Diagnosing Death with Diatoms: A Retrospective Study of Forensic Cases in Himachal Pradesh, India

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Background: Diatoms found inside the body of a drowned victim may serve as corroborative evidence in the diagnosis of cause of death. Diatom has proved to be the only golden standard for diagnosis and confirmation of drowning deaths whether the drowning was ante-mortem or post-mortem.

Methods: The study was based on the cases of death due to drowning received from three districts of Northern Range of Himachal Pradesh, India during the period of five years from Ist January, 2010 to 31st December, 2015 for diatom test. A total of 66 human cases were examined for detection of diatoms. The detailed information regarding cause of death, socio-demographic factors and other associated information was gathered. The acid digestion method accepted worldwide for diatom extraction was used.

Results: Male victims predominated (75.75%). Most common affected age group was 21-40 years (53.02%). The youngest victim was a girl of 4 years age who drowned accidentally in a water tank, while the oldest victim being a 86 years old who fell accidently in a river. Married victims contributed to 33 (50.0%) and unmarried to 21 (31.81%). 13 (19.69%) drowning cases belonged to students followed by labourers (18.18%) and housewives (15.15%). The highest reported cases of drowning were from the rural areas (65.15%) followed by urban areas (16.66%). Majority (81.81%) of the victims drowned in fresh water. The incidences of drowning were more in water of natural flowing streams (khuds) (31.81%) followed by rivers (22.72%), nullahs/rivulets (12.12%), wells (9.09%), kuhls/water channels (7.57%), canals (6.06%), ponds (3.03%), water tanks (3.03%) and check dam, dam and waterfall in one each (4.54%) respectively. Accidental drowning was the most common cause of death (37.87%). Highest (51.51%) percentage of drowning cases was noticed during the months of monsoon/rainy season. Blood on mouth and nostrils was present in 14 (21.21%), froth from mouth, nose, larynx and trachea in 35 (53.03%) cases. Diatom-test was found positive in 62 (93.93%) cases. The results of the study revealed the occurrence of various varieties of diatoms in water bodies of northern region of Himachal Pradesh. The most common diatom genera detected were *Navicula* (86.36%).

Conclusion: The study concluded that diatoms are amongst the important biological forensic evidences in diagnosing the cause and place of death due to drowning**.** Gender based examination revealed higher percentage of males involved in drowning fatalities and the accidental submersion was the commonest manner of death.

►*Implication for health policy/practice/research/medical education:* Diagnosing Death with Diatoms

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1. Introduction:

Diatoms are among the well-known water planktons. They are autotrophic and found in both marine and fresh waters. The shape of the diatom plays a role in drowning. When the shape is more elongated, thin and small in size, it is presumed to penetrate lung tissues and enter into blood stream easily. However, with physical force almost all types of diatoms can be associated in the blood stream (1).

The theory behind the diatom test is that when any person drowns, the diatoms present in that water will reach to the lungs and some of them because of their size penetrate into the alveoli. If the heart is still beating, the diatoms that have entered into the blood stream travel around the body and may lodge in distant organs such as the kidneys, brain and bone marrow before death (2, 3).

Diatom was first detected in lung fluid by Hoffmann in the year 1896 (4). However, Rovenstorf had successfully utilized it to solve a case of a drowning mystery in 1904 (5). The method of its extraction was improved by acid digestion of the tissues (6). Incze successfully detected diatoms in blood and parenchymal organs in 1942 (7). Tamasaka detected diatom in bone marrow in 1949 (8). In the 1960's and 1970's, Timperman using large series of drowning cases, provided evidence for the validity of diatom test (9). Pollanen (3), Peabody (10), and Ludes *et al* (11) strongly supported diatom test in diagnosis of drowning.

According to World Health Organization (WHO), the drowning ranks third biggest cause of unintentional injury-related death around the world, representing 7% of all such incidents (12). Chronological literature has also disclosed drowning as a common cause of death in India (13-16). National Crime Records Bureau (NCRB), India has accounted number of accidental and suicidal deaths in India. This report has mentioned drowning responsible for 6.6% deaths in 2014. Drowning took 29903 (6.6%) lives in 2014 of which 23166 (77.5%) and 6736 (22.5%) were males and female respectively (17). India ranks 32nd in the incidents of drowning in the world (18).

Himachal Pradesh- a mountainous region famous for its natural beauty is called fruit bowl of the country. There are many perennial rivers flowing in the state and it has lakes, canals/sub-canals, water tanks, ponds and wells which enhance the rate of drowning. Every year heavy rainfall takes place which results in a large number of accidental drowning in the area (19). Therefore, drowning is a one of the leading cause of death in the state of Himachal Pradesh (13). Patterns of drowning vary from one geographic area to other, depending on the local physical and social environment (20). A tragic incident occurred in year 2014 which took 24 lives of engineering students from Hyderabad at the Beas River in Himachal Pradesh due to sudden surge of river water released from Larji hydroelectric power project (21).

The objective of the study was to identify the drowning associated diatoms amongst the samples referred to RFSL, Northern Range,

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Dharamshala, India. 66 samples (belonging to different suspected drowning cases) received for detection of diatoms with a history of drowning were analysed with a view to identify the diatoms present. The biological samples included bones, lungs, kidney, heart, spleen, and control water samples from the site of drowning.

2. Materials and Methods:

Study design

The study was conducted at Biology & Serology Division of Regional Forensic Science Laboratory, Northern Range, Dharamshala, India. It was based on the cases of death due to drowning received from three districts of Northern Range of Himachal Pradesh during the period of five years from Ist January 2010 to 31st December 2015 for diatom test. A total of 66 cases were examined. The specimens of bones (sternum, femur and tibia), lungs, spleen, kidney, heart, and control water samples from the site of drowning were received for examination.

Data collection

The detailed information regarding cause of death, socio-demographic factors (age, sex, religion, marital status, place of residence, profession, place of incidence etc.) and other associated information was gathered from police forwarding letter/records, inquest reports, first information reports, autopsy examination reports (deaths suggestive of drowning), and results of forensic laboratory examination of the specimens. Various sites of drowning such as river, dam, water of natural flowing stream (*khud)*, well, pond, check dam, *kuhl*/water channel etc. were also recorded. The details were entered on a semi-structured questionnaire developed by the researchers after a review of the related literature.

Specimen/sample analysis

The cases were opened and processed following standard methodology (22). The acid digestion method for diatom extraction accepted worldwide was used. The Lugol's iodine was added in water sample to inhibit microbe growth. The Lugol's iodine digested water sample was centrifuged thrice at 4000 rpm for 10 minutes and washed with double distilled water.

The material was extracted from the bones, lungs, spleen, kidney and heart. 50 ml. of nitric acid was added to the beaker containing extracted material and was simmered on a hot plate at 60-70^{0}C for approximately 48 hours in a fume hood. The material extracted was centrifuged thrice at 4000 rpm for 10 minutes and washed with double distilled water. The acid resistant extracted material isolated and control water samples were examined microscopically. After microscopic observation of diatoms in both samples, a correlation of diatoms present in the water sample in which drowning took place and material extracted from biological specimen was drawn out accordingly (23). The diatoms were identified according to the diatoms biology and morphology of the genera.

Data analysis

The data was put into the master-chart, which was prepared and feed into the computer in Excel worksheet and then analysed.

3. Results:

In the present study, total 66 specimens of bones, lung, spleen, kidney, heart and control water samples from the site of drowning during the period of five years were examined for detection of diatoms.

Demographic details

Out of 66 cases of drowning, 50 (75.75%) were male and 16 (24.24%) were female indicating a male: female ratio of nearly 3:1. The most common affected age group was 21-40 years (53.02%). Married victims contributed to 33 (50.0%) and unmarried 21 (31.81%). 43 (65.15%) drowning cases were reported from rural areas and 11 (16.66%) from urban areas. 13 (19.69%) drowning cases belonged to students followed by labourer in 12 (18.18%) and housewife in 10 (15.15%) cases (Table 1).

Place of drowning

The incidences of drowning were more in water of natural flowing streams (*khuds)* 21 (31.81%) followed by rivers in 15 (22.72%), *nullahs*/rivulets in 8 (12.12%), wells in 6 (9.09%), *kuhls*/water channels in 5 (7.57%),

Table 1: Socio-demographic characteristics of drowning cases

Characters	No. of cases	Percentage
Sex		
Male	50	75.75
Female	16	24.24
Age group (years)		
Below 10	01	1.51
11-20	11	16.66
21-30	20	30.30
31-40	15	22.72
41-50	11	16.66
51-60	03	4.54
61-70	02	3.03
Above 70	01	1.51
Not mentioned	02	3.03
Religion		
Hindu	54	81.81
Muslim	02	3.03
Not mentioned	10	15.15
Marital status		
Married	33	50.0
Unmarried	21	31.81
Not mentioned	12	18.18
Residential status		
Rural	43	65.15
Urban	11	16.66
Not mentioned	12	18.18
Education		
Up to tenth standard	04	6.06
10+2	10	15.15
Graduate	04	6.06
Not mentioned	48	72.72
Occupation		
Housewife	10	15.15
Student	13	19.69
Farmer	02	3.03
Employed	04	6.06
Business	02	3.03
Skilled laborer	03	4.54
Laborer	09	13.63
Shepherd	01	1.51
Private job	03	4.54
Not mentioned	19	28.78

canals in 4 (6.06%), ponds in 2 (3.03%), water tanks in 2 (3.03%) cases and others in 3 (4.54%) cases (Table 2).

Season wise incidence

Season wise distribution of the drowning death showed that 34 (51.51%) victims died during the months of monsoon/rainy season followed autumn, winter and summer seasons, almost in equal distribution (Table 3).

Manner of death

In 25 (37.87%) cases, the causative factors of drowning were accidental followed by alcoholic in 5 (7.57%), mental disorder in 4 (6.06%), suicidal drowning in 4 (6.06%) and poisoning, homicidal drowning and seizure disorder/epilepsy in 3 (4.54%) cases (Table 4).

Autopsy findings

The autopsy findings showed that blood on mouth and nostrils was present in 14 (21.21%), froth from mouth, nose, larynx

Table 2: Distribution of cases according to place of drowning

Place of drowning	Number of cases	Percentage
Well	06	9.09
Pond	02	3.03
Water of natural flowing stream (*khud)*	21	31.81
Nullah/rivulet	08	12.12
River	15	22.72
Waterfall	01	1.51
Water tank	02	3.03
Kuhl/water channel	05	7.57
Check dam	01	1.51
Dam	01	1.51
Canal	04	6.06

Table 3: Season wise distribution of drowning cases

Year/ season	Number of cases	Percentage
Winter (December - February)	11	16.66
Monsoon/Rainy (June - September)	34	51.51
Summer (March - May)	10	15.15
Autumn (October - November)	11	16.66

and trachea in 35 (53.03%), froth and dust/mud particles in mouth larynx and trachea in 7 (10.60%), lungs voluminous, oedematous and swollen in 16 (24.24%), and lungs voluminous, heavy indented with ribs impression in 3 (4.54%) cases (Table 5).

Results of diatom test

After examination and conducting test on biological samples of 66 human cases suspected for drowning, diatom-test was found positive in 62 (93.93%) cases. The diatoms present in the biological samples showed significant similarities to the diatoms present in control water samples from the putative site of drowning. In 4 (6.06%) cases diatoms were present only in water and absent in bone marrow (Table 6).

Diatom genera detected

The results of the study revealed the occurrence of various varieties of diatoms in drowning deaths. The most common diatom genera found were *Navicula* (Table 7).

4. Discussion:

The study in northern region of Himachal Pradesh was conducted to ascertain the cause of death. A positive diagnosis of drowning can be very difficult, particularly in decomposed bodies. Even when there are only skeletal remains, diatoms can be

Table 4: Manner of death in cases of drowning

Cause	Number of cases	Percentage
Mental disorder	04	6.06
Alcoholic	05	7.57
Accidental	25	37.87
Poisoning	01	1.51
Suicide	04	6.06
Homicide	01	1.51
Seizure	01	1.51
Not mentioned	25	37.87

detected in the bone marrow of drowned victims. Though the diatom test has certain limitations, like diatoms could have been inhaled or ingested with material containing diatoms before death or contamination of the glassware and reagents that are used to detect diatoms. Yet, the quantitative and qualitative identification of diatoms in experienced hands provides the most reliable proof of drowning (24).

According to Modi (25), accidental drownings occurs often in India. In our study, the incidence of drowning cases was higher (53.02%) in third and fourth decade of life in Hindus (81.81%) and males predominated (75.75%). Our observations in this regard are consistent with Majumdar (26), Chaudhary (27), Pandey (28), Brenner *et al* (290, and Pathak *et al* (30). It can also be explained by the fact that major population of Himachal Pradesh belongs to the Hindu community. Similar findings have been reported by Saini *et al* (31) where gender based difference revealed high mortality (71.85%) in males, particularly adults between 26-40 years. A study by Rao *et al* (32) showed that there were 57 (63.33%) males and 33 (36.67%) females and maximum number of cases appeared in the third and fourth decades (56.66%). Our findings in this regard are in concordance with Shetty (14), Tan (33), Wirthwein *et al* (34) and Palimar *et al* (35) where male were at higher risk than females to be drowned. According to the NCRB report, there were 77.5% males and 22.5% females (17). Similar findings of male predominance were also noted in observations made by Mukherjee *et al*. (36) (71.43%), Sayed *et al*. (37) (75.68%), Chormunge *et al* (38) (73.53%), Singh *et al* (39) (67.56%), Salachin (40) (75.6%), Gross *et al* (41) (90%), Momamchand (42) (80.3%) and Reddy *et al* (43) (59.14%). Our study is in agreement with a study by Thakar and Guleria (44) where 74.2 % drowned deceased were males while 25.7% were females. Our study is similar to findings of the study done by Morris *et al* (45) which attributed 219 (79%) males and 59 (21%) females, Chidanand and Satish (46) which showed 68% males and 32% females, Sheikhazadi and Ghadyani (47) which reported 87% males and 13% females and Chakraborty *et al* (48) where incidences of drowning deaths were much higher in males (80%). Our study is in contrast with a study by Buri *et al* (49) where maximum incidence of drowning was in 32% cases in the age group of 11-20 years. Chaudhury (27), Pandey (28) and Momonchand *et al* (42) observed almost similar findings.

In our study, 19.69% of victims were students with no specific occupation. Our study is closely related to a study done by Chakraborty *et al*. (48) where 33% victims were students.

In our study, married victims contributed to 33 (50.0%) and unmarried 21 (31.81%). A study by Rao *et al*. (32) showed that 41 (56.16%) cases were married and 32 (43.83%) cases were unmarried.

Many authors indicate that the increase or decrease of drowning death in a year is closely related to seasons, climatic factors and geographic zone (50-53). In the present study, majority of the deaths due to drowning were noticed during the months of monsoon (June to September), as compared to other months of the year. Our findings in this regard are consistent with Majumdar (26), Pandey (28), Brenner *et al* (29), Pathak et al (30) and Thakar et al (44). This may be due to monsoon rain resulting in increased water levels in water bodies in hilly state. Our study is in agreement with the study of Saini *et al* (31) where percentage of drowning cases was noticed highest in monsoon season and Anand & Unmesh (54) which showed increase in drowning cases during rainy season. Our study is in contrast with a study of Rao *et al* (32) which showed that maximum number of cases appeared in April to June. However, there may be variations depending on the geographic zones.

It was observed in the present study that freshwater drowning accounted for 54 (81.81%) cases followed by 12 (18.18%) cases in well, pond, water tank and check dam/dam as these are the major water sources in north India. Our study is consistent with a most sensational Shopian rape and murder case of two young women Neelofar Jan and her 17-year-old sister-in-law whose bodies were found in Rambiara nallah, Shopian district in the Indian state of Jammu & Kashmir. The case was solved on the basis of diatom test by Vinayak (55). Our study is concordance with studies of Kumar *et al* (56) where cases of rivers, ponds, and canals were studied and Palimar and Manjunath (35) where every fourth victims drowned in fresh water. Our study correlates to the results of a study by Sheikhazadi and Ghadyani (47) where freshwater settings were the sites of about 83% of all drownings and Patetta and Biddinger (57) which showed 39% deaths from fresh water drowning mainly due to submersion in lakes and ponds. This can be explained due to the fact that Himachal Pradesh is a hilly and mountainous tract and there is tremendous boost in water level in monsoon season as most of water bodies swells during this period.

With regard to the forensic etiology, three possibilities are usually considered: accidental, suicidal and homicidal submersion (47). In the present study, 25 (37.87%) persons lost their life accidentally followed by alcoholic in 5 (7.57%), mental disorder in 4 (6.06%), suicidal drowning in 4 (6.06%) and poisoning, homicidal drowning and seizure disorder/epilepsy in 1 case each i.e., 3 (4.54%). Our observations in this regard are consistent with Chidanand and Satish (46) who reported that most of deaths were accidental and accounted for 55.5% of drowning deaths. They noticed only 3 cases of homicidal drowning and 15 cases of suicidal drowning. A study by Thakar and Guleria (44) reported 73.9% cases due to drowning while suicidal and homicidal were 16.1% and 0.15% respectively. Sheikhazadi and Ghadyani (47), observed that the most common manner of death was accidental drowning in 85.1% cases which is consistent with our study. They reported seizure disorder in 8.5% cases, suicidal drowning in 1.7% cases and homicidal drowning in 0.3% case. A study by Rao *et al*. (32) showed accidental drowning in 42 (46.66%) cases followed by suicide in 16 (33.33%) cases. The manner of drowning was accidental in 74.1% cases in a study by Palimar and Manjunath (35). The study done by Wintemute *et al* (58), Giersten *et al* (59) and Copeland (60) mention the accidental drowning to be in the range of 80-90%. Our findings in this regard are consistent with Shetty (14), Modi (25), Mukherjee *et al* (36), Mason (61), Singh (62), Knight (63), and Apurba (64). Our study is close to the observations made by Chakraborty *et al* (48), where the suicidal submersion was 10% (6 out of 60). Our study is in concurrence with a study of Chakraborty *et al* (48) where homicidal submersions were seen in only two cases. However, a study from New York reveals suicidal drowning to

Table 5: Autopsy findings in cases of drowning

Post-mortem findings	Number of cases	Percentage
Blood in mouth and nostrils		
Present	14	21.21%
Absent	52	78.78%
Injuries on body		
Abrasions on hands, elbow, shoulder, chest, and abdomen	11	16.66%
Laceration on eye, pinna ear, hand, and knee	05	7.57%
Hematoma on head/occipital region	05	7.57%
Absent	45	68.18%
Froth from mouth, nose, larynx and trachea		
Froth from mouth, nose/nostrils, larynx, and trachea	35	53.03%
Froth and dust/mud particles in mouth larynx and trachea	07	10.60%
Not mentioned	24	36.36%
Lungs		
Lungs voluminous, oedematous and swollen	16	24.24%
Lungs voluminous heavy indented with ribs impression	3	4.54%
Lungs with fluid froth	13	19.69%
Lungs oedematous and congested	05	7.57%
Lungs oedematous with mucus	01	1.51%
Lungs collapsed/ swollen	03	4.54%
Lungs putrefied	01	1.51%
Lungs congested and floppy	06	9.09%
Lungs with air bubble, soft discolored, blotted appearance, ballooning, and distended	06	9.09%
Lungs oedematous with petechial hemorrhages	01	1.51%
Not mentioned	11	16.66%
Weed/stone/soil in hands		
Weed/stone/soil in hands	03	4.54%
Not mentioned	63	95.45%

be more common (65). Our study is in agreement with a study of Morris *et al* (45) where 9 (3.2%) cases of epilepsy were reported. It is noteworthy to mention that counseling of the patients with epilepsy and

Table 6: Distribution of drowning cases according to diatom-test

Results of Drowning-Test	Number of cases	Percentage
Diatoms present in sternum and water both	43	65.15%
Diatoms present in humerus and water both	03	4.54%
Diatoms present in tibia and water both	01	1.51%
Diatoms present in lungs and water both	04	6.06%
Diatoms present in femur and water both	11	16.66%
Diatoms not present in sternum	03	4.54%
Diatoms not present in sternum due to insufficient sample	01	1.51%

patients suffering from mental illness may reduce the submersions.

The typical signs of drowning are froth from nose and mouth, clenching of hands as victim struggle for survival, washerwoman hands and feet, oedematous lungs, petechial hemorrhages, indentation of ribs and mud and water in the stomach. The fine froth from mouth and nose is pathognomonic of drowning. Problems are faced by the investigating agencies to know the exact cause of death in case body is putrefied/ decomposed/skeletonised/recovered from the water body or having being transported to the place by drifting along the waves of water (66). In our study, the autopsy findings showed that blood on mouth and nostrils was present in 14 (21.21%), froth from mouth, nose, larynx and trachea in 35 (53.03%), froth and dust/mud particles in mouth larynx and trachea in 7 (10.60%), lungs voluminous, oedematous and swollen in 16 (24.24%), lungs with fluid, froth 13 (19.69%), lungs oedematous and congested in 5 (7.57%), lungs voluminous, heavy indented with ribs impression in 3 (4.54%), lungs oedematous with mucus, collapsed/swollen, putrefied, congested and floppy, air bubble, blotted appearance, ballooning, distended and petechial hemorrhages in 18 (27.27%) cases. Our observations in this regard are in agreement with a study of Morris *et al* (45) where a plume of froth at the nostrils was recorded in 85 (31%) cases. Our study is in agreement with Chidanand and Satish (46) where the presence of froth was noticed in 58% of drowning deaths. Our findings are consistent with Modi (25), Mukherjee *et al* (36), Mason (61), Knight (63), Apurba (64) and Mukherjee (67). Our study is closely related to a study by Mukherjee *et al* (36) where external petechial hemorrhages were observed over face, forehead, conjunctiva was observed in 9 (12.85%) cases. Our observations are in concordance with the opinion of Farrugia and Ludes (68) and Rao (69).

According to Farrugia and Ludes (68), the body having sunk to the bottom of the site of drowning, will show a pattern of post mortem injuries such as post-mortem abrasion over the forehead, the prominent points of the face, the anterior trunk, the backs of the hands and the fronts of the lower legs. Accidental or suicidal injuries due to the way the person falls or enters into the water may also be observed. In our study, injuries on body (abrasions, lacerations and hematoma) were found in 21(31.81%) cases. This observation is consistent with the opinion of Farrugia and Ludes (68).

The presence of mud, sand, gravel and weed in the hand is one of the confirmatory signs of death due to drowning. Presence of sand, weed etc. in the hand indicated that, just before death victim tried to grab the soil at

Table 7: Diatom genera detected in water and bones/tissue

Place of occurrence	Dominating Diatom (Water sample)	Dominating Diatom (Bone marrow/tissue)	Least Occurring
Well	*Navicula, Nitzschia, Diatoma, Asterionella, Cymbella and Epithemia*	*Navicula, Cymbella, Nitzschia and Asterionella*	*Pinnularia*
Pond	*Navicula, Diplonesis, Nitzschia and Cymbella*	*Navicula, Nitzschia and Diatoma*	*Coconesis*
Water of natural flowing stream (*khud)*	*Navicula, Nitzschia, Cyclotella, Asterionella, Cymbella and Epithemia*	*Navicula, Nitzschia, Asterionella and Cyclotella*	*Diplonesis, Melosira and Tabuleria*
Nullah/ rivulet	*Navicula, Nitzschia, Asterionella, Cyclotella and Epithemia*	*Navicula, Nitzschia and Asterionella*	*Gyrosigma, Diplonesis and Pinnularia*
River	*Navicula, Nitzschia, Asterionella, Cyclotella and Diplonesis,*	*Navicula, Nitzschia, Asterionella, Cyclotella and Pinnularia*	*Gyrosigma, Aulacoseria, Stauronesis, Coconesis and Tabuleria*
Waterfall	*Navicula, Nitzschia, Asterionella and Cyclotella*	*Navicula, Nitzschia*	--
Water tank	*Navicula, Nitzschia, Diatoma, Asterionella and Diatoma*	*Navicula, Nitzschia, Asterionella and Diatoma*	*Pinnularia and Cyclotella*
Kuhl/water channel	*Navicula, Nitzschia, Asterionella, Pinnularia and Cyclotella*	*Navicula, Nitzschia and Cyclotella*	*Epithemia and Cymbella*
Check dam	*Navicula, Nitzschia, Pinnularia, Asterionella, Cymbella and Tabularia*	*Navicula, Nitzschia and Asterionella*	--
Dam	*Cyclotella, Navicula, Diplonesis and Epithemia*	*Navicula, Cyclotella and Epithemia*	--
Canal	*Cyclotella, Navicula, Nitzschia and Asterionella*	*Navicula, Cyclotella and Asterionella*	*Aulacoseria, Tabuleria and Pinnularia*

the bottom of the water. This means that at the time of death, the person was alive. In our study weeds, soil, stones were found in hands in 3 (4.54%) cases. The observations of the present study are in agreement with the opinion of Farrugia and Ludes (68) and Rao (69).

In the present study, the diatom-test was positive in 62 (93.03%) human cases and thus a correlation between the diatoms extracted from biological specimen and the samples obtained from drowning medium was established. The diatoms found in biological specimen were showing significant similarities approximately in the same proportions with the diatoms present in control water sample from the site of drowning. The similar findings were also noted in observations made by Ludes *et al* (11), Timperman (24), Modi (25), Vij (70), Mathiharan *et al* (71), Singh et al (72), Reddy (73), Nandy (74) but there were differences in the diatom diversity. Our findings are in agreement with a study by Rao *et al* (32) wherein diatom test was positive in 52 (57.77%) cases. Almost similar findings have been reported by Pollanen (3) where out of 52 cases of fresh water drowning, 47 (90%) were found positive for diatoms. Our observations in this regard are consistent with observations of Kumar *et al* (56) where out of seven human cases, four (57.14%) were found positive for diatoms. Mitali *et al* (75) concluded that out of 20 cases, 13 (65%) cases of death were confirmed due to drowning, which showed consistency with the findings of present study. Our study is in concurrence with a study by Magrey *et al* (76) in which out of 11 cases, 9 (81.81%) were attributed to drowning in lakes, wells, rivers, canals, and water tank. Sitthiwong *et al* (77) reported

diatoms in all 12 (100%) cases where corpses were found submerged in water, but there were differences in the diatom diversity, which is similar to findings of our study in terms of death due to drowning. The variation of diatoms was mostly seen in ponds in our study which is in agreement with a study by Anand and Unmesh (54) who reported that the samples from house well did not show much change as that of the ponds and rivers.

The results of the present study revealed the occurrence of various varieties of diatoms in northern region of Himachal Pradesh. The common diatom genera which were found to be most frequent in this study were Navicula (86.36%) followed by Nitschzia, Asterionella, Cyclotella and Cymbella. Our findings are in agreement with Gunatilake et al (1). and Kumar et al (78). Similar findings have been presented by Vinayak et al (66) who found almost similar genera of diatoms in water of rivers. The present study concur with the findings of Malik et al (79) where bodies were recovered from water tank, canals, rivers and well. Our findings are further in concordance with studies of Purohit (80) who studied different distributional pattern of diatoms in baudis and wells and Sharma (81) who studied water samples of some rivers of district Kangra of Himachal Pradesh.

5. Conclusion:

The study concluded that diatoms are amongst the important biological forensic evidences which have great forensic significance in solving the crime cases pertaining to drowning and may serve as corroborative evidence in diagnosing the cause and place of death**.** Death by drowning remains one of the most difficult diagnosis and positive diatom findings in the bone marrow indicated valuable diagnostic information about death by drowning and can be useful for medico-legal purposes. Characteristics of drowning incidents vary greatly in water bodies, which may be due to the difference in the geographical conditions. Drowning is a significant cause of mortality in Himachal Pradesh. Gender based examination revealed higher percentage of males involved in drowning fatalities. Monsoon/rainy season definitely acted as a supplement for the drowning cases as highest percentage of drowning cases was noticed in this season. The accidental submersion was the commonest manner of death.

Differences in the diatom diversity were found in northern part of Himachal Pradesh. Though some diatoms studies have been conducted by the scientists, but forensic and medico-legal value in crime cases has not been investigated so far in this region. The diagnostic of drowning can be achieved when qualitative, quantitative and morphological criteria are fulfilled during laboratory investigations. With the advent of new technologies and techniques like Nuclear Magnetic Resonance (NMR), Fluorimetry, Automatic Diatom Identification and Classification (ADIAC) and Polymerase Chain Reaction (PCR-based identification of drowning), there are some hopes of revival of diatom test in near future which may overcome the shortcomings associated with diatom analysis in the present scenario.

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