Morphological Identification of Diatoms And Their Physical And Chemical Analysis From Different Water Bodies of Punjab, India

Palika Sharma[1], Tapas Giri[1], Manjot Kaur[1] and Shristy Gautam[1*]

[1]Department of Molecular biology and Genetic Engineering, School of Bio Engineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India, 144411.

*Corresponding author email id: shristy.23772@lpu.co.in

Abstract

Diatoms played a definitive part in the ecosystem for a long time as one prominent arrangement of oxygen synthesizers on earth and as a key source of biomass in water bodies and because of their particular adaptations and biology, they are utilized in different fields like paleoecology, nanotechnology, Biomonitoring and forensic sciences. Various environmental factors, for example, physical-chemical factors and organic components impact the bounty and species lavishness of diatoms, which is reflected in their seasonal variations. In the present study, morphological identification of diatoms from different water bodies of Punjab, India during different seasons (autumn, pre- winter, winter and spring) has been done for identification of site specific diatoms followed by diatom counting for the study of species richness and the effect of physical-chemical factors on the diatom growth and structure.

Keywords: Diatoms, Phytoplankton, Diatom counting, Physical-chemical, Shannon weaver index

Introduction

Diatoms alluded to as "algae in glass houses" are unicellular (~1-500mm), photosynthetic algae belonging to the division Chrysophyta (form endoplasmic cysts and store oils), class Bacillariophyceae [1], are most abundant phytoplankton and primary producers in oceans responsible for up to 40% of marine primary productivity due to their photosynthetic activity, silicon recycling and carbon fixation [2]. The trademark feature of the diatoms is its silicified cell wall (frustule) which shows a decent variety in shape, frame, and ornamentation, which has been the premise of customary diatom scientific categorization, for instance, organelle arrangement, cell motility and method of sexual propagation [3]. Owing to the morphology and natural surroundings, they are delegated centrale and pennate diatoms [4]. They exist in both wet and moist areas where the photosynthesis process is feasible. Diatoms are either benthic, attached to the substrate with no delicate structures or planktonic free floating, have fine frustules [5] and can form colonies or be solitary that adds to right around ~40% of the essential profitability in the seas and are the key players in the biogeochemical cycle of carbon (C) and also the other macronutrients such as nitrogen (N), silicon (Si), phosphorus (P) as well as responsible for ~25% oxygen production globally which is almost every fourth breath of oxygen that we uptake [6].

Diatoms have been studied since the end of the 18th century, but major advances in this area took place in the mid-19th century. Some European specialists have created monographs on diatoms in the late 19th century. In the mid-twentieth century, fossil diatoms were first examined and conducted an orderly and natural investigation of diatoms, which remains an essential reference today [7]. Presently, over 8000 species are documented in water bodies all over the world and approximated that there are 20,000–200,000 existing diatom species worldwide, making it an extremely diverse group with about 200 genera comprising about 10 to 12 thousand known species one of the most successful existing taxa of photosynthetic eukaryotic unicellular organisms [3]. However, several studies intensify the estimated number of species to be around 0.2-10 million, which can furnish data about an entire diversity of divergent changes occurring in the surroundings as they are vernacular and reciprocate rapidly than other bio-indicators to changes in hydrology and can possibly offer grounds for amplitude alterations. Most diatom species manifest a global distribution is mainly pollution tolerant in nature [8]. Despite, plenty of endemic taxa from various regions across the world are proclaimed chiefly from ancient lakes such as African Rift Valley lakes, Baikal Lake, and Tropical areas [9]. Generally, there are analogous diatoms in almost all types of bodies of water. Some physical, chemical and local environmental factors, including the mineral content (especially silicon) of water and soil, temperature, stratification of water, pH and bond related to contamination can lead to considerable variations in diatom diversity compared to seasonal changes [10]. This enormous variation in the estimate due to the limited knowledge of its diversity.

Diatoms naturally produce food substances, antibiotics and active pharmaceutically substances this makes them a useful resource for the purchase of food supplements and replacement for synthetic substances, also been established as a natural environmental indicator and a rich source of HVMs (High-Value Molecules) diatoms [11]; have a broad arena of applications in water quality management, forensic sciences, climate control changes [12] and paleo-ecological reconstruction where researchers utilize learning about natural surroundings of present taxa to decipher the historical conditions from the fossil record [9]. Industrial applications for commercial diatom use include fuel renewable energy production, the production of raw materials and detoxification of natural and industrial waste using biological waste as a substrate [13]. They utilize silicon from the environment and deposit it in their cell walls forming a perplexing, hard, mesoporous, siliceous nanostructures called as frustules that can be customized to improve their application in the field of bioreactors, biosensors, nano-medicine, photonic devices and biofuel by genetic modification which could easily compete with other biological solar energy factories, without the added disadvantage of competing with food resources as some diatoms easily thrive on waste such as nutrients from wastewater and carbon dioxide exhaust gases, all without stealing food or harvesting from fertile soils [14]. Climatic conditions contribute significantly to the qualitative and quantitative distribution of diatoms in bodies of water. The forensic biologist studies this botanical material (diatoms) to establish the mode (ante mortem/post-mortem drowning), the probable supposed drowning sites and regarded as the most valuable indicator for the ecological evaluation of rivers around the world in the last fifty years and are extremely sensitive to changes in nutrient concentration, organic pollution and aquatic productivity [15].

Diatoms in India have been studied since the 19th century. Several Indian and alternative country researchers have studied the fresh, fossilized marine diatoms of the Indian mass. The landmass of

India has about 7,000 seaweed taxa from marine, brackish, saline and fossil environments. In recent years, several species and genera of new algae have been reported around the world, especially in the tropics [16]. Estimation of different environmental conditions in rivers using diatoms has a long history which can be followed back to the work of Kroth [17]. Recent studies of the western currents of Ghats have given science several new diatom species, however, different unexplored regions of India need to be studied to obtain baseline data on diatom diversity which will not only help to improve the understanding of their distribution, but in addition explore enthusiastic prospects for new biotechnology diatom applications.

2. Material and Methodology

Ten water bodies from different regions of Punjab such as Cantt Lake, Jalandhar; Sutlej River, Ludhiana; Beas River, Kapurthala; Buddha Nullah, Ludhiana; Ravi River, Pathankot; Sukhna Lake, Chandigarh; Srihind Canal, Ropar; Devi Talab, Jalandhar; Golden temple, Amritsar; Talwara River, Talwara were selected. One liter of water sample was collected from the littoral region of selected water bodies in sterile and properly labeled plastic bottles covered with a cotton plug during different seasons having a two-month interval i.e. during September-October (2018) and November-December (2018), January-February (2019) and March-April (2019). Several precautions should be taken while sample collection, such as water should not be collected from shaded area or sites invaded by hydrophytes [18]. The temperature of the water was measured during the collection process using a digital thermometer (Techicon YES PLUS digital thermometer).

Physiochemical analysis of the water sample was done in the laboratory. pH of samples was measured using the pH meter. One liter (1000mL) of water sample was treated with formalin (2%) and mixed properly by vigorous shaking followed by the addition of a few drops of Lugol's iodine in 1000 ml beaker which was covered by brown paper and kept overnight [19]. Settled sediments were collected from the beaker using pasture pipette and was then transferred to 15 ml falcon tubes. Centrifugation was done at 5000 RPM for 3-5 minutes, the supernatant was removed, distilled water was added to the tubes having pellet and homogenized. Centrifugation process was repeated three times for proper washing, the supernatant was decanted followed by the addition of a few drops of 30% analytical grade hydrogen peroxide (H_2O_2) to the pellet in the tube and was left undisturbed overnight [20]. The next day few drops of concentrated HCl (99.99%, analytical grade) were added to the pellet [21]. Centrifugation was done at 5000 RPM for 3-5 minutes, centrifugation process was done as previously described in order to remove the residues of hydrogen peroxide [18]. 50µL of pellet obtained after the centrifugation process was taken on the clean glass slide, dried on the slide warmer (45°C) and mounted in a DPX mountant medium [21]. Coverslip was properly placed on the glass slide to avoid air bubbles, the slide was observed under a compound microscope at 40X and 100X using immersion oil and photographs were taken using Magnus MIPS with DC-200 camera, software- Magnus live camera. Three slides were prepared for each water sample. For diatom identification, the images of diatom acquired from microscopical examination were compared with the diatom databases such as a Diatom Image database of India (DIDI) [22], Automatic Diatom Identification and Classification (ADIAC) [23-25] and Database of United States [26].

The diatoms were counted for each water sample (P01-P10) during all the four seasons using Sedgewick-Rafter counting chamber [27]. The slide was divided into four sectors as A, B, C and D and then observed under the microscope at 40X [18]. The original volume of the sample was one liter and the volume of sample concentrate was one ml. Three slides were prepared for each sample. The sampling was done for four seasons and eight months and the data was examined for seasonal changes. Further, the mean, percentage of relative abundance/ml and the Shannon weaver index were calculated using formulas in Microsoft Excel. The mean was calculated by adding diatom cell counts in three slides and then dividing it by three using Microsoft Excel [28]. Shannon Weaver index (eH') was calculated in the Microsoft Excel by firstly, calculating the relative abundance (Pi) and then multiplying the value discrete genera with a log of relative abundance, when we get a negative value, it is multiplied by -1 to get the e^H value by taking exponential of Shannon's index (EXP). Percentage of relative abundance/ml was calculated by taking the average/ mean of discrete diatom cell counts, then dividing it by average/ mean of all diatoms cell counts and multiplying by hundred [29].

3. Result and Disscussion

3.1 Physical and chemical analysis of water samples:

The physical and chemical analysis of selected water samples was performed during the four seasons i.e. autumn, pre-winte, winter and spring (September 2018-April 2019). The temperature, pH and conductivity for the selected water bodies were checked during the time of sample collection using a digital thermometer. The temperature, pH and conductivity for all the water bodies during the four seasons is mentioned in Table 1, 2, 3, 4 for the month September-October, November-December, January-February and March-April respectively. For the month of September-October, the highest temperature was found in the site P05 and lowest in site P09 and P06. The highest pH range was examined in site P06 and the lowest in site P04. The conductivity of site P05 was the highest and the lowest in site P02. During the month of November-December, the highest temperature range was observed in site P02 and the lowest in site P06. For the pH, the highest range was in site P05 and the lowest in site P03. The conductivity of site P05 was the higher and the lower in site P03. In the case of January-February, the maximum temperature was observed at site P02 and P06 and minimum at site P08. The highest pH range was examined on site P09 and the lowest on site P10. The conductivity of site P05 was the highest and the lowest in site P03. During the month of March-April, the highest temperature range was observed on site P05 and the lowest on site P06 and P09. For the pH the highest range was in site P06 and the lowest in site P05. The conductivity of site P07 was the highest and the lowest in site P10. According to the study by Pearsall [30], physical-chemical factors affected the diatom species richness and diversity and are exhibited as seasonal diversification. Maximal diatom density was reported during summers when the temperature is the highest as described in a study done by Agale and Patil [31].

Table 1: *Physical-Chemical analysis for month September-October, 2018*

S.No.	CODE	SITE	TEMPERATURE (°C)	Ph	CONDUCTIVITY (20 S/m)
1	P01	Cantt Lake	26	6.9	0.451
2	P02	Sutlej River	26	7.2	0.204
3	P03	Beas River	25	7.0	0.454

4	P04	Buddha Nullah	25	6.4	0.253
5	P05	Ravi River	27	7.2	0.621
6	P06	Sukhna Lake	24	7.3	0.490
7	P07	Srihind Canal	28	7.0	0.404
8	P08	Devi Talab	26	6.8	0.436
9	P09	Golden Temple	24	6.9	0.480
10	P10	Talwara River	26	6.72	0.420

Table 2: Physical-Chemical analysis for month November-December, 2018

S.No.	CODE	SITE	TEMPERATURE (°C)	pH	CONDUCTIVITY (20 S/m)
1	P01	Cantt Lake	21	7.0	0.631
2	P02	Sutlej River	24	6.7	0.622
3	P03	Beas River	22	6.4	0.436
4	P04	Buddha Nullah	22	7.2	0.521
5	P05	Ravi River	23	7.3	0.634
6	P06	Sukhna Lake	20	6.4	0.387
7	P07	Srihind Canal	22	6.7	0.471
8	P08	Devi Talab	21	7.1	0.451
9	P09	Golden Temple	23	7.0	0.492
10	P10	Talwara River	22	6.9	0.624

Table 3: Physical-Chemical analysis for month January-February, 2019

S.No.	CODE	SITE	TEMPERATURE (°C)	pH	CONDUCTIVITY (20 S/m)
1	P01	Cantt Lake	27	6.5	0.496
2	P02	Sutlej River	29	6.7	0.631
3	P03	Beas River	27	6.5	0.682
4	P04	Buddha Nullah	24	6.7	0.629
5	P05	Ravi River	27	6.8	0.541
6	P06	Sukhna Lake	29	7.1	0.647
7	P07	Srihind Canal	25	6.9	0.410
8	P08	Devi Talab	28	7.0	0.623
9	P09	Golden Temple	27	7.2	0.652
10	P10	Talwara River	25	6.4	0.253

Table 4: Physical-Chemical analysis for month March – April, 2019

S.No.	CODE	SITE	TEMPERATURE (°C)	pH	CONDUCTIVITY (20 S/m)
1	P01	Cantt Lake	28	7.0	0.451
2	P02	Sutlej River	27	6.8	0.202
3	P03	Beas River	28	6.7	0.490
4	P04	Buddha Nullah	28	6.4	0.629
5	P05	Ravi River	29	6.5	0.253
6	P06	Sukhna Lake	26	7.2	0.256
7	P07	Srihind Canal	27	7.0	1.951
8	P08	Devi Talab	28	6.6	0.243

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

9	P09	Golden Temple	26	6.8	0.622
10	P10	Talwara River	28	6.9	0.200

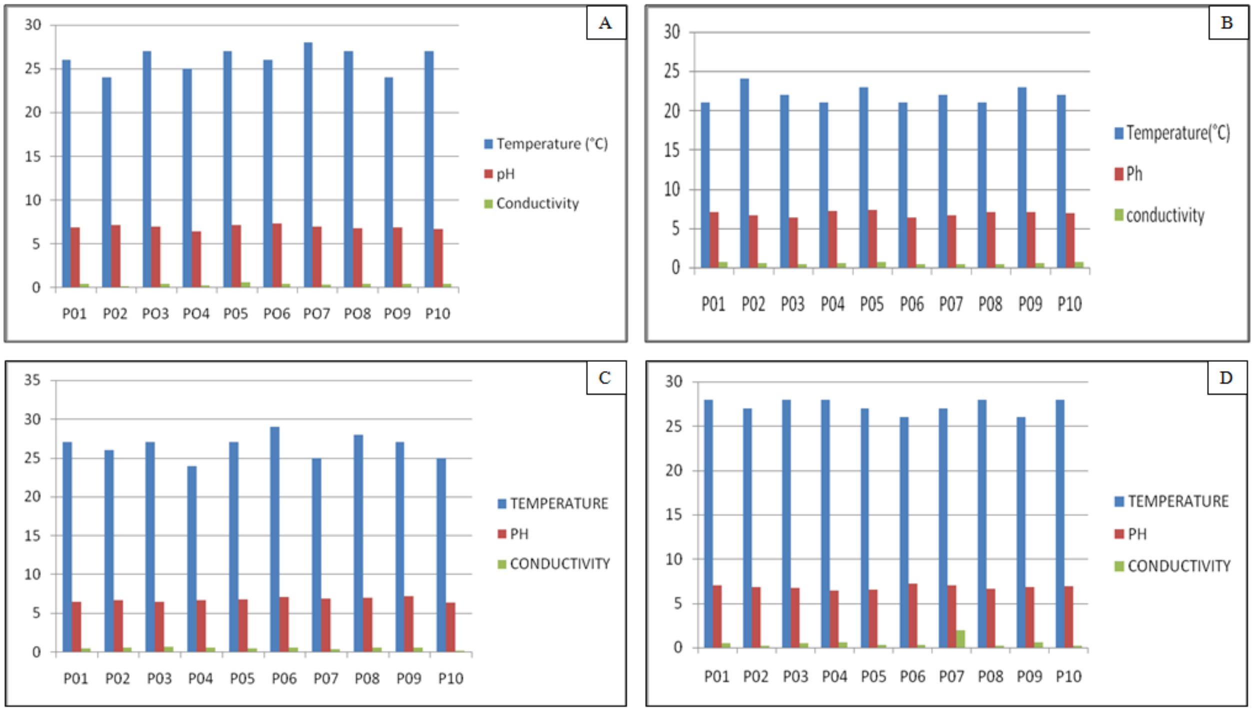

Figure 1: Physical and chemical analysis (pH, temperature, conductivity) of selected ten water bodies of Punjab, India for different months of the year 2018-2019 (A) September-October, 2018 (B) November-December, 2018 (C) January-February, 2019 (D) March-April, 2019

3.2 Morphological analysis of water samples:

Morphological identification of the diatoms was done after the microscopical examination of the water samples. Twelve different diatom species were identified from the site P01-P10 which are *Gomphonema sp., Achnanthes sp., Tryblinella sp., Nitzschia sp., Denticula sp., Melosira sp., Craticula sp., Cocconeis sp., Rhopalodia sp., Cyclotella sp., Opephora sp. and Diatoma sp.* are shown in Figure 2.

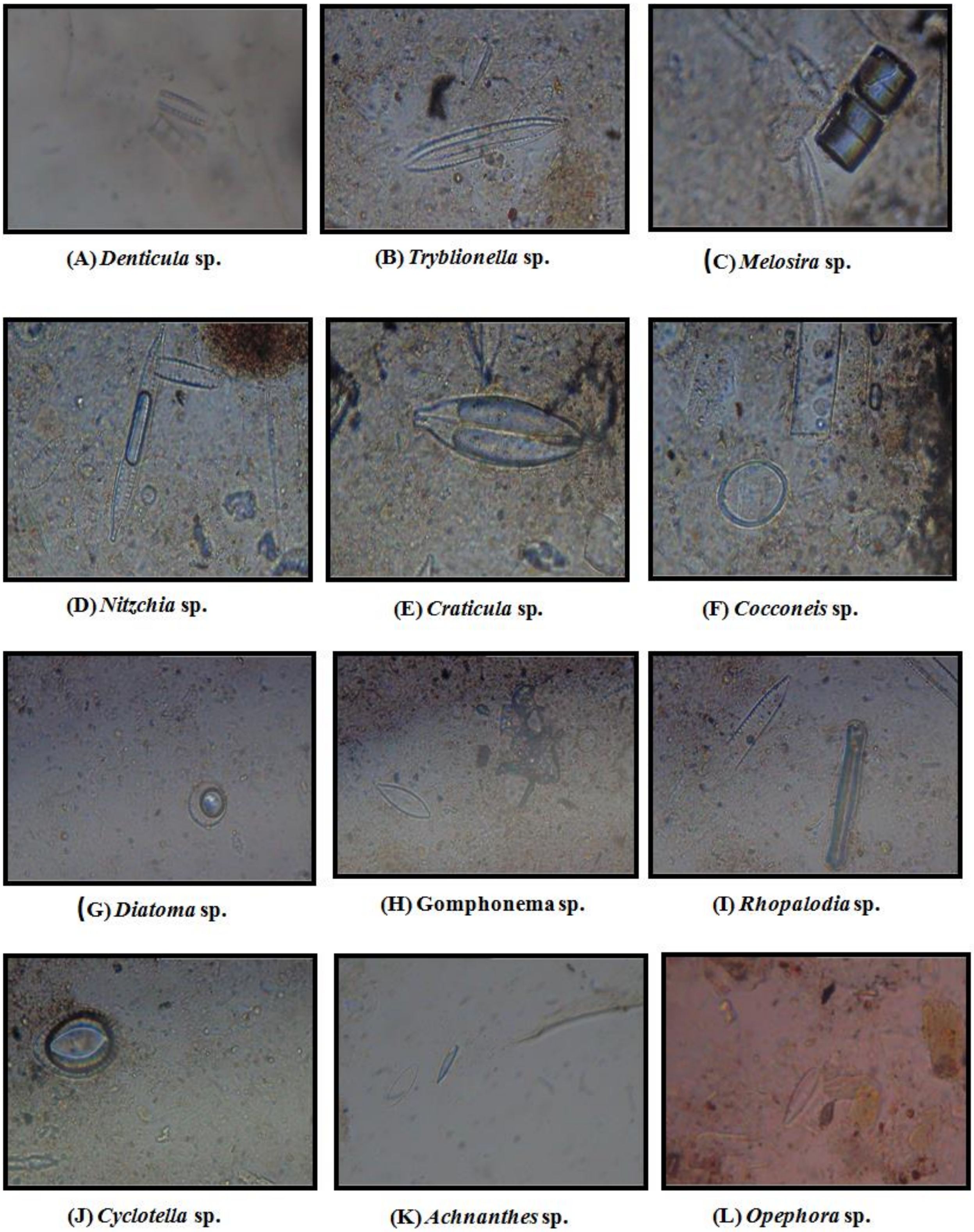

Figure 2: Pictorial representation of diatom species identified in water bodies of Punjab, India.

3.3 Diatom counting identified from water samples:

The diatoms were counted for each water sample (P01-P10) during all the four seasons using the Sedgewick-Rafter counting chamber [27]. The original volume of the sample was one liter and the volume of sample concentrate was one ml. The count of diatoms for each water sample from all the seasons were observed. The maximum diatom count was observed in the spring season, i.e. during the month of March-April, 2019 and the minimum diatom count were observed during the autumn season, i.e. September-October, 2018. According to Thakar *et al.* [28], there is a decline in the diatom growth during autumn seson as the environmental conditions are not favourable for growth and diatoms bloom occur exists during summer and spring season because of the high sufficiency of sunlight and temperature ranges (25°C-30°C). Maximal diatom density was reported during summers as described in a study done by Agale and Patil [31].

3.4 Statistical analysis of water samples:

The Shannon weaver index was calculated for each water samples in order to determine the diatom species richness and diversity. The calculated index for all the water samples during the four seasons are mentioned in Table-5. The Shannon weaver index calculated was the highest for site P03 (Beas River) for the spring season as 4.33 and the lowest index was determined for the site P01 (Cantt Lake) for the autumn season as 0.14. The higher the value of the Shannon weaver index, the species richness is determined to be the most [32]. The species richness and diversity of the diatoms was found to be the highest in Site P03 (Beas River) in the spring season and the lowest in site P01 (Cantt Lake) for the autumn season. In a study done by Agale and Patil [31], highest diatoms species richness was observed in summer season, whereas the moderate richness during the winter and the minimum species richness were observed during monsoon season.

Table 5: Shannon weaver index for water bodies

S. No	Code	Site	Autumn season	Pre- winter season	Winter Season	Spring season
1	P01	Cantt Lake	0.14	0.80	0.48	2.92
2	P02	Sutlej River	1.99	0.39	3.30	1.97
3	P03	Beas River	2.73	0.25	3.89	4.33
4	P04	Buddha Nullah	1.87	0.31	0.31	3.46
5	P05	Ravi River	2.73	0.21	0.79	0.21
6	P06	Sukhna Lake	0.98	0.60	0.39	0.57
7	P07	Srihind Canal	2.65	0.18	0.63	4.14
8	P08	Devi Talab	1.83	1	3.59	0.39
9	P09	Golden temple	1.88	0.31	0.20	0.80
10	P10	Talwara River	2.18	0.42	0.28	1

3.5 **Diatomological Mapping:**

Diatom mapping was done to characterize the distribution of diatoms on a various basis, such as; commonly occurring diatoms, rarely occurring diatoms, site-specific diatoms and seasonal diatoms as shown in Table 6. The diatom species identified in all the water bodies are listed in Table 7, where (+) shows the presence of particular diatom in the water sample and (–) shows the absence of the diatom species. The qualitative mapping analysis has determined 12 diatom species from ten different water bodies during four different seasons. The most dominating and commonly occurring species observed in all the water samples during all the seasons were *Cyclotella sp*. and *Nitzschia sp*. whereas *Gomphonema sp., Achnanthes sp., Tryblinella sp., Denticula sp., Melosira sp., Craticula sp., Cocconeis sp. and Diatoma sp.* was observed in few water bodies. Diatom species such as *Rhopalodia sp., Opephora sp* were characteristic to only two water bodies which were site P03 (Beas River) and site P05 (Ravi River) respectively during a specific season. The nature of the existed diatom species and the effect of climatic situations on its bloom are observed to be comparable with observations made by the scientists especially associated with Indian region. The diatom diversity of lakes of Gujarat was studied by the scientists of which heterogeneous diatom floras were present due to the environment, organic and edaphic constitutes [28].

Table 6: Distribution of Diatoms

S.No	Occurrence	Diatom species
1	**Commonly occurring diatoms**	*Cyclotella sp*. and *Nitzschia sp.*
2	**Seasonal diatoms**	*Rhopalodia sp., Opephora sp*
3	**Rarely occurring diatoms**	*Gomphonema sp., Achnanthes sp., Tryblinella sp., Denticula sp., Melosira sp., Craticula sp., Cocconeis sp., and Diatoma sp*

Table 7: Diatomological mapping of diatom species in different water bodies of Punjab

S.No.	Diatom species	Site P01	Site P02	Site P03	Site P04	Site P05	Site P06	Site P07	Site P08	Site P09	Site P10
1	*Gomphonema sp.*	-	+	+	+	+	-	+	-	-	+
2	*Achnanthes sp.*	-	+	-	+	+	+	-	-	-	-
3	*Tryblinella sp.*	-	+	+	+	+	+	+	-	-	+
4	*Nitzschia sp.*	+	+	+	+	+	+	+	+	-	+
5	*Denticula sp.*	-		-	-	+	-	+	-	+	-
6	*Melosira sp.*	-	+	-	-	+	+	+	+	+	-
7	*Craticula sp.*	-	+	+	+	+	-	+	-	-	-
8	*Cocconeis sp.*	+	-		+	+	-	+	-	-	-
9	*Rhopalodia sp*	-	-	+	-	-	-	-	-	-	-

ISSN: 0971-1260

Vol-22-Issue-17-September-2019

10	*Cyclotella sp.*	+	+	+	+	+	+	+	+	-	+
11	*Opephora sp.*	-	-	-	-	+	-	-	-	-	-
12	*Diatoma sp.*	-	+	-	+	+	+	-	-	-	+

Conclusion

The present study was to apprehend the diatom distribution in different water bodies of Punjab, India during four different seasons as well as the understanding the species richness and diversity. Diversification of the diatoms in exclusive water bodies can be beneficial in forensic sciences and the study of the provincial diatom distribution may be used in the reassessment of drowning cases. Results have shown differences in the diatom range, diversity and richness which would possibly have happened due to the difference within the geographical situations and physical-chemical factors which can affect the nutrient content of the water in diatoms growth.

Acknowledgement: Authors are thankful to Lovely Professional University for providing necessary facilities for the research study.

Conflict of Interest: Authors declare no conflict of interest

References

1. J.S. Patil and A.C. Anil, "Influence of diatom exopolymers and biofilms on metamorphosis in the barnacle Balanus Amphitrite," Marine Ecology Progress Series, 301, pp. 231-245, 2005.
2. P.J. Lopez, J. Descles, A.E. Allen, and C. Bowler, "Prospects in diatom research," Current opinion in Biotechnology, 16, pp. 180-186, 2005.
3. F.E. Round, R.M. Crawford, and D.G. Mann, "Diatoms: biology and morphology of the genera, Cambridge university press, 1990.
4. M. Mishra, A.P. Arukha, T. Bashir, D. Yadav, and G. Prasad, "All new faces of diatoms: potential source of nanomaterials and beyond," Frontiers in microbiology, 8, pp. 1239, 2017.
5. T. Fuhrmann, S. Landwehr, M. El Rharbi-Kucki, and M. Sumper, "Diatoms as living photonic crystals," Applied Physics B., 78, pp. 257-260, 2004.
6. J.P. Smol and E.F. Stoermer, "The diatoms: applications for the environmental and earth sciences", Cambridge University Press, 2010.
7. P.G. Falkowski, R.T. Barber, and V. Smetacek, "Biogeochemical controls and feedbacks on ocean primary production," Science, 281, pp. 200-206, 1998.
8. L. Triest, P. Kaur, S. Heylen, and N. De Pauw, "Comparative monitoring of diatoms, macroinvertebrates and macrophytes in the Woluwe River (Brussels, Belgium)," Aquatic Ecology, 35, pp. 183-194, 2001.
9. A. Kale and B. Karthick, "The diatoms," Resonance, 20, pp. 919-930, 2015.
10. B. Ludes, M. Coste, A. Tracqui, and P. Mangin, "Continuous river monitoring of the diatoms in the diagnosis of drowning," Journal of Forensic Science, 41, pp. 425-428, 1996.

11. S. Gautam, M. Kashyap, S. Gupta, V. Kumar, B. Schoefs, R. Gordon, C. Jeffryes, K.B. Joshi, and V. Vinayak, "Metabolic engineering of TiO_2 nanoparticles in *Nitzschia palea* to form diatom nanotubes: an ingredient for solar cells to produce electricity and biofuel," RSC Advances, 6, pp. 97276-97284, 2016.
12. D. Losic, J.G. Mitchell, and N.H. Voelcker, "Diatomaceous lessons in nanotechnology and advanced materials," Advanced Materials, 21, pp. 2947-2958, 2009.
13. A. Bozarth, U.-G. Maier, and S. Zauner, "Diatoms in biotechnology: modern tools and applications," Applied microbiology and biotechnology, 82, pp. 195-201, 2009.
14. I. Atazadeh, M. Sharifi, and M.G. Kelly, "Evaluation of the Trophic Diatom Index for assessing water quality in River Gharasou, western Iran," Hydrobiologia, 589, pp. 165-173, 2007.
15. M.K. Malik, P. Jakhar, and A. Kadian, "Role of diatoms in forensic investigation: Case studies from Haryana," International Journal of Forensic Science & Pathology, 1, pp. 302-304, 2013.
16. B. Karthick, P.B. Hamilton, and J.P. Kociolek, "An illustrated guide to common diatoms of Peninsular India," Gubbi Labs, 2013.
17. P.G. Kroth, A. Chiovitti, A. Gruber, V. Martin-Jezequel, T. Mock, M.S. Parker, M.S. Stanley, A. Kaplan, L. Caron, and T. Weber, "A model for carbohydrate metabolism in the diatom *Phaeodactylum tricornutum* deduced from comparative whole genome analysis," PloS one, 3, e1426, 2008.
18. V. Vinayak, V. Mishra, and M.K. Goyal, "Diatom fingerprinting to ascertain death in drowning cases," J Forensic Res, 4, pp. 1-6, 2013.
19. D.G. Mann and S.J.M. Droop, "Biodiversity, biogeography and conservation of diatoms," Biogeography of freshwater algae, Springer, pp. 19-32, 1996.
20. R. Singh, R. Singh, R. Singh, and M.K. Thakar, "Diatomological studies from three water bodies of Jaipur," Indian Internet Journal of Forensic Medicine & Toxicology, 4, pp. 69-76, 2006.
21 G. Martín and M.A. de los Reyes Fernandez, "Diatoms as indicators of water quality and ecological status: Sampling, analysis and some ecological remarks," Ecol. Water Qual., 9, pp. 183-204, 2012.
22. 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. Environmental Technology & Innovation. 5:148-160 (2016).
23. H. Du Bufand M.M. Bayer. Automatic diatom identification, World Scientific, 2002.
24. C. Cheung, D.A. Chiasson, and M.S. Pollanen. The diagnostic value of the diatom test for drowning, I. Utility: a retrospective analysis of 771 cases of drowning in Ontario, Canada. Journal of Forensic Science. 42:281-285 (1997).
25. D.B. Czarneckiand D.W. Blinn. Diatoms of the Colorado River in Grand Canyon National Park and Vicinity: Diatoms of the Southwestern USA. II, Lubrecht & Cramer, Limited, 1978.
26. K.E. Camburnand D.F. Charles. Diatoms of low-alkalinity lakes in the northeastern United States, Academy of Natural Sciences, 2000.
27. B.J. McAlice. PI-IYTOPLANKTON SAMPL. ING WITH THE SEDGWICK-RAFTER CELL1. Limnology and Oceanography. 16: (1971).

 ISSN: 0971-1260

Vol-22-Issue-17-September-2019

28. M.K. Thakar, D. Luthra, and R. Singh. A fluorescent survey of diatom distribution patterns in some small water bodies (lakes and Sarovars). J Punjab Acad Forens Med Toxicol. 11:81-86.
29. C. Phiri, J. Day, M. Chimbari, and E. Dhlomo. Epiphytic diatoms associated with a submerged macrophyte, Vallisneria aethiopica, in the shallow marginal areas of Sanyati Basin (Lake Kariba): a preliminary assessment of their use as biomonitoring tools. Aquatic ecology. 41:169-181 (2007).
30. W.H. Pearsall. A theory of diatom periodicity. Journal of Ecology. 11:165-183 (1923).
31. M.C. Agaleand N.G. Patel. Impact of Biotic and Abiotic Factors on Chlorophyceae in Budki MI Tank Water. Magnesium (Mg). 196:465.
32. L.K. Pandey, D. Kumar, A. Yadav, J. Rai, and J.P. Gaur. Morphological abnormalities in periphytic diatoms as a tool for biomonitoring of heavy metal pollution in a river. Ecological indicators. 36:272-279.

