A Study on Spatio-Temporal Variations in Physicochemical Parameters and Ciliate Community Structure of Three Freshwater Bodies from Delhi, India.

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Research Article

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Abstract

The present investigation studied the spatial and temporal variation of the physicochemical parameters and their correlation with the ciliate community structure. Samples were collected from three freshwater sites viz., Okhla Bird Sanctuary (OBS), Sanjay Lake (SL) and Raj Ghat (RJ) in alternate months for one year from September 2018 to August 2019. Physicochemical parameters studied were temperature, pH, dissolved oxygen (DO), total dissolved solids (TDS), water hardness (WH), chloride, sulphate, phosphate, nitrite, nitrate and ammonia. Ciliate diversity, structure and abundance were studied using various diversity indices. Multiple statistical methods such as Pearson correlation coefficients, principal component analysis (PCA) and non-metric multidimensional scaling (NMDS) were applied to analyze the data. Maximum ciliate diversity, richness and evenness were observed in the OBS site. Maximum ciliate diversity was observed from class Spirotrichea (47%), followed by Oligohymenophorea (20%), Prostomatea (16%), Phyllopharyngea (6%), Colpodea (5%), Heterotrichea (3%), Litostomatea (2%) and Karyorelictea (1%). Maximum diversity was observed during the monsoon and post monsoon season and lowest during the winter and summer season. The present study on the dynamics of the environment driven community assembly suggests that ciliates are good indicators for assessing community-level responses and can be useful in predicting the water quality as well as environmental degradation in future.

Introduction

Freshwater habitats support about 6% of all described species representing 0.01% of the world's water (Dudgeon et al. 2006). Unfortunately, these ecosystems are the most vulnerable in the world, and even those that are well protected are at risk due to different kinds of anthropogenic activities, such as industrial and domestic sewage and runoff from farms (Pander and Geist 2013; Xiong et al. 2018), hence causing a significant level of pollution in the ecosystem. Therefore, habitat quality and corresponding biodiversity are severely compromised (Hadwen et al. 2003; Soller et al. 2010). Freshwater biodiversity provides valuable ecosystem services, such as climate stabilization, drinking water, irrigation, nutrient recycling, and recreational amenities (Covich et al. 2004; Strayer and Dudgeon 2010). In order to maintain a healthy and sustainable ecosystem, it is important to have proper knowledge as well as systematic documentation of the ecosystems (World Health Organization 2005).

Various physicochemical parameters corresponding to the habitat of the ciliate species are important in community studies. Numerous studies have shown the effect of various physicochemical parameters on the ciliate diversity and their distribution (Andrushchyshyn et al. 2007; Debels et al. 2005; Dhib et al. 2013; Xiong et al. 2019). Physical parameters such as temperature, conductivity, salinity, pH, transparency, depth, and chemical parameters such as dissolved oxygen, nitrite, nitrate, ammonia, total phosphorous, sulphate and calcium have been studied profoundly in the marine ecosystems and their correlations with the ciliate community have been found to be significant (Grinienė et al. 2019; Xu et al. 2011; Ustaoglu et al. 2020). Several reports suggest a prevalence of oligotrich ciliate during spring season along with a low concentration of total phosphorous, total nitrogen, orthophosphate and nitrite (Balkis 2004; Dhib et al. 2013; Dolan and Marassé 1995; Nogueira et al. 2005; Pérez et al. 2000; Xu and Cronberg 2010). A study from the coastal waters of Port Blair, Andaman showed that the abundance of various loricate ciliate species and microzooplankton is correlated to the environmental variables such as temperature, pH, salinity, dissolved oxygen, and rainfall (Elangovan et al. 2017). A long term study of six years from the Lake Ringsjön showed that the ciliate species composition in the lake did not change in the year 1988–1990 and in 1998–2000 but mean abundance and biomass of ciliates decreased with respect to minor fluctuations in the physicochemical parameters (Xu and Cronberg 2010). Another study from lake Donghu, China showed that the ciliate communities composed of larger carnivorous and herbivorous species were replaced by smaller bacterivorous species (Gong 1986). Also, in another study from the Mediterranean Sea, it was found that temperature, total phosphate, nitrogen, nitrate, and orthophosphate were significantly related to the ciliate abundance (Dhib et al. 2013). The study also showed that spatial variation had no significant correlation with ciliate abundance but temporal variation had significant effect on physicochemical parameters (Dhib et al. 2013). Also, in another study from Delhi, it was found that there was significant difference in the physicochemical parameters and the taxonomic diversity of ciliate communities in polluted and non-polluted sites (Abraham et al. 2019). These studies suggest that spatial and temporal variations in the ciliate community structure were significantly correlated with certain environmental variables. These findings also suggest that the spatial patterns of ciliate communities reflect the habitat (water or terrestrial) conditions and have the potential for assessing environmental status. Such studies at global level may be useful in better predicting the environment related changes (Sonntag et al. 2006; Sunagawa et al. 2020).

The main objectives of the present study were (1) to study the physicochemical parameters and ciliate diversity of the three sites (2) to reveal the spatial and temporal variations in the physicochemical parameters and ciliate diversity (3) to correlate the variations in physicochemical parameters with ciliate community structure of the three sites. Three selected sites, OBS, SL and RJ were studied for a period of one year from September, 2018 to August, 2019 in the present study.

Materials And Methods

Study area

(1) Okhla Bird Sanctuary (OBS) (28°34'12"N, 77°18'8.28"E), is a bird sanctuary at the Okhla barrage over River Yamuna. The site is located at the point where the River enters Uttar Pradesh. It spreads over 4 km², with 20% area having depth of 2–3 m and rest of the area with depth of more than 3 m.

(2) Sanjay Lake (SL) (28°36'51.12"N, 77°18'14.04"E) is an artificial lake developed by the Delhi Development Authority (DDA). It is located in Trilokpuri in East Delhi and surrounded by the residential areas of Kalyanpuri on the eastern side and Mayur Vihar on the western side. Its surface area is about 0.17 km², depth of 1 m to 2.5 m and has extensive growth of water hyacinth.

(3) Raj Ghat Pond (RJ) (28°38'26.16"N, 77°14'57.84"E) is a man-made pond located inside Raj Ghat which is a memorial to Mahatma Gandhi. It is a major tourist spot in India. The pond is small and shallow with a surface area of about 0.01 km² and depth of about 2 m.

Sampling

Sampling was carried out in alternate months from September 2018 to August 2019 to have representation from all seasons of Delhi. Different month corresponds to different seasons viz. September, November, January, March, May and July represents Post-monsoon, Autumn, Winter, Spring, Summer and Monsoon, respectively. The water samples were collected from the edge of the water bodies near the land using a beaker. Samples were brought to the laboratory in 500–1,000 ml bottles after which the samples were passed through a Nytex net/mesh of 120 µm to filter out large crustaceans, debris, and other unwanted materials. These filtered samples were immediately transferred to 500–1,000 ml beakers and protected from artificial light by covering with aluminium foil papers. About 250 ml of the samples were kept in 4 °C for further physicochemical analyses. Rest of the samples were maintained at room temperature for studying ciliate diversity, richness and evenness.

Physicochemical Analyses

Atmospheric temperature, temperature inside water (TIW), pH and dissolved oxygen (DO) were measured at the time of collection. Atmospheric and temperature inside water (TIW) was measured by a standard mercury thermometer, pH was measured by standard pH meter (EUTECH instruments), dissolved oxygen (DO) was determined using the Winkler's method (APHA 2005; Gautam et al. 2011; Montgomery et al. 1964; Winkler 1888). Electrical conductivity (EC) was measured by an HM digital AP-2 AquaPro Water quality tester (EC tester). Total dissolved solids (TDS) were measured using Wellon digital LcdTds meter Waterfilter tester. Water hardness (WH) was determined by the EDTA titration method (Gautam et al. 2011; Goetz and Smith 1959). Chloride, sulphate, nitrite, nitrate and phosphate were estimated by standard methods (APHA 2005; Gautam et al. 2011; Rossum and Villarruz 1961). Ammonia concentration in the sample was estimated using Sera Ammonium/Ammonia-Test kit (NH₄/NH₃) according to the manufacturer's protocol. All the physicochemical parameters were analysed in triplicates and one control or blank solution was also analysed for each test.

Ciliate Diversity Analysis

The samples were maintained at room temperature for diversity analysis. Samples were subjected to periodic microscopic examination for about 15–20 days. Total species number and abundance per ml were analyzed using samples collected from different sites in triplicates. The ciliate diversity in different samples was determined by Shannon-Weiner index and Simpson index (Shannon and Wiener 1949; Simpson 1949). Species richness was calculated by Margalef's Index (Margalef, 1966) and species evenness was estimated by the Pielou's Index (Pielou 1966). The biodiversity calculator used for the study was available online (http://alyoung.com/labs/biodiversity_calculator.html) (Young 2017). Percent relative abundance (RA) was also calculated for each species and dominant or rare species were determined from the collected data (Achacoso et al. 2016). Dominant species were identified as the species with the relative abundance more than or equal to 5% (RA ≥ 5%) and rare species were identified as species with relative abundance less than or equal to 0.5% (RA ≤ 0.5%).

Statistical analysis

Analysis of variance (ANOVA) was done to measure the differences among the means of the values of physicochemical parameters as well as the diversity indices (Fisher 1954; Fisher and Yates 1963). Two way ANOVA was done to estimate the difference in the means of physicochemical parameters and diversity indices of different sites and seasons. A post-hoc analysis Tukey's test was performed to determine which mean pairs were significant (Vajravelu et al. 2018). A p value less than 0.05 (≤ 0.05) was considered statistically significant (Fisher 1922; Lehmann 2011; Wooditch et al. 2020). Correlations between physicochemical parameters and ciliate communities were estimated using Pearson correlation, Principal component analysis (PCA) and non-metric multidimensional scaling (NMDS). This was used to estimate relationships between different seasons and sites in terms of physicochemical and diversity indices (Clarke and Warwick 2001; Hure et al. 2020; Li et al. 2018). All statistical analyses were performed using IBM SPSS 22.0 statistics software.

Results And Discussion

Spatial and temporal variations in physicochemical parameters

The physicochemical parameters were compared site wise (spatial variations) as well as during different seasons (temporal variations). The mean values of the physicochemical parameters studied for the three sites Okhla Bird Sanctuary (OBS), Sanjay Lake (SL) and Raj Ghat (RJ) pond for different seasons are given in Table 1 and Fig. 2.

Table 1
Physicochemical parameters (mean $\pm$ SD) of Okhla Bird Sanctuary (OBS), Sanjay Lake (SL) and Raj Ghat Pond (RJ), Delhi recorded during the study period.

Parameters (n = 3)	Sites	Sept 2018	Nov 2018	Jan 2019	Mar 2019	May 2019	July 2019
Temperature (inside water) (°C)	OBS	28 $\pm$ 1	22 $\pm$ 1	14 $\pm$ 1	23 $\pm$ 1	30 $\pm$ 1	33 $\pm$ 1
	SL	29 $\pm$ 1	24 $\pm$ 1	16.3 $\pm$ 1.5	25 $\pm$ 1	30 $\pm$ 1	35.3 $\pm$ 0.57
	RJ	29 $\pm$ 1	25.3 $\pm$ 1	18 $\pm$ 1.5	25 $\pm$ 1	32 $\pm$ 1	35 $\pm$ 1
Temperature (atmospheric) (°C)	OBS	30 $\pm$ 1	25 $\pm$ 1	15 $\pm$ 1	25.3 $\pm$ 1.57	32.6 $\pm$ 0.57	35.3 $\pm$ 1.52
	SL	30 $\pm$ 1.15	25 $\pm$ 0.57	18 $\pm$ 1	28 $\pm$ 1	32 $\pm$ 1	36 $\pm$ 1
	RJ	30 $\pm$ 1	26 $\pm$ 1	20 $\pm$ 1.52	28 $\pm$ 1	33 $\pm$ 1	36 $\pm$ 1
pH	OBS	7.7 $\pm$ 0.01	7.38 $\pm$ 0.12	7.34 $\pm$ 0.13	7.39 $\pm$ 0.04	7.77 $\pm$ 0.08	6.85 $\pm$ 0.12
	SL	7.7 $\pm$ 0.02	8.0 $\pm$ 0.11	7.7 $\pm$ 0.04	7.8 $\pm$ 0.08	7.6 $\pm$ 0.08	7.7 $\pm$ 0.12
	RJ	7.8 $\pm$ 0.09	8.3 $\pm$ 0.02	7.8 $\pm$ 0.1	8.1 $\pm$ 0.12	8.2 $\pm$ 0.15	8 $\pm$ 0.06
Dissolved oxygen (mg/L)	OBS	2.5 $\pm$ 0.5	3.3 $\pm$ 0.5	2.5 $\pm$ 0	3.8 $\pm$ 0.28	6.3 $\pm$ 0.28	2 $\pm$ 1
	SL	7 $\pm$ 1.32	4.6 $\pm$ 1.15	2 $\pm$ 0.5	4.6 $\pm$ 0.57	3.3 $\pm$ 0.28	4 $\pm$ 1
	RJ	6.33 $\pm$ 1.52	5 $\pm$ 1.8	4.8 $\pm$ 0.28	5.1 $\pm$ 0.28	6.66 $\pm$ 0.57	4.83 $\pm$ 0.28
Total dissolved solids (mg/L)	OBS	304.3 $\pm$ 2.08	351.6 $\pm$ 2.3	272 $\pm$ 2.64	302.6 $\pm$ 2.08	353 $\pm$ 2.64	317.3 $\pm$ 2.08
	SL	310.3 $\pm$ 1.52	368 $\pm$ 0.57	308 $\pm$ 0.57	416.6 $\pm$ 2.08	566 $\pm$ 2	663 $\pm$ 2
	RJ	573.33 $\pm$ 2.08	697 $\pm$ 2	894.6 $\pm$ 1.5	1180.3 $\pm$ 1.5	1362.3 $\pm$ 2.51	931.3 $\pm$ 2.3
Electrical conductivity (μ S/cm)	OBS	549.3 $\pm$ 1.52	693.6 $\pm$ 1.52	542 $\pm$ 2	592.3 $\pm$ 2.51	654.6 $\pm$ 2.08	573.6 $\pm$ 1.52
	SL	612 $\pm$ 1	719 $\pm$ 2.6	729.6 $\pm$ 2.08	826 $\pm$ 1	922.3 $\pm$ 2.5	973 $\pm$ 2
	RJ	1044 $\pm$ 2.64	1249.3 $\pm$ 3.2	1549.3 $\pm$ 1.52	1688.3 $\pm$ 2.08	1581.3 $\pm$ 2.08	1058.3 $\pm$ 1.5
Water hardness (mg/L)	OBS	150 $\pm$ 0	201.6 $\pm$ 2.88	175.3 $\pm$ 4.6	164.0 $\pm$ 0.98	86.8 $\pm$ 2.8	15 $\pm$ 0
	SL	90 $\pm$ 0	215 $\pm$ 5	197.6 $\pm$ 3.78	206.5 $\pm$ 2.08	82.2 $\pm$ 222	15 $\pm$ 0
	RJ	229 $\pm$ 4	286.6 $\pm$ 5.03	398.3 $\pm$ 2.88	648.6 $\pm$ 2.3	182.5 $\pm$ 1.64	100 $\pm$ 0
Chloride (mg/L)	OBS	43.4 $\pm$ 1.5	64.0 $\pm$ 5.41	48.6 $\pm$ 2.04	57.5 $\pm$ 3.26	94.1 $\pm$ 2.8	111.8 $\pm$ 0.81
	SL	60.1 $\pm$ 0.36	73.4 $\pm$ 4.02	81.7 $\pm$ 5.4	116.5 $\pm$ 3.62	166.1 $\pm$ 0.81	221.9 $\pm$ 0.59
	RJ	149.21 $\pm$ 0.55	194.89 $\pm$ 3.55	250.1 $\pm$ 3.37	337.60 $\pm$ 2.25	445.22 $\pm$ 2.38	321.85 $\pm$ 3.59
Sulphate (mg/L)	OBS	94.5 $\pm$ 3.53	106 $\pm$ 1.76	77.7 $\pm$ 7.01	75.5 $\pm$ 6.61	82.6 $\pm$ 7.83	83.5 $\pm$ 7.53
	SL	89.4 $\pm$ 9.85	90.1 $\pm$ 15.13	100 $\pm$ 20.13	49.7 $\pm$ 5.55	88.2 $\pm$ 7.49	176.3 $\pm$ 11.84
	RJ	177.3 $\pm$ 13.56	143.5 $\pm$ 4.03	182.2 $\pm$ 12.35	373.7 $\pm$ 10.59	109.4 $\pm$ 10.86	103.8 $\pm$ 10.42
Phosphate (mg/L)	OBS	0.33 $\pm$ 0.09	2.39 $\pm$ 0.06	1.61 $\pm$ 0.06	0.63 $\pm$ 0.018	0.17 $\pm$ 0.02	0.12 $\pm$ 0.016
	SL	1.70 $\pm$ 0.179	0.06 $\pm$ 0.04	0.03 $\pm$ 0.016	0.70 $\pm$ 0.143	0.06 $\pm$ 0.04	0.03 $\pm$ 0.016
	RJ	1.98 $\pm$ 0.02	0.17 $\pm$ 0.016	0.04 $\pm$ 0.024	0.17 $\pm$ 0.024	0.23 $\pm$ 0.04	0.36 $\pm$ 0.024
Nitrite (mg/L)	OBS	0.38 $\pm$ 0.014	0.17 $\pm$ 0.08	0.133 $\pm$ 0.028	0.090 $\pm$ 0.004	0.414 $\pm$ 0.11	0.014 $\pm$ 0.004
	SL	0.361 $\pm$ 0.039	0.04 $\pm$ 0.002	0.047 $\pm$ 0.04	0.566 $\pm$ 0.229	0.007 $\pm$ 0.008	0.008 $\pm$ 0.0007
	RJ	0.73 $\pm$ 0.05	0.059 $\pm$ 0.026	0.106 $\pm$ 0.03	0.479 $\pm$ 0.189	0.846 $\pm$ 0.02	0.029 $\pm$ 0.014
Nitrate (mg/L)	OBS	1.89 $\pm$ 0.78	2.49 $\pm$ 0.42	2.73 $\pm$ 0.26	3.07 $\pm$ 0.099	1.00 $\pm$ 0.07	1.404 $\pm$ 0.07
	SL	0.507 $\pm$ 0.07	0.468 $\pm$ 0.419	1.17 $\pm$ 0.16	0.19 $\pm$ 0.59	1.55 $\pm$ 0.64	1.76 $\pm$ 0.05
	RJ	5.34 $\pm$ 2.9	6.19 $\pm$ 0.29	1.88 $\pm$ 0.234	0.89 $\pm$ 0.983	2.38 $\pm$ 0.35	0.89 $\pm$ 0.79
Ammonia (mg/L)	OBS	5.00 $\pm$ 0	10.00 $\pm$ 0	10.00 $\pm$ 0	10.00 $\pm$ 0	10.00 $\pm$ 0	10.00 $\pm$ 0
	SL	0.16 $\pm$ 0.14	0.50 $\pm$ 0	0.50 $\pm$ 0	5.00 $\pm$ 0	1.00 $\pm$ 0	5.00 $\pm$ 0

Lowest and highest values for each parameter are marked with bold letters; n = number of samples.

Parameters (n = 3)	Sites	Sept 2018	Nov 2018	Jan 2019	Mar 2019	May 2019	July 2019
	RJ	0.25 ± 0	0.50 ± 0	0.50 ± 0	0.50 ± 0	0.50 ± 0	0.50 ± 0
Lowest and highest values for each parameter are marked with bold letters; n = number of samples.							

Comparison of physicochemical characteristics of the three sites in different seasons using two-way ANOVA suggests that there is a significant difference in pH ($F = 11.151$, $p < 0.005$), TDS ($F = 24.203$, $p < 0.005$), EC ($F = 31.369$, $p < 0.005$), WH ($F = 7.800$, $p < 0.05$), chloride ($F = 28.982$, $p < 0.005$), and ammonia ($F = 52.515$, $p < 0.005$). Other parameters such as TIW, DO, sulphate, phosphate, nitrite and nitrate had no significant variations. Post-hoc Tukey test suggest that pH, TDS, EC, WH, chloride and ammonia of OBS and SL are significantly different from RJ ($p < 0.005$). Comparison of physicochemical parameters among different months of all three sites using two-way ANOVA suggests that there is a significant difference in TIW ($F = 234.99$, $p < 0.005$), WH ($F = 4.378$, $p < 0.05$), and chloride ($F = 4.293$, $p < 0.05$) in different months. Other parameters such as pH, DO, TDS, EC, sulphate, phosphate, nitrite, nitrate, and ammonia showed no significant difference among different months. Post-hoc Tukey test comparing different parameters individually among different seasons suggests a significant difference in the TIW among all the months ($p < 0.005$). Rest of the parameters does not show significant variation seasonally when compared individually.

Correlation among various physicochemical parameters with each other was also analysed in the present study. Pearson correlation coefficients suggested positive and negative correlations of physicochemical parameters with each other as given in Table 2. Principal component analysis plot showed that the parameters such as TIW, pH, DO, TDS, EC, WH, chloride, sulphate correlated with each other and phosphate, nitrite, nitrate and ammonia correlated with each other (Fig. 3). pH is positively and directly correlated with factors like temperature (TIW) and DO, as the photosynthetic activity responsible for the assimilation of bicarbonates and carbon dioxides is responsible for the increase in the pH (Manjare et al. 2010). Dissolved oxygen (DO) in water is attributed to the fact that oxygen is dissolved more during the period of catabolic activity by photosynthesis. Temperature has direct influence on DO and the duration of sunlight, since during the summers when the water temperature increases, it increases the amount of photosynthesis and production of O_2 thereby increasing the DO levels (Manjare et al. 2010). The electrical conductivity (EC) of the water depends upon the concentration of ions and its nutrient load. EC is the measure of a solution to conduct electricity hence shows positive correlation with temperature (TIW), pH, DO, TDS, WH, chloride, sulphate, nitrite and nitrate, whereas it showed a negative correlation with phosphate and ammonia (Joshi et al. 2009). Water hardness (WH) is contributed from the evaporation of water during summers leaving the water concentrated (Dubey and Ujjania 2013; Yogesh 2020), hence WH showed a positive correlation with pH, DO, TDS, EC, chloride, sulphate, nitrite and nitrate, whereas it showed a negative correlation with TIW, phosphate and ammonia. Total dissolved solids (TDS) include total inorganic and organic solutes of the water such as organic wastes, industrial effluents, calcium, magnesium, chloride, sulphate, nitrate and nitrite (Tajmunnaher and Chowdhury 2017). TDS is positively correlated with TIW, pH, DO, EC, WH, chloride, sulphate, nitrite and nitrate, whereas it showed a negative correlation with phosphate and ammonia. Chloride concentration is influenced by presence of ionic compounds such as sodium, potassium, nitrate and nitrite which ultimately increases the pH and electrical conductivity of the water (Joshi et al. 2009). Chloride showed a positive correlation with TIW, pH, DO, TDS, EC, WH, sulphate, nitrite and nitrate, whereas it showed a negative correlation with phosphate and ammonia. Sulphate accumulation in water leads to increase in pH, total dissolved solids, and electrical conductivity (Asamoah and Amorin 2011; Popoola et al. 2019). In this study, sulphate showed a positive correlation with TIW, pH, DO, TDS, EC, WH, chloride, nitrite and nitrate, whereas it showed a negative correlation with phosphate and ammonia. Phosphate and nitrate show positive correlation with each other since both acts as nutrients for living organisms (Golder and Chattopadhyay 2016). It has been reported that phosphate availability in water decreases with increase in pH since high pH induces phosphorous to bind with other cations forming insoluble compounds (Cerozi and Fitzsimmons 2016). Phosphate showed a positive correlation with DO, nitrite, nitrate and ammonia whereas it showed a negative correlation with TIW, pH, TDS, EC, WH, chloride and sulphate. Ammonia is converted to nitrite and nitrate by nitrifying bacteria due to which ammonium and nitrite show negative correlation in nitrification-dominant period (Sepehri and Sarrafzadeh 2019). Nitrite showed a positive correlation with TIW, pH, DO, TDS, EC, WH, chloride, sulphate, phosphate and nitrate whereas it showed a negative correlation with ammonia. Nitrate showed a positive correlation with pH, DO, TDS, EC, WH, chloride, sulphate, phosphate and nitrite whereas it showed a negative correlation with TIW and ammonia. Ammonia similar to nitrate, act as nutrient along with phosphate showing positive correlation with phosphate but negative correlation with nitrate especially in nitrification-dominant period (Sepehri and Sarrafzadeh 2019). Ammonia showed a positive correlation with phosphate, whereas it showed a negative correlation with TIW, pH, DO, TDS, EC, WH, chloride, sulphate, nitrite and nitrate.

Table 2
Correlation between physicochemical parameters of three sites sampled during one year from September 2018 to August 2019.

Parameters	TIW	pH	DO	TDS	EC	WH	Chloride	Sulphate	Phosphate	Nitrite	Nitrate	Ammonia
TIW	1											
pH	0.110	1										
DO	0.349	0.620**	1									
TDS	0.291	0.642**	0.464	1								
EC	0.094	0.668**	0.454	0.940**	1							
WH	-0.453	0.468*	0.185	0.493*	0.672**	1						
Chloride	0.388	0.586*	0.429	0.983**	0.891**	0.359	1					
Sulphate	0.008	0.384	0.228	0.612**	0.718**	0.778**	0.530*	1				
Phosphate	-0.222	-0.283	0.139	-0.322	-0.310	-0.053	-0.375	-0.130	1			
Nitrite	0.168	0.360	0.611**	0.363	0.326	0.256	0.313	0.183	0.279*	1		
Nitrate	-0.098	0.143	0.087	0.076	0.179	0.110	0.034	0.115	0.275	0.087	1	
Ammonia	-0.111	-0.731**	-0.426	-0.546*	-0.609**	-0.369	-0.511*	-0.380	0.253	-0.173	-0.026	1
Values are Pearson correlation coefficients, *correlation is significant at $p < 0.05$ level and **correlation are significant at $p < 0.01$ level. TIW = temperature (inside water), DO = dissolved oxygen, EC = electrical conductivity, TDS = total dissolved solids, WH = water hardness.												

Spatial and temporal variations in Ciliate diversity, richness and evenness

Okhla Bird Sanctuary (OBS) exhibited highest diversity, followed by Sanjay Lake (SL) and Raj Ghat pond (RJ) as represented by Shannon, Simpson, Margalef's and Pielou's indices in Table 3 and Fig. 4. In OBS, Shannon index (H') was found to be maximum during September 2018 (2.21 ± 0.40) and minimum during March 2019 (1.63 ± 0.22) and May 2019 (1.63 ± 0.49). In SL, Shannon index corresponding to species diversity (H') was found to be maximum in September 2018 (1.64 ± 0.02) and minimum in January 2019 (0.18 ± 0.32). In RJ, Shannon index corresponding to species diversity (H') was found to be maximum in September 2018 (1.33 ± 0.20) and minimum in July 2019 (0.50 ± 0.53).

Table 3
Species diversity, richness and evenness of ciliate community present in Okhla Bird Sanctuary (OBS), Sanjay Lake (SL), Raj Ghat pond (RJ), Delhi during the study period.

Month of collection (n = 3)	Sites	Species diversity		Species richness	Species evenness
		Shannon Index (H')	Simpson Index (D)	Margalef's Index (d)	Pielou's Index (J')
Sep-18	OBS	2.21 ± 0.40	0.16 ± 0.05	3.51 ± 1.36	0.48 ± 0.05
	SL	1.64 ± 0.02	0.23 ± 0	1.69 ± 0.31	0.36 ± 0.02
	RJ	1.33 ± 0.20	0.35 ± 0.10	1.23 ± 0.15	0.28 ± 0.05
Nov-18	OBS	2.14 ± 0.39	0.12 ± 0.06	2.46 ± 0.74	0.53 ± 0.06
	SL	0.80 ± 0.28	0.39 ± 0.10	0.72 ± 0.215	0.38 ± 0.10
	RJ	1.05 ± 0.28	0.40 ± 0.11	0.83 ± 0.315	0.29 ± 0.09
Jan-19	OBS	1.76 ± 0.43	0.20 ± 0.09	2.07 ± 0.98	0.46 ± 0.07
	SL	0.18 ± 0.32	0.20 ± 0.34	0.12 ± 0.20	0.06 ± 0.11
	RJ	0.93 ± 0.28	0.43 ± 0.17	0.81 ± 0.17	0.32 ± 0.08
Mar-19	OBS	1.63 ± 0.22	0.21 ± 0.045	1.57 ± 0.407	0.38 ± 0.028
	SL	0.90 ± 0.69	0.51 ± 0.29	0.84 ± 0.59	0.27 ± 0.15
	RJ	1.07 ± 0.36	0.35 ± 0.11	0.77 ± 0.33	0.32 ± 0.04
May-19	OBS	1.63 ± 0.49	0.21 ± 0.10	1.31 ± 0.65	0.38 ± 0.07
	SL	1.11 ± 0.38	0.39 ± 0.19	0.98 ± 0.40	0.30 ± 0.11
	RJ	0.94 ± 0.31	0.41 ± 0.11	0.47 ± 0.19	0.22 ± 0.05
Jul-19	OBS	1.86 ± 0.52	0.19 ± 0.09	1.84 ± 1.18	0.42 ± 0.100
	SL	1.57 ± 0.24	0.23 ± 0.07	1.25 ± 0.37	0.37 ± 0.06
	RJ	0.50 ± 0.53	0.37 ± 0.35	0.30 ± 0.30	0.11 ± 0.10
Lowest and highest values for each index are marked with bold letters; n = number of samples.					

Comparison of diversity indices among all three sites by two-way ANOVA suggests that there is a significant difference in the species diversity indices, Shannon ($F = 12.665$, $p < 0.005$) and Simpson ($F = 10.839$, $P < 0.005$), species richness index ($F = 17.712$, $p < 0.005$), and species evenness index ($F = 6.712$, $p < 0.05$). Post-hoc Tukey test suggests that Shannon and Simpson diversity index, Margalef's richness index and Pielou's evenness index of OBS is significantly different from SL and RJ ($p < 0.005$). Comparison of diversity indices among different seasons in all three sites by two-way ANOVA followed by Post-hoc Tukey tests suggest that there is no significant seasonal difference in the species diversity, richness and evenness indices of all the three sites. Though, ciliate community composition varied during different seasons in the three sites.

In the previous reports the diversity of biological community was studied using the diversity indices as they prove to be an effective way to study the diversity in environmental samples. Various studies such as the ciliate diversity of different sites of river Yamuna (Kaur et al. 2021), the phytoplankton diversity from the coastal regions of Tamil Nadu (Vajravelu et al. 2018), microzooplankton community from the Kochi backwaters (Anjusha et al. 2018), etc were studied using diversity indices. These studies have also suggested that the diversity of microzooplankton communities were significantly high during the pre and post monsoon periods similar to the present study (Anjusha et al. 2018; Vajravelu et al. 2018).

Spatial and temporal correlation between physicochemical parameters and ciliate diversity indices

Pearson correlation coefficients were calculated to determine the correlation between physicochemical parameters and species diversity, richness and evenness (Table 4). Ciliate species diversity calculated by the Shannon index (H') was found to be positively correlated with temperature (inside water), the concentration of phosphate, nitrite, nitrate and ammonia, whereas it was negatively correlated with pH, DO, TDS, EC, WH, concentration of chloride and sulphate. Ciliate species richness calculated by the Margalef's index (d) was found to be positively correlated with EC, concentration of phosphate, nitrite, nitrate and ammonia whereas it was negatively correlated with TIW, pH, DO, TDS, WH, concentration of chloride and sulphate. Ciliate species evenness calculated by the Pielou's index (J') was observed to be positively correlated with the concentration of phosphate, nitrate and ammonia, whereas it was negatively correlated with TIW, pH, DO, TDS, EC, WH and concentration of chloride, sulphate, and nitrite.

Table 4
Correlation between physicochemical parameters and species diversity, richness and evenness from three sites sampled for one year from September 2018 to August 2019.

Physicochemical parameters	Pearson correlation coefficients		
	With species diversity (H')	With species richness (d)	With species evenness (J')
Temperature (inside water)	0.111	-0.036	-0.093
pH	-0.571*	-0.536*	-0.494*
Dissolved Oxygen	-0.170	-0.344	-0.202
Total dissolved solids	-0.432	-0.525*	-0.430
Electrical conductivity	-0.475*	0.558*	-0.411
Water hardness	-0.284	-0.245	-0.095
Chloride	-0.463	-0.573*	-0.496*
Sulphate	-0.137	-0.214	-0.080
Phosphate	0.458	0.436	0.378
Nitrite	0.049	0.007	-0.075
Nitrate	0.158	0.104	0.072
Ammonia	0.696**	0.593**	0.653**
*Correlation is significant at $p < 0.05$ level and **correlation is significant at $p < 0.01$ level.			

Environmental changes have a significant correlation with the ciliate communities in the aquatic environments. Because of their small size and life span, they can resist or adapt to the environmental changes and therefore, the community is shaped according to the environmental conditions (Abdullah et al. 2018; Curds 1992). The environmental factors such as temperature, light, nutrients, salt concentration, food resources are major driving forces in establishing a community structure and slight changes in these variables can cause changes in the community composition of ciliates (Abdullah et al. 2018; Jiang et al. 2011; Sikder et al. 2019; Xu et al. 2014).

Temperature showed a positive correlation with the number of ciliates. The ciliate diversity represented by Shannon index (H') was relatively high in all the three sites in the post-monsoon months, i.e., September and November 2018 (Table 3 and Fig. 4). Similarly, species richness represented by Margalef's index (d) was highest in September and November 2018 in all the three sites. The temperature ranged from 22–29°C during these months. This temperature range was observed to be desirable for the ciliate growth as reported in the previous studies (Babu et al. 2013; Bera et al. 2014; Kedar et al. 2008; Vajravelu et al. 2018). Pielou's evenness index was highest in September (0.48 ± 0.05) and November 2018 (0.53 ± 0.06) in case of OBS, highest in November 2018 (0.38 ± 0.10) and July 2019 (0.37 ± 0.06) in case of SL and in case of RJ it was highest in January 2019 (0.32 ± 0.08) and March 2019 (0.32 ± 0.04). A slight drop in the ciliate diversity, ciliate richness and ciliate evenness was observed in May 2019 (summer season) and July 2019 (monsoon season) where the temperature ranged from 30–35°C in all the sites. Similarly, in January 2019 (winter season), low ciliate diversity ($H' = 1.76 \pm 0.43$ in OBS and $H' = 0.18 \pm 0.32$ in SL), ciliate richness ($d = 2.07 \pm 0.98$ in OBS and $d = 0.12 \pm 0.20$ in SL) and ciliate evenness ($J' = 0.46 \pm 0.07$ in OBS and $J' = 0.06 \pm 0.11$ in SL) was observed in OBS and SL as the temperature was low ranging from 14–16°C. But, species evenness was observed to be high in case of RJ ($J' = 0.32 \pm 0.08$) during the winter season, i.e. in January 2019. It has been previously reported that evenness may increase at low temperature if the sample contains more taxonomically distinct species (Passy et al. 2016). Previous reports suggest that the temperature, within the desirable range (20 to 31°C), shows a positive correlation with species abundance (García et al. 2018; Limberger et al. 2014; Luna-Pabello et al. 1992). A temperature within this range helps in increasing the metabolism of the species thereby increasing the population density (Parain et al., 2019). The present study as well as the previous reports show that extremely high and low temperatures have a negative impact on the growth and replenishment of ciliates in the freshwater bodies (García et al. 2018; Limberger et al. 2014; Luna-Pabello et al. 1992).

pH correlated negatively with ciliate diversity, richness and evenness. High ciliate diversity, richness and evenness were observed during the post-monsoon season, i.e., September 2018 in all the sites with the pH in the range of 7.7–7.8. In OBS, the ciliate diversity was recorded to be lowest in May (summer) with a pH of 7.77 ± 0.08 . In SL, the diversity was lowest in January (winter) with a pH of 7.7 ± 0.04 and in RJ, the lowest diversity was noticed in July (monsoon season) with pH of 8 ± 0.06 . Thus, this indicates that increase in pH (from neutral to slightly alkaline) decreased the ciliate diversity since it has been reported that increase in pH or alkalinity decreases the growth of ciliate species (Abraham et al. 2019). On the other hand, extremely low pH creates an acidic environment which also negatively impacts the species diversity (Thirunavukkarasu et al. 2014; Vajravelu et al. 2018). The pH of freshwater bodies has been reported to decrease during autumn or monsoon seasons (Salim et al. 2015). This has been well correlated in the present investigation as well. Increase in pH indicates an increase of CO₂ uptake by phytoplanktons leading to algal blooms (Thirunavukkarasu et al. 2014; Vajravelu et al. 2018). This gradually depletes the nutrient contents in the ecosystem thereby affecting the species diversity at higher pH (Abraham et al. 2019).

Dissolved oxygen (DO) content showed a negative correlation with the ciliate diversity, richness and evenness when compared among sites. There was no significant difference in the overall mean and range of DO values of all the three sites, with slightly low value in OBS (3.4 ± 1.55 mg/L), followed by SL (4.25 ± 1.66 mg/L) and comparatively high in case of RJ (5.45 ± 0.82 mg/L). Seasonal data suggests that in OBS, low DO was observed in September 2018 (2.5 ± 0.5 mg/L; postmonsoon), January 2019 (2.5 ± 0 mg/L; winter) and July 2019 (2 ± 1 mg/L; monsoon) and was high in May 2019 (6.3 ± 0.28 mg/L; summer). In SL, the lowest DO was observed in January 2019 (2 ± 0.5 mg/L; winter) and highest DO was observed in September 2018 (7 ± 1.32 mg/L; post-monsoon). In RJ, lowest DO was observed in January 2019 (4.8 ± 0.28 mg/L; winter) and July 2019 (4.83 ± 0.28 mg/L; monsoon) and highest DO was observed in May 2019 (6.66 ± 0.57 mg/L; summer). Overall, DO was low in the post-monsoon and winter months and was high during summer months. In all the sites and all the months, the threshold value of dissolved oxygen was higher than the classic definitions of hypoxia (< 2.0 mg/L), suggesting favourable conditions for microbial growth (Spietz et al. 2015). The previous report on the DO levels of OBS suggests a low level of DO (average value of 1.6 ± 0.84 mg/L) in the year 2009–2010 (Manral and Khudsar 2013). DO levels have been reported to be low during the winter season due to low species diversity (Araoye 2007, 2009). Another study reported high DO levels in the monsoon season (Babu et al. 2013). This correlates with the present study where DO was low in all the sites during winter and high DO was observed in monsoon and summer season. The desirable DO concentration results in the availability of more oxygen to the organism thereby increasing species metabolism which positively influences the species diversity (Rajagopal et al. 2010).

Total dissolved solids (TDS) represent dissolved materials including inorganic salts and organic matter as well as toxic contaminants from industrial effluents, waste materials etc. present in the water body (Jayakumar et al. 2009; Weber-Scannell and Duffy 2007). The present study indicated a negative correlation between ciliate diversity, richness and evenness and TDS concentration. Among the three freshwater sites, the total mean value of TDS from all the months showed that OBS had the lowest TDS concentration (316.80 ± 31.26 mg/L), followed by SL (438.65 ± 145.30 mg/L) and RJ (939.81 ± 293.84 mg/L). Ciliate diversity was also maximum in OBS ($H' = 1.63 - 2.21$), followed by SL ($H' = 0.18 - 1.64$) and RJ ($H' = 0.50 - 1.33$). Seasonal data suggests that in OBS lowest TDS was observed in January 2019 (272 ± 2.64 mg/L; winter) and highest TDS was observed in May 2019 (353 ± 2.64 mg/L; summer). In SL, the lowest TDS was observed in January 2019 (308 ± 0.57 mg/L; winter) and highest TDS was observed in July 2019 (663 ± 2 mg/L; monsoon). In RJ, lowest TDS was observed in September 2018 (573.33 ± 2.08 mg/L; post-monsoon) and highest in May 2019 (1362.3 ± 2.51 mg/L; summer). Overall seasonal data suggests that the lowest TDS was observed in winter and highest in summer. TDS correlates negatively with the species diversity, richness and evenness as it induces toxicity by increasing salinity in the water (Ivanova and Kazantseva 2006; Weber-Scannell and Duffy 2007). Thus, a high amount of TDS indicates poor quality of water and is directly proportional to the degree of water pollution (Bharati and Krishnamoorthy 1990; Tripathy and Adhikary 1990). The present study suggests that high concentration of TDS in a water body inhibits the growth and enrichment of ciliates.

Electrical conductivity (EC), similar to pH and TDS, correlates negatively with ciliate diversity, richness and evenness. OBS, having high ciliate diversity, showed the lowest EC mean value (600.90 ± 60.70 μ S/cm) followed by SL (793.93 ± 135.86 μ S/cm) and highest was in RJ (1361.70 ± 281.25 μ S/cm). It has been reported that high EC indicates the hypersaline condition of the water which inhibits ciliate diversity (Abraham et al. 2019). EC is considered to be an important factor for characterizing water quality (Esteves 1988). High EC content in water suggests that there may be an increased sewage load and/or industrial effluents in the freshwater bodies thereby increasing water pollution level (Dias et al. 2008). High EC content in the RJ pond may be due to effluents from the adjacent sewage treatment plants and thermal power station.

Water hardness (WH) also correlated negatively with the ciliate diversity, richness and evenness in the present study. OBS having highest ciliate diversity, richness and evenness showed the lowest value of WH (132.11 ± 68.99), followed by SL (134.38 ± 83.23), and highest was observed in RJ (307.5 ± 194.87). WH was observed to be lowest in July 2019 (monsoon season) which favoured ciliate diversity and WH was highest in November 2018 and March 2019 where ciliate diversity was comparatively low. WH represents the presence of dissolved minerals such as inorganic salt contents (calcium and magnesium) in water. WH generally remains high in summers than in monsoon or winter season as high-temperature increases solubility of calcium and magnesium in water (Vyas and Sawant 2008). This correlates with the present study since WH is maximum during March and lowest in monsoon season. High WH again signifies more alkaline water which lowers species diversity (Boyd et al. 2016). In the present study, chloride and sulphate showed a negative correlation with the ciliate diversity, richness and evenness where OBS (with high ciliate diversity) had lowest mean value of the chloride concentration (69.90 ± 27.15 mg/L) and sulphate (86.63 ± 11.54 mg/L), followed by SL (119.95 ± 62.84 mg/L of chloride and 98.95 ± 41.70 mg/L of sulphate) and RJ (283.12 ± 107.29 mg/L of chloride and 181.66 ± 99.62 mg/L of sulphate). Seasonal data of chloride and sulphate provide no significant correlation with species diversity, richness and evenness and it varied according to dynamics of different sites. In OBS, chloride was lowest in September 2018 (43.4 ± 1.5 mg/L; winter) and highest in July 2019 (111.8 ± 0.81 mg/L; monsoon); sulphate was lowest in January 2019 (77.7 ± 7.01 mg/L; winter) and highest in November 2018 (106 ± 1.76 mg/L; winter). In SL, chloride was lowest in September 2018 (60.1 ± 0.36 mg/L; post-monsoon) and highest in July 2019 (221.9 ± 0.59 mg/L; summer); sulphate was lowest in March 2019 (49.7 ± 5.55 mg/L; spring) and highest in July 2019 (176.3 ± 11.84 mg/L; monsoon). In RJ, chloride was lowest in September 2018 (149.21 ± 0.55 mg/L; post-monsoon) and highest in May 2019 (445.22 ± 2.38 mg/L; summer); sulphate was lowest in July 2019 (103.8 ± 10.42 mg/L; monsoon) and highest in March 2019 (373.7 ± 10.59 mg/L; spring). Nutrients such as chloride and sulphate have been reported to show a negative correlation with species diversity (Mohan et al. 2013; Rath et al. 2016). Chloride and sulphate salts are known to affect microorganisms at higher concentration since the presence of these salts increases water salinity (Rath et al. 2016). These soluble salts lower the microbial activity as they induce osmotic stress (Rath et al. 2016). This affects the nutrient intake of these microorganisms and makes them permissible to toxic soluble salts (Greaves 1992; Yan et al. 2015). Chloride and sulphate are known to enter the cells and disrupts the proper functioning of the microorganisms by interfering with their key enzyme activity (Greaves 1992; Rath et al. 2016). Also, chloride is reported to be more toxic than sulphate since it is known to inhibit protein synthesis by interfering with the binding of ribosomes to mRNA (Choquet et al. 1989; Rath et al. 2016; Weber et al. 1977).

The present investigation showed a positive correlation between ciliate diversity and phosphate, nitrite, nitrate and ammonia. Among the three freshwater sites, OBS, which had highest ciliate diversity, showed a significantly high concentration of phosphate (0.87 ± 0.92 mg/L) and ammonia (9.16 ± 2.04 mg/L) as compared to SL (0.43 ± 0.67 mg/L of phosphate and 2.02 ± 2.31 mg/L of ammonia) and RJ (0.49 ± 0.73 mg/L of phosphate and 0.45 ± 0.102 mg/L of ammonia). In all the sites, phosphate was highest in September 2018 (post-monsoon) and November 2018 (autumn) and ammonia was low in September 2018. Nitrite and nitrate showed no specific pattern seasonally it varied according to the dynamics of the site. Phosphate and inorganic nitrogen (nitrite, nitrate and ammonia) are known to correlate positively with species diversity by increasing ciliate biomass since they act as nutrients and help in the growth of microorganisms (Dopheide et al. 2009; Wang et al. 2014). Phosphate and inorganic nitrogen have been reported to directly favour the growth of photosynthetic ciliates and indirectly favour the growth of heterotrophic ciliates (Wang et al. 2014). The inorganic nitrogen parameters (nitrite, nitrate and ammonia) are generally present in abundance in areas having nitrifying bacteria which act as primary feeders for ciliates (Prast et al. 2007).

Physicochemical and ciliate diversity data for all the seasons and all the sites were combined and plotted using nonmetric multidimensional scaling (NMDS) (Fig. 5). The plot and the distance values suggest that the physicochemical parameters and diversity data estimated for September 2018 are distinct from the other months, hence lies separated at the upper right quadrant of the plot. May 2019 and July 2019 lie at the lower right quadrant of the plot. January 2019 and November 2019 lie at the lower left quadrant. March 2019 lies separately at the lower left quadrant. The distance values also suggest that the characteristics of September 2018 were distinct from the other months.

Physicochemical and ciliate diversity data for all the sites studied for different months were combined and plotted using nonmetric multidimensional scaling (NMDS)

(Fig. 6). The three sites OBS, SL and RJ were placed separately in different quadrants on the plot. The distances of sites with each other suggest that the physicochemical parameters and ciliate diversity data of OBS were closely related to SL than with the RJ. SL and RJ were more closely related to each other than with OBS.

Spatial And Temporal Variations In Ciliate Community Composition

Ciliate community composition varied in each site and during different seasons. A total of 44 species belonging to 8 classes from OBS, 18 species belonging to 6 classes from SL, and 13 species belonging to 6 classes from RJ were identified from September 2018 to August 2019 (Table 5). Percent relative abundance (RA%) of each species belonging to different classes identified in each month for all the three sites were calculated and summarized in Figs. 7–9.

Table 5
Cumulative numbers of five taxonomic levels of ciliate communities observed at three sampling sites, Okhla Bird Sanctuary (OBS), Sanjay Lake (SL), and Raj Ghat Pond (RJ) in Delhi during the study period.

Taxonomic levels	OBS	SL	RJ
Species	44	18	13
Genus	35	13	11
Family	28	12	10
Order	15	9	8
Class	8	6	6

In OBS, seven dominant species were identified as *Tetmemena pustulata*, *T. saprai*, *Gonostomum strenua* and *Oxytricha granulifera* belonging to class Spirotrichea, *Paramecium multimicronucleatum* and *Paramecium* sp. belonging to class Oligohymenophorea, *Coleps* sp. belonging to Prostomatea, and *Chilodonella* sp. belonging to class Phyllopharyngea. 13 rare species were identified as *Hemiamphisiella terricola*, *Uroleptus longicaudatus*, *Pseudokeronopsis erythrina*, *Urostyla grandis*, *Aspidisca* sp., *Euplotes woodruffi* belonging to class Spirotrichea, *Frontonia* sp. belonging to class Oligohymenophorea, *Blepharisma sinosum*, *B. undulans*, and *Blepharisma* sp. belonging to class Heterotrichea, *Dileptus* sp. and *Spathidium* sp. belonging to class Litostomatea, *Loxodes* sp. belonging to class Karyorelictea (Fig. 7).

In SL, six dominant species were identified as *Coleps* sp. belonging to class Prostomatea, *Tetmemena saprai* and *Gonostomum strenua* belonging to class Spirotrichea, *Paramecium multimicronucleatum* and *Paramecium* sp. belonging to class Oligohymenophorea, *Colpoda magna* belonging to class Colpodea, and *Chilodonella* sp. belonging to class Phyllopharyngea. Two rare species were identified as *Uroleptus* sp. and *Euplotes aediculatus* belonging to class Spirotrichea (Fig. 8). In RJ, seven dominant species were identified as *Tetmemena saprai*, *Gonostomum strenua*, and *Oxytricha granulifera* from class Spirotrichea, *Coleps* sp. from the class Prostomatea, *Paramecium multimicronucleatum* and *Paramecium* sp. belonging to class Oligohymenophorea, and *Chilodonella* sp. from class Phyllopharyngea. Three rare species were identified as *Anteholosticha* sp. and *Euplotes aediculatus* from class Spirotrichea and *Colpoda* sp. from class Colpodea (Fig. 9).

In OBS, species with maximum relative abundance belonged to the class Spirotrichea (47%) followed by class Oligohymenophorea (20%), Prostomatea (16%), Phyllopharyngea (6%), Colpodea (5%), Heterotrichea (3%), Litostomatea (2%) and Karyorelictea (1%). In SL, species with maximum relative abundance belonged to the class Prostomatea (38%), followed by Spirotrichea (23%), Oligohymenophorea (21%), Colpodea (7%), Phyllopharyngea (6%), Litostomatea (5%). In RJ, species with maximum relative abundance belonged to the class Spirotrichea (38%), followed by Prostomatea (37%), Oligohymenophorea (17%), Phyllopharyngea (5%), Heterotrichea (2%), and Colpodea (1%) (Fig. 10).

In the present study, significant temporal variations in the ciliate composition was observed i.e. some ciliates were observed throughout the year and some were observed only once or twice in the year. Their temporal variations were analysed based on the number of species and their percent relative abundance for each site and each month (Figs. 7–9). Total abundance corresponds to the approximate number of individuals observed per ml which was calculated for each site for each month and summarised in Fig. 11. In OBS, the highest number of species were observed in September 2018 (29 species), followed by November 2018 (23 species), July 2019 (18 species), January 2019 (15 species), March 2019 (12 species), and May 2019 (10 species). Species that were observed in all the months (6 out of 6 months) were from the genera *Aponotophymena*, *Tetmemena* and *Paramecium*. Species observed in 5 out of 6 months sampled belonged to the genera *Oxytricha*, *Gonostomum*, and *Coleps*. In SL, the highest number of species were observed in September 2018 (11 species), followed by March 2019 (9 species), November 2018 (5 species), July 2019 (5 species), May 2019 (4 species), and January 2019 (3 species). Some species that were observed in most of the sampled months i.e. 6 out of 6 months was *Coleps*, *Tetmemena* (5 out of 6 months) and *Gonostomum* (4 out of 6 months). In RJ, the highest number of species was observed in September 2018 (7 species), followed by March 2019 (6 species), November 2018 (5 species), January 2019 (5 species), July 2019 (5 species) and May 2019 (4 species). Species that were observed in most of the months were members of the genera *Coleps* (6 out of 6 months), *Gonostomum* (4 out of 6 months), and *Paramecium* (4 out of 6 months). In all the three sites, the total number of species showed a peak in September 2018 and there was a significant drop in January 2019 and May 2019. Abundance was observed to be highest in September 2018 and lowest in January 2019 (Fig. 11).

This study evidently shows that ciliate diversity and composition depends on season as well as dynamics of each site. Also, the major contributors to the community structure were ciliates belonging to the classes Spirotrichea, Oligohymenophorea, and Prostomatea which were predominantly present in the monsoon (July 2019) and post-monsoon seasons (September 2018 and November 2018). The ciliates of these classes were comparatively low in the summer (May 2019) and winter (January 2019) seasons in all the three sites. The present study is similar to the previous reports which indicated that the ciliate abundance was low in the summer and winter seasons (Sikder and Xu 2020; Zhang and Xu 2015; Zhang et al. 2013). This could be due to the extreme water temperature and/or inadequate food supply (Sikder and Xu 2020). Class Spirotrichea was predominantly observed in all the three sampling sites which is similar to other reported studies from Delhi (Kaur et al. 2021), Tamil Nadu (Basuri et al. 2020), Tunisia (Dhib et al. 2013), and the Yellow Sea, China (Sikder and Xu 2020). OBS site had the highest percentage of spirotrich ciliates as compared to the other two sites. Spirotrich ciliates, especially urostylid group was observed only in OBS. Growth of Spirotrich ciliates including urostylids is generally encouraged in sites enriched with nutrients such as phosphate and inorganic nitrogen contents (Basuri et al. 2020; Dhib et al. 2013). Since OBS site had high concentration of phosphate (0.87 ± 0.92 mg/L) and ammonia (9.16 ± 2.04 mg/L), that could be related to high percent of spirotrich ciliates.

Blepharisma spp. was observed only in OBS as it has been reported earlier that *Blepharisma* spp. is generally present in low or neutral pH (7.40 ± 0.32) and shows sensitivity towards the alkaline conditions. It has been reported in earlier studies that the growth of *Blepharisma* spp. is favoured in the pH range of 7.2–7.8 (Repak 1991). OBS had neutral pH (7.40 ± 0.32) favouring the growth of *Blepharisma* spp. whereas SL and RJ had slightly alkaline pH. *Tetmemena* spp. and *Gonostomum strenua*, had high relative abundance in comparison to other species belonging to class Spirotrichea. In class Oligohymenophorea, the genus *Paramecium* was observed to be present predominantly. These ciliate species were observed to be present in all the seasons and in all three freshwater sites but predominantly noticed to be present in September 2018 and July 2019 which are the post monsoon and monsoon seasons, respectively. The high abundance of *Paramecium* spp. in September 2018 was similar to previously reported study where *Paramecium* spp. was found to be abundantly present during September (Dhib et al. 2013). It has been observed that during these two months (July and September), temperature, pH and electrical conductivity are desirable in all the three sites. These factors could have encouraged the growth of *Tetmemena* spp., *Gonostomum strenua* and *Paramecium* spp.

Loxodes sp. belonging to class Karyorelictea was found only in the OBS site predominantly in January 2019. This could be because of low DO concentration at the OBS site (2–6 mg/L) as compared to the other two sites (2–7 mg/L in SL and 5–7 mg/L in RJ). *Loxodes* sp. belonged to a community called “Metopetum” (Foissner and Berger 1996). The community “Metopetum” contains anaerobic organisms which do not have mitochondria, thus, indicating that *Loxodes* sp. prefers low oxygen content for its survival (Czapik 1982; Dias et al. 2008). Since, OBS site had low DO concentration as compared to the other two sites, this may have favoured the growth of *Loxodes* sp.

Coleps sp. of class Prostomatea and *Colpoda* sp. of class Colpodea were observed to be present predominantly in all the three sites in January 2019 (winter). The survival of *Colpoda* sp. in adverse condition (very low temperature and high pH) could be due to their ability to form cyst very frequently (Kaur et al. 2021; Bhamare et al. 2012; Funadani et al. 2013, 2016). Similarly, the survival of *Coleps* sp. in extreme conditions could be due to the presence of the calcium carbonate containing alveolar plates assembled to form an armor on its body (Buonanno et al. 2014; Lemloh et al. 2013). *Coleps* sp. and *Colpoda* sp. were observed to be more abundant in SL and RJ site as compared to the OBS site, suggesting their ability to survive with less favourable conditions.

Chilodonella sp. of class Phyllopharyngea was observed to be present predominantly in all the three freshwater sites in May 2019 (summer). It has been reported that *Chilodonella* sp. exhibits a wide temperature tolerance and can survive well in warmer climates (Gomes et al. 2016). *Chilodonella* sp. exists as parasitic and free-living ciliates thus, showing high tolerance towards warmer climates (Gomes et al. 2016).

Conclusion

Three-dimensional approach in the present study, offers an account of the spatial and temporal variations in physicochemical gradients and their correlation with the ciliate diversity, richness and evenness. This study indicates the inter-relationship of desirable conditions for increased species diversity, richness and abundance as temperature (22–29 °C), neutral pH (7–7.5), dissolved oxygen level more than 2 mg/L, lower levels of total dissolved solids, water hardness, chloride, and sulphate concentration and, increased levels of phosphate, nitrite, nitrate and ammonia levels. These conditions support the growth and enrichment of the ciliates as also reported in previous studies (Abdullah et al. 2018; Vajravelu et al. 2018; Xu et al. 2014). The physicochemical conditions at OBS site favour ciliate growth conditions, hence maximum diversity was observed at this site. The high value of diversity indices (species diversity, richness and evenness) at the OBS site indicates presence of desirable conditions for ciliate growth as compared to SL and RJ site. Ciliate diversity, richness and evenness were maximum during the monsoon and post-monsoon season, whereas diversity and richness were lowest during summer and winter seasons. The present study on the dynamics of the environment driven community assembly suggests that ciliates are good indicators for assessing community-level responses and can be useful in predicting the water quality as well as environmental degradation in the future.

Declarations

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Author contribution

S Makhija, R Toteja and R Gupta designed and supervised the research study. JS Abraham, S Somasundaram, and S Maurya collected samples, performed experiments and analyzed the data. JS Abraham wrote the original draft. S Makhija, R Toteja and R Gupta revised and improved the manuscript.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

All authors read and approved the final version of the paper to be published.

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Figures

Figure 1

GPS location of sampling sites, Okhla Bird Sanctuary, Sanjay Lake and Raj Ghat.

Figure 2

Variation in the physicochemical parameters of the three sites, Okhla Bird Sanctuary (OBS), Sanjay Lake (SL) and Raj Ghat Pond (RJ), Delhi during the one year study period. Blue stars represent parameters that showed significant difference among the three sites, black stars represent parameters that showed significant difference among different months, and remaining parameters did not show significant difference among sites or months.

Figure 3

Principal component analysis (PCA) based on the physicochemical parameters examined during one year from three freshwater sites. TIW = temperature (inside water), DO = dissolved oxygen, TDS = total dissolved solids, EC = electrical conductivity.

Figure 4

Species diversity represented by Shannon Index and Simpson index (A and B), species richness represented by Margalef's index (C) and species evenness represented by Pielou's index (D). Blue stars represent indices that varied significantly among the three sites. OBS = Okhla Bird Sanctuary, SL = Sanjay Lake, RJ = Raj Ghat Pond.

Figure 5

Nonmetric Multidimensional scaling (NMDS) showing the placement of different seasons analysed during the study period based on physicochemical parameters and ciliate diversity indices.

Figure 6

Nonmetric Multidimensional analysis (NMDS) showing the placement of different sites analysed during the study period based on physicochemical and ciliate diversity data. OBS = Okhla Bird Sanctuary, SL = Sanjay Lake, RJ = Raj Ghat Pond.

Figure 7
Relative abundance (RA) (%) of ciliates observed in Okhla Bird Sanctuary, Delhi during the study period. Size and colour of the circles represent %RA.

Figure 8
Relative abundance (RA) (%) of ciliates observed in Sanjay Lake, Delhi during the study period. Size and colour of the circles represent %RA.

Figure 9

Relative abundance (RA) (%) of ciliates observed in Raj Ghat Pond, Delhi during the study period. Size and colour of the circles represent %RA.

Figure 10

Pie chart depicting relative abundance (%) of different ciliate classes observed during the study period in three sites. OBS = Okhla Bird Sanctuary, SL = Sanjay Lake, RJ = Raj Ghat Pond.

Figure 11

Variation in total species number (S) and abundance (N) recorded from three sites, Okhla Bird Sanctuary (OBS), Sanjay Lake (SL), and Raj Ghat Pond (RJ) during the study period.