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Diatomological mapping of eight waterbodies of Himachal Pradesh by using Scanning Electron Microscopy- A forensic perspective

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ABSTRACT

Diatom test is widely used for ascertaining the place of drowning as well as the manner of drowning i.e. ante or post mortem drowning. In this paper, we report Diatomological mapping of eight waterbodies of Himachal Pradesh across *Una, Bilaspur, Mandi, Kullu, Sirmaur* and *Kinnuar* districts during across all the seasons using SEM, which would be helpful in the correct identification of diatoms in drowning cases. Diatoms from water samples were extracted using acid digestion and analyzed using Scanning electron microscopy. The results of present study have generated database of diatom diversity in context to different seasons i.e. Summer, Autumn, Winter and Spring seasons. It has been found that majority of diatoms was observed in the Spring season due to favorable environmental conditions while the adverse effect of light and temperature in the winter season declines the growth of diatom flora. It has been concluded that during the morphological examination of diatoms, sometimes the finer details of diatoms frustules such as their valve number, striae number, raphe details cannot be examined by LM even at higher magnification. SEM is required to examine them and correct identification of diatoms can be done up to the species level.

Keywords: Scanning electron microscopy, Diatoms, Forensic Science, Drowning and Season

Introduction

The diatom test is of paramount importance, whenever a dead body is recovered from water and cause of death is under suspicion. The diatoms are the significant evidence to confirm that death has happened due to submersion in water and to ascertain the drowning site. In the drowning process, the water is distended and inhaled into the living alveoli. The diatoms present in the water perforate the alveolar capillary barrier and enter into the blood stream and via circulation travels to other body organs and get trapped in tissues such as bone marrow. Therefore, the presence of diatoms in the bone marrow is the best indicator of ante-mortem drowning and gives the positive test for diatom. The comparison of diatom profile of species found in the bodily tissue and that recovered from reference samples from the suspected drowning medium gives the information about the drowning site.

The diatom composition is water body specific and vary from one waterbody to another waterbody depending upon various physiochemical factors [6-7]. The basic principle of diatom test relies on the continuous monitoring of waterbodies which can help to generate the Diatomological profile of waterbodies by applying a positive diatom test in which the presence of significant number of diatoms in the vital organs of the individual as well as matching with the putative drowning site. Therefore, this positive approach can prove its usefulness in searching the specific drowning site.

Light microscopy (LM) has been employed extensively for the examination of diatoms from different tissues i.e. from lungs [20], brain and heart samples. [21] A number of studies have been carried out on the diatom test, which can be used for the diagnosis of drowning deaths. Diatoms were extracted from various body organs [1-5] and were subsequently analyzed by LM [22- 26] Sometimes, Scanning electron microscopy (SEM) is employed to study the finer details of diatoms to identify them as much higher magnification and resolution is achievable with SEM as compared to LM.

A number of research publications have been reported in the forensic literature to study various evidences of botanical origin such as pollens [8] Insects [9] and diatoms [10], [19] using SEM. Comparative studies on Light and Electron microscopy have also reported by number of authors. [10-12], [19] The finer details of diatoms frustules like number of pores, valve details, girdle- view, areolae and their striae number which are very important for the identification of diatom taxa up to the species level requires SEM.

Himachal Pradesh, a hilly state in northwestern Himalaya, many tourist places are waterbodies or situated near waterbodies. Every year tourists in large numbers visit these places and increase in number of drowning cases has been seen in the recent past. [18] The chances of washing away in the waterbodies during exploring the adventurous spot are increasing. The present study has been carried out with an objective of season-wise Diatomological mapping (D-mapping) to generate a reference database of the distribution of diatoms in eight waterbodies of Himachal Pradesh situated at different altitudes using SEM.

2. Materials and Methodology

2.1 *Sample Collection*: The water samples were collected from eight waterbodies of Himachal Pradesh across various districts namely Gobind Sagar Lake in Una and Bilaspur district, Renuka Lake in Sirmaur district, Macchial Lake, Pondoh dam, Prashar Lake and Rewalsar lake in Mandi district, Manikaran in Kullu district and Nako lake in Kinnaur district. The waterbodies include

lakes, reservoir and dams. The details regarding different sites of sample collection are given in map in **Fig. 1**

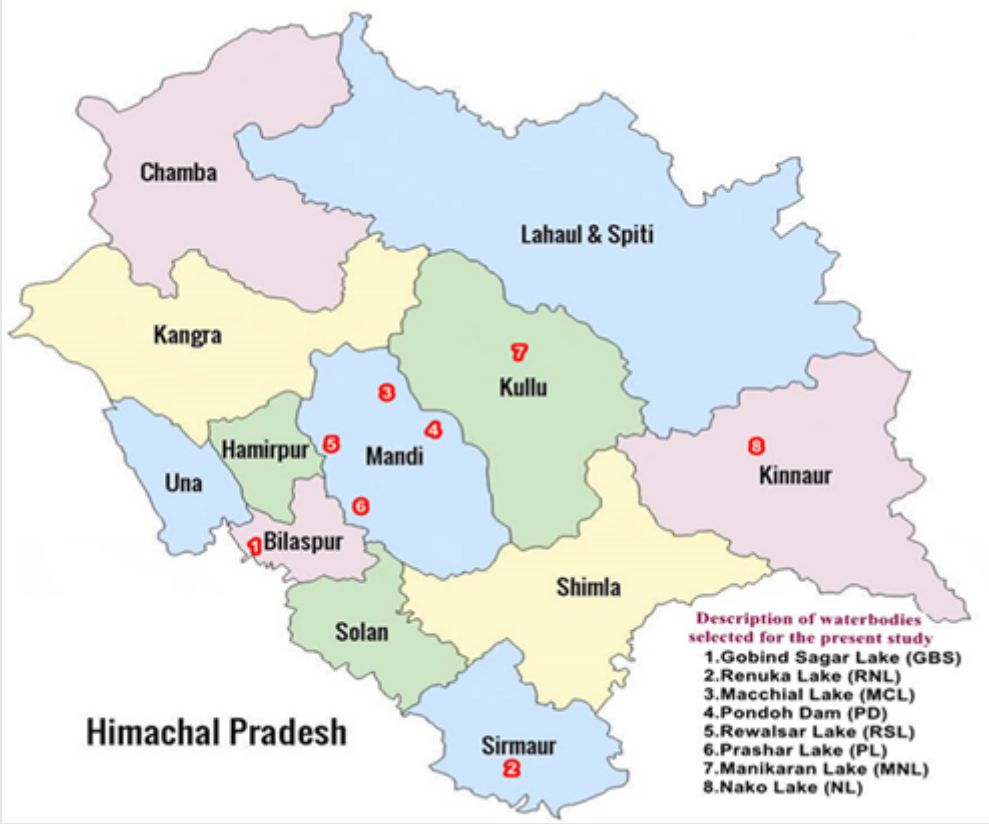


Fig.1 Map of Himachal Pradesh showing selected waterbodies

One litre water sample was collected from the banks and mid region of waterbodies during all the seasons i.e. summer, autumn, winter and spring continuously up to two years from June 2014 to March 2016. The samples were properly labelled and stored separately at room temperature to prevent any cross contamination. Various parameters such as temperature, pH were also recorded during the time of sampling. At least three replicates of samples were collected from the surface of each waterbody appx. distance 10 m apart. to check the intra-site variation in diatoms.

2.2 Methodology

2.2.1 Extraction of diatom from water sample

In the present study, the extraction of diatoms from the water samples was done by Acid Digestion method [14] for SEM. 100 ml of water sample was taken in 250 ml beaker and 2 mg of Potassium dichromate followed by 20 ml of Conc. Nitric acid was added to it. After through mixing, it was left undisturbed for 24 hours to ensure sample digestion. The samples were transferred to fresh tubes and centrifuged at 3000 rpm for 15 minutes. The supernatant was pipette out, discarded and the pellet at the bottom was retained. This step was repeated thrice to remove all the traces of acid and organic matter. Then the final pallet was put onto the microscopic slide, and dried at room temperature. The samples were analysed twice to check the standardization and reproducibility in results.

Then samples were prepared for SEM by drying at the room temperature (26°C) to remove all the traces of moisture. Then coated with gold by fine auto coater (JFC-1600) fixed onto stub and

scanned by Scanning Electron Microscope (JEOL JSM 6510LV). All the electron micrographs were saved in bmp. format and diatoms were identified by using the keys given by (Hartley *et al.* 1996) [15]. Abundance / frequency of diatoms occurrence was also checked by counting number of diatoms per microscopic field view.

3. Results

3.1.1 Morphological examination of diatoms using SEM

47 diatom genera were observed across the selected waterbodies. Details are summarized in **Table 1** and electron micrographs are shown in **Photographic plate 1.** The detailed observations of morphological characteristics such as presence of areolae (perforations), spines (pointed projections), pores, striae number (row of areolae), raphe details (slit like channel across the apical axis), fibulae (internal structural support), sternum (thickened silica valve), keel (raised portion), rimoportula (tubular process), valve and girdle view of diatom frustules were made with SEM images. It has been observed that assessment of some morphological features like striae pattern details in case of *Cocconeis pediculus, Aulacoseria granulata* and *Cocconeis placentula* can be easily be resolved and examined using SEM which are not achievable with LM.

Keel, another fine characteristic feature of species *Bacillaria paradoxa, Cymatopleura solea* and *Rapholodia gibberulla* and rimoportula present in *Diatoma vulgaris* and *Aulacoseria granulata* can be easily analyzed through their respective SEM images.

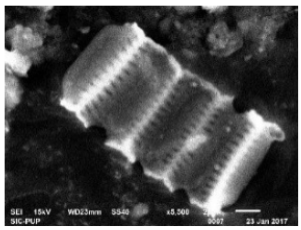


Fig.1 ***Fragillaria crotonensis***

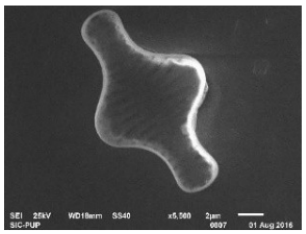


Fig.2 ***Staurosira construens***

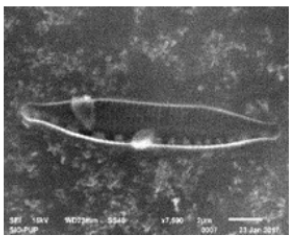


Fig.3 ***Hantzschia amphioxys***

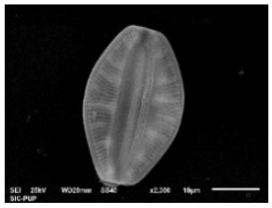


Fig.4 *Rapholodia gibberula*

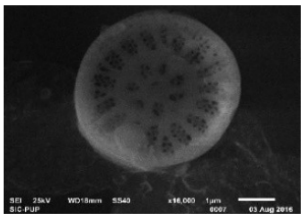


Fig.5 Cyclotella *meneghiniana*

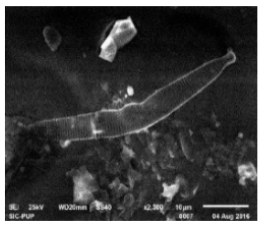


Fig.6 Hannaea *arcus*

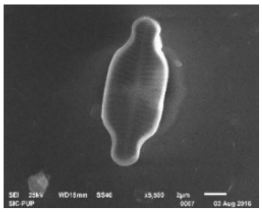


Fig. 7 *Achnanthidium exiguum*

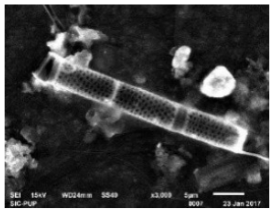


Fig. 8 *Aulacoseria granulata*

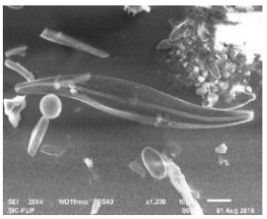


Fig. 9 *Gyrosigma attenuatum*

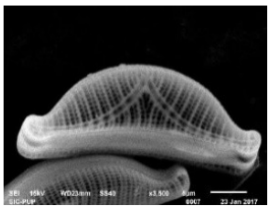


Fig. 10 *Epithermia sorex*

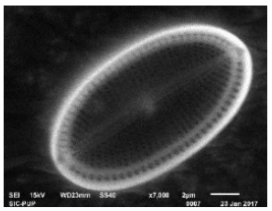


Fig. 11 *Cocconeis placentula*

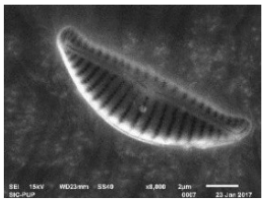


Fig.12 *Encyonema lange-bertaloti*

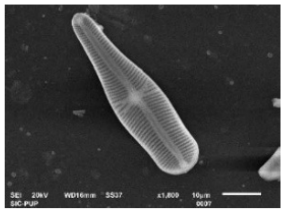


Fig. 13*Gomphonema turgidum*

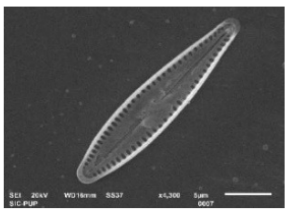


Fig.14 *Gomphonema germainii*

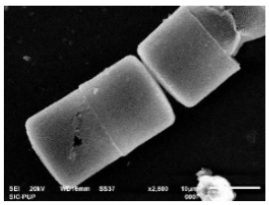


Fig.15 *Meloseria varians*

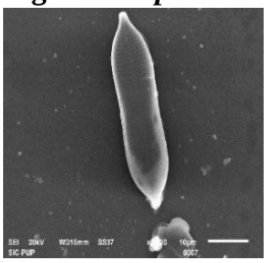


Fig. 16 *Caloneis cleve*



Fig. 17 *Eunotia minor*

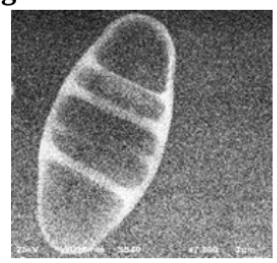
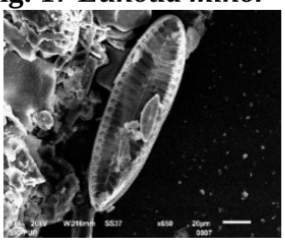


Fig. 18 *Diatoma vulgaris*





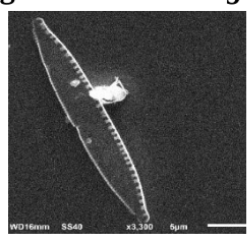


Fig. 19 ***Synedra famelica***

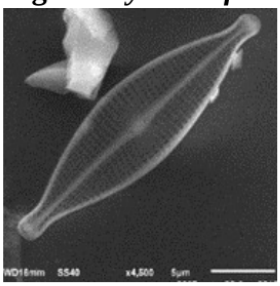


Fig. 20 ***Surirella tenera***

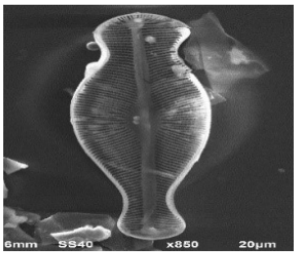


Fig. 21 ***Nitzschia maniuta i***

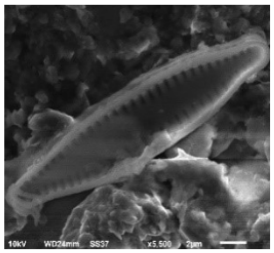


Fig. 22 ***Navicula bory***

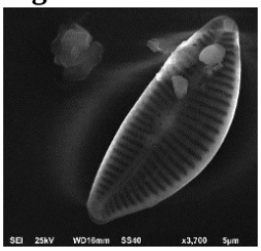


Fig. 23 ***Didymosphenia geminata***

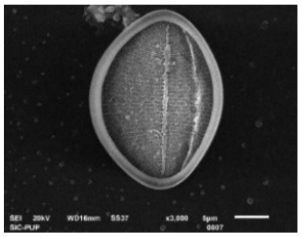


Fig. 24 ***Ulnaria contracta***

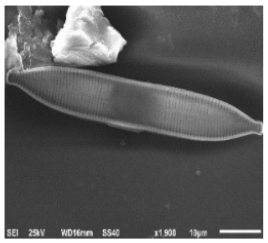


Fig. 25 ***Cymbella affinis***

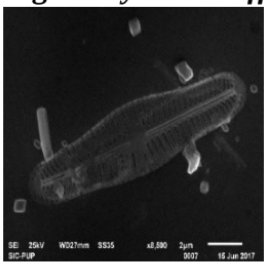


Fig. 26 ***Cocconeis pediculus***

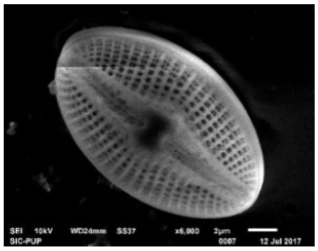


Fig. 27 ***Tabularia fasciculata***



Fig. 28 ***Pinnularia borealis***

Fig. 29 ***Diploneis elliptica***

Fig. 30 ***Pleurosigma lange bertalotii***

Photographic plate 1. Photomicrographs of identified diatoms by Scanning electron microscopy (SEM)

Table 1. Details of morphological characteristic observed by SEM

Characterstic	*Cymbella affinis*	*Achnanthidium exiguum*	*Cocconeis pediculus*	*Gomphonema turgidum*	*Gomphonema germainii*	*Hannaea arcus*	*Nitzschia minuta*	*Ulnaria contracta*	*Aulacoseria granulata*	*Diatoma vulgaris*	*Didymosphenia geminata*	*Encyone lange-bertalo*
Valve shape	Valves are dorsi - ventral with rostrate apices	Valves having linear- elliptical and capitate apices.	Valves are saddle-shaped frustules and having slightly pointed apices.	Valves are asymmetrical and broad.	Valves are linear clavate	Valves are asymmetric to the apical axis.	Valves are tapering gently to narrow rounded apices.	Valves are elongated and linear with constricted central area	Valves having cylindrical in shape	Eliptical valves with round ends	Valves are large and robust	Valve havin curve subrostr apice
No. of striae (in 10um)	20-25	24-25	16-22	10-14	10-12	13-14	36-39	10-12	-	40-45	7-9	14-16
Raphe	-	Raphe valve having distinct fascia while rapheless valve having small rectangular central area.	The raphe valves is narrow and irregular central area	Having complex raphe.	Undulate raphe	-	Eccentric raphe	-	-	-	Raphe is lateral with expanded proximal raphe and deflected distal raphe	Raphe fulifor
Striae	-	Striae more dense on raphe valve than rapheless valve.	Striae are Uniseriate	Striae are uniseriate	Striae are punctate	Straie are parallel present in the central swelling	Striae are fine and evident	Pseudo straie present	-	uniseriate	Radiate and irregular	Straie a radiate the dor side ar less pres on vent side
No. of fibulae (in 10 um)	-	-	-	-	-	-	present	-	-	-	-	-
Areola	25-30	50-55	20-25	20-26	-	-	-	-	10-20	-	-	-

Characterstic	*Cymbella affinis*	*Achnanthidium exiguum*	*Cocconeis pediculus*	*Gomphonema turgidum*	*Gomphonema germainii*	*Hannaea arcus*	*Nitzschia minuta*	*Ulnaria contracta*	*Aulacoseria granulata*	*Diatoma vulgaris*	*Didymosphenia geminata*	*Encyone lange-bertalo*
Keel	-	-	-	-	-	-	present	-	-	-	-	-
Rimoportula	-	-	-	-	-	present	-	present	present	present	-	-
Stigma	present	-	-	present	present	-	-	-	-	-	present	Prese
Spines	-	-	-	-	-	-	-	-	present	-	-	-
Sternum	-	-	-	-	-	present	-	-	-	-	-	-

Table 1 (continued)

Characterstics	*Diploneis elliptica*	*Rapholodia gibberula.*	*Cyclotella meneghiniana*	*Epithermia sorex*	*Punctastriata sp.*	*Gyrosigma attenuatum*	*Pleurosigma lange-bertalotii*	*Eunotia minor*	*Cocconeis placentula*	*Staurosira construens*	*Pseudostaurosira sp.*
Valve shape	Elliptic in shape	Valves having cresent shape	Valves are disc shaped	Valves having arched shaped	Having compex valves	Valves having S or Sigmoid and narrow shape	Valves having sigmoid - lanceolate	Having short raphe on side margin	Valves are linear and flat	Valves are cruciform	Valves elliptical
No. of striae (in 10um)	9-10	12-20	6-10	12-15	9-10	12-14	26-30	10-15	20-24	12-14	11-15
Raphe	-	Raphe present on dorsal margin	-	Eccentric raphe	-	Sigmoid and perpendicular.	Fuliform raphe	Present towards margin	Raphe valve having small circular and oval central area	-	-
Striae	Radiate and uniseriate	Straie present in between costa.	Present on circumference	Striae present in between costae.	Distinct straie	sigmoid	Straight and parallel	uniserate	Straie are present on the marginal	Straie are alternate present on	Mainly present at valve margin.

Characterstics	*Diploneis elliptica*	*Rapholodia gibberula.*	*Cyclotella meneghiniana*	*Epithermia sorex*	*Punctastriata sp.*	*Gyrosigma attenuatum*	*Pleurosigma lange-bertalotii*	*Eunotia minor*	*Cocconeis placentula*	*Staurosira construens*	*Pseudostaurosira sp.*
									sides. These are radiate in both raphae and rapheless valve	each side of valve.	
No. of fibulae (in 10 um)	-	-	-	-	-	-	-	-	-	-	-
Areola	10-14	-	6- 8	-	-	-	-	-	-	-	-
keel	-	present	-	-		-	-	-	-	-	-
Rimoportula	-	-	-	-		-	-	present at the apex of valve	-	-	-
Stigma	-	-	-	-		-	-	-	-	-	-
Spines	-	-	Marginal spines present	-		-	-	-	-	present	present
Sternum	-	-	-	-		-	-	-	-	present	-
Characterstics	***Stauroneis sp.***	***Mastogloia sp.***	***Rhicosphenia stoermeri***	***Sellaphora americana***	***Pinnularia borealis.***	***Caloneis cleve***	***Tabularia fasciculata***	***Surirella tenera***	***Synedra famelica***	***Trybilonella calida***	***Navicula bory***
Valve shape	Valves are elliptical - lanceolate	Valves elliptical and having rounded apices	Valves are heteropolar and clavate shape.	Valves are broadly linear	Valves are larger and round apices	Valves linear to linear lenceolate	Valves linear lanceolate to lanceolate	Valves are heteropolar	Having narrow valves	Linear valves and having narrow apices	Valves are lanceo
No. of striae (in 10um)	10-15	15-20	10-15	16-18	5-7	15-17	12-15	15-18	16-20	30-32	12-15
Raphe				Filliform		present	-	-	-	-	

Characterstics	*Diploneis elliptica*	*Rapholodia gibberula.*	*Cyclotella meneghiniana*	*Epithermia sorex*	*Punctastriata sp.*	*Gyrosigma attenuatum*	*Pleurosigma lange-bertalotii*	*Eunotia minor*	*Cocconeis placentula*	*Staurosira construens*	*Pseudostaurosira sp.*
	curved and inflated	Undulate and straight	Having minor undulations		Having complex raphe						Raphe is straigt a fuliform
Striae	Uniseriate	Punctate striae	More radiate towards the center of valve.	radiate throughout	Straie more radiate towards center	Straie are parallel to radiate	Striae are costate	-	Straie are parallel throughout	Indistinct striae.	Straie are more ra towards cente
No. of fibulae (in 10 um)	-	-	-	-	-	-	-	-	-	7-9	-
Areola	18-20	-	-	-	-	-	-	-	-	-	30-35
keel	-	-	-	-	-	-	-	present	-	-	-
Rimoportula	-	-	-	-	-	-	-	-	-	-	-
stigma	-	-	-	-	-	-	-	-	-	-	-
spines	-	-	-	-	-	-	-	-	-	-	-
sternum	-	-	-	-	-		present	-	-	-	present

Table 1 (continued)

Characterstics	*Achnanthes* mauiensis	*Ampipleura pellucida*	*Amphora ovalis*	*Bacillaria paradoxa*	*Craticula ambigua*	Cymatopleura solea	*Diatomella sp.*	*Gomphoneis minuta*	*Hantzschia amphioxys*	*Haslea sp.*	*Mulleria sp.*	*Nedium sp.*	Tabe fene
Valve shape	Linear in shape	Narrow valves	Semi elliptical valves having arched dorsal margin	Isopolar, linear in shape having narrow apices.	Lanceolate valves having rostrate ends.	Broad valves	Linear valves.	Valves having clavate shape, footpole rounded ends.	Valves are asymmetric	Lanceolate valves.	Valves are eliptical	Valves are linear	Valv li
No. of striae (in 10um)	15-19	40-48	9-12	20-24	13-21	20-34	20-25	10-12	20-25	11-25	20-26	10-12	14

Characterstics	*Achnanthes* mauiensis	*Ampipleura pellucida*	*Amphora ovalis*	*Bacillaria paradoxa*	*Craticula ambigua*	Cymatopleura solea	*Diatomella sp.*	*Gomphoneis minuta*	*Hantzschia amphioxys*	*Haslea sp.*	*Mulleria sp.*	*Nedium sp.*	Tabe fene
Raphe	Raphe having proximal ends deflected towards in one direction and with distal ends having curved in opposite direction.	Having Longitudinal ribs present surrounding the raphe.	Raphe are arched dorsally	-	Fuliform raphe present	Raphe present along the margin of valve.	-	Undulate raphe	Eccentric raphe present	Complex raphe	Straight raphe	Fuliform raphe present	
Striae	Punctate striae.	Having fine striae	Striae are denser towards center	Striae are fine and parallel.	Striae dense on one valve than other.	-	Parallel striae present	Multiseriate striae present	Uniseriate striae	Uniseriate	Striae radiate throughout.	Uniseriate	Pa st
No. of fibulae (in 10 um)	-	-	-	-	-	-	-	-	-	-	-	-	
Areola	-	-	-	-	-	-	-	-	-	-	-	-	
keel	-	-	-	present	-	present	-	-	-	-	-	-	
Rimoportula	-	-	-	-	-	-	-	-	-	-	-	-	pr
Stigma	-	-	-	-	-	-	-	present	-	-	-	-	
Spines	-	-	-	-	-	-	-	-	-	-	-	-	
Sternum	-	-	-	-	-	-	-	-	-	present	-	-	

3.1.2 D-mapping of selected waterbodies

D-mapping were generated based on intra and inter waterbody variations in the selected waterbodies. The results of the present study show that diatom composition varies from one site to another within a waterbody as well as among different waterbodies though levels of variation are different for different waterbodies. Seasonal variation of diatom within and among selected waterbodies was also recorded and has been summarized in **Table 2, 3 and 4.**

3.2 Discussion

3.2.1 Intra waterbody variations in diatoms

In **Gobind Sagar Lake**, 7 genera have been observed. Among these few diatom species i.e. *Aulacoseria granulata, Cymbella affinis* and *Fragillaria crotonensis* were observed in the bank region whereas some species such as *Bacillaria paradoxa* and *Gyrosigma attenuatum* were found to be mid region specific. The other species which were commonly seen in both bank and mid regions were *Cyclotella meneghiniana* and *Navicula bory.*

In **Renuka Lake**, 22 genera of diatom were identified in all seasons. Among these some diatom species i.e. *Cyclotella meneghiniana, Cocconeis placentula, Eunotia minor, Epithermia sorex, Melosira varians, Synedra famelica* and *Pleurosigma lange-bertalotii* were observed in bank region while few diatoms such as *Diploneis elliptica, Encyonema lange-bertalotii, Gomphonema turgidum* and *Stauroneis* were found only in the mid region. Other species such as *Amphora ovalis, Cymbella affinis, Fragillaria crotonensis, Gomphoneis minuta, Nitzschia minuta, Navicula bory, Pinnularia borealis, Pseudostaurosira sp., Rhicosphenia stoermeri* and *Rhapholodia gibberula* were commonly seen in both bank and mid regions.

In **Macchial Lake,** 24 genera were observed in all seasons. Among these some genera i.e. *Achanathes manuiensis, Cymatopleura solea, Diatomella sp., Fragilaria crotonensis, Hantzschia amphioxys, Mulleria sp., Nitzschia minuta, Navicula bory, Rhicosphenia stoermeri, Sellaphora americana* and *Ulnaria contracta* were observed in the bank region while one of the diatom i.e. *Didymosphenia geminate* was only found as mid region specific. Other diatom such as *Cymbella affnis, Gyrosigma attenuatum, Gomphonema germainii, Melosira varians, Surrirella tenera* were found in both regions. Maximum number of diatoms were observed in this waterbody.

In **Pondoh dam**, 22 genera were observed in all the seasons. Among these, some diatom species like *Achnanthes manuiensis, Cyclotella meneghiniana, Cymatopleura solea, Diatoma vulgaris, Encyonema lange-bertalotii, Nedium sp., Nitzschia minuta, Tabularia fasciculata* and *Ulnaria contracta* were seen in the bank region whereas few diatoms such as *Gomphonema turgidum* and *Pinnularia borealis* were found in mid region only. The common occurring diatom seen in both sites are *Nitzschia minuta, Stauroneis, Cymbella affinis, Caloneis cleve, Didymosphenia geminata, Fragillaria crotonensis* and *Hanneae arcus.*

In **Rewalsar Lake**, 14 genera were observed in all the seasons. Among these *Cyclotella meneghiniana, Encyonema lange-bertalotii, Eunotia minor, Fragilaria crotonensis, Hantzschia amphioxys* and *Tabularia fasciculata* were found in the bank region, while few species such as *Gomphonema turgidum* and *Rhicosphenia stoermeri* were seen in mid region only.

In **Manikaran Lake**, 14 genera were identified during all seasons as well. Among these diatoms such as *Cyclotella meneghiniana, Diatoma vulgaris, Epithermia sorex, Fragilaria crotonensis* and *Hantzschia amphioxys* were seen in bank region, while other diatom such as *Aulacoseria granulata,*

Eunotia minor and *Didymosphenia geminate* were found in mid region specific. It was observed that diatom *Cocconeis placentula* was richly present in the Manikaran Lake.

In **Prashar Lake,** 19 genera were observed in all seasons. Among these *Amphora ovalis, Nedium sp., Pleurosigma lange-bertalotii, Stauroneis sp., Surirella tenera* and *Tryblionella calida* were found in bank region, while other genera such as *Diatoma vulgaris* and *Fragilaria crotonensis* were found in the mid region. The commonly occurring diatom such as *Eunotia minor, Epithermia sorex, Hanneae arcus* and *Hantzschia amphioxys* were seen in both bank and mid region. *Pinnularia borealis* found abundantly in Prashar lake.

In **Nako Lake**, 9 genera have been observed across all the seasons. Among these one of the diatom i.e. *Gomphonema germainii* was seen in bank region, while other diatom species such as *Melosira varians* and *Navicula bory* were found in mid region only. Few diatoms such as *Cocconeis pediculus, Diatoma vulgaris, Rhicosphenia stoermeri, Epithermia sorex, Nitzschia minuta* and *Gomphoneis minuta, which* were observed in both the regions.

From the above observations, it is clear that majority of diatom were observed in the low altitude waterbodies (up to 1000 meters) like in Macchial Lake in both bank and mid regions because of temperature and light conditions were favorable for growth of diatoms, while less diatoms were seen in high altitude waterbody (above 2000 meters) such as Nako lake, where low temperature and less sunlight conditions were recorded [13].

3.2.2 Inter waterbody variations in diatoms

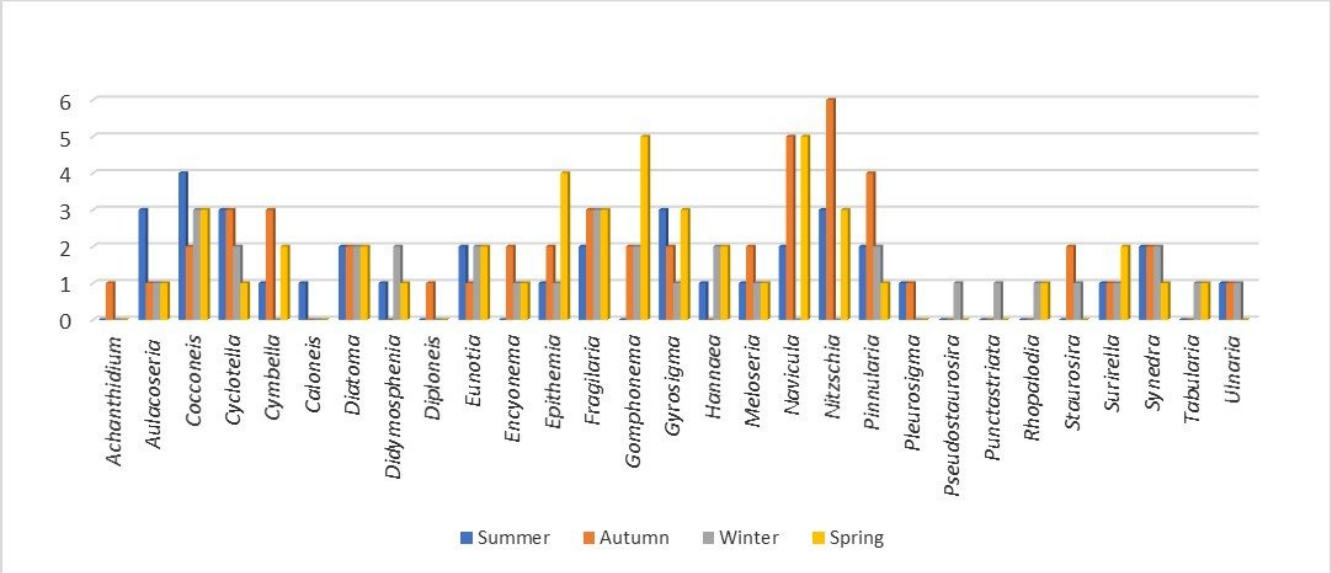
In the present study, the inter waterbody variation in diatoms was observed. The D-maps were generated on the basis of *commonly occurring diatoms, rare occurring diatoms, site-specific diatoms* and *seasonal diatoms.* The details are summarized in **table 5**.

In the present study, the commonly occurring diatom which were present in selected waterbodies during all the seasons i.e. *Cyclotella meneghiniana* (Gobind sagar), *Cocconeis placentula* (Renuka lake), *Melosira varians* (Macchial lake), *Hanneae arcus* (Pondoh dam), *Aulacoseria granulata* (Rewalsar lake), *Cocconeis placentula* (Manikaran) and *Epithermia sorex* (Nako lake). The site-specific diatoms which were seen only a particular waterbody i.e. *Bacillaria paradoxa* for (Gobind sagar), *Punctastriata mimetica, Diploneis elliptical, Pseudostaurosira sp*., for (Renuka lake), *Achanthidium exiguum, Craticula ambigua, Diatomella sp., Sellaphora americana* for (Macchial lake), *Didymosphenia geminata, Caloneis sp., Mastogolia sp*. for (Pondoh dam), *Staurosira sp*. for (Rewalsar lake), *Trybilonella calida* and *Amphipleura pellucida* for(Prashar lake) were observed in specific waterbody respectively. Other rare occurring diatoms such as *Stauroneis sp, Amphora ovalis* and *Achnanthes mauiensis* were seen in Renuka and Pondoh, Prashar and Nako, and in Macchial lake respectively.

The seasonal specific diatoms which were observed in all the seasons i.e *Aulacoseria granulata, Cyclotella meneghiniana* and *Cocconeis placentula* were found in Rewalsar, Gobind sagar and Manikaran lake respectively. The seasonal variation of diatoms was also thoroughly examined. The change in the diversity of the diatom across the entire seasons was based on the change in temperature difference and other local factors such as light, rainfall, pH and mineral requirements. [16] It was observed that the maximum diversity of diatoms species was found in the spring season. In the summer season, the maximum temperature recorded was 36°C due to the warm temperature less diversity of diatom were seen in this season as compared to autumn and spring season, [17] While in Autumn season the 30°C temperature was recorded. Large number of diatom diversity

was also seen in this season. In the winter season, the minimum temperature was recorded at 7°C and due to low temperature, the growth of diatom declined and less number of diatom species was found. Spring season, a bloom of diatoms was observed due to the presence of natural light conditions, which helps in photosynthesis of diatoms and abundance in diatom species was observed in this season [16]. The details of frequency of occurrence/ abundance of diatoms is given in **Graph 1.**

Diatom composition across various seasons



Graph 1. Graphical representation of diatom abundance in various seasons

Table 2. Intra -waterbody variation in Diatom composition of Gobind Sagar, Renuka and Macchial Lake

Waterbodies	*Gobind Sagar lake*								*Renuka lake*								*Macchial lake*							
Genera	*Summer*		*Autumn*		*Winter*		*Spring*		*Summer*		*Autumn*		*Winter*		*Spring*		*Summer*		*Autumn*		*Winter*		*Spring*	
	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M
Aulacoseria	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Achnanthes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Amphora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
Bacillaria	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cymbella	-	-	+	-	-	-	+	-	+	+	-	-	-	-	-	-	+	+	-	+	-	-	+	-
Cyclotella	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cymatopleura	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
Cocconeis	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-	+	+	-	-
Diploneis	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Diatomella	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Diatoma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Didymosphenia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Eunotia	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+
Encyonema	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Epithermia	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
Fragilaria	-	-	+	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-
Gyrosigma	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-
Gomphonema	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	+	+
Gomphoneis	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hantzschia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Melosira	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	+	+	-	+	+

Mulleria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Nitzschia	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	+	-	-	-	-	-
Navicula	+	-	+	+	-	-	+	-	-	-	+	+	-	-	+	+	-	-	+	-	-	-	-	-
Pinnularia	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Pleurosigma	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudostaurosira	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Punctastriata	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Rhicosphenia	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-
Rhapalodia	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
Stauronies	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Sellaphora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Synedra	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	-	+	-
Surirella	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+
Ulnaria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-

B= Bank region
M= Middle region

Table 3. [Pondoh dam, Rewalsar lake and Manikaran lake; untitled in the manuscript]

Waterbodies	*Pondoh dam*								*Rewalsar lake*								*Manikaran lake*							
Genera	*Summer*		*Autumn*		*Winter*		*Spring*		*Summer*		*Autumn*		*Winter*		*Spring*		*Summer*		*Autumn*		*Winter*		*Spring*	
	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M
Achanthes	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aulacoseria	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	+	-	+	-	-	-	-	-	-
Cymbella	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cocconeis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-
Cyclotella	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-
Cymatopleura	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Caloneis	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diatoma	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Didymosphenia	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Encyonema	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Eunotia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-
Epithermia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Fragilaria	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
Gyrosigma	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gomphoneis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Gomphonema	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Hantzschia	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
Haslea	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Hanneae	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nedium	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Navicula	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+

Nitzschia	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-
Pinnularia	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-
Rhicosphenia	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Stauroneis	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Staurosira	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Synedra	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Tabularia	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Tabellaria	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
Ulnaria	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. Intra -waterbody variations in Diatom composition of Prashar and Nako Lake

Waterbodies	***Prashar lake***								***Nako lake***							
Genera	***Summer***		***Autumn***		***Winter***		***Spring***		***Summer***		***Autumn***		***Winter***		***Spring***	
	B	**M**	**B**	**M**	**B**	**M**	**B**	**M**	**B**	**M**	**B**	**M**	**B**	**M**	**B**	**M**
Amphipleura	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Amphora	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-
Cocconeis	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Diatoma	-	-	-	+	-	-	-	-	-	+	-	+	+	-	-	-
Eunotia	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Epithermia	-	-	-	-	-	+	+	+		+	+	+	-	-	-	+
Fragilaria	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Gyrosigma	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Gomphonema	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	-
Gomphoneis	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Hanneae	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-
Hantzschia	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-

Melosira	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Nitzschia	-	+	-	-	-	-	-	-	+	+	+	-	-	-	+	+
Navicula	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Nedium	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Pinnularia	-	-	+	+	+	+	-	+	-	-	-	-	-	-	-	-
Pleurosigma	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Rhicosphenia	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-
Stauroneis	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Surirella	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Synedra	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tryblionella	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-

Table 5. Characteristic diatoms observed in the selected waterbodies

Characteristic Diatom	Selected waterbodies of present study							
	Gobind Sagar	***Renuka lake***	***Macchial lake***	***Pondoh dam***	***Rewalsar lake***	***Manikaran***	***Prashar lake***	***Nako lake***
Commonly occurring diatom	*Cyclotella meneghiniana* *Navicula caterva*	*Cocconeis placentula*	*Melosira varians*	*Hanneae arcus*	*Aulacoseria granulata*	*Cocconeis placentula*	-	*Epithermia sorex*
Rare occurring diatom	*Cymbella affinis* *Fragillaria sp.*	*Stauroneis sp.* *Amphora ovalis*	*Achanthes mauiensis* *Diatoma vulgaris* *Rhicosphenia stoermeri*	*Achnanthes mauiensis* *Stauroneis sp*	*Cyclotella meneghiniana* *Encyonema lange-bertalotii.* *Haslea sp.* *Navicula sp.*	*Aulacoseria granulata* *Diatoma moniliformis* *Eunotia minor*	*Aulacoseria granulata* *Cocconeis placentula* *Diatoma moniliformis* *Amphora ovalis.*	*Amphora ovalis*
Site specific	*Bacillaria paradoxa*	*Punctastriata mimetica* *Diploneis elliptical* *Pseudostaurosira sp.* *Synedra femilca*	*Achanthidium exiguum* *Craticula ambigua* *Diatomella sp.* *Sellaphora americana*	*Didymosphenia geminata* *Caloneis sp.* *Mastogolia sp*	*Staurosira sp*	-	*Trybilonella calida* *Amphipleura pellucida*	-

Seasonal diatoms	*Aulacoseria granulata* *Gyrosigma attenuatum*	*Cyclotella meneghiniana,* *Cymbella affinis*	*Hantzschia amphioxys*	*Pinnularia borealis*	*Aulacoseria granulata*	*Nitzschia minuta*

Conclusion

In the present study it is concluded that during the morphological examination of diatoms, sometimes the finer details of diatoms frustules such as their valve number, striae number, raphe details cannot be examined by LM even at higher magnification. SEM is required to examine them and correct identification of diatoms can be done up to the species level.

It is also concluded that variations occur within a waterbody during different seasons. Majority of diatoms was observed in the Spring season due to favorable environmental conditions while the adverse effect of light and temperature in the winter season declines the growth of diatom flora. Common, rare and specific diatoms have also been found in all the selected waterbodies, which will be helpful in establishing site of drowning.

Therefore, it is strongly recommended that investigators and forensic analysts must keep in mind the season while collecting and analyzing reference samples respectively in order to avoid false results.

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