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SHORT COMMUNICATION

Algal bloom, hypoxia, and mass fish kill events in the backwaters of Puducherry, Southeast coast of India

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Abstract The Chunnambar backwater of Puducherry experienced changes in water quality over a period. The most significant impact was the sudden mass fish kill event coincided with the *Pseudo-nitzschia* bloom. On 25th September 2019, a mass fish kill event was reported, i.e. about a 0.25 metric ton (MT) floating on the water surface. On 29th September 2019, a much larger (~1 MT) than the earlier incident had occurred. Sampling was carried out to assess the causes thereof. The results indicate that high organic matter and bacterial loads accumulated in the water and sediment due to the closure of the river mouth for an extended period. High ammonia (61.4 μM) and phosphate (6.2 μM) levels attributed to eutrophic conditions in the water column and hypoxemia due to low dissolved oxygen (1.62 mg/L at St.1 and 2.4 mg/L at St.5) and algal bloom were the proximate cause of these sudden fish kills. We recommend periodic dredging to facilitate proper water exchange between the backwater and the sea.

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The mass fish kill in the rivers, lakes, estuaries, and coastal waters are (Hoyer et al., 2009; La and Cooke, 2011), usually triggered by abrupt changes in environmental parameters, i.e., salinity, temperature, pH, and dissolved oxygen (DO), and the introduction of chemicals, toxicants or pollutants, toxic algal blooms and pathogens (Eissa et al., 2013; Meyer and Barclay, 1990). These changes can hinder coastal and marine environments and influence the local economy, i.e., loss of tourism, fisheries, aesthetic value, and negatively impact the food web dynamics and nutrient balance (Holmlund and Hammer, 1999). In many cases, the primary reasons attributed to mass fish kill are natural and anthropogenic hypoxia (Ram et al., 2014). The other important factor that triggers fish kill is toxic algal blooms (Kangur et al., 2005). Certain species of diatom like the genus *Pseudo-nitzschia* produce neurotoxin domoic acid (DA) is responsible for the neurological disorder known as amnesic shellfish poisoning (ASP) (Pulido, 2008), can affect fish and other organisms (Galvao et al., 2009). Excess nutrient input is a primary concern in many coastal areas (Carpenter et al., 1998; Rabalais et al., 2002) and induce the growth of phytoplankton, some harmful algal blooms, high organic matter, hypoxic or anoxic conditions, and mass mortality of biota (Anderson et al., 2008; Glasgow and Burkholder, 2000). Various developmental activities introduce a significant amount of organic matter and nutrients, typically untreated or partially treated sewage released into the rivers and estuaries, accelerate hypoxia and the eutrophication processes (Villate et al., 2013). Coastal hypoxia (DO $<$ 2.0 mg/L, or approximately 30% saturation) is a major threat to coastal ecosystems globally (Ram et al., 2014; Zhang et al., 2010). Mortality occurs if oxygen levels drop below a critical concentration depending on the fish species. Fishes in the water bodies likely evade adverse environmental conditions to overcome the harmful surroundings. However, if a large proportion of the water bodies are suddenly affected, fishes cannot absorb the ecological stress and relocate, resulting in mass mortality. In the past, mass fish kill events in the Indian coastal waters have been reported, i.e., Tapti Estuary of Gujarat (Ram et al., 2014), Kokilamedu, Ennore, and Adyar estuary of Tamil Nadu (Raja et al., 2019; Sachithanandam et al., 2017; Venugopalan et al.,1998).

Puducherry, a union territory (UT) coastal city, is one of India's most visited tourist destinations, surrounded by the Bay of Bengal on the eastern side and Tamil Nadu state on the other three sides. A backwater known locally as Chunnambar lies in the southern part of the city adjacent to the popular Paradise Beach, attracts many tourists for recreational activities, i.e., swimming, sailing, boating, sunbathing, and wading. The city has a population of around 735,000 (as per the 2011 census). There are six major industries in the UT of Puducherry manufacturing paper, alcoholic beverages, chemicals, and pharmaceuticals. Around 40 million liters per day (MLD) of domestic sewage is being generated, of which 31 MLD is treated in situ and the remaining 9 MLD is lost into the drains and rivers (Govt. of Puducherry, 2019). The region experiences a tropical sub-humid climate with an annual mean temperature of 25°C and average yearly precipitation of 1200 mm (Solai et al., 2010).

On 25th September 2019, the Puducherry Pollution Control Committee (PPCC) was informed by the locals about the incident of a large number of dead fishes floating near the boathouse (St.1) of Chunnambar backwater (Figure 1). To assess the immediate cause of the fish kill incident, PPCC carried out a field survey and surface water sampling at four locations (St.1 to St.4) on 26th September 2019 (Figure 1). On 29th September 2019, another fish kill incident larger than the previous one occurred at a different location in the backwater at St.5 (Figure 1). On 1st October 2019, National Centre for Coastal Research (NCCR), Chennai, took cognizance of the incident and sampled water and sediment from 6 locations between 9 AM and 1 PM. The sampling locations are spatially distributed i.e., St.1 to St.5 in the Chunnambar backwater, and St.6 at the shore, covering about 1.8 km^2 of backwater area (Figure 1). Surface water samples were collected in pre-cleaned polyethylene bottles using a 5L Niskin's water sampler, then preserved in an icebox and transported to the laboratory for analysis. A mercury thermometer measured the temperature with an accuracy of $\pm$ 0.1°C. The water quality probe (*In Situ* AQUA TROLL, USA) measured pH and oxygen saturation. The dissolved oxygen and biochemical oxygen demand (BOD) were estimated following Winkler's titration method. Salinity was determined by Mohr Knudsen's titration method (Grasshoff et al., 2009). Millipore membrane, 47 mm diameter filter (porosity 0.45 μm) was used for gravimetric determination of total suspended matter (TSM). Dissolved inorganic nutrients (NO_3^--N, NO_2^--N, NH_4^+-N, PO_4^{3-}-P, SiO_4^{4-}-Si, TN, and TP) and the analytical precision of each nutrient parameters such as (nitrate+nitrite $\pm$ 0.02 µM, ammonium $\pm$ 0.02 µM, phosphate $\pm$ 0.01 µM and silicate $\pm$ 0.02 µM) was adopted by following the standard protocol (Grasshoff et al., 2009). One liter of water sample for Chl-*a* and pheophytin were filtered through Whatman GF/F, 47-mm-diameter filter (porosity 0.7 mm), and pigment extraction was performed using 90% acetone. Chlorophyll-*a* (Chl-*a*) and pheophytin concentrations were measured by using a UV-visible spectrophotometer (Strickland and Parsons, 1972). One liter of phytoplankton sample was collected and preserved with Lugol's iodine solution. The samples were settled for 48 hours after that, the supernatant was siphoned to the volume of approximately 250 ml. 1 ml of an aliquot of the sample was taken in a Sedgewick-Rafter counting chamber for enumeration of cells under a phase-contrast microscope (Nikon ECLIPSE N*i*), and species were identified following standard identification manuals (Desikachary, 1987; Sournia, 1970; Subrahmanyan, 1971). The microbial density from the surface waters was analyzed by the spread plating method on selective medium plates with 0.1ml of suitable dilutions. The final counts were expressed in the colony-forming unit (CFU/mL) followed by APHA (1998). Statistical analyses included Factor Analysis (FA) and Canonical Correspondence Analysis (CCA) on the present water quality data to understand the relationships between variables. FA and CCA were carried out by using *Past 3* statistical software.

The spatial variations of physicochemical and biological parameters are shown in Table 1. Automatic weather station (AWS) data on air temperature, rainfall, wind speed, and direction for the period 20th to 30th September 2019 provided by PPCC were analysed to understand local meteorological influence. The wind speed was in the range of 0.5 to 4 m/s with an average of 2.2 m/s, and wind direction varied from 200° to 250°, predominantly south-westerly. About

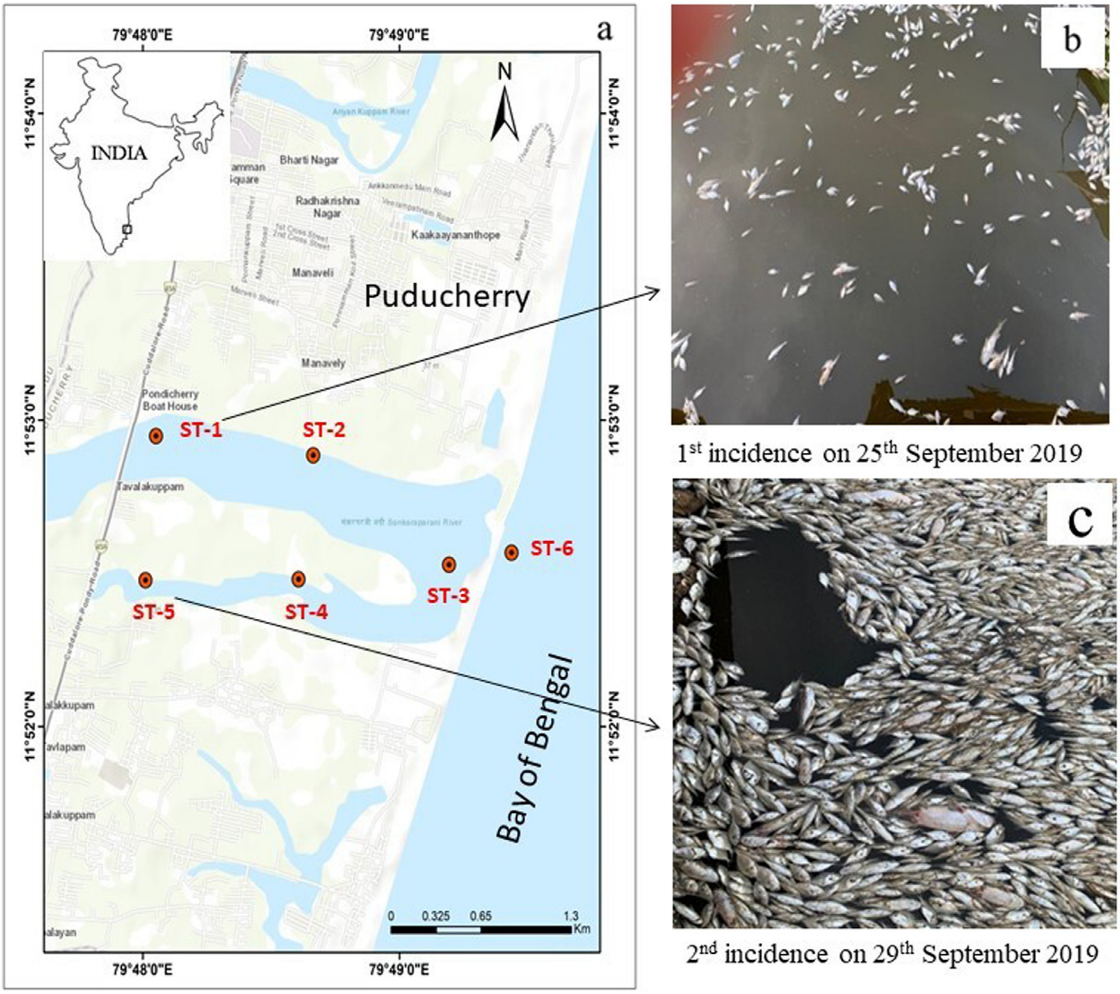

Figure 1 a) Study map with sampling locations; b– c) Fish kill incidents.

15 mm rainfall was recorded on 26th September 2019. The daily air temperature followed high and low values consistent with the diurnal variations, between 28 to 32°C except for two days (22nd and 23rd September 2019) with the minimum value of more than 30°C. Water temperature varied from 28.6 to 32.9°C. Maximum temperature was recorded at St.5 during second fish kill incident (Table 1). The pH ranged from 6.9 to 8.4; the lowest and highest pH was noticed at St.1 on 26th September and 1st October 2019, respectively (Table 1). Salinity ranged from 26 to 34.9 PSU, and the highest salinity was observed at St.6 (shore). Total suspended matter (TSM) varied from 42 to 201.2 mg/L; high values were observed during the first fish kill incident. The DO ranged from 1.62 to 10.5 mg/L were recorded during the study period. Low DO (1.62 mg/L) was recorded at St.1 in the first fish kill incident whereas, DO was 2.4 mg/L at St.5 in the second incident (Table 1). The maximum DO (10.5 mg/L) was recorded at St.4 might be due to high oxygen saturation (177.1%) in the water column (Table 1). Nitrite concentration ranged from 0.2 to 0.5 μM, with an average of 0.33 μM. Nitrate and ammonia ranged from 1.2 to 4.7 μM and 2.5 to 61.4 μM, respectively. The maximum value was recorded at the mass fish kill location (St.5, Table 1). High nitrate and ammonia values revealed the degradation and decomposition of fishes after the fish kill incident. Similarly, phosphate values varied from 1.2 to 6.2 μM, and the maximum was observed at St.5 (Table 1). High phosphate characterizes the decomposition process, and the adjacent location at St.4 (4.35 μM) signifies the impact of the incident. Silicate varied from 4.9 to 148.2 μM, and the maximum value was recorded at St.5 (Table 1), indicating the riverine input of silica into the water.

During the sampling period, a prominent discolouration of the surface water was noticed, attributed to the bloom of *Pseudo-nitzschia,* resulting in long, dense golden-brown patches (Figure 2a,b,c) spreading over about 1 km^2 in the backwater. Phytoplankton density ranged from 94,800 Nos/L to 589,600 Nos/L (Table 1). A total of 31 species of phytoplankton belonging to four groups, such as diatoms, dinoflagellates, green algae, and cyanobacteria, were recorded. Diatoms (67%) constituted the most dominant group, followed by dinoflagellates (17%), green algae (11%), and cyanobacteria (5%). Eutrophic waters consequently favor algae proliferation, causing algal bloom and the onset of hypoxic and anoxic conditions (Karim et al., 2002). The bloom of *Pseudo-nitzschia* contributed 75% of the total abundance and was widely distributed in all locations in the backwater except at St.6 shore water (Table 2).

Table 1 Physicochemical and biological parameters in the study area.

Parameters	Backwater (BW)									Shore (SH)
	St.1		St.2		St.3		St.4		St.5	St.6
	I	II	I	II	I	II	I	II	II	II
Depth (m)	-	1.4	-	2.6	-	1.3	-	2.5	1.2	-
AT (°C)	28	29.5	28	32	29	30.6	28.5	31.2	30.6	30
WT (°C)	29	28.6	29.5	30.8	31	29.7	30.5	31.7	32.9	28.8
pH	6.9	8.4	7.1	8.2	7.7	8.2	7.8	8.2	7.6	8.0
Salinity (PSU)	29	28.7	32	28.8	28	28.9	26	29.2	29.5	34.9
TSM (mg/L)	164.7	50	164.3	54.0	201.2	50.0	167.7	42.0	52.0	42.0
DO (mg/L)	1.62	8.3	2.91	6.2	3.90	9.8	4.11	10.5	2.4	6.2
Oxygen Sat %	-	149.4	-	98.7	-	153.3	-	177.1	39.3	99.1
BOD (mg/L)	-	3.7	-	2.9	-	3.5	-	4.5	2.2	3.0
NO_2 (μM)	-	0.3	-	0.2	-	0.3	-	0.4	0.3	0.3
NO_3 (μM)	-	4.4	-	2.9	-	1.2	-	1.9	4.7	4.1
NH_4 (μM)	-	6.8	-	8.2	-	4.9	-	2.5	61.4	2.8
PO_4 (μM)	-	2.9	-	2.4	-	2.8	-	4.3	6.2	1.2
SiO_4 (μM)	73.6	9.3	80.2	73.8	66.4	90.8	62.0	113.1	148.2	4.9
Chl-*a* (mg/m^3)	-	188.8	-	210.9	-	203.0	-	98.9	43.2	34.7
Phaeo (mg/m^3)	-	46.9	-	15.6	-	10.7	-	11.8	1.4	4.1
Phytoplankton (x 10^5 Nos/L)	-	3.82	-	5.90	-	4.85	-	3.33	4.57	0.95
THB (×10^5CFU/mL)	-	3.44	-	5.28	-	8.54	-	4.6	4.36	3.96
TC (×10^3CFU/mL)	-	9.5	-	1.3	-	15.6	-	6.1	3.1	9.2
FC (×10^1 CFU/mL)	-	5.0	-	ND	-	2.0	-	13.0	ND	9.0
ECLO (×10^2 CFU/mL)	-	9.0	-	ND	-	2.0	-	ND	14.0	3.0
SHLO (×10^4 CFU/mL)	-	1.08	-	2.04	-	1.24	-	3.32	1.38	2.0
VPLO (×10^4 CFU/mL)	-	1.12	-	0.38	-	6.4	-	1.56	9.3	1.92

I — Fish kill incident on 25.09.2019; Sampling date: 26.09.2019.
II — Fish kill incident on 29.09.2019; Sampling date: 01.10.2019.

Some species of phytoplankton had occurred commonly in both shore and backwater. The common species such as diatoms *Rhizosolenia setigera, Navicula longa,* and green algae *Chlorella marina* were recorded. A high density of *Pseudo-nitzschia* cells were found in the backwater and the gills of the dead fishes. The presence of many cells might have suffocated the fish by clogging or irritating the gills leading to asphyxiation. Samples of six fish kill species were collected and identified (Figure 3a—f). DO levels were below 3 mg/L during fish kill events, and low DO (<2 mg/L) can cause suffocation of fish and one of the common causes of fish kills. The decay of algae exhausted the oxygen, and the decomposition process depleted the available DO result in massive fish kill (San Diego-McGlone et al., 2008). The Chl-*a* ranged from 34.7 to 210.9 mg/m^3, and the highest was recorded at St.2 in concomitant with high phytoplankton population density (Table 1). The values of pheophytin in the study area varied between 1.4 and 46.9 mg/m^3, and the highest pheophytin concentration was recorded at St.1 (Table 1).

High microbial abundance recorded in the backwater was attributed to the enrichment of organic matter associated with sewage effluent. Total heterotrophic bacteria (THB) ranged from 3.44 to 8.54 $\times 10^5$ CFU/mL, and the maximum density was encountered at St.3. Total coliform (TC) and Faecal coliform (FC) varied from 1.30 to 15.60 $\times 10^3$ CFU/mL and ND (not detected) to 13.0 $\times 10^1$ CFU/mL, respectively. The pathogenic bacteria such as *Vibrio parahaemolyticus* like organisms (VPLO) varied from 0.38 to 9.30 $\times 10^4$ CFU/mL, *Shigella* like organisms (SHLO) from 1.08 to 3.32 $\times 10^4$ CFU/mL and, *Escherichia coli* like organisms (ECLO) from ND to 14.0 $\times 10^2$ CFU/mL, respectively. Maximum counts of VPLO and ECLO were found at St.5 (Table 1). In the present case, oxygen depletion in the backwater might have occurred due to the increasing uptake of available dissolved oxygen by bacteria during the breaking down of high organic matter.

The factor analysis was carried out to elucidate the pattern of variation existing in the dataset. Four significant factors (eigenvalue>1) indicate the total variance of 85.14% (Table 3). Factor 1 explains 39.52% of the total variance and strong positive loadings of PO_4, NH_4, SiO_4, and TN moderate positive loading of water temperature. High phosphate and nitrogenous substances specify the sewage pollution re-

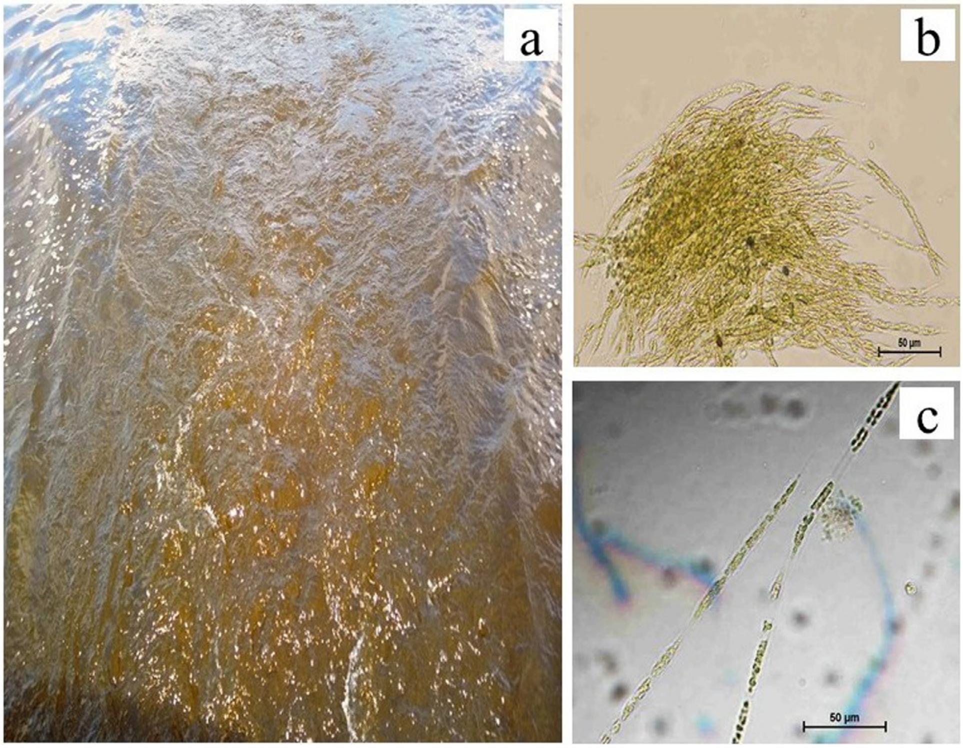

Figure 2 a) Discoloration of backwater; b—c) microscopic images of *Pseudo-nitzschia*.

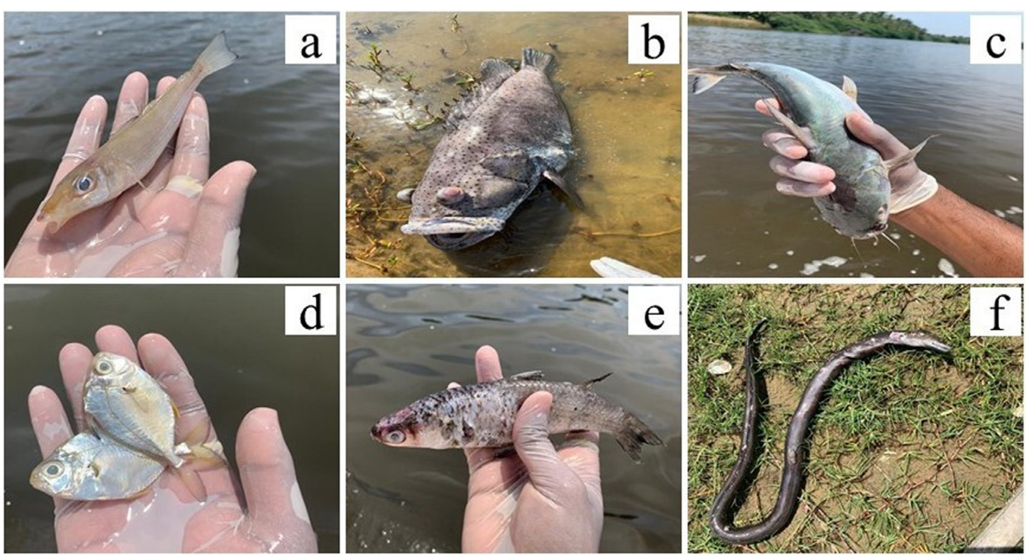

Figure 3 a—f) Six fish species killed; a) *Silago sihama*, b) *Cephalopholis sonerrati*, c) *Arius arius*, d*) Leiognathus equidens*, e) *Mugil cephalus* and, f) *Strophiodonsathate*.

sulting from the untreated sewage drained into the backwater. Factor 2 explains the 25.71% of the total variance with strong positive loading of NH_4, and NO_3, whereas negative loading of DO%, DO, BOD, and pH. Depletion of DO and increase of BOD indicate the eutrophic condition in the water column due to mostly land and agricultural runoff, domestic and urban sewage. Factor 3 explained 19.91% of the total variance and detected strong positive loading of phytoplankton, Chl-*a,* and TP. In contrast, a negative salinity load indicates the presence of excess nutrients, and favorable water temperature supports the phytoplankton growth in the backwater. The tri-plot of CCA was performed to understand the relationship between the phytoplankton and environmental variables are presented in Figure 4. The first three axes explained about 99% of the relationship between the environmental variables. Axis-1 accounted for 75.85% of the total variation, was positively correlated with salinity and pheophytin but negatively correlated with WT, NO_2, SiO_4, and phytoplankton. Axis-2 explained about 16.43% of the total variation, and it was positively correlated with

Table 2 Phytoplankton taxa (Nos/ml) in the Chunnambar backwater.

Sl.No.	Taxa	Backwater (BW)					Shore (SH)	% Comp.	
		St.1	St.2	St.3	St.4	St.5	St.6	BW	SH
1	*Asterionellopsis glacialis*	X	X	X	X	X	56	0.0	11.8
2	*Bacteriastrum hyalinum*	X	X	X	X	25	X	0.4	0.0
3	*Ceratium furca*	X	X	32	29	42	X	1.8	0.0
4	*Ceratium tripos*	X	X	X	46	X	X	0.8	0.0
5	*Chaetoceros curvisetus*	X	56	X	X	X	X	1.0	0.0
6	*Chaetoceros diadema*	X	X	X	64	X	X	1.1	0.0
7	*Chlamydomonas* sp.	X	X	34	X	X	X	0.6	0.0
8	*Chlorella marina*	X	X	X	X	78	34	1.3	7.2
9	*Chlorella salina*	30	84	X	X	X	X	1.9	0.0
10	*Coscinodiscus centralis*	X	X	X	X	X	72	0.0	15.2
11	*Dinophysis caudata*	X	X	X	X	X	28	0.0	5.9
12	*Gonyaulax minima*	29	X	X	X	56	X	1.4	0.0
13	*Guinardia striata*	X	X	X	X	X	24	0.0	5.1
14	*Leptocylindrus danicus*	X	X	X	31	X	X	0.5	0.0
15	*Lyngbya elegans*	X	X	X	35	X	X	0.6	0.0
16	*Navicula clavata*	X	X	X	X	X	19	0.0	4.0
17	*Navicula longa*	24	X	X	X	X	38	0.4	8.0
18	*Odontella mobiliensis*	X	X	42	X	X	X	0.7	0.0
19	*Oocystis* sp.	X	36	45	X	X	X	1.4	0.0
20	*Oscillatoria australis*	28	X	X	X	X	X	0.5	0.0
21	*Peridinium pentagonum*	X	X	18	X	X	X	0.3	0.0
22	*Pinnularia alpina*	X	X	X	X	X	26	0.0	5.5
23	*Planktoniella sol*	X	X	23	X	X	X	0.4	0.0
24	*Prorocentrum micans*	X	X	X	20	X	X	0.3	0.0
25	*Pseudo-nitzschia*	729	1026	956	572	1135	X	**75.1**	0.0
26	*Rhizosolenia alata*	X	X	X	X	X	43	0.0	9.1
27	*Rhizosolenia setigera*	57	106	75	128	92	76	7.8	16.0
28	*Skeletonema costatum*	X	X	X	X	X	58	0.0	12.2
29	*Thalassionema nitzschioides*	12	X	X	X	X	X	0.2	0.0
30	*Thalassiothrix longissima*	X	X	52	X	X	X	0.9	0.0
31	*Triceratium reticulum*	X	32	X	X	X	X	0.5	0.0
	Total taxa	**909**	**1340**	**1277**	**925**	**1428**	**474**	**100.0**	**100.0**

NO_3, NH_4, PO_4, turbidity, and TSM and negatively correlated with pH, DO, DO%, BOD, and Chl-*a*. The study revealed that the anthropogenic nutrients and salinity were the most important environmental factors influencing the phytoplankton bloom and hypoxic conditions in the water column resulted in the mass fish kill.

Usually, fish kills incidents are a major cause of concern for the coastal water quality and ecosystem health. Our study demonstrates the impact of untreated sewage load in the backwater declined the water quality as THB counts were overall high in the water column. Pollution indicators such as FC and TC were several folds above the permissi-

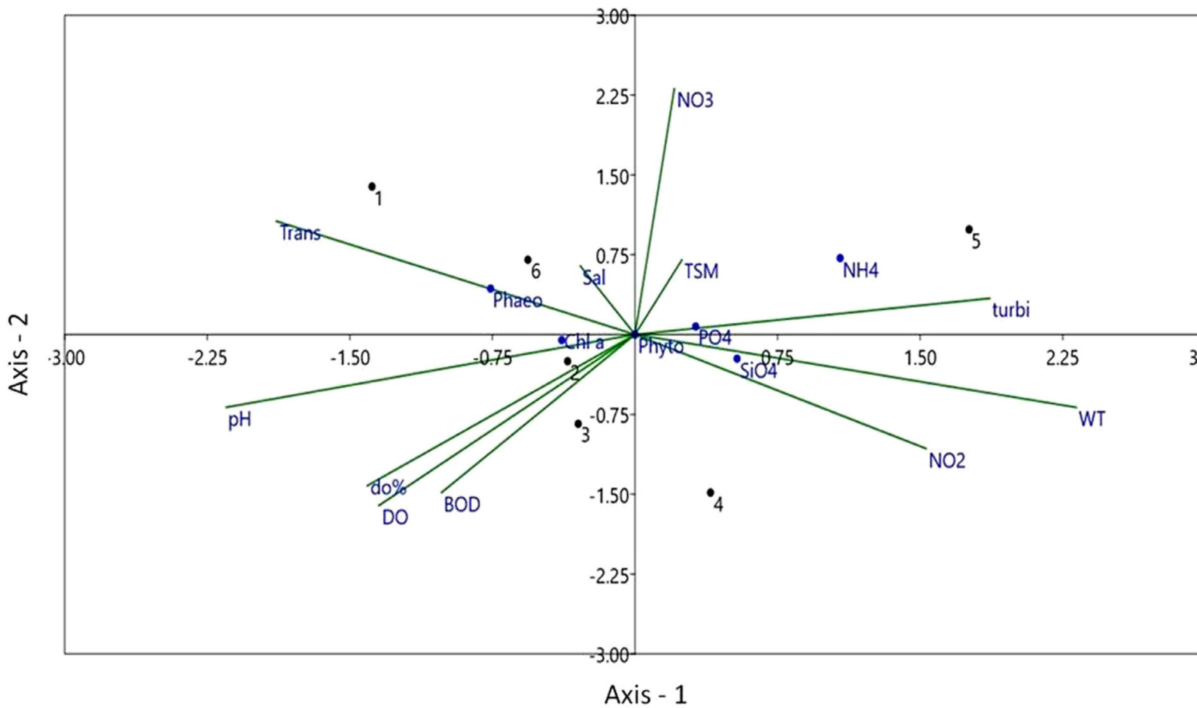

Figure 4 Canonical Correspondence Analysis (CCA) for environmental variables.

Table 3 Factor Loadings > 0.650000 (Varimax normalized). Extraction: Principal Component Analysis.

Variables	F-1	F-2	F-3
AT	0.08	0.03	0.3
WT	**0.79**	0.31	0.02
pH	−0.49	**−0.75**	0.41
DO%	−0.1	**−0.99**	0.11
salinity	−0.48	0.22	**−0.84**
DO	0.01	**−0.85**	−0.21
BOD	0.04	**−0.98**	−0.01
PO_4	**0.96**	0.23	0.09
NO_2	**0.65**	−0.36	−0.2
NO_3	−0.05	**0.69**	−0.17
SiO_4	**0.84**	0.16	0.12
NH_4	**0.88**	**0.75**	0.01
TP	0.21	0.51	**0.73**
TN	**0.83**	−0.08	0.45
Chl-*a*	−0.3	−0.36	**0.87**
Phaeo	−0.23	−0.35	0.55
Phyto	0.27	0.14	**0.89**
Eigenvalue	7.11	4.63	3.58
% of Variance	39.52	25.71	19.91
Cumulative %	39.52	65.23	85.14

ble level [100 CFU/100ml, Central Pollution Control Board (CPCB), New Delhi], and the variability of pathogenic and vibrio forms was more pronounced during the study. There is a dire requirement to develop a framework for safe backwater use for amusement, contact sports, and fishing. The intrusion of a huge load of nutrients, *viz.*, nitrate, ammonia, and phosphate levels, were associated with sewage discharge and other anthropogenic and recreational activities in the backwater. High nitrate, phosphate, ammonia, and silicate were recorded at the location of the major fish kill. In addition to this, the algal bloom of *Pseudo-nitzschia*, high bacterial loads, and the hypoxic conditions (1.62 mg/L) attributed to the episodic mass fish kill events in the Chunnambar backwater.

The present study demonstrates that the discharges from nearby residential hamlets, tourism activities, untreated point sources such as Sankaraparani River waters must be maintained as per CPCB water quality criteria and guidelines. Even though several measures have been implemented by the local administration and pollution control agencies, it is still necessary to focus on long-term solutions. It is suggested that appropriate water exchange between the backwater and the sea through periodic dredging and maintenance of the inlet, proper wastewater treatment facilities, and installation of good aeration systems are some of the immediate measures that need to be implemented for improving the Chunnambar backwater quality. The regulatory authorities need to regularly monitor the water bodies to assess the changes happening in the water bodies to prevent pollution.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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