga (A3) site. 13 species were common at three sites of Shahjahanpur.

Table 4: Identified species (A1, A2, A3) and their respective orders

S.No.	Name of Diatom genera.	Average no. of diatom observed from each site of Shahjahanpur		
	(A1)	(A2)	(A3)	
1.	*Nitzschia sp.*	*Nitzschia sp.*	*Nitzschia sp.*	Pennales
2.	*Achnanthidium sp.*	*Achnanthidium sp.*	*Achnanthidium sp.*	Pennales
3.	*Cyclotella sp.*	*Cyclotella sp.*	*Cyclotella sp.*	Centrales
4.	*Fragilariforma sp.*	*Fragilariforma sp.*	*Fragilariforma sp.*	Pennales
5.	*Fragilaria sp.*	*Fragilaria sp.*	*Fragilaria sp.*	Pennales
6.	*Aulucoseira sp.*	*Aulucoseira sp.*	*Aulucoseira sp.*	Pennales
7.	*Synedra sp.*	*Synedra sp.*	*Nymphia*	Pennales
8.	*Amphora sp.*	-	*Amphora sp.*	Pennales
9.	*Cymbella sp.*	*Cymbella sp.*	*Cymbella sp.*	Pennales
10.	*Rhopalodia sp.*	*Rhopalodia sp.*	*Rhopalodia sp.*	Pennales
11.	*Melosera sp.*	-	*Melosera sp.*	Centrales
12.	*Nupela sp.*	*Nupela sp.*	*Flagilariforma*	Pennales
13.	*Diploneis sp.*	*Diploneis sp.*	*Diploneis sp.*	Centrales
14.	*Surirella sp.*	*Surirella sp.*	*Surirella sp.*	Pennales
15.	*Triblionella sp.*	-	*Triblionella sp.*	Pennales
16.	*Actinocyclus sp.*	*Actinocyclus sp.*	*Actinocyclus sp.*	Centrales

Discussion

Diatoms are unicellular, eukaryotic, microscopic algae that draw attention of many researchers worldwide due to its unique morphological feature and site specific nature. Its occurrence is generally in water bodies therefore has significant place in the investigation of cases where bodies of individuals are submerged in water bodies, mainly in drowning and dumping cases and anti-mortem and post mortem cases. Diatom test is generally found useful corroborative test to identify the cause along with other factors. The potential to provide an independent ecological assessment of trace evidence make it even more useful. This study represents identification of diatom and observed data to provide a preliminary evidence base in order to be able to understand the nature of diatom.

The complete examination of different species throughout this study, 18 species of diatom was identified, in which 5 species were site specific. The complete work can be discussed as according to the correlation of the work carried out by the Garima *et al*., (2013) [3] reported that one or more than one water sample should be taken from the site of drowning. The study is done in each an every aspect of identification including quantitative, qualitative and morphological detail analysis of the collected samples. In the present research, different types of diatoms were identified on the basis of cell wall structure, color and size and distinction has been made. The diatom species shows the seasonal variation in different season, Mishra *et al*., (2014) [5].

Conclusion

From the present study, conclusion can be made that the alternation in diatom diversity at different sites of Garra River at Shahjahanpur region play the most significant role in the investigation of corpses which were obtained from the water bodies. The time since death can be calculated due to changes in seasonal variation. The location of drowning, and determination of suspect can be made due to site specific nature. Thorough examination of lungs and other internal organs for the presence of diatoms will therefore yield supporting evidence for drowning if diatom valves are found. Significantly, diatom test proved its worth in forensic science, medico legal aspects and in solving the drowning and dumping cases.

Future Aspects

Further studies can be done by considering the other factors that may affect diatom test and question its reliability, limitations and drawbacks to draw more appropriate conclusions. There is a lot more research can be done to explore new facts and findings, its future environmental change scenarios to preserve this unique ecosystem

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