Research Article

Diatom Fingerprinting to Ascertain Death in Drowning Cases

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Abstract

Diatoms are unicellular microorganisms which are commonly found in almost all water bodies. Their silica wall plays important tool in forensic diatomology. Diatom test has been widely applied to detect post mortem or antemortem drowning and comparing the diatoms found in biological sample with those found in water sample confirms that death took place, probably in same water medium. However, in skeletonized stage or dismemberment of body parts at different location, diatom of specific kind helps to link the place of drowning. Diatom fingerprinting was thus created studied for 2 years in water bodies of Haryana to differentiate commonly, least commonly found and seasonal diatoms. A total of 111 species and their 55 genera of diatoms were identified. Diatom genera which were commonly found at all the ten sites were *Achnanthes, Asterionella, Aulacoseira, Cyclotella, Gomphonema, Navicula, Nitzschia, Synedra* and *Tabularia*. Some of the diatoms were site specific such as, *Cymatopleura, Opephora,* and *Capartogramma* and *Cyclotella*. A new genus of diatom was discovered *Eunocymbellarania vandana*, which thus make it site specific. Study of seasonal and site specific diatoms at different sites may help in indicating time of drowning along with the location of drowning which would be very helpful for the scientific forensic investigation of drowning cases.

Keywords: Diatom fingerprinting; Haryana; Drowning

Introduction

Every year in India, along with other cause of death "Drowning" plays a major role; it may be Accidental, Suicidal or Homicidal Drowning. Drowning is a type of asphyxial death in which the respiration is inhibited by submersion in a fluid, and it is not necessary whether the fluid is aspirated into the lungs or not.

The diagnosis of drowning for bodies freshly retrieved from water is mainly based on some "drowning signs", such as the presence of fine froth at the mouth or nostrils, petechial haemorrhages, impression of ribs on lungs, oedematous lungs and some other histo-pathological findings. For the decomposed corpses and skeletonised body found in water, however, the diagnosis of drowning is rather difficult because those "drowning signs" were destroyed. Here diatom test stands as the only direct screening test for drowning [1].

The principle behind the "diatom test" is when drowning takes place the diatoms present in medium, enter through ruptured capillaries via penetration into the alveolar system, subsequently to blood stream and further distant organs and other body tissues, it is here diatom test seeks as golden standard test in forensic investigation to detect antemortem or postmortem drowning. The utility of the diatom test for drowning using femoral bone marrow was calculated in a series of 771 cases of drowning that occurred during the summer, late spring and early autumn in Ontario, Canada. The diatom test for drowning was positive in 205 cases (28%) of the freshwater drowning cases but was rarely positive if drowning occurred in water from a domestic source that lacked diatoms due to water filtration and processing. Only four cases (12%) of domestic drowning had a positive diatom test. The relationship of test outcome with diatom population dynamics is important corroborating evidence for the validity of the test since it is predictable that the diatom content of the water would influence test outcome [2,3]. The postmortem analysis of drowning is a classical problem in the pathology of sudden violent death. From a historical perspective, one of the most important issues in the study of drowning has been the search for a sensitive, specific and easily applicable test

for this cause of death. On this basis, the diatom test has emerged as the foremost laboratory procedure for the detection of drowning [3]. 29 cases of drowning were analyzed and emphasize for the usefulness of the diatom-test to find the manner of death and stressed on the collaboration between forensic pathologists and specialist- biologists, who could work together to generate the diatomologic map of the water bodies of that region. In this way, some rare types of diatoms can be identified and located, which can be of great help in pinpointing the actual site of drowning [4].

The presence of diatoms can be established and analyzed both quantitatively and qualitatively through a diatom test. This can lead not only to a more direct determination of the cause of death, but also can help to pinpoint the site of a suspected drowning [5].

Continuous monitoring of water bodies for the percentage bloom of diatom flora and its taxonomy is very essential every year. Since due to climate changes there is variability in diatom flora, which serves as an effective tool to diagnose death due to drowning [6]. Many time body get drifted to some other place either due to water current or otherwise. In such cases if a thorough monitoring of diatom flora of a particular body is recorded, it becomes possible to locate site and season of drowning. For this purpose database of most commonly occurring, site specific, and seasonal occurring diatoms was constituted along with the shape and size resulting into the formation of a fingerprinting system for diatoms.

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Materials and Methods

Sampling of water from different water bodies of Haryana

Two liter water sample was collected in a sterilized plastic bottle from ten different water bodies (Table 1) of Haryana (Figure 1) every month for a period of two years (2008-2010). At the time of sampling, temperature, pH and conductivity was measured using pH-conductivity probe (Swastika ltd., India). Site of sample collection was located by landmarks such as tree, stone or simply a flag.

The water sample was brought in the laboratory and was stored in a cool dark place, to it was added 1 ml of 0.2% formalin to restrict further division and growth. Slide was prepared by Lugol's iodine method given by Witzel and Likens [7] and under Compound microscope attached with camera (Leica DM 2500) at 100x (Oil immersion) slide was observed and there morphological and morphometric studies were done using LAS EZ software provided with the microscope.

Morphological identification

Acquired images of diatoms were identified using identification keys of Hans [2,8-19].

Distribution pattern and variation of different types of diatoms in all the three types of water bodies (canal, lake and pond) was characterized as follows:

- Commonly occurring or Dominating diatoms

- Least occurring or Site specific diatoms

- Seasonal or Rarely occurring diatoms

Commonly occurring (dominating) diatoms, are defined as the diatoms found in large percentage than others, and mostly they were present in all seasons at all the sites. Least occurring diatoms were those which were generally present in all the seasons but there count was less than the dominating ones thus are site specific. Rarely occurring diatoms one those which were observed only in particular season thus also known as seasonal diatom (Plate 1).

Results and Discussion

Diatoms are sensitive to changes in pH, conductivity, temperature, and thus variation in the water quality enables them to function as environmental, ecological and forensic indicators. For two consecutive years study of Diatoms from different water bodies of Haryana from ten water bodies (Table 1) was done and a total 111 diatom taxa were studied [6]. The most commonly found diatom at all the ten sites were *Achnanthes, Asterionella, Aulacoseira, Cyclotella, Gomphonema, Navicula, Nitzschia, Synedra* and *Tabularia*.

Canal sites (H1, H2 and H6)

The commonly occurring diatoms at the canal water bodies were *Asterionella, Cocconeis, Cymbella, Navicula*. Site specific diatom at H1 was *Cymatopleura, Opephora,* and *Capartogramma* at H2 *Catenula, Didymosphenia, Stenopterobia* and at H6 it was *Denticula, Luticola,* and *Staurosira*. Seasonal diatom at H1 was *Opephora,* at H2 was *Catenula,* and at H6 it was *Luticola*.

Lake sites (H4, H5 and H9)

The commonly occurring diatoms at lake water bodies were *Asterionella, Cyclotella, and Cymbella* where as the site specific at H4 was *Brachysira* and *Thalassiosira,* at H5 it was *Licmophora* and *Rhopalodia,* and at H9 it was *Denticula* and *Reimeria*. Seasonal diatom at H4 was *Actinocyclus,* at H5 was *Eunocymbellarania* [6] and at H9 it was *Epithemia*.

Pond sites (H3, H7, H8 and H10)

The commonly occurring diatom genera at pondwater bodies were *Cyclotella, Navicula* and *Fragilaria*. Site specific at H3 were *Coscinodiscus, Diploneis,* and *Eunotia,* at H7 *Rhopalodia* at H8 it was *Cyclotella* and at H10 it was *Coscinodiscus*. Seasonal diatom at H3 was *Eunotia,* at H7 was *Eunocymbellarania* [6] at H8 was *Coscinodiscus* and at H10 the seasonal diatom was *Gomphonema*. Figure 2 shows few commonly occurring diatoms, Figure 3 shows some least occurring, and Figure 4 shows some seasonal diatoms). Diatom fingerprinting (Plate 1) of different diatom taxas at different water body would thus help in generating a database to link scene of actual drowning and thus would be of great aid in criminal justice delivery system.

Station Code	Water body	Source	Geographical area type	Location
H-1	Canal	Western Eastern Yamuna Canal, Panipat	Plain	29°21'41.44"N 76°57'05.58"E
H-2	Canal	Western Eastern Yamuna Canal, Karnal	Plain	29°37'18.29"N 76°58'56.16"E
H-3	Pond	Muncipal park, Ambala	Plain	30°22'38.78"N 76°46'19.95"E
H-4	Lake	Bhramsarover Lake, Kurukshetra	Plain	29°57'43.10"N 76°49'40.32"E
H-5	Lake	Rani Taalab Lake, Jind	Plain	29°18'53.06"N 76°19'03.00"E
H-6	Canal	Bhakra Canal, Hisar	Plain	29°07'51.86"N 75°42'33.41"E
H-7	Pond	Ghaseda village, Mewat	Foot hills	28°07'57.26"N 77°03'53.41"E
H-8	Pond	Baghot village, Mahendergarh	Foot hills	28°25'31.72"N 76°15'55.69"E
H-9	Lake	Tilyar Lake, Rohtak	Plain	28°52'52.60"N 76°38'16.20"E
H-10	Pond	Baund village, Bhiwani	Plain	28°46'44.64"N 76°20'09.59"E

Table 1: Selected water stations for sampling from Haryana (India).

Sr. No.		Diatom genera	Station code										Order	Shape	Size (µm)	
			H1	H2	H3	H4	H5	H6	H7	H8	H9	H10			Length	Width
1.	1	Achnanthes											Pennale	Elliptical	8-16	4-6
2.	2	Actinocydus											Centrale	Circular	36-40	-
3.	3	Amphora											Pennale	Elliptical	75-80	15-20
4.	4	Asterionella											Pennale	Elongated	80-300	5-8
5.	5	Aulacoseira											Centrale	Circular	45-50	-
6.	6	Brachysira											Pennale	Elliptical	20-48	12-20
7.	7	Caloneis											Pennale	Elliptical	22-50	10-14
8.	8	Capartogramma											Pennale	Elliptical	18-45	12-18
9.	9	Catenula											Pennale	Elliptical	10-15	2-5
10.	10	Craticula											Pennale	Elliptical	44-200	25-75
11.	11	Cocconeis											Pennale	Oval	35-45	16-17
12.	12	Coscinodiscus											Centrale	Circular	36-120	-
13.	13	Cyclotella											Centrale	Circular	6-40	-
14.	14	Cymatopleura											Pennale	Shoe	80-300	10-45
15.	15	Cymbella											Pennale	Elliptical	20-200	5-50
16.	16	Denticula											Pennale	Elliptical	11-14	3-5
17.	17	Diatoma											Pennale	Elliptical	18-60	8-18
18.	18	Didymosphenia											Pennale	Elliptical	80-150	30-50
19.	19	Diploneis											Pennale	Elliptical	10-40	6-10
20.	20	Entomoneis											Pennale	Sigmoid	95-120	40-60
21.	21	Epithemia											Pennale	`Elliptical	40-50	8-10
22.	22	Encyonema											Pennale	Elliptical	20-60	4-35
23.	23	Eunocymbellarania											pennale	Elliptical	20-150	5-25
24.	24	Eunotia											Pennale	Elliptical	22-28	6-8
25.	25	Fragilaria											Pennale	Elliptical	95-119	6-7.2
26.	26	Frustulia											Pennale	Elliptical	60-85	10-18
27.	27	Gomphoneis											Pennale	Elliptical	20-130	12-20
28.	28	Gomphonema											Pennale	Elliptical	74-90	16-20
29.	2 9	Gyrosigma											Pennale	Sigmoid	150-240	26-30
30.	30	Gomphocymbella											Pennale	Elliptical	18-30	5-8
31.	31	Hantzschia											Pennale	Elongate	80-120	18-22
32.	32	Licmophora											Pennale	Elliptical	45-60	5-7
33.	33	Luticola											Pennale	Elliptical	15-20	5-10
34.	34	Melosira											Centrale	Circular	23-25	-
35.	35	Navicula											Pennale	Elliptical	10-60	5-16
36.	36	Neidium											Pennale	Elliptical	40-50	12-20
37.	37	Nitzschia											Pennale	Elliptical	25-115	5-10
38.	38	Opephora											Pennale	Elliptical	30-35	5-7
39.	39	Pinnularia											Pennale	Elliptical	20-250	8-18
40.	40	Placoneis											Pennale	Elliptical	20-30	10-12
41.	41	Pseudostaurosira											Pennale	Elliptical	12-22	6-8
42.	42	Reimeria											Pennale	Elliptical	8-12	4-6
43.	43	Rhoicosphenia											Pennale	Elliptical	16-46	8-10
44.	44	Rhopalodia											Pennale	Elliptical	18-150	4-8
45.	45	Sellaphora											Pennale	Elliptical	18-45	4-10
46.	46	Stauroneis											Pennale	Elliptical	40-75	14-20
47.	47	Staurosira											Pennale	Elliptical	7-22.5	5-7
48.	48	Stenopterobia											Pennale	Sigmoid	80-100	8-16
49.	49	Staurosirella											Pennale	Elliptical	5-10	2-5
50.	50	Surirella											Pennale	Elliptical	40-70	30-40
51.	51	Synedra											Pennale	Rod	93-150	7-9
52.	52	Tabularia											Pennale	Elliptical	20-120	4-6
53.	53	Tryblionella											Pennale	Elliptical	18-40	5-10
54.	54	Thalassionema											Pennale	Elongate	20-66	2-4
55.	55	Thalassiosira											Centrale	Circular	15-20	-

Symbols used:
- (H1 to H10): Sites of water sample collection.
- (■): Present.
- (■): Absent.
- (■): Restricted (rarely occurring and seasonal).

Plate 1: Diatom fingerprinting.

Figure 1: Geographical Map of Haryana.

Achnanthes minutissima

Asterionella formosa

Melosira varians

Cyclotella meneghiniana

Gomphonema clavatum

Navicula capitoradiata

Nitzschia angustata

Tabularia fasciculata
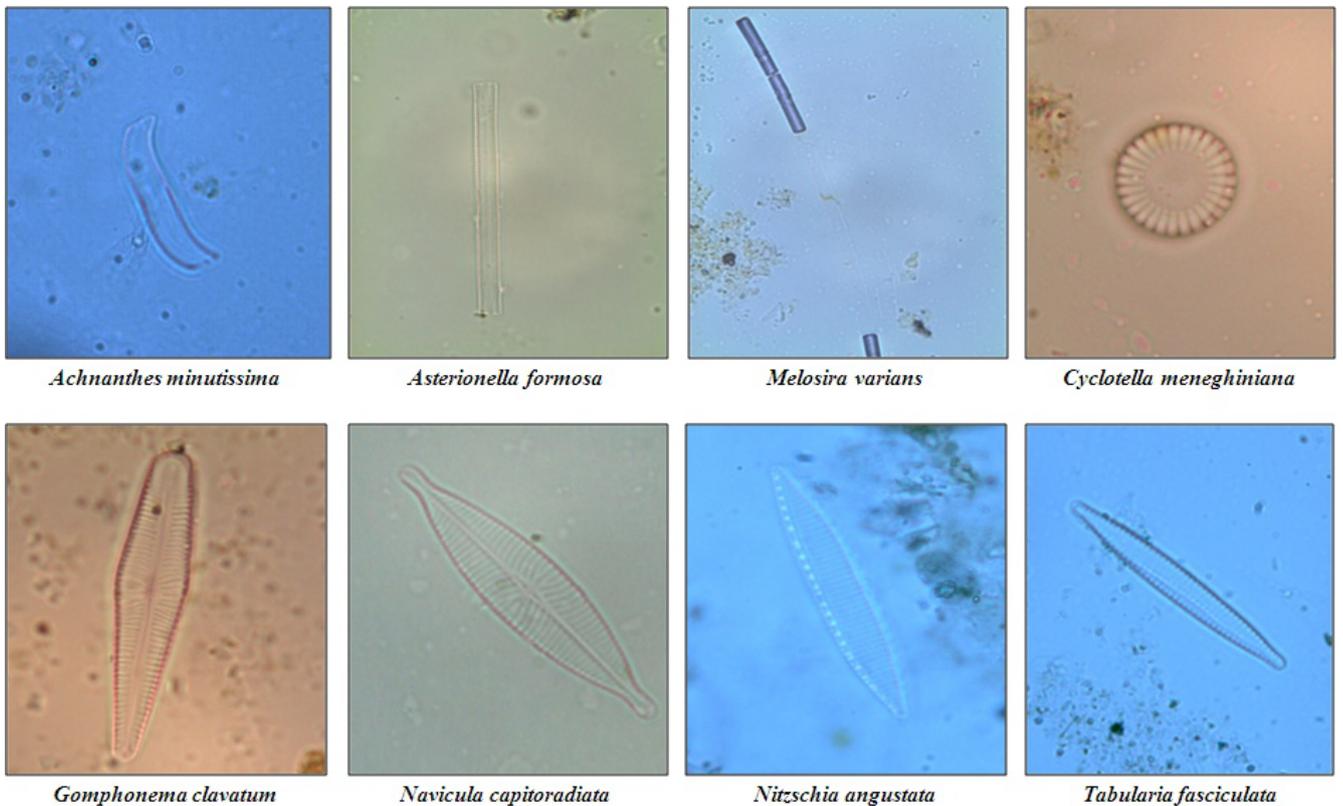
Figure 2: Few Commonly occurring diatoms.
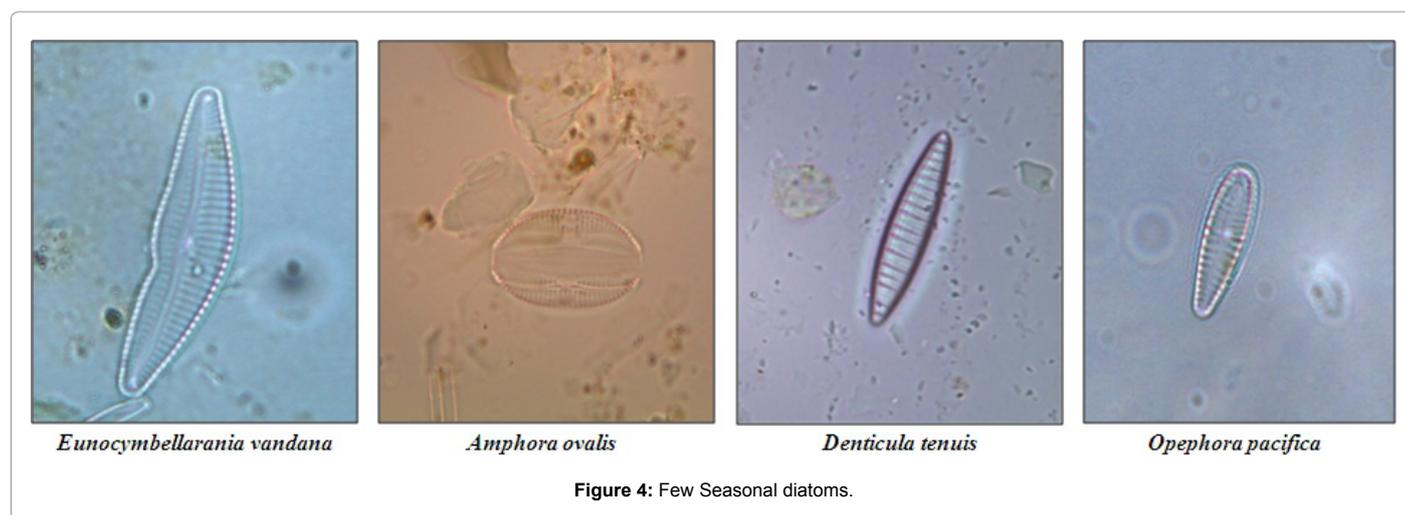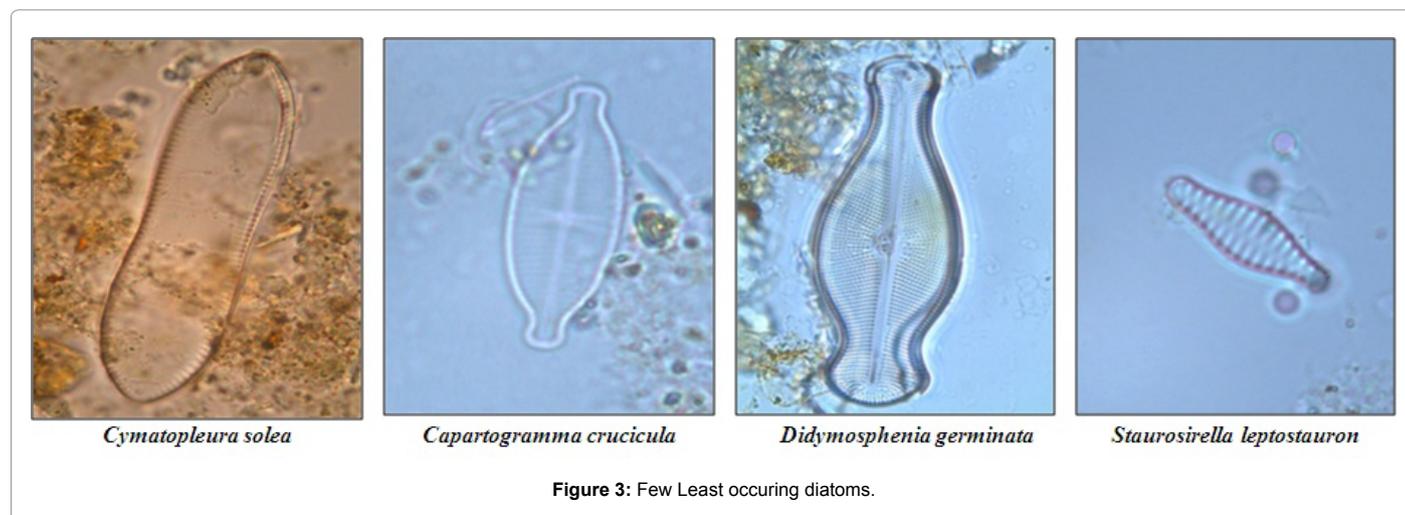
Figure 3: Few Least occuring diatoms.

Figure 4: Few Seasonal diatoms.

Acknowledgements

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