Diatomological Mapping of Aurangabad Region for Unrevealing Drowning Mystery

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Abstract

Purpose:

Drowning is the third unnatural cause of death in India. Drowning death is still considered as one of the most difficult to investigate, it requires the expertise in the pathophysiology and limnology. Long procedural protocol in laboratory analysis of vital organs and the site to obtain the victim and site-relation that creates a burden on the investigating agency. The presence of diatoms in the vital organs of the body helps to establish the pre- or post-mortem drowning death and the appearance of site-specific/unique diatoms for a region aids into locating the exact drowning site. The onsite identification can be solved by studying the diversity and preparing the diatoms map (D-map). Availability of D-map can help to reduce the investigation time and helps in confirmation of the drowning site.

Methods:

In the present study, samples were collected from the 12 water bodies in Aurangabad region M.S. India, and were acid digested, using Aqua regia solution containing HNO_3 : H_2SO_4 in 1:3 proportion in 20ml of water sample. Slides were prepared, mounted and microscopic analysis was performed using light and inverted phase microscope.

Results:

The D-map was generated and it featured 55 identified species, amongst the members of *Nitzschia*, *Navicula* and *Cymbella* were commonly found in many water bodies, while *Craticula*, *Gomphonema*, *Tibetiella*, *Melosira*, *Pleurosira*, and *Mastogloia* were restricted to only single water body. These unique diatoms can be used as site-specific indicators in drowning investigation of Aurangabad region. This study generated diatomic map for experimental water body present in Aurangabad region.

Conclusion:

These D-maps will be helpful in dispense a significant information about water bodies and their common as well as unique flora with a qualitative and quantitative distribution of diatoms. D- Map generated in this study can be harnessed to update knowledge of diatomology or diatoms database and generated data will aid in drowning investigation.

1. Introduction

Diatoms are unicellular eukaryotic microalgae inhabited in the silica glasshouse. They are cosmopolitan, profoundly seen in fresh and marine habitats. Being phytoplankton, they are the major source of atmospheric oxygen and are a key player in organic carbon fixation on earth. Diatoms are very sensitive to the nutrient content, temperature, seasonal variation [1], pH (Potential of hydrogen), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and the availability of silica in water. These factors have an impact on the growth of diatom biomass and show specificity towards all these parameters. Though some species are commonly found across the world, some are unique to location and season. Thus, they are used as one of the “biotic indices” i.e., water quality, aquatic ecosystem health flora and fauna etc. [2]. The global studies have estimated that the number of diatom taxa is around 200,000 [3]. India harbors one of the seventh largest biodiversity of the world, 14,700 taxa from freshwater and marine aquatic [4]. Diatoms are classified according to the structure of their siliceous cell wall, based on their valve shapes into centric and pennate diatoms. The centric diatoms have valves with radial symmetry and ribs, central stellate ornamentation, spins, mantle, and stria on the frustules are the features for its identification. The Pinnate have narrow elliptical valve shapes such as diverse raphe, apex-shapes, areolae pattern, longitudinal lines, lineolate, puncta features of their frustules. Further based on the symmetry they are classified into Asymmetric Biraphid, Symmetric Biraphid, Monariphid, Epithelioid and Nitzsoid.

The above said species are mainly encountered in the drowning Investigation, the presence or absence of diatoms in the drowned body indicates the suicidal or homicidal death but many times it needs further investigation.

Long procedural protocol in laboratory analysis of vital organs and the site to obtain the victim and site-relation that creates a burden on the investigating agency.

Drowning death possesses a significant challenge to forensic investigators, as it requires expertise in the pathophysiology, autopsy report [5]. Three major factors that impede the investigations include putrefaction, pre- and post-mortem drowning and drowning site. Since the diatoms were first discovered in the lungs by Hofmann in 1896 and were first used by Revenstorfin in 1904 for diagnostic purposes. Since then, diatoms role has been extensively explored and evolved with time. Diatoms plays a significant role in the forensic investigation of drowning cases. In such investigations, questions arise about the pre and post mortem drowning, along with suspicion on the drowning site [6]. Diatom test is the “gold standard” method [7]. Many other tests are available for drowning investigation, but they are not widely accepted and appreciated, rarely used as compared to the diatom test [8]–[10].

Diatom test is theoretically based on the presence of diatom in the vital organs of the body such as lungs, bone marrow, kidney and brain [11], [12]. When a person tries to breathe inside water bodies such as lake, ocean and river, the diatoms along with water penetrates in the alveolar system of the human body and gets deposited in vital organs by blood circulation. However, when the dead body is thrown in the water, diatoms enters in the body but cannot be deposited in the vital organs [13], [14]. The occurrence or recovery of diatoms mainly from the organs such as bone marrow and lungs in the body is caused due to aspiration of water and is considered as premortem drowning and the absence indicates non-drowned death. Thus, the accuracy in the diatom identification is very much important in drowning death investigation [5].

In the diatom test, a drowning site is located by comparing the species of diatoms extracted from tissue samples and the water samples of suspected water bodies as they are site-specific [15]. In numerous instances, there is a possibility that the body which has been drowned and moved away from the actual site of drowning, where no other references are available to locate [6], [13] After extraction from the tissue, the forensic scientist will have to compare the species present in the tissue over the putative sample.

As per the National Crime Record Bureau (NCRB) drowning is 3rd leading cause of unnatural death in India. Drowning death recorded 7.3% (30,187) and 7.8% (32,671) in India while 4,165 and 4,516 in the State of Maharashtra of total cases of unnatural death in 2018 and 2019 respectively [16] [17]. Since the numbers of drowning cases are growing, it is necessary to have a regional diatomic distribution map to validate, identify the drowning site and increase the rate of investigation. However, as per literature search the current diatomological data is not available for Aurangabad (India) region. Aurangabad region is on the Godavari river basin and the district is located between 19° and 20° north longitudes, and 74° and 76° east latitudes (Figure 3). The region has several large and small water bodies where numerous drowning cases are reported. The present study was intended to study diatom distribution, prevalence and diversity. D-Map is the diatomological map that defined the diatom diversity of defined region.

2. Methods

Sample collection was performed using the following standard collection protocol [18]

2.1 Selection of Water Bodies and Sample Collection:

Twelve major suicidal prone water bodies (see Table-14) from Aurangabad region M.S. (India) were selected and the samples were collected (Benthic, Phytoplankton, Epilithon, Epiphyton and surface) from different sites of water bodies by standard modified collection protocol [18] in the autumn season. All the information was documented including sample collection time, the geographical location of the water body i.e., longitude and latitude. Approximately 200-300ml surface

water samples were collected and directly transferred into sterile sealed plastic bags. To extract diatoms from substrata stones were collected in bags & were vigorously shaken. The samples were brought to the laboratory for further analysis.

2.2 Sample Preparation:

2.2.1 Aqua Regia Acid Digestion Method for Diatom Extraction:

A modified standard acid digestion protocol was used for diatom extraction [18]. The increased acid concentration reduced the time required to digest all organic material in the sample

Aqua regia is a mixture of two strong acids i.e. nitric acid and sulphuric acid in a 1:3 ratio to digest all the organic material in a sample (HNO_3 : H_2SO_4) (1:3) [18]. About 20ml (milliliter) stored sample was vigorously mixed, then transferred to 100ml clean beaker, to which equal volume of saturated Potassium Permanganate (KMnO_4) solution was added, and incubated at room temperature for 3 hours. Later, 20ml of aqua regia was added and kept for 24 hours undisturbed for sediment digestion. Next day the solution was placed at 90°C on a hot plate for 2 hours until the solution turns colorless and subsequently transferred into the 15ml centrifuge tube (Tarson Products, Kolkata, India). Later these samples were centrifuged in an Eppendorf tube (Eppendorf Hamburg, Germany) at 3000 (Revolutions per minute) RPM for 10 minutes and the pellets were resuspended and collected with 2ml aqua regia. This step was repeated twice for concentrating the diatoms for slide preparation and analysis.

2.2.2 Slide Preparation and Microscopic Analysis:

A drop of diatom sample was evenly spread over a clean grease-free glass slide, covered with a coverslip and air dried. Subsequently, the microscopic slide was permanently mounted with Dibutylphthalate Polystyrene Xylene (DPX), (Thermo Fisher Scientific, Massachusetts, United States) and observed under the inverted phase contrast compound microscope (Magnus OPTO systems Microscope, New Delhi, India) 100x, oil immersion objective for species identification. The slides were scanned and analyzed by the inverted phase contrast compound microscope. Photographic analysis for the identification of taxa of the diatom was carried out using available literatures of commonly found diatoms of India and the available guides from web portals such as diatoms.org, diatombase.org and An illustrated guide to a Common Daitoms of Peninssular India [19]–[21]

3. Results

In current study generated data shows the distribution pattern of diatom species in 12 different sites (Table No. 1 to 12) that were taken for sampling. Distribution of diversity helps to identify the key indicator species of the drowning site. The unique and key indicator species such as *Craticula*, *Gomphonema*, *Tibetiella*, *Melosira*, *Pleurosira*, and *Mastogloia* were identified in (Water body) WB1, WB4, WB5, WB8 and WB12 respectively (Figure No. 4, Table No. 13). This study has generated data that will assist in the investigation and to locate a site of drowning in the Aurangabad region (Table No.14) [22].

While the characterization of all sites, we found that WB1 has the highest diversity with 17 identified species of 8 genus, while in WB3, 11 species were identified. However, in both sites, we noticed *Nitzschia*, *Navicula*, and *Cymbella* were the most common genus. In WB7 and WB9, 6 species were detected at both the sites. In WB8 the 5 species were identified. In WB4, WB10, WB11 and WB12, 4 diatom species were reported. 3 Species were identified in WB6 with 2 species in WB2 and WB5 (Figure No. 1)

In the genus distribution *Nitzschia* was the most common species found in 10 different sites followed by *Navicula* and *Cymbella*, observed in 7 and 8 different sites. *Ulnaria* and *Cyclotella* were found in the 6 different sites whereas *Pinnularia*, *Diploneis* and *Encyonema*, were comparatively rare as were found in only in 2 sites. *Craticula*, *Gomphonema*, *Tibetiella*,

Melosira, *Pleurosira*, and *Mastogloia* were unique diatoms and their presence is restricted to only one location (Table number 13, Figure No. 2).

4. Discussion

The clear image of diatom thus obtained helped in accurate identification of the species even without the highly sophisticated instruments. A lucid and conspicuous image are required to identify the taxa and demarcated based on cell wall (valves) shape as well as the number stria present.

D-map is a concept of making representative information of all known and unknown identified species in the area of study, all data regarding the particular species

Microscopic and invisible diatoms accidentally adhere to the victims' clothing in addition to their footwear that can also lead to identification or to co-relate the drowning site and also can discover the victim/suspect relation [23], [24]. The availability of the D-map results can assist in quick correlation to the victim/suspect as well as to the drowning site. Diatoms are only evidence to be considered in the putrefied body to conclude the drowning death [25]. The autopsy contamination and non-vegetarian foodstuff can give false positive diatom tests in case of non-drowned death [26].

5. Conclusion

Diatoms are the key indicators of drowning death and can be used to locate the site of the drowning. The present study demonstrates that the acid concentration is inversely related to the time required for organic matter digestion and helps to improve image clarity. The current study intends to identify predominant diatoms in suicide prone water bodies of the Aurangabad region and prepare a database.

The diatoms restricted to only single water body are unique diatoms and can be used as site-specific indicators and marker in drowning investigation for the Aurangabad region. This study generated diatomic map for experimental water body present in Aurangabad region. These D-maps will be helpful in dispense a significant information about water bodies and their common as well as unique flora with a qualitative and quantitative distribution of diatoms. D- Map generated in this study can be harnessed to update knowledge of diatomology or diatoms database and generated data will aid in drowning investigation. Further studies are required to generate D-map data at global level to speed up the investigation and reduce the burden of investigators. Further the method of extraction and identification of diatoms in both putative and the visceral sample is arduous and time consuming, requires experience and knowledge in the field of limnology to minimize the errors in the Diatomic test.

Abbreviations

D-Map- Diatomological map

HNO₃- Nitric acid

H₂SO₄- Sulfuric acid

ml- Milliliter

pH- Potential of hydrogen

BOD- Biological oxygen demand

COD- Chemical oxygen demand

NCRB- National crime record bureau

KMnO₄- Potassium permagnet

RPM- Revolution per minute

DPX- Dibutylphthalate polystyrene xylene

WB- Water body

UN- Unidentified

VD- Valve dimeter

Declarations

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Conflicts of interest/Competing interests:

The authors declare that they have no competing interests

Availability of data and material:

All data generated or analyzed during this study are included in this published article.

Code availability:

Not applicable

Authors' contributions:

JA and SB author performed lab experimentation and helped in manuscript preparation. AD / corresponding author conceived the idea of manuscript preparation, design, data analysis, interpretation and critical revision of the article. SG helped in design, data analysis and gave valuable inputs in manuscript writing. All the authors read, discussed the results and gave approval for submission.

Ethics approval:

Not applicable

Consent to participate

Not applicable

Consent for publication

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References

1. M. P. Stoyneva, J. P. Descy, and W. Vyverman, "Green algae in Lake Tanganyika: Is morphological variation a response to seasonal changes?," in *Hydrobiologia*, 2007, vol. 578, no. 1, pp. 7–16, doi: 10.1007/s10750-006-0428-1.
2. A. De Wever *et al.*, "Differential response of phytoplankton to additions of nitrogen, phosphorus and iron in Lake Tanganyika," *Freshw. Biol.*, vol. 53, no. 2, pp. 264–277, 2008, doi: 10.1111/j.1365-2427.2007.01890.x.
3. D. G. Mann and S. J. M. Droop, "Biodiversity, biogeography and conservation of diatoms," in *Hydrobiologia*, 1996, vol. 336, no. 1–3, pp. 19–32, doi: 10.1007/BF00010816.
4. L. K. Pandey, K. K. Ojha, P. K. Singh, C. S. Singh, S. Dwivedi, and E. A. Bergey, "Diatoms image database of India (DIDI): A research tool," *Environ. Technol. Innov.*, vol. 5, no. Didi, pp. 148–160, 2016, doi: 10.1016/j.eti.2016.02.001.
5. N. Fucci, V. L. Pascali, C. Puccinelli, S. Marcheggiani, L. Mancini, and D. Marchetti, "Evaluation of two methods for the use of diatoms in drowning cases," *Forensic Sci. Med. Pathol.*, vol. 11, no. 4, pp. 601–605, 2015, doi: 10.1007/s12024-015-9708-2.
6. M. K. Thakar and R. Singh, "Diatomological mapping of water bodies for the diagnosis of drowning cases," *J. Forensic Leg. Med.*, vol. 17, no. 1, pp. 18–25, 2010, doi: 10.1016/j.jflm.2009.07.016.
7. M. H. A. Piette and E. A. De Letter, "Drowning: Still a difficult autopsy diagnosis," *Forensic Sci. Int.*, vol. 163, no. 1–2, pp. 1–9, 2006, doi: 10.1016/j.forsciint.2004.10.027.
8. N. P. M. piette, J. Timperman, "Serum strontium estimation as a drowning indicator: Statistical evidence revised," *Med. Sci. Law*, vol. 29, no. 4, p. 347, 1989.
9. B. M. R. Jeanmonod, CH. Staub, "The reliability of cardiac haemodilution as a diagnostic test of drowning," *Forensic Sci. Int.*, vol. 52, pp. 171–180, 1992.
10. Z. J. Z. C. Y. Chuan, D. Z. Ke, "The significance of detecting serum flourine level n the diagnosis of drowning," *Forensic Sci. Int.*, vol. 164, pp. 29–294, 1990, doi: 10.1016/j.forsciint.2011.02.006.
11. E. J. Armstrong and K. L. Erskine, "Investigation of Drowning Deaths: A Practical Review," *Acad. Forensic Pathol.*, vol. 8, no. 1, pp. 8–43, 2018, doi: 10.23907/2018.002.
12. A. Kumar, A. Singh, R. Joshi, S. Mittal, and I. Tayal, "Role of study of diatoms in forensic investigations - two year retrospective study," *J. Indian Acad. Forensic Med.*, vol. 38, no. 3, pp. 321–325, 2016, doi: 10.5958/0974-0848.2016.00081.6.
13. D. Luthra, M. K. Thakar, and J. S. Khattar, "Forensic study of the distribution of diatoms in Bhakra canal near Narwana, Haryana, India," *AIP Conf. Proc.*, vol. 2142, no. August, 2019, doi: 10.1063/1.5122649.
14. N. Kaushik, K. S. Pal, A. Sharma, G. Thakur, and B. Marrow, "Role of Diatoms in Diagnosis of Death Due to Drowning: Case Studies," *Int. J. Med. Toxicol. Forensic Med.*, vol. 7, no. 1, pp. 59–65, 2017, doi: 10.22037/ijmtfm.v7i1(Winter).14047.
15. S. M. Ajay Rana¹ *, "Significance of Diatoms in Diagnosis of Drowning Deaths: A Review," *Peer Rev. J. Forensic Genet. Sci.*, vol. 1, no. 5, pp. 77–81, 2018, doi: 10.32474/prjfgs.2018.01.000121.
16. National Crime Records Bureau, "Accidental Deaths and Suicides in India," 2019.
17. National Crime Records Bureau, "Accidental Deaths and Suicides In India," 2018. doi: 10.1017/CBO9781107415324.004.
18. T. R. B. Karthick, J. C. Taylor, "Protocols for Collection, Preservation and Enumeration of Diatoms from Aquatic Habitats for Water Quality Monitoring in India," *IUP J. Soil Water Sci.*, vol. III, no. January, pp. 25–60, 2010.

19. "Taxa Identification Guide for the practioner," 2016. <https://diatoms.org>.
20. J. P. Kociolek, "The Diatom: A brief Overview and Serch Taxa," 2000. <https://http//diatombase.org>.
21. P. B. H. & J. P. K. B.Karthick, *An illustrarted uide To a Comman Daitoms Of Peninssular India*. 2013.
22. M. K. Thakar, D. Luthra, and J. S. Khattar, "Forensic studies of phytoplankton ecology of two water bodies of Kurukshetra area of Haryana, State in India," 2018.
23. K. R. Scott, R. M. Morgan, V. J. Jones, and N. G. Cameron, "Reply to A. Dragutinovic, 'A reply to: The transferability of diatoms to clothing and the methods appropriate for their collection and analysis in forensic geoscience Forensic sci. Int. 241 (2014) 127–137,'" *Forensic Sci. Int.*, vol. 247, pp. e26–e27, 2015, doi: 10.1016/j.forsciint.2014.11.019.
24. S. Bargu, E. Smith, and K. Ozhan, "The Diatom World," *The Diatom World*, vol. 19, no. 1998, pp. 519–534, 2011, doi: 10.1007/978-94-007-1327-7.
25. P. Lunetta, A. Miettinen, K. Spilling, and A. Sajantila, "False-positive diatom test: A real challenge? A post-mortem study using standardized protocols," *Leg. Med.*, vol. 15, no. 5, pp. 229–234, 2013, doi: 10.1016/j.legalmed.2013.03.002.
26. L. Y. Yen and P. T. Jayaprakash, "Prevalence of diatom frustules in non-vegetarian foodstuffs and its implications in interpreting identification of diatom frustules in drowning cases," *Forensic Sci. Int.*, vol. 170, no. 1, pp. 1–7, 2007, doi: 10.1016/j.forsciint.2006.08.020.

Tables

Table 1: Distribution of Identified Genus and Species at Hirannaya Lake (WB1)

Sr. No.	Category	Class	Order	Family	Genus	Species	Size (microns)		Stria/ Fibula per 10 µm
							Length	Breadth	
1	Symmetrical Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Naviculaceae</i>	<i>Navicula</i>	<i>Cryptocephala Kützing</i>	21-28	4-6	15-18
2	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Umbonata (Ehrenbrg) Lang-Bertalot</i>	38-64	6-8	8-10
3	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Naviculaceae</i>	<i>Navicula</i>	<i>Cf. radiosa Kützing</i>	33-45	6-8	12-14
4	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Palea (Kützing) Smith</i>	27-47	3-5	10-14
5	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Reversa W Smith</i>	63-67	3-4	7-9
6	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	UN*	-	-	-
7	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Amphibia Grunow</i>	14-26	2-5	7-9
8	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Naviculaceae</i>	<i>Navicula</i>	<i>Rostellata Kützing</i>	25-40	7-9	13-15
9	Asymmetric Biraphid	<i>Bacillariophyceae</i>	<i>Cymbellales</i>	<i>Cymbellaceae</i>	<i>Cymbella</i>	<i>Kolbei Hustedt</i>	18-25	6-7	12-14
10	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Diploneidaceae</i>	<i>Diploneis</i>	<i>Smithii cleve (Brebisson in W. Smith)</i>	24-48	11-21	16-24
11	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Lorenziana Grunow</i>	98-116	3-4	14-17
12	Araphid	<i>Fragilariophyceae</i>	<i>Fragilariales</i>	<i>Fragilariaceae</i>	<i>Ulnaria</i>	<i>Ulna (Nitzsch) Compere</i>	74-103	6-8	8-11
13	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Stauroneidaceae</i>	<i>Craticula</i>	<i>Accaomodiformis Lange-Bertalot</i>	36-40	13-14	22-27
14	Centric	<i>Coscinodiscophyceae</i>	<i>Thalassiosirales</i>	<i>Stephanodisceae</i>	<i>Cyclotella</i>	<i>Meneghiniana Kützing</i>	VD#10-34		6-8
15	Centric	<i>Coscinodiscophyceae</i>	<i>Thalassiosirales</i>	<i>Stephanodisceae</i>	<i>Cyclotella</i>	<i>Bifacialis jurilj</i>	VD#10-34		6-8
16	Centric	<i>Coscinodiscophyceae</i>	<i>Thalassiosirales</i>	<i>Stephanodisceae</i>	<i>Cyclotella</i>	UN*	-		-
17	Centric	<i>Bacillariophyceae</i>	<i>Triceratiales</i>	<i>Triceratiaceae</i>	<i>Pleurosira</i>	<i>Pleurosira laevis (Ehrenberg) compere</i>	VD#35-70		14-18

UN* = Unidentified VD# = Valve Diameter WB*= Water Body

Table 2: Distribution of Identified Genus and Species at Kagzipura Lake (WB2)

Sr. No.	Category	Class	Order	Family	Genus	Species	Size (microns)		Stria/ Fibula per 10 µm
							Length	Breadth	
1	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Triceratiales</i>	<i>Triceratiaceae</i>	<i>Pinnularia</i>	<i>Acrosphaeria W.Smith</i>	38-54	8-12	12-16
2	Asymmetric Biraphid	<i>Bacillariophyceae</i>	<i>Cymbellales</i>	<i>Cymbellaceae</i>	<i>Cymbella</i>	<i>Turgidula Grunow</i>	35-38	9-10	12-15
3	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Lorenziana Grunow</i>	98-116	3-4	14-17

UN* = Unidentified WB*= Water Body

Table 3: Distribution of Identified Genus and Species at Lamgaon Lake. (WB3)

Sr. No.	Category	Class	Order	Family	Genus	Species	Size (microns)		Stria/ Fibula per 10 µm
							Length	Breadth	
1	Asymmetrical Biraphid	<i>Bacillariophyceae</i>	<i>Cymbellales</i>	<i>Cymbellaceae</i>	<i>Cymbella</i>	<i>Turgidula Grunow in Schmidt ET. 1875</i>	25- 38	9-11	12-15
2	Symmetrical Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Diploneidaceae</i>	<i>Diploneis</i>	<i>Smitii (Brebisson in W. Smith) Cleve</i>	24-48	11-12	16-24
3	Asymmetrical Biraphid	<i>Bacillariophyceae</i>	<i>Cymbellales</i>	<i>Cymbellaceae</i>	<i>Encyonema</i>	<i>Javanicum (hustedt) D. G. Mann</i>	15-22	4-5	11-14
4	Symmetrical Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Naviculaceae</i>	<i>Navicula</i>	<i>Heimansioides Lange-Bertalot</i>	45-58	6-7	11-13
5	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Naviculaceae</i>	<i>Navicula</i>	<i>Cryptocephala Kützing</i>	21-28	4-6	15-18
6	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Umbonata (Ehrenberg) Lange -Bertelot</i>	38-64	6-8	8-10
7	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Amphibia Grunow</i>	19- 26	2-4	15-18
8	Araphid	<i>Fragilariophyceae</i>	<i>Fragilariales</i>	<i>Fragilariaceae</i>	<i>Ulnaria</i>	<i>Ulna (Nitzsch) compere</i>	74-103	7-8	8-11
9	Asymmetric Biraphid	<i>Bacillariophyceae</i>	<i>Cymbellales</i>	<i>Cymbellaceae</i>	<i>Encyonema</i>	UN*	-		
10	Asymmetric Biraphid	<i>Bacillariophyceae</i>	<i>Cymbellales</i>	<i>Cymbellaceae</i>	<i>Cymbella</i>	UN*	-		
11	Centric	<i>Coscinodiscophyceae</i>	<i>Thalassiosirales</i>	<i>Stephanodisceae</i>	<i>Cyclotella</i>	UN*	-		

UN* = Unidentified WB*= Water Body

Table 4: Distribution of Identified Genus and Species at Lake Mahismal (WB4)

Sr. No.	Category	Class	Order	Family	Genus	Species	Size (microns)		Stria/ Fibula per 10 µm
							Length	Breadth	
1	Araphid	<i>Fragilariophyceae</i>	<i>Fragilariales</i>	<i>Fragilariaceae</i>	<i>Ulnaria</i>	<i>Ulna (Nitzsch) compere</i>	74-103	7-8	8-11
2	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	UN*	-	-	-
3	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Naviculaceae</i>	<i>Navicula</i>	<i>Germainii wallace</i>	33-39	7-8	13-15
4	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Cymbellales</i>	<i>Gomphonemataceae</i>	<i>Gomphonema</i>	<i>Affine Kützing</i>	46-70	8-9	10-13

UN* = Unidentified WB*= Water Body

Table 5: Distribution of Identified Genus and Species at Kala Lake (WB4)

Sr. No.	Category	Class	Order	Family	Genus	Species	Size (microns)		Stria/ Fibula per 10 µm
							Length	Breadth	
1	Araphid	<i>Fragilariophyceae</i>	<i>Fragilariales</i>	<i>Fragilariaceae</i>	<i>Tibetiella</i>	<i>Pulchra li., William and Metzeltin</i>	-	-	-
2	Araphid	<i>Fragilariophyceae</i>	<i>Fragilariales</i>	<i>Fragilariaceae</i>	<i>Ulnaria</i>	<i>Ulna (Nitzsch) compere</i>	74-103	7-8	8-11

UN* = Unidentified WB*= Water Body

Table 6: Distribution of Identified Genus and Species at Jalna Road Lake (WB5)

Sr. No.	Category	Class	Order	Family	Genus	Species	Size (microns)		Stria/ Fibula per 10 µm
							Length	Breadth	
1	Araphid	<i>Fragilariophyceae</i>	<i>Fragilariales</i>	<i>Fragilariaceae</i>	<i>Ulnaria</i>	<i>Ulnaria delicatissima</i>	158-262	5-6	10-11
2	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Umbonata (Ehrenberg) Lange-Bertalot</i>	38-64	6-8	8-10
3	Asymmetric Biraphid	<i>Bacillariophyceae</i>	<i>Cymbellales</i>	<i>Cymbellaceae</i>	<i>Cymbella</i>	<i>Turgidula Grunow</i>	35-38	9-10	12-15

UN* = Unidentified WB*= Water Body

Table 7: Distribution of Identified Genus and Species at MIDC Phase 3 Lake (WB7)

Sr. No.	Category	Class	Order	Family	Genus	Species	Size (microns)		Stria/ Fibula per 10 µm
							Length	Breadth	
1	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Umbonata (Ehrenberg) Lange- Bertalot</i>	38-64	6-7	8-10
2	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Naviculaceae</i>	<i>Navicula</i>	<i>Rostellata Kützing</i>	25-40	7-9	13-15
3	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Lorenziana Grunow</i>	98-116	3-4	14-17
4	Asymmetric Biraphid	<i>Bacillariophyceae</i>	<i>Cymbellales</i>	<i>Cymbellaceae</i>	<i>Cymbella</i>	<i>Bengalensis Grunow</i>	97-112	22-25	7-9
5	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Naviculaceae</i>	<i>Navicula</i>	UN*	-	-	-
6	Centric	<i>Coscinodiscophyceae</i>	<i>Thalassiosirales</i>	<i>Stephanodisceae</i>	<i>Cyclotella</i>	UN*	-	-	-

UN* = Unidentified WB*= Water Body

Table 8: Distribution of Identified Genus and Species at Ghanewadi Lake, Jalna (WB8)

Sr. No.	Category	Class	Order	Family	Genus	Species	Size (microns)		Stria/ Fibula per 10 µm
							Length	Breadth	
1	Asymmetric Biraphid	<i>Bacillariophyceae</i>	<i>Cymbellales</i>	<i>Cymbellaceae</i>	<i>Cymbella</i>	<i>Turgidula Grunow</i>	35-38	9-10.5	12-15
2	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Naviculaceae</i>	<i>Navicula</i>	<i>Cryptocephala Kützing</i>	21-28	4.5-6.5	15-18
3	Araphid	<i>Fragilariophyceae</i>	<i>Fragilariales</i>	<i>Fragilariaceae</i>	<i>Ulnaria</i>	<i>Ulnariadelicatissima</i>	158-262	43987	10-11
4	Centric	<i>Coscinodiscophyceae</i>	<i>Thalassiosirales</i>	<i>Stephanodisceae</i>	<i>Cyclotella</i>	<i>Meneghiniana Kützing</i>	VD#10-34		6-8
5	Centric	<i>Bacillariophyceae</i>	<i>Melosirales</i>	<i>Melosiraceae</i>	<i>Melosira</i>	<i>Lineata (dillwyn) C. Agardh</i>	VD# 17-25		-

UN* = Unidentified VD# = Valve Diameter WB*= Water Body

Table 9: Distribution of Identified Genus and Species at Moti Talab Jalna (WB9)

Sr. No.	Category	Class	Order	Family	Genus	Species	Size (microns)		Stria/ Fibula per 10 µm
							Length	Breadth	
1	Asymmetric Biraphid	<i>Bacillariophyceae</i>	<i>Cymbellales</i>	<i>Cymbellaceae</i>	<i>Cymbella</i>	UN*	-	-	-
2	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Naviculaceae</i>	<i>Navicula</i>	UN*	-	-	-
3	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Solita Hustedt</i>	22-24	4-5	11-13
4	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Williamsii Alakananda, Hamilton and Karthick</i>	38-49	2-5	12-14
5	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Reversa w smith</i>	60-66	3-5	7-9
6	Centric	<i>Coscinodiscophyceae</i>	<i>Thalassiosirales</i>	<i>Stephanodiscaeae</i>	<i>Cyclotella</i>	<i>Meneghiniana Kützing</i>	VD# 10-34		6-8

UN* = Unidentified VD# = Valve Diameter WB*= Water Body

Table 10: Distribution of Identified Genus and Species at Jaikwadi Dam Paithan (WB10)

Sr. No.	Category	Class	Order	Family	Genus	Species	Size (microns)		Stria/ Fibula per 10 µm
							Length	Breadth	
1	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Hantzschia rabenhorst</i>	27-38	3-4	9-11
2	Centric	<i>Coscinodiscophyceae</i>	<i>Thalassiosirales</i>	<i>Stephanodiscaeae</i>	<i>Cyclotella</i>	UN*	-	-	-
3	Asymmetric Biraphid	<i>Bacillariophyceae</i>	<i>Cymbellales</i>	<i>Cymbellaceae</i>	<i>Encyonema</i>	<i>Hustedtii krammer</i>	37-68	10-13	7-11
4	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	UN*	-	-	-

UN* = Unidentified WB*= Water Body

Table 11: Distribution of Identified Genus and Species at Salim Ali Lake Aurangabad (WB11)

Sr. No.	Category	Class	Order	Family	Genus	Species	Size (microns)		Stria/ Fibula per 10 µm
							Length	Breadth	
1	Centric	<i>Coscinodiscophyceae</i>	<i>Thalassiosirales</i>	<i>Stephanodiscaeae</i>	<i>Cyclotella</i>	<i>Meneghiniana Kützing</i>	VD#10-34		6-8
2	Centric	<i>Coscinodiscophyceae</i>	<i>Triceratiales</i>	<i>Triceratiaceae</i>	<i>Pleurosira</i>	<i>Laevis (Ehrenberg) compere</i>	VD#35-50		14-18
3	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Umbonata (Ehrenbrg) Lange-Bertalot</i>	38-49	6-8	8-10
4	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Naviculales</i>	<i>Naviculaceae</i>	<i>Navicula</i>	UN*	-	-	-

UN* = Unidentified VD# = Valve Diameter WB*= Water Body

Table 12: Distribution of Identified Genus and Species at Sai Tekdi Lake, Aurangabad (WB12)

Sr. No.	Category	Class	Order	Family	Genus	Species	Size (microns)		Stria/ Fibula per 10 µm
							Length	Breadth	
1	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Cf. pumila Hustedt</i>	56-60	2-5	13-15
2	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Elegantula Grunow</i>	12-17	2-4	10-15
3	Nitzschoid	<i>Bacillariophyceae</i>	<i>Bacillariales</i>	<i>Bacillariaceae</i>	<i>Nitzschia</i>	<i>Williamsi Alakananda Hamilton and Karthick</i>	38-49	2-5	12-14
4	Symmetric Biraphid	<i>Bacillariophyceae</i>	<i>Mastogloiales</i>	<i>Mastogloiaceae</i>	<i>Mastogloia</i>	<i>Elliptica (Agardh) Cleve</i>	25-45	11-14	12-14

UN* = Unidentified WB*= Water Body

Table 13: Observed prevalence and diatom diversity pattern of Genus in selected water bodies

Sr. No.	Genus	WB1	WB 2	WB 3	WB 4	WB 5	WB 6	WB 7	WB 8	WB 9	WB 10	WB 11	WB 12	Prevalence
1	Navicula	+	-	+	+	-	-	+	+	+	-	+	-	7
2	Nitzschia	+	+	+	+	-	+	+	-	+	+	+	+	10
3	Cymbella	+	+	+	-	-	+	+	+	+	-	-	-	7
4	Diploneis	+	-	+	-	-	-	-	-	-	-	-	-	2
5	Ulnaria	+	-	+	+	+	+	-	+	-	-	-	-	6
6	Craticula	+	-	-	-	-	-	-	-	-	-	-	-	1
7	Cyclotella	+	-	-	-	-	-	+	+	+	+	+	-	6
8	Encyonema	-	-	+	-	-	-	-	-	-	+	-	-	2
9	Gomphonema	-	-	-	+	-	-	-	-	-	-	-	-	1
10	Pinnularia	-	+	-	-	+	-	-	-	-	-	-	-	2
11	Tibetiella	-	-	-	-	+	-	-	-	-	-	-	-	1
12	Melosira	-	-	-	-	-	-	-	+	-	-	-	-	1
13	Pleurosira	+	-	-	-	-	-	-	-	-	-	+	-	2
14	Mastogloia	-	-	-	-	-	-	-	-	-	-	-	+	1
Diatom diversity/WB		8	3	6	4	3	3	4	5	4	3	4	2	49

WB*= Water Body

Table 14: Geo location of twelve water bodies

Name of water body	Codes for water bodies	Co-ordinates	Direct link for maps
Hirannaya lake	WB1	19°57'43.0" N 75°13'21.6" E	https://goo.gl/maps/DDEs9gw8Ngx5xsHy9
Kagzipura	WB2	19°58'33.3" N 75°12'32.2" E	https://goo.gl/maps/q5uHHfPzvBuTZwZF6
Lamgaon Chincholi	WB3	20°04'33.2"N 75°11'05.9"E	https://goo.gl/maps/toJx9xdn7q9MkcC18
Mahismal road lake	WB4	20°04'50.7"N 75°11'50.9"E	https://goo.gl/maps/yrch66kry1YAAjsY8
Kala Lake	WB5	20°02'48.5"N 75°11'51.5"E	https://goo.gl/maps/oBLbZUr8TJLapLeD9
Jalna MIDC are lake	WB6	19°52'32.2"N 75°29'51.3"E	https://goo.gl/maps/igcBrCEttWutZG1GA
MIDC Phase three lake	WB7	19°51'55.2"N 75°31'42.2"E	https://goo.gl/maps/HEp7WECT7weA8Q5c6
Ghanewadi lake	WB8	19°54'32.9"N 75°51'41.1"E	https://goo.gl/maps/LMdx5cpmSPzrQCug8
Moti Talab	WB9	19°50'47.7"N 75°52'04.5"E	https://goo.gl/maps/LzQb4QAC2h5iKQtB9
Jaikwadi dam	WB10	19.4851° N, 75.3701° E	https://goo.gl/maps/RcwGapdoG2Wmz1Hx6
Salim Ali lake	WB11	19°53'55.9"N 75°20'30.7"E	https://goo.gl/maps/zF12zNHkTu5sXX1B8
Sai Tekdi lake	WB12	19°47'25.5"N 75°24'07.8"E	https://goo.gl/maps/7c3bjAcuPGNpPpwW9

Figures

Figure 1

Comparison of diatoms diversity in selected water bodies: "Y axis represents no. of different species" reported, X axis represent water bodies from where the samples were collected. WB1 has most species diversity followed by WB3, WB5. Least diversity of diatom species were observed in WB12.

Figure 2

Genus distribution of diatoms: x axis represents “periodic diversity of genus in all water bodies”, y axis represents “Identified genus”. The genus *Nitzschia* have been identified in 10 Water Bodies that concludes it is most diverse genus present in the region followed by *Navicula*, *Cymbella*, and *Cyclotella*. *Mastogloia*, *Melosira*, *Tibetiella*, *Craticula* and *Gomphonema* are concluded as the marker genus for the respective water body.

Waterbodies Location

Figure 3

Map of water bodies location: Google map showing location of twelve studied water bodies. Study area were marked showing the details about experimental water body and its google location. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 4

Representative photograph of identified Diatom genus under the phase inverted microscope 100x (A. Araphid- Ulnaria, B. Colony of Asymmetric Biraphid- Cymbella, C. Nitzschiod- Nitzschia-Reversa, D. Centric –Pleurosira, E. Nitzschiod- Nitzschia, F. Araphid- Ulnaria, G. Nitzschiod, H. Symmetric Biraphid- Craticula, I. Nitzschiod- Nitzschia, J. Asymmetric Biraphid- Cymbella. K. Symmetric Biraphid- Navicula. L. Centric- Cyclotella.