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DIATOMOLOGICAL PROFILING OF SIX WATER BODIES OF RAJASTHAN

Pooja Rawat[1*], Gunjit Singhal[2], Dr. Umema Ahmed[3], Surya Shekhar Daga[4], Dr Ajay Sharma[5] Dr. Rajesh Singh[6] Sahar Zehra Naqvi[7]

[1]Department of Forensic Science, Vivekananda Global University, Jaipur
[2,3,4,5,6,7]State Forensic Science Laboratory, Jaipur,Rajasthan

ABSTRACT

Forensic Phycology plays a very important role in drowning cases where the site of drowning or the cause of death is suspected. Diatom profiles of various water bodies have been formulated by different researchers for different region for the same purpose. Apart from this, it also helps in analyzing the water quality index and diatom specificity of the region. The main aim of this study is to evaluate the diatoms of the major water bodies of tourist attractions sites of Rajasthan, which are more susceptible to drowning accidents. Ana Sagar, Fateh Sagar, Foy Sagar, Jaisamand Lake, Pichola Lake, Nakki Lake and Sambhar Lake were selected for this study. This study also aims to identify the site- specific diatoms and commonly occurring diatoms. The samples were collected during January-March and further analysis was done by centrifugation and slide preparation. The results of the qualitative estimation showed the presence of *Nitzschia* and *Aulacoseira* as commonly occurring diatom whereas *Navicula* was rarely seen. Two different species of *Cyclotella* were also observed like *Cyclotella ocellata, Cyclotella menghiniana. Encyonopsis* and *Encyonema* were the species which were reported for the first time in Rajasthan. Only *Meloseira* was reported in the Sambhar, the largest saline lake.

Keywords: Phycology, Water, Saline-lake, Tourist Attractions, Centrifugation, Diatom profile., etc.

INTRODUCTION

Diatoms are the basic association between primary (autotrophic) and secondary (heterotrophic) production with unicellular structure, mostly living as single cells or sometimes colonial, filamentous, ribbons, fans, zigzags, or stellate algae which are eukaryotic with amorphous silica [(SiO2)n(H2O)] cell wall [1]. Diatoms belong to the class of Bacillariophyceae with the feature of frustules having siliceous cell walls and have unique photosynthetic pigments and oil and chrysolaminarin as storage products [2]. They are very diverse, with 250 genera existing presently in every environment ranging from water to soil to ice and even volcanoes and 1,00,000 species in both aquatic and terrestrial habitat[3]. Based on shape, diatoms can be of two types i.e. Centric and Pennate diatoms. The centric have radial symmetry while penates have bilateral symmetry [4]. Centric diatoms are usually round or circular with an adaptation of living as phytoplankton in water column whereas Pennate diatoms are adapted to live in benthic condition, but sometimes found re-suspended in the water column[5, 6].

Diatoms are found to have diverse roles in different-different fields such as nanotechnology,

***Corresponding Author Email:** pooja.rawat@vgu.ac.in

biotechnology, ecology, forensics, pharmacology, etc. Due to its property of heat resistance and high sensitivity, it is used in boilers, blast furnaces and used in bioactive molecules attachment for modification [7]. It is very useful in ecological applications such as it can be used as a bio-filter for water purification since it is better than diatomaceous earth [8] and also acts as a biosensor for assessing water quality index by botanist and ecologists [9]. In forensic and environmental science, it can be used to generate flora profiles which can be used for identification of probable crime scene, drowning victims and approximation of time since death[10]. Forensic phycology is the application of diatoms in solving crime and establishing a link between the crime scene and crime[11]. It plays a very vital role in drowning cases in answering the questions like whether the drowning was anti-mortem or post-mortem, where did the drowning take place or the location of the crime scene, the time since death, etc.[12].

The principal behind finding whether the drowning was anti-mortem or post-mortem is that when the individual is drowned in water and is alive, during breathing a lot of water enters the lungs and exerts high pressure on the lungs wall[13]. This heavy pressure ruptures the alveoli in the lungs and therefore the water enters the blood circulation and now the water gets mixed with blood and also enters the vital organs like the liver, spleen, kidney, brain, femur, and bone marrow till the heart does not stop working[14]. Whereas in the case of post-mortem drowning, the respiration and circulation has already ceased, so the diatoms cannot be detected in the distant body organs, but can be present in lungs due to long time exposure of water and passive absorption of water[15].

Rajasthan, in a stereotypic view is land of deserts but still it has almost 80 artificial lakes, including the largest saline lake “Sambhar”, 7 medium dams and 5 major irrigation projects. Majority of the artificial lakes are the tourist attraction, hence can be a putative site for drowning accidents.

MATERIALS AND METHODS

Keeping in mind the sensitivity and probability of drowning cases, following water bodies which are major tourist attraction in Rajasthan are selected:

- Ana Sagar
- Fateh Sagar
- Foy Sagar
- Jaysamand Lake
- Nakki Lake
- Pichola Lake
- Sambhar Lake

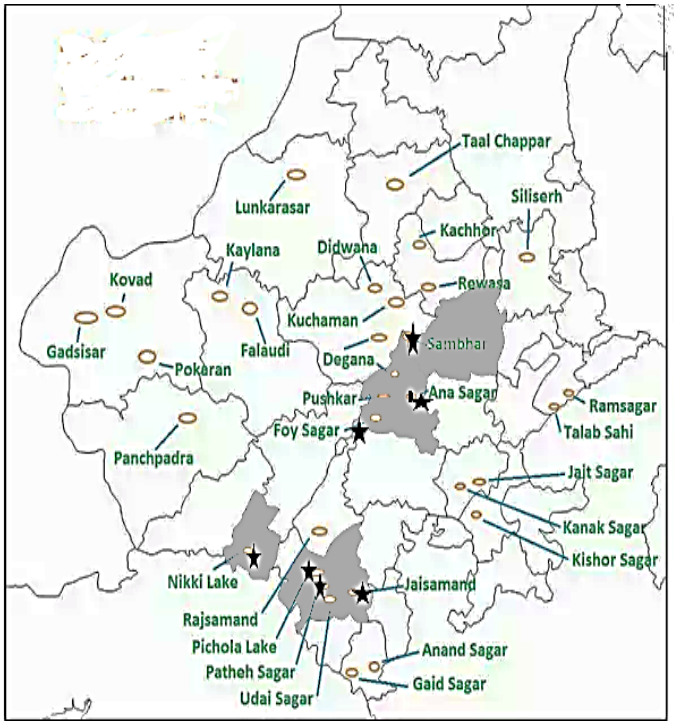


Figure. 1: Map showing Selected water-bodies and covered region

Table 1: Characteristics of selected water bodies

Name of Water Body	Nature of water body	pH	Depth	Temperature (approx.)	Sample Collection Month
Ana Sagar	Fresh water	7.1	4.4 m	23° C	February
Fateh Sagar	Fresh water	7.4	13.4 m	20° C	January
Foy Sagar	Fresh water	7.1	74 m	23° C	February
Jaysamand Lake	Fresh water	7.2	31 m	25° C	March
Nakki Lake	Fresh water	7.2	24 m	21° C	January
Pichola Lake	Fresh water	7.2	8.5 m	20° C	January
Sambhar Lake	Saline	7.5	3 m	30° C	March

SAMPLE COLLECTION

From the selected water bodies of different districts of Rajasthan, the water samples were collected in neat and clean water bottles with tightly fitted cap so as to avoid contamination. The surface of the water bodies was disturbed before collecting the sample to allow the passage of the mud while collecting the water.1L of water samples were collected from each of the water bodies. The bottles were labeled properly with the location of sampling area along with date and month.

EXTRACTION AND ANALYSIS OF DIATOMS

The samples were transferred to properly sterilized and labeled plastic centrifuge tubes and centrifuged at 3500 rpm for 10 minutes. The supernatant water was pipette out and only residual material was left at the bottom side. Then, again water sample was transferred into the centrifuge tubes and it was again centrifuge in the same way for 2 times.

Finally, the supernatant containing diatom frustules were spread on serially marked slides (1-5) for each sample. They were allowed to dry and after that permanently mounted with DPX. Slides were examined under compound microscope fitted with light source at different magnifications up to 1500X under oil immersion. Photomicrographs were captured using a computerized photo capturing device (Q-Win Leica) attached to microscope. Diatom species were identified on the basis of available literature [5,13-15]

RESULTS AND DISCUSSION

Table 2: Diatom Species Observed in Different Sample

Diatom	Water Bodies						
	Ana Sagar	Fateh Sagar	Foy Sagar	Jaysamand	Nakki Lake	Pichola	Sambhar
Achnanthidium	+	-	-	-	-	-	-
Asterionella	-	-	-	-	-	+	-
Aulacoseira	+	+	++	+	+	-	-
Chaetoceros	++	-	-	-	-	-	-
Cocconeis	-	+	-	-	-	-	-
Cyclotella	++	-	+	-	+	+	-
Cymbella	-	-	+	-	-	-	-
Encyonema	-	-	-	+	-	+	-
Encyonopsis	-	-	+	+	-	-	-
Eunotia	+	-	+	-	-	-	-
Fragilaria	+	++	+	+	+	++	-

Frustulia	-	+	-	-	-	-	-
Gomphonema	-	+	-	+	-	+	-
Halamphora	-	-	-	+	-	-	-
Melosira	-	-	+	-	-	+	+
Navicula	-	+	-	-	-	+	-
Nitzschia	+	+ +	+	+ +	+ +	+	-
Rhopalodia	-	-	-	+	-	+	-
Sellaphora	-	-	+	-	-	-	-
Stauroneis	-	-	+	-	-	+	-
Tabellaria flocu-losa	-	-	-	+	-	+	-
Tabularia	-	+	-	-	-	-	-

***Symbol description (+ = Present, + + = Dominant species, - = Absent)**

ANALYSIS OF DIATOM DISTRIBUTIONS

A significant variation has been observed on analyzing the diatom distribution pattern of the selected sites. *Nitzschia, Fragilaria* and *Aulacoseira* are the most common diatoms of freshwater bodies, although their quantity varies. Few diatoms are restricted to particular site only therefore known as site-specific diatom species or indicator species such as *Halamphora* in **Jaysamand,** *Tabularia* in **Fateh Sagar** etc. In Sambhar, only *Melosira* was observed as it is tolerant species of high salinity. *Cymbella, Aulacoseira granulate, Nitzschia, Rhopalodia gibba* are the good indicators of pollution. Therefore, it can be concluded that almost every water body is polluted due to the major tourist attraction of its region.

1.1 Diatom distribution of selected water bodies.

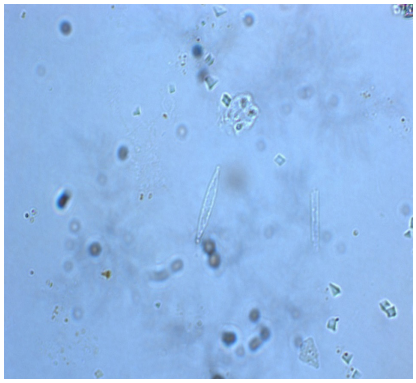
. 1.2.1 Ana Sagar:

The species which dominant here are *Chaetoceros* (indicator-species) and ***Cyclotella*** indicating that water is rich in electrolyte and many more minerals. Other species found here are *Eunotia minor, Fragilaria, Nitzschia palea, Aulacoseira granulate, Achnanthidium*. Two species varieties of *Cyclotella* are observed i.e. *Cyc. meneghiniana* and *Cyc. ocellata. Achnanthidium* is the specific to this site only among the selected water-bodies.

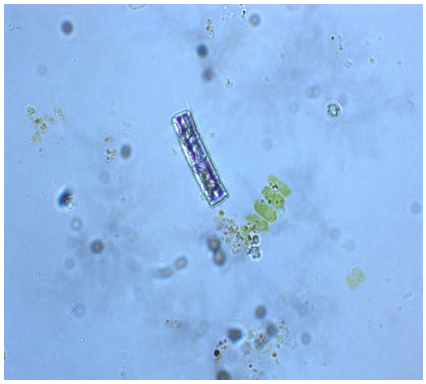
Table 3: Most Common Diatoms



[A] *Fragilaria*



[B] *Nitzschia*



[C] *Aulacoseira granulata*

Table 4: Diatoms of Ana Sagar

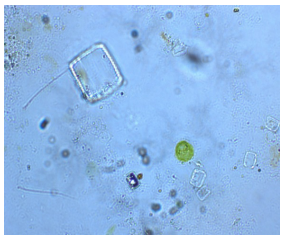
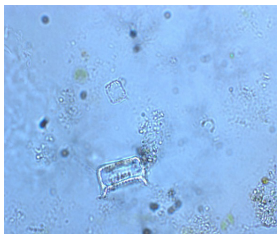
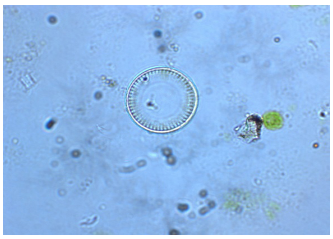
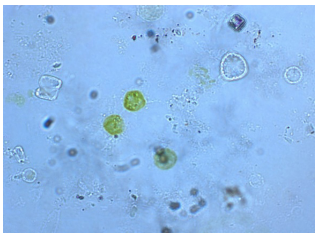
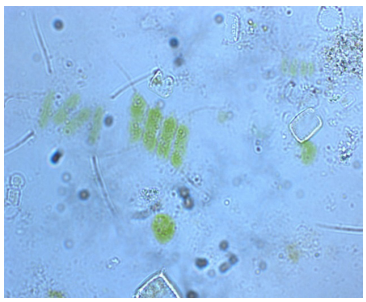
			
[A] *Eunotia minor*	**[B]** *Achnanthidium*	**[C]***Cyclotella meneng-hiniana*	**[D]** *Cyclotella ocel-lata*

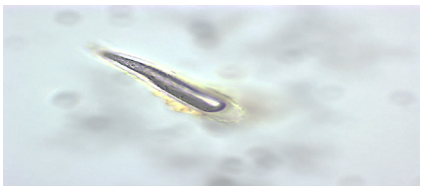
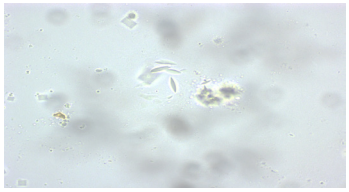
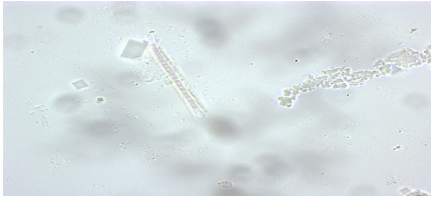
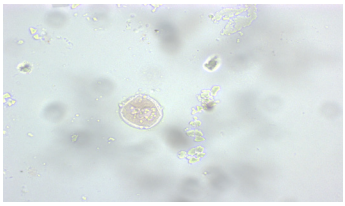
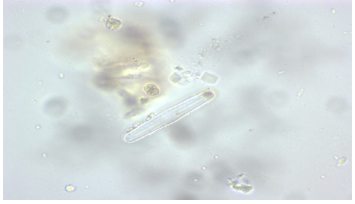
Table 5: Indicator species of Ana Sagar

	*Chaetoceros* (Indicator species)	

. 1.2.2 Fateh Sagar

The diatom species which are observed here are *Gomphonema, Fragilaria, Tabularia, Frustulia, Cocconeis, Navicula* and *Nitzschia.* The most dominant species here are *Nitzschia* and *Fragilaria. Nitzschia* being the most dominant indicate that this water body is rich in electrolyte and is brackish in nature and *Fragilaria* suggests that the water is planktonic rich. Cocconeis, *Frustulia* and *Tabularia* are the site-specific diatom here indicating that water is modestly polluted by industries and tourism around it.

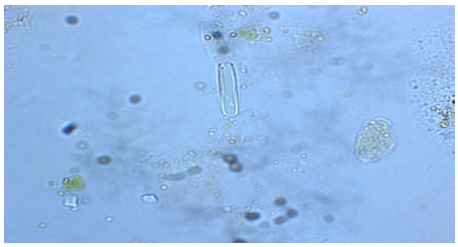
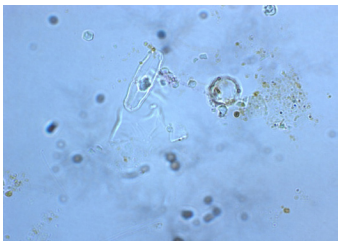
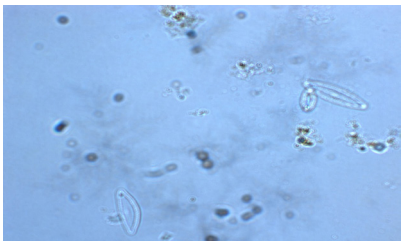
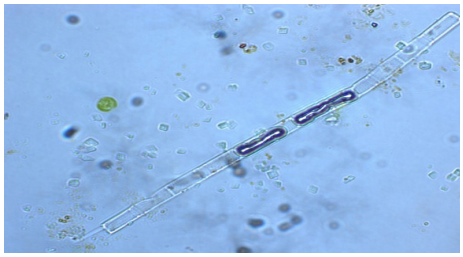
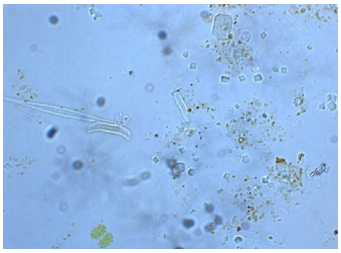
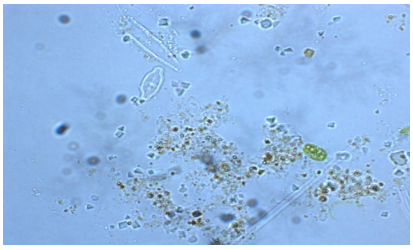
Table 6: Diatoms of Fateh Sagar

		
[A] *Gomphonema*	**[B]** *Navicula*	**[C]** *Nitzschia*
		
[D] *Tabularia*	**[E]** *Cocconeis*	**[F]** *Frustulia*

. 1.2.3 Foy Sagar

Aulacoseira granulata is the freshwater diatom which is found in abundance here. Other species found here are *Stauroneis, Nitzschia, Meloseira, Cyclotella meneghinian, Eunotia minor, Cymbella, Encyonopsis* and *Sellaphora.* According to this research, *Eunotia minor* is the species which is present here and also in the Ana Sagar which is also present in Ajmer, indicating that this species is specific to the Ajmer district . *Sellaphora* and *Cymbella* are the site-specific diatoms here.

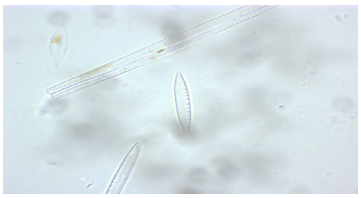
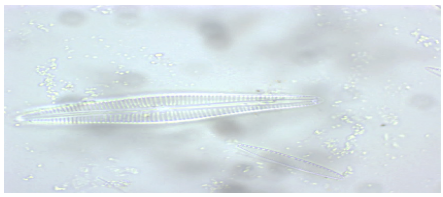
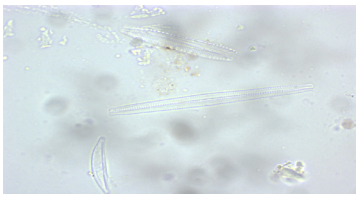
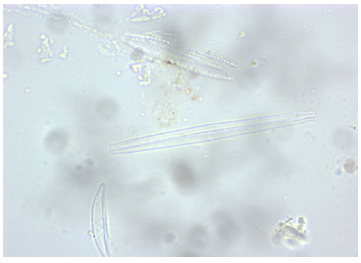
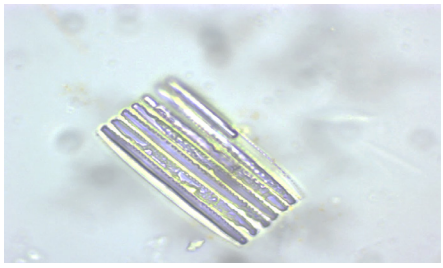
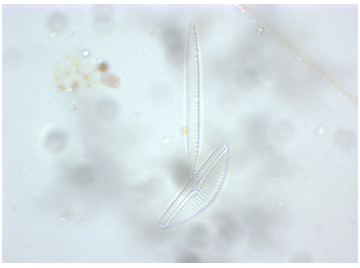
Table 7: Diatoms of Foy Sagar

		
[A] *Encyonopsis*	**[B]** *Sellaphora*	**[C]** *Cymbella*
		
[D] *Aulacoseira granulata*	**[E]** Upper one *Nitzschia* and lower one *Eunotia minor*	**[F]** *Stauroneis*

. 1.2.4 Jaysamand Lake

Nitzschia dissipata, Rhopalodia gibba, Fragilaria, Encyonopsis, Gomphonema augur, Nitzschia palea,Nitzschia linearis, Gomphonema gracile, Encyonema, Halamphora and *Tabularia flocculosa* are the species of diatoms found here. It can be therefore concluded that *Halamphora* is the indicator species of this sample. *Nitzschia* is the dominant species here whereas, *Navicula* was negligible here.

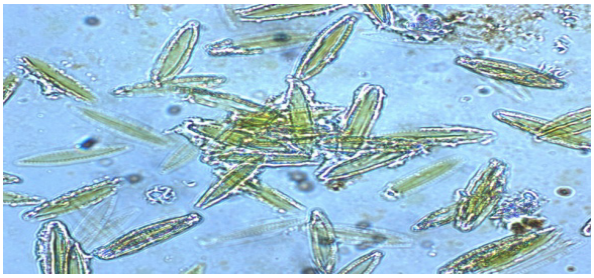
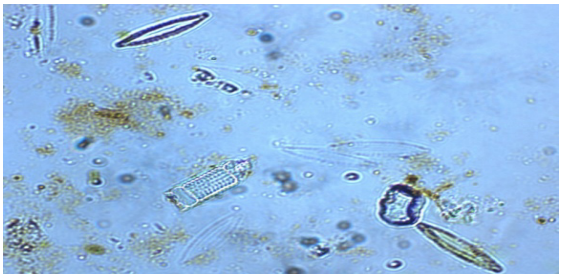
Table 8: Diatoms of Jaysamand Lake

		
[A] *Fragilaria and Nitzschia*	**[B]** *Gomphonema augur*	**[C]** *Nitzschia palea upper one and Nitzschia linearis*
		
[D] *Hallamphora*	**[E]** Tabellaria flocculosa	**[F]** *Encyonema*

. 1.2.5 Nakki Lake

Nitzschia here is also dominant species here whereas Cyclotella is rarely found here suggest that it is not found at heights usually. *Nitzschia palea* which is a tolerable strain here due to its height above the sea level. Other species found here are *Aulacoseira granulata and Fragilaria*

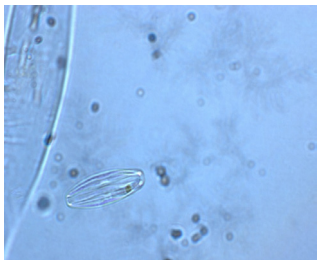
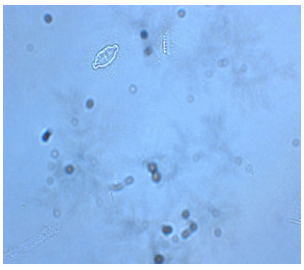
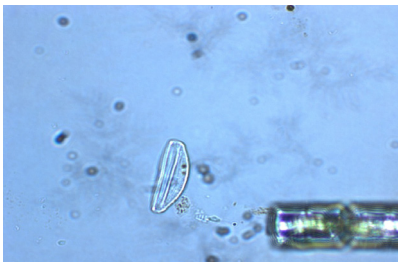
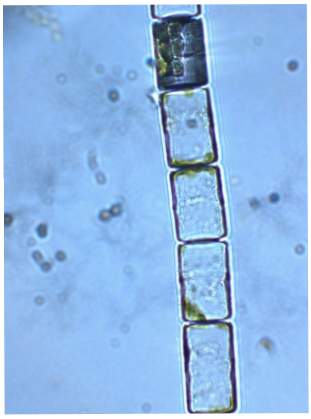
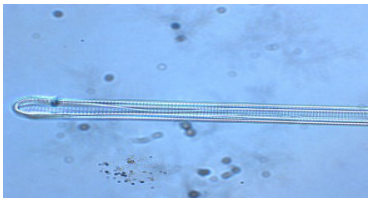
Table 9: Diatoms of Nakki Lake

 **[A]** *Nitzschia*	 **[B]** *Nitzschia palea and Aulacoseira granulata*

. 1.2.6 Pichola Lake

This lake has variety of diatoms present here. *Fragilaria, Stauroneis, Asterionella, Nitzschia, Tabellaria flocculosa, Nitzschia amphibian, Gomphonema augur, Cyclotella menenghniana, Rhopalodia, Encyonema, Fragilaria, Navicula Stauroneis, Rhopalodia gibba* and *Melosira* are observed here. *Asterionella* is the site-specific diatom here and *Fragilaria* is the dominant one.

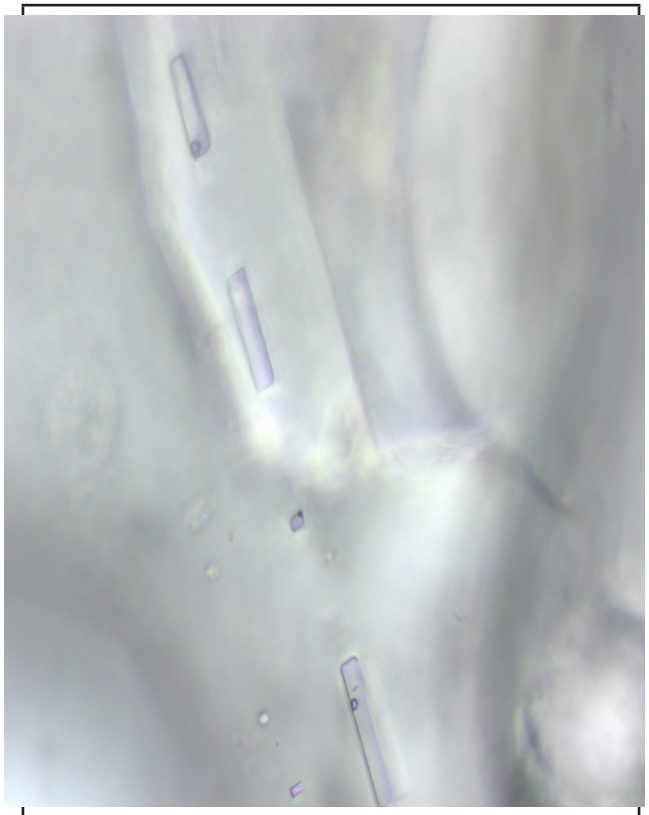
Table 10: Diatoms of Pichola Lake

 **[A]** *Rhopalodia*	 **[B]** *Stauroneis*	 **[C]** *Encyonema*
 **[D]** Meloseira	 **[E]** *Gomphonema*	   **[F]** *Asterionella*

. 1.2.7 Sambhar Lake:

Due to the heavy presence of NaCl in the sample, crystals were formed on the slide and only the species of *Melosira* was observed. Some crystal like structure were also observed. In future, further studies can be done on this sample by removal of NaCl prior to centrifugation.

Table 11: Diatom of Sambhar Lake



[A] *Meloseira*

Table 12: Observation

Commonly occurring diatoms	Rarely occurring Diatoms	Site specific diatoms	Remarks
Nitzschia *Aulacoseira*	*Encyonema* *Encyonopsis* *Stauroneis* *Tabellaria flocculosa* *Eunotia minor* *Rhopalodia gibba*	*Achnanthidium* *Asterionella* *Chaetoceros* *Cocconeis* *Cymbella* *Frustulia* *Halamphora* *Tabularia*	*Meloseira* is found in the saline lake and more research can be done for the comparison by removing the NaCl from the sample

CONCLUSION

From the present study it can be inferred that almost all the major tourist attraction lake's have *Nitzschia, Aulacoseira* however their number varies. Some lakes have site specific diatoms like *Tabularia* in Fateh Sagar, *Sellaphora* in Foy Sagar etc. All the results have been formulated in table and further studies for generating D-maps can be done by studying seasonal varations in future.This D-Maps will aid in forensic investigation of drowning cases and will provide useful lead to the forensic experts.

REFERENCES

1. Rahman, T.T., et al., *Diatoms in wastewater treatment: Potentials, applications, and values of biomass.* 2024. **54**(7): p. 557-580.
2. Mann, D.G. and R.J.P. Trobajo, *Diatoms as dandelions: convergent evolution in the reproductive biology of small Nitzschia species (Bacillariophyta) and its possible taxonomic consequences.* 2024: p. 1-17.
3. Spaulding, S.A., et al., *Diatoms. org: supporting taxonomists, connecting communities.* 2021. **36**(4): p. 291-304.
4. Medlin, L.K.J.P., *Evolution of the diatoms: major steps in their evolution and a review of the supporting molecular and morphological evidence.* 2016. **55**(1): p. 79-103.
5. Kooistra, W.H.J.H.o.B.B.A. and S. Formation, *The evolution of the diatoms.* 2007: p. 95-111.
6. Janousek, C.N., *Functional diversity and composition of microalgae and photosynthetic bacteria in marine wetlands: spatial variation, succession, and influence on productivity*. 2005: University of California, San Diego.
7. Levkov, Z., et al., *The use of diatoms in forensic science: advantages and limitations of the diatom test in cases of drowning.* 2017: p. 261-277.
8. Md Anawar, H. and R.J.S. Chowdhury, *Remediation of polluted river water by biological, chemical, ecological and engineering processes.* 2020. **12**(17): p. 7017.
9. B-Béres, V., et al., *Ecosystem services provided by freshwater and marine diatoms.* 2023. **850**(12): p. 2707-2733.
10. Metcalf, J.L., et al., *Microbiome tools for forensic science.* 2017. **35**(9): p. 814-823.
11. Pal, S., N. Bhardwaj, and A. Ahluwalia, *Role of Diatoms in Forensics: A Molecular Approach*, in *Insights into the World of Diatoms: From Essentials to Applications*. 2023, Springer. p. 143-163.
12. Morabito, M. and R.J.A.d.A.P.d.P.-C.d.S.F. Somma, Matematiche e Naturali, *The crucial role of Forensic Botany in the solution of judicial cases.* 2023. **101**(S1): p. 11.
13. Bhardwaj, N., et al., *Potential of golden brown algae in forensic analysis: a review.* 2021: p. 353-373.
14. Somma, R., et al., *RECENT ADVANCES IN FORENSIC GEOLOGY AND BOTANY FOR THE RECONSTRUCTION OF EVENT DYNAMICS IN OUTDOOR CRIME SCENES: A CASE STUDY.* 2023. **101**.
15. Marshall, M.A., *Diatom Interactions with Bone as a Substrate: Ecological Considerations and Implications for Forensics*. 2022, Tarleton State University.