

Phytoplankton communities in Pinang River, Malaysia: Exploratory study in the profoundly polluted river

Azma Hanim Ismail*, Nurul Atikah Shahul Hamid

School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia

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Abstract: Phytoplankton is an important biotic component that interacts with other communities in an aquatic ecosystem. This study explored the zooplankton communities and their interactions among various water quality parameters that may affect their abundance and distribution in Pinang River. Pinang river is known to the locals as the dirtiest river in Penang due to its role in the assimilation of industrial, municipal wastewater and manure discharge. Phytoplankton and water samples were collected from three different sampling locations in the river. A total of 81 phytoplankton taxa belonging to seven phyla were identified. Bacillariophyta was the most dominant phylum of the total phytoplankton communities (43 taxa), followed by Chlorophyta (23 taxa), while Cyanophyta, Euglenophyta, and Chrysophyta were represented by 8, 3 and 2 taxa, respectively. The dominant species of Bacillariophyta were *Nitzschia* sp., *Navicula* sp. and *Pinnularia* sp. which were recorded as the highly abundant species found at all stations. Our results clearly showed that there was a strong, positive correlation between ammonium-nitrogen ($\text{NH}_4^+\text{-N}$) concentration and phytoplankton abundance ($p < 0.01$), indicating the importance of ammonia to phytoplankton growth.

Keywords: Bacillariophyta, Chlorophyta, Phytoplankton, Pinang River

1 Introduction

Water bodies such as rivers and lakes provide drinking water supply and aid irrigation. Many organisms live in those water bodies that function critically according to their roles. Phytoplankton or microalgae are one of the organisms that are vital as they become a major primary producer in the aquatic ecosystem. Phytoplankton undergo photosynthesis by converting the light energy into chemical energy using chlorophyll. They are phototrophic

*e-mail: azmahanim@usm.my

organisms and make their own food from inorganic materials present in their environment and convert them to organic matter and make it available for the ingestion of other organisms.

Phytoplankton is divided into 6 main divisions which are Bacillariophyta (diatoms), Cyanophyta (blue-green algae), Euglenophyta (euglenoid), Chlorophyta (green algae), Pyrrophyta (dinoflagellates) and Chrysophyta whereby diatoms is the dominant group because they usually present in high abundance in an aquatic ecosystem (Onyema, 2013). Diatoms, dinoflagellates and cyanobacteria are some of the important and common groups of phytoplankton that usually inhabit the water column.

The key element of phytoplankton is that it can respond rapidly to any environmental changes, and its fast reproduction rate makes it a valuable indicator of water quality (Thakur et al., 2013). Their characteristics of being abundant over a wide area and sensitive to changes in water quality, planktonic microalgae fulfil the requirement of being a good indicator (Onyema, 2013). The assessments of water quality using biological indicators are extensively developing because they could provide information on the surrounding physical and chemical environment through their availability and frequency of abundance sites (Singh et al., 2013).

According to Poniewozik and Lenard (2022), phytoplankton assemblage is one of the elements used to assess the ecological status of the water body (Poniewozik and Lenard, 2022). Therefore, studies on phytoplankton species abundance and distribution would provide the benchmarks of impacted water bodies. The present study is aimed to identify the abundance and species distribution of phytoplankton and the factors affecting their abundance and distribution. This study is vital to provide information on how water bodies at particular sites deteriorate if any.

2 Materials and Methods

2.1. Study area

The Pinang River is a meandering river located northeast of Penang Island (5°24'N 100°19'E) (Figure 1). It consists of six major tributaries, which are Air Terjun River, Kecil River, Air Hitam River, Air Putih River, Dondang River and Jelutong River. The total area of the river basin is approximately 50.97 km² and meanders eastward is estimated at 3.1 km (Naemah & Norulaini, 2006). The locals know the Pinang River as the dirtiest river in Penang and one of the most polluted rivers in Malaysia. The Pinang River flows through an immensely developed and populated area of George Town which is the capital city of Penang. This river has been categorized as Class IV (very polluted) due to its polluted water quality in accordance proposed Interim National Water Quality Standards for Malaysia (Naemah & Norulaini, 2006).

2.2. Methodology

The collection of water samples was conducted monthly for consecutive months, starting from October 2018 to December 2018. The three replicates of water samples were collected at each station using 5 litres (L) Van Dorn Sampler, preferable as it is more reliable, easy to trigger and can be used at various water depths (Howmiller & Sloey, 1969). The feature of the Van Dorn Sampler, which is made of acrylic, allows the user to see the sample making it more

suitable and convenient to be used. 40 L water was collected 0.5 m below the surface using a Van Dorn Sampler and then filtered with a Wisconsin plankton net of 35 μm mesh size. The filtrate was then transferred into a 60 ml polyethylene bottle. The filtrate was preserved with a few drops of Lugol Iodine Solution (Lugol's) immediately after obtaining the water samples. Lugol is used to maintain the quality of the sample other than to minimize the cell from deteriorating and having morphological changes (Williams et al., 2016).

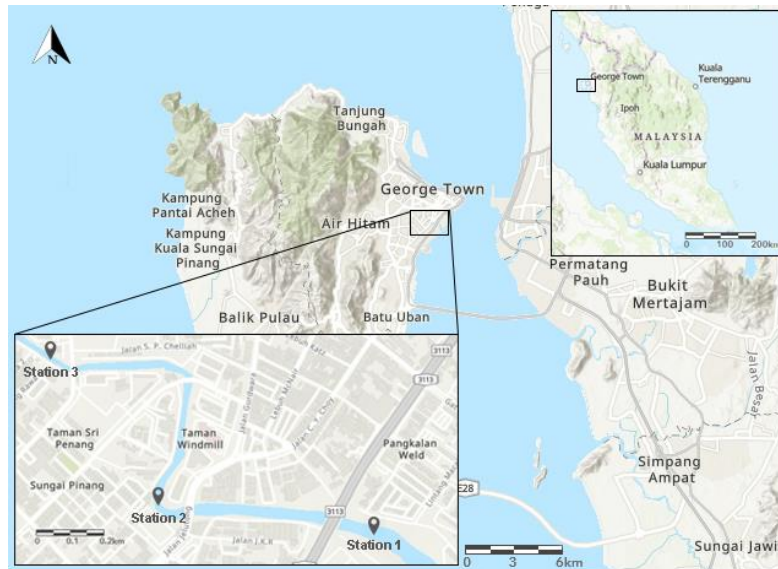


Figure 1. Map of Pinang River with the sampling stations

Measurements for water quality parameters, including water temperature (WT), conductivity, total dissolved solids (TDS), and dissolved oxygen (DO), were conducted *in-situ* with a multi-parameter YSI 556 Multi Probe System. pH was measured using the HACH sensION1 Portable pH meter, while turbidity was measured using the Vernier Turbidity Sensor (TRB-BTW) after calibrating the sensor using the 100 NTU StabCal® Formazin Standard. Water transparency was determined using a Secchi disk. Other parameters, including chlorophyll-a, total suspended solids (TSS), ammonium-nitrogen ($\text{NH}_4^+\text{-N}$), nitrite-nitrogen ($\text{NO}_2\text{-N}$) and orthophosphates ($\text{PO}_4^{3-}\text{-P}$), were analyzed in the laboratory following the methods from Adams (1990) (Adams, 1990).

The Sedgwick-Rafter (S-R) counting cell was used to count the cell number (Woelkerling et al., 1976). Next, the sample bottle was shaken gently to avoid bias and to make the phytoplankton sample in suspension. By using a micropipette, 1 mL of the sample was dispensed on the Sedgwick-Rafter counting cell and then covered with a cover slip gently to ensure that no air bubble was trapped in the slide.

A compound microscope model Olympus BX41 was used to observe the phytoplankton with various magnifications. The camera model used was Xcam Alpha, and the software for this microscope was AnalySIS Image Processing version 5.1. At random, 30 squares of S-R counting cell were chosen to count the number of the cells present. The S-R counting cell has an area of 1000 mm^2 and occupies a volume of 1 mL. The grids were examined using the compound microscope equipped with a camera attached to capture images. The phytoplankton

specimens were identified using the relevant taxonomic literature (Baker & Fabbro, 2002; Janse van Vuuren et al., 2006; Bellinger & Sigee, 2010) while the abundance was calculated using the Lobban et al. (1988) formula (Lobban et al., 1988).

A repeated measures two-way analysis of variance (ANOVA) was performed to distinguish the significant differences in physicochemical parameters and phytoplankton abundance among the sampling stations and monthly intervals. The possible correlation between the parameters and phytoplankton abundance was determined using Spearman's correlation. In addition, Shannon Wiener Diversity Index (H') and the Margalef Index (R) were used to measure the species diversity and the richness of the phytoplankton community at the sampling stations. In contrast, Pielou's Evenness Index (J') was used to measure the equitability and uniformity of the species observed.

3 Results and Discussions

3.1. Phytoplankton species abundance

A total of 81 phytoplankton taxa were quantified in samples collected from 3 stations in Pinang River. Table 1 presents a list of phytoplankton identified in this study. Bacillariophyta was the highest taxa recorded (43 taxa) with the highest abundance at all sampling stations indicating that Bacillariophyta was the dominant phylum in the water samples, followed by Chlorophyta (23 taxa). Cyanophyta, Euglenophyta, and Chrysophyta were represented by 8, 3 and 2 taxa, respectively. Cryptophyta and Dinophyta were represented by only one taxon. In the phylum of Bacillariophyta, *Nitzschia* sp. (14.7 %; 1160 cells/L), *Navicula* sp. (11.2 %; 880 cells/L) and *Pinnularia* sp. (9.9 %; 783 cells/L) were recorded as the highly abundant species found at all stations. *Oscillatoria* sp. (10.3 %; 813 cells/L) was a highly abundant species in the phylum of Cyanophyta, while in Chlorophyta, *Chlorella* sp. was found to be highly abundant, particularly at Station 2 (9.2 %; 723 cells/L). *Trachelomonas* sp. was recorded as the abundant taxa in the phylum of Euglenophyta (0.8 %; 63 cells/L), while some taxa under Cryptophyta, Dinophyta and Chrysophyta occurred at low abundance during the sampling period. On the other hand, a high diversity of phytoplankton (69 taxa) was recorded at Station 1, followed by 63 taxa at Station 2, and 55 taxa at Station 3. Other than that, 68 taxa were rare, with a relative abundance of < 1 %, but they were vital components as they controlled the levels of species diversity at the study sites.

Based on the two-way repeated measures ANOVA analysis, there was no significant difference in phytoplankton abundance between sampling stations and months ($F_{4,6} = 4.19$, $P > 0.01$, $\eta_p^2 = 0.74$). In addition, there was also no significant effect in phytoplankton species number between sampling stations and months ($F_{4,6} = 1.43$, $P > 0.01$, $\eta_p^2 = 0.49$). This study failed to reject the null hypothesis that there is no difference in phytoplankton abundance and species number in the sampling stations throughout the study periods. This effect indicated that the rating profile for abundance and species number in sampling stations were similar for Stations 1, 2 and 3.

Table 1. The occurrence of phytoplankton species at all sampling stations in the Pinang River

Phylum/Species	Station/Mean Abundance (cells/L)		
	1	2	3
CYANOPHYTA			
<i>Anabaena</i> sp.	10	0	10
<i>Arthospira</i> sp.	130	100	110
<i>Calothrix</i> sp.	10	10	20
<i>Gloeocapsa</i> sp.	10	0	0
<i>Lyngbya</i> sp.	10	10	30
<i>Microcystis</i> sp.	10	210	90
<i>Oscillatoria</i> sp.	990	510	940
<i>Planktolyngbya</i> sp.	40	10	70
CHRYSOPHYTA			
<i>Dynobryon</i> sp.	0	0	10
<i>Synura</i> sp.	0	20	10
BACILLARIOPHYTA			
<i>Achnantheidium minutissimum</i>	50	180	250
<i>Actinella</i> sp.	10	20	10
<i>Amphipleura pellacida</i>	30	0	0
<i>Amphora</i> sp.	10	0	0
<i>Aulacodiscus</i> sp.	10	0	10
<i>Aulacoseira granulata</i>	10	0	30
<i>Bacillaria</i> sp.	10	70	60
<i>Chaetoceros</i> sp.	370	60	0
<i>Cocconeis pediculus</i>	0	60	0
<i>Coscinodiscus</i> sp.	0	10	0
<i>Craticula</i> sp.	110	90	60
<i>Cyclotella meneghiniana</i>	460	230	270
<i>Cymbella</i> sp.	10	10	0
<i>Diadismis confervacea</i>	60	60	20
<i>Diatoma vulgare</i>	70	0	50
<i>Diploneis</i> sp.	20	20	10
<i>Ditylum sol</i>	60	0	0
<i>Eucampia</i> sp.	530	180	20
<i>Eunotia</i> sp.	30	60	30
<i>Fragilaria</i> sp.	20	60	160
<i>Gomphonema</i> sp.	30	90	270
<i>Guinardia striata</i>	40	10	0
<i>Gyrosigma</i> sp.	40	0	0
<i>Hantzschia</i> sp.	0	20	10
<i>Hyalodiscus</i> sp.	120	20	40
<i>Lauderia borealis</i>	10	0	0
<i>Leptocylindrus</i> sp.	20	20	0
<i>Melosira varians</i>	60	0	0
<i>Navicula</i> sp.	680	740	1220
<i>Neidium</i> sp.	0	10	0
<i>Nitzschia</i> sp.	890	690	1900
<i>Odontella</i> sp.	70	20	0
<i>Pinnularia</i> sp.	560	670	1120

Phylum/Species	Station/Mean Abundance (cells/L)		
	1	2	3
<i>Pleurosigma elongatum</i>	120	20	10
<i>Rhizosolenia</i> sp.	100	10	0
<i>Skeletonema costatum</i>	30	0	0
<i>Stephanodiscus</i> sp.	40	20	0
<i>Striatella unipunctata</i>	50	20	0
<i>Surirella</i> sp.	50	500	20
<i>Synedra ulna</i>	20	30	110
<i>Tabellaria flocculosa</i>	30	20	10
<i>Tetracyclus</i> sp.	10	30	0
<i>Thalassiosira</i> sp.	20	10	10
CRYPTOPHYTA			
<i>Cryptomonas</i> sp.	0	10	0
DINOPHYTA			
<i>Ceratium hirudinella</i>	20	0	10
EUGLENOPHYTA			
<i>Euglena</i> sp.	10	70	50
<i>Phacus</i> sp.	20	10	70
<i>Trachelomonas</i> sp.	140	10	40
CHLOROPHYTA			
<i>Actinastrum hantzchii</i>	20	10	0
<i>Chlamydomonas</i> sp.	0	10	0
<i>Chlorella</i> sp.	10	2160	0
<i>Closterium</i> sp.	10	20	10
<i>Coelastrum</i> sp.	0	20	20
<i>Cosmarium</i> sp.	40	20	40
<i>Crucigenia</i> sp.	70	0	10
<i>Crucigeniella</i> sp.	10	0	30
<i>Desmidium</i> sp.	30	10	170
<i>Dictyosphaerium</i> sp.	40	60	10
<i>Micractinium</i> sp.	10	30	10
<i>Monoraphidium</i> sp.	40	10	20
<i>Pandorina</i> sp.	20	0	10
<i>Pediastrum simplex</i>	60	390	130
<i>Penium</i> sp.	450	10	30
<i>Pleurotaenium</i> sp.	0	10	0
<i>Scenedesmus</i> sp.	20	40	10
<i>Staurastrum</i> sp.	50	370	310
<i>Stigeoclonium</i> sp.	20	20	20
<i>Tetraedron</i> sp.	0	10	10
<i>Tetrastrum</i> sp.	40	60	130
<i>Ulothrix</i> sp.	0	20	10
<i>Volvox</i> sp.	10	0	0

Notes: Absent = 0 cell/L; Present = 1-30 cell/L; Abundant = 31-300 cell/L; Highly abundant = 301-2200 cell/L

The result of ecological indices is shown in Table 2. For Shannon Weiner's index, the highest value was recorded at Station 1 in October (3.34) while the lowest value was recorded at Station 2 in November (1.87). Similarly, the highest value of Margalef's index was recorded at Station 1 in October (4.85), but the lowest value was recorded at Station 3 also in October (3.23). The phytoplankton species were evenly distributed based on the evenness index with the highest value at Station 1 in October (0.89), while the lowest value occurred at Station 2 in November (0.54).

Table 2. Temporal and spatial variations of phytoplankton ecological indices in Pinang River

Month	Station	Diversity Index	Richness Index	Evenness Index
		Shannon Wiener (H')	Margalef (R)	Pielou (J')
October	1	3.34	4.85	0.89
	2	2.88	3.45	0.85
	3	2.32	3.23	0.68
November	1	2.47	3.99	0.69
	2	1.87	3.31	0.54
	3	2.57	3.66	0.73
December	1	2.75	4.31	0.75
	2	2.84	4.68	0.75
	3	2.60	3.76	0.73

Spearman's correlation coefficient test was analyzed each month at each sampling station. The results (Table 3) show that phytoplankton abundance had a strong negative correlation with turbidity ($r = -0.651$, $P < 0.01$) and $\text{PO}_4^{3-}\text{-P}$ ($r = -0.534$, $P < 0.01$) values, a moderate negative correlation with DO ($r = -0.458$, $P < 0.01$), but a significantly strong positive correlation with $\text{NH}_4^+\text{-N}$ ($r = 0.712$, $P < 0.01$).

3. 2. Phytoplankton assemblages in relation to physicochemical parameters

Physicochemical parameters are an important factor in determining the abundance of phytoplankton and indicating the status of water quality in an aquatic ecosystem. Ajayan and Ajit Kumar (2017) suggested that phytoplankton diversity and abundance effectively monitor deteriorating water conditions. On the other hand, water quality parameters such as temperature, pH, salinity and dissolved oxygen regulate the species composition of phytoplankton (El Gammal et al., 2017).

Generally, the abundance of phytoplankton in this study was high at a higher temperature (except for measurements at Station 1 in November). The findings of this study are consistent with the study conducted by Babu et al. (2013) in Muthupettai, India which stated that a lower abundance of phytoplankton was observed during monsoon months due to intense rainfall, reduced temperature, salinity, and pH. According to Rasconi et al. (2017), it can be expected that, with rising temperatures, the growth rate of phytoplankton will increase. In contrast, Striebel et al. (2016) claimed that increasing water temperature would reduce phytoplankton biomass. Therefore, temperature variability may have an inconsistent effect on the phytoplankton abundance.

The DO in the water body of the Pinang River at all sampling sites varied from 3.66 to 0.22 mg/L. DO recorded relatively low measurements in December, with the highest abundance of cyanobacteria (Cyanophyta) species recorded. This result agrees with Noyma et al. (2015) who suggested that massive and harmful blooms often contribute by cyanobacteria species to the depletion of the water quality as DO is depleted in the water. Previous results stated that the DO level declined with an increase in temperature and salinity (El Gammal et al., 2017).

In this study, DO showed a strong negative correlation with phytoplankton abundance ($r = -0.458$). The negative correlation of DO was probably due to the decomposition of organic matter, detritus and other aquatic life that used oxygen and produced carbon dioxide (El Gammal et al., 2017). Besides, Hu et al. (2018) reported that DO in river water, which was negatively correlated with phytoplankton abundance, was affected by the reproductive status of phytoplankton, as the increased rate of growth and reproduction of phytoplankton necessitated an enormous consumption of DO.

Throughout the sampling period, salinity, conductivity and TDS data showed a similar trend, with a high value of salinity resulting in high conductivity and TDS values. According to Sukumaran et al. (2013), high salinity is caused by the intrusion of neritic water and river discharge, while inflows of freshwater and tide fluctuation are responsible for reduced salinity (Vajravelu et al., 2018) besides rainfall intensity (Vinayachandran et al., 2002). According to Nursuhayati et al. (2013), some freshwater phytoplankton species have evolved into tolerance to fluctuations in the salinity of the water body.

Another study conducted in the Bay of Bengal, India, found that the river discharge from the Ganges with low salinity, TDS and nutrients prefers the growth of diatoms *Chaetoceros* sp., *Nitzschia* sp. and *Thalassiosira* sp. with the same species present at higher salinity and TDS water (Bharathi et al., 2018; Prasad et al., 2015). Therefore, the researchers suggested that the phytoplankton community should control the source of the river discharge and associated physicochemical parameters. This present study is also relevant to the findings of the studies, as three species were found abundantly at sampling stations with higher salinity. However, *Chaetoceros* sp. was absent in November, which may be related to other environmental parameters that hindered the growth of this species.

Inyang et al. (2016) stated that TDS values were lower in dry months and higher in wet months. The TDS values in this study fluctuated between 0.09 and 24.54 g/L and significantly differed between sampling months and the stations. In the study, there is no correlation between the TDS phytoplankton abundance ($r = -0.229$). The contribution of TDS to the phytoplankton growth rate is unclear (Kang et al., 2019). However, TDS showed a strong correlation with salinity and conductivity, which indirectly affects the distribution and abundance of phytoplankton. Some studies have proposed that a higher concentration of TDS may reduce the productivity of phytoplankton and that the species may be more sensitive to the toxicity of TDS (Duffy & Weber-Scannell, 2007).

Table 3. Spearman's correlation coefficients between physicochemical parameters and phytoplankton abundance in Pinang River

Parameter	r _s / Sig.	Temperature	DO	TDS	Conductivity	Salinity	pH	Turbidity	TSS	Chl. <i>a</i>	NH ₄ ⁺ -N	NO ₂ -N	PO ₄ ³⁻ -P	Phyto
Temperature	r _s	1.000	-0.456*	0.648**	0.471*	0.718**	0.250	-0.187	-0.182	0.163	0.274	-0.045	0.074	0.043
	Sig.	.	0.017	0.000	0.013	0.000	0.208	0.349	0.363	0.417	0.166	0.825	0.713	0.014
DO	r _s	-0.456*	1.000	-0.248	0.053	-0.285	0.285	0.473*	0.473*	0.217	-0.633**	-0.183	0.307	-0.458*
	Sig.	0.017	.	0.212	0.794	0.150	0.149	0.013	0.013	0.277	0.000	0.362	0.119	0.016
TDS	r _s	0.648**	-0.248	1.000	0.768**	0.982**	0.548**	-0.127	-0.404*	-0.104	-0.052	0.080	-0.056	-0.229
	Sig.	0.000	0.212	.	0.000	0.000	0.003	0.527	0.036	0.607	0.798	0.690	0.781	0.250
Conductivity	r _s	0.471	0.053	0.768**	1.000	0.762**	0.585**	0.035	-0.278	-0.159	-0.356	-0.311	0.163	-0.370
	Sig.	0.013	0.794	0.000	.	0.000	0.001	0.861	0.161	0.428	0.068	0.114	0.415	0.057
Salinity	r _s	0.718**	-0.285	0.982**	0.762**	1.000	0.538**	-0.137	-0.397*	-0.081	-0.013	0.056	-0.068	-0.190
	Sig.	0.000	0.150	0.000	0.000	.	0.004	0.495	0.040	0.687	0.947	0.782	0.736	0.344
pH	r _s	0.250	0.285	0.548**	0.585**	0.538**	1.000	-0.026	-0.511**	-0.071	-0.330	-0.043	-0.173	-0.240
	Sig.	0.208	0.149	0.003	0.001	0.001	.	0.898	0.006	0.726	0.093	0.830	0.387	0.228
Turbidity	r _s	-0.187	0.473*	-0.127	0.035	-0.137	-0.026	1.000	0.665**	-0.218	-0.763**	-0.152	0.823**	-0.651**
	Sig.	0.349	0.013	0.527	0.861	0.495	0.898	.	0.000	0.275	0.000	0.449	0.000	0.000
TSS	r _s	-0.182	0.359	0.404*	-0.278	-0.397*	-0.511**	0.665**	1.000	0.188	-0.355	-0.137	0.776**	-0.280
	Sig.	0.363	0.066	0.036	0.161	0.040	0.006	0.000	.	0.348	0.069	0.497	0.000	0.157
Chl. <i>a</i>	r _s	0.163	0.217	-0.104	-0.159	-0.081	-0.071	-0.218	0.188	1.000	0.418*	0.135	-0.027	0.333
	Sig.	0.417	0.277	0.607	0.428	0.687	0.726	0.275	0.348	.	0.030	0.501	0.894	0.089
NH ₄ ⁺ -N	r _s	0.274	-0.633**	-0.052	-0.356	-0.013	-0.330	-0.763**	-0.355	0.418*	1.000	0.380	-0.571**	0.712**
	Sig.	0.166	0.000	0.798	0.068	0.947	0.093	0.000	0.069	0.030	.	0.051	0.002	0.000
NO ₂ -N	r _s	-0.045	-0.183	0.080	-0.311	0.056	-0.043	-0.152	-0.137	0.135	0.380	1.000	-0.169	0.254
	Sig.	0.825	0.362	0.690	0.114	0.782	0.830	0.449	0.497	0.501	0.051	.	0.400	0.200
PO ₄ ³⁻ -P	r _s	0.074	0.307	-0.056	0.163	-0.068	-0.173	0.823**	0.776*	-0.027	-0.571**	-0.169	1.000	-0.534**
	Sig.	0.713	0.119	-0.781	0.415	0.736	0.387	0.000	0.000	0.894	0.002	0.400	.	0.004
Phyto	r _s	0.043	-0.458*	-0.229	-0.370	-0.190	-0.240	-0.651**	-0.280	0.333	0.712**	0.254	-0.534**	1.000
	Sig.	0.832	0.016	0.250	0.057	0.344	0.228	0.000	0.157	0.089	0.000	0.200	0.004	.

Notes.

** Correlation is significant at 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

3. 3. Species composition of phytoplankton phyla

Bacillariophyta (diatom) is the dominant phylum and has contributed to the highest number of taxa (43 taxa) in the Pinang River. This also resulted in an agreement with previous studies in Malaysia that diatoms are a common group found in most water bodies (Sidik et al., 2008; Nursuhayati et al., 2013). Muhammad Adlan et al., (2012) found that Bacillariophyta was a common phylum, followed by Chlorophyta (green algae) and Cyanophyta (cyanobacteria) at selected stations in the coastal waters of Manjung, Perak. Similarly, the abundance of phytoplankton species in the Pinang River also constitutes diatoms as the main phylum followed by green algae and cyanobacteria as the second and third highest abundance groups.

Diatom has also been shown to be one of the dominant groups commonly found in most water bodies worldwide (Gao & Song, 2005; Babu et al., 2013). *Chaetoceros* sp. was one of the dominant genera identified in both studies of Sidik et al. (2008) and Muhammad Adlan et al. (2012). In the Pinang River, this species was found to be abundant at Station 1 with the highest temperature and salinity recorded at the station. According to Nurul Salma et al. (2013), *Chaetoceros* sp. is abundant in high-salinity habitats, such as marine, and may tolerate low salinity. Renaud et al. (2002) reported *Chaetoceros* sp. performed the best within 27-30 °C and the temperature recorded at Station 1 in the Pinang River lies within this range.

The Bacillariophyta found in the Pinang River was *Nitzschia* sp., followed by *Navicula* and *Oscillatoria* sp. Vajravelu et al. (2018) and Gharib et al. (2011) listed Bacillariophyta as the main phylum and *Nitzschia* as the most common genus seen throughout the study period, respectively, in the Parangipettai coastal waters of India and the Mediterranean Sea, Egypt. Vajravelu et al. (2018) also suggested that a suitable environmental condition would promote the proliferation and growth of diatoms. This may also indicate that the Pinang River basin has a favourable growth condition for diatoms since this group of species dominated the river. Mixson (2007) claimed that Nitzchioids and Naviculoids are some of the dominant groups present in samples taken from Maple River, Northern Michigan. This finding is consistent with the current study, as *Navicula* sp. and *Nitzschia* sp. were highly abundant at all sampling stations.

Chlorophyta, the second dominant phylum recorded in this study, was mainly contributed by *Oscillatoria* sp., which was highly abundant in all the sampling stations. The adaptive characteristics of *Oscillatoria* sp. as a thermotolerant that grew at both high and low temperatures by changing pigmentations accordingly to changes in temperature may contribute to its successful abundance growth (Zhao et al., 2019). In a study by Huertas et al. (2011), *Scenedesmus* sp. proliferated at 40 °C and *Dicotyphaerium* sp. can tolerate a wide range of temperatures supported by this study where both species were found to be highly abundant at fluctuating temperatures.

Other than that, the excessive growth of some phytoplankton genera, such as *Oscillatoria*, *Pediastrum*, *Scenedesmus*, *Melosira*, and *Anabaena* displays nutrient enrichment of aquatic bodies (Zargar & Ghosh, 2006). Therefore, the water body of the Pinang River can be considered rich in nutrients, as the above-mentioned species were present either as highly abundant and abundant at all; or at some stations, except for *Anabaena*, which was only present in small numbers, and *Melosira*, which was only abundant at Station 1 and absent at both Stations 2 and 3.

Furthermore, Chlorophyta was once found at higher abundance in Station 2 in November, which contributed to the occurrence of *Chlorella* sp. At Station 2, where *Chlorella* sp. was found

in high abundance, the salinity level was very low. Therefore, the present results contradict the previous study by Nurul Salma et al. (2013) which suggested that *Chlorella* sp. showed a higher growth rate with increased salinity. However, according to Kang et al. (2019), phytoplankton abundance is not regulated by single factors but by several environmental parameters.

On the other hand, some of the phytoplankton genera and species found throughout the sampling period, such as *Cyclotella meneghiniana*, *Coelastrum* sp., *Closterium* sp., *Gomphonema* sp., *Scenedesmus* sp., *Nitzschia* sp., *Navicula* sp., *Euglena* sp., *Phacus* sp., *Pediastrum* sp., *Synedra ulna*, and *Tracheolomonas* sp., were significant species used to indicate the conditions of the Pinang River due to their characteristics as a pollutant-tolerant species (Ajayan & Ajit Kumar, 2017). Other than *Coelastrum* sp. and *Closterium* sp., all of the phytoplankton species mentioned above were found either highly abundant or abundant at each sampling station.

Cyclotella sp. is claimed to be common freshwater bloom phytoplankton by Soja-Wozniak et al. (2018). It may be proposed that the water body of the Pinang River is at the risk of occurrence of *Cyclotella* sp. bloom since it was found to be highly abundant at Station 1 and abundant at Stations 2 and 3. *Scenedesmus* sp. from phylum Chlorophyta and *Synedra ulna* from Bacillariophyta were found abundantly at Stations 2 and 3, respectively, and were collectively known as algae bloom inducers (Zhao et al., 2019).

Bouhaddada et al. (2016) found *Microcystis* sp. bloom contains harmful toxins and is known to bioaccumulate in common aquatic phytoplankton feeders that pose a high risk to human and animal health when consumed. Despite being only present at Station 1, *Microcystis* sp. was observed in abundance at both Stations 2 and 3. Therefore, according to the results obtained, the probability of *Microcystis* bloom occurring at the study site cannot be denied even though no bloom can be observed during the sampling period.

Ceratium sp. is one of the harmful species of Dinophyta and may cause intensive dinoflagellates to bloom if found in high abundance (Aktan et al., 2005). *Ceratium hirudinella* was found to be only about three individuals throughout the sampling period in the Pinang River, suggesting that dinoflagellate's bloom may be neglected at present. Most phytoplankton species found in the Pinang River may be used as bioindicators of water quality in the Pinang River basin. Palmer (1969) stated that diatoms, green algae, and blue-green algae are well-established as pollution-tolerant genera and species. The leading eight genera according to Palmer (1969), are *Oscillatoria*, *Euglena*, *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula* and *Stigeoclonium*. Therefore, the presence of these genera indicates the organic pollution of the water body (Jose et al., 2011).

Ecological indices can provide greater insight into the interactions in a system, particularly for environmental monitoring and conservation (Morris et al., 2014). The indices have become part of the standard methodology in many applied studies of ecology, such as to compare biological components and to be a complement in conjunction with multivariate analyses. In order to understand the interactions between plankton communities and physicochemical parameters in the study site including their changes in population diversity and abundance, ecological indices are essential. Nevertheless, the interpretation of community dynamics likely depends on the index chosen as it is predicted to interact in different ways and slightly different aspects. Thus, it will be useful to compare several indices for other assemblages, such as specific structural differences.

Zargar and Ghosh (2006) stated that the Shannon-Wiener Diversity Index (H') is the most widely used index to calculate species diversity, while the Evenness Index (J') is used to identify how evenly the species were distributed in the sampling area. Station 1 in October (3.34) showed the highest H' during the sampling period. Station 1 also recorded the highest number of species (S) of 42 species. This may also indicate that, at greater H' , more diverse the phytoplankton species in the Pinang River. The lowest value of H' was recorded at Station 2 in November (1.87) but included many species. *Microcystis* sp. was found at the highest abundance at Station 2 in November (540 cells/L). The occurrence of *Microcystis* may influence the species diversity at Station 2 since Hu et al. (2018) stated that microcystin toxin produced by *Microcystis* sp. at high concentrations has changed the water quality and affected the growth of other aquatic organisms. Telesh (2004) demonstrated that the trophic state of the water body influenced the species diversity within aquatic communities.

According to Gao and Song (2005), the H' of phytoplankton community was correlated in several cases with the degree of pollution, whereby the authors suggested that the value of H' would be greater in less populated water. They found that the sites with red tide blooms in the Changjiang estuary were recorded with low diversity. However, Zargar and Ghosh (2006) noted that it is widely accepted that the decrease of H' may be relevant to more severe pollution due to environmental stress. This is earlier supported by Green and Vascotto (1978), who argued that the relationship between large H' and better environmental quality was not always reliable.

In addition, Station 2 was recorded in November with the highest abundance of phytoplankton (11760 cells/L) but had the lowest H' value. Therefore, phytoplankton abundance and species diversity may not be related. The present result was further supported by a previous study by Gharib et al. (2011), that the phytoplankton abundance and species diversity indices were insignificant.

Based on the Evenness Index (J') value, the distribution of phytoplankton species in a population can be determined by the increase in J' , indicating that the phytoplankton species in the sample are evenly distributed. Species evenness also indicates the ecosystem's health when no single species dominates the phytoplankton community in the sample and no invasive species in that sample. Station 1 in October has the highest J' value (0.89) and Station 2 in November has the least evenly distributed phytoplankton community (0.54). This is probably due to the dominant species of *Chlorella* sp. that appeared in abundance only at Station 2 in November (6480 cells/L). Lehtinen (2017) stated that the species evenness was inversely proportional to the phytoplankton abundance which applies to the current study in the Pinang River.

Similar trends were seen between H' and J' whereby H' increased with J' throughout the sampling period at all sampling stations. There was a positive relationship between J' and H' , but ecological processes, such as competition and predation, can modify the species diversity index by changing the species evenness without altering the species richness of the sample (Gao & Song, 2005). According to Zhang et al. (2017), environmental parameters such as water temperature, pH and nutrients affect the species richness of phytoplankton in the community and the pH is an important factor that influences Bacillariophyta. The findings of this study are relevant to the Margalef index (R) in the Pinang River as it was the highest at Station 1 in

October (4.85), where Bacillariophyta was the most abundant and pH was the highest (7.74) throughout the sampling period. Overall, the species diversity, evenness, and richness of the phytoplankton community may express the status of the water body in the Pinang River.

4 Conclusion

Phytoplankton communities have been utilized as a useful tool for accessing the ecological status of the river ecosystem. As a sensitive ecosystem, various environmental stressors can affect river structure and function, including the phytoplankton community. This study suggested that the water body in the Pinang River is likely to be polluted at higher intensity due to anthropogenic activities. Changes in physicochemical parameters may affect the distribution and abundance of phytoplankton. Thus, in order to preserve the Pinang River from losing its function as a habitat for aquatic organisms, a food source and a clean water resource, the monitoring of phytoplankton abundance by researchers would help to determine the water quality of the Pinang River. Human attitudes that neglect the function of the river should be punished, and awareness should be triggered from time to time. The state council should consider reviewing existing laws with a view to more intense punishment for those who still intend to pollute the river.

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Conflict of interests

The authors state that there are no conflicts of interest regarding the publication of this article.

References

- Adams, V. D. (1990). *Water and Wastewater Examination Manual*. Lewis Publishers, Michigan.
- Ajayan, A. P., & Ajit Kumar, K. G. (2017). Phytoplankton as biomonitors: A study of Museum Lake in Government Botanical Garden and Museum, Thiruvananthapuram, Kerala India. *Lakes and Reservoirs: Research and Management*, 22(4), 403-415. <https://doi.org/10.1111/lre.12199>
- Aktan, Y., Tufekci, V., Tufekci, H., & Aykulu, G. (2005). Distribution patterns, biomass estimates and diversity of phytoplankton in İzmit Bay (Turkey). *Estuarine, Coastal and Shelf Science*, 64(2-3), 372-384. <https://doi.org/10.1016/J.ECSS.2005.03.003>

- Babu, A., Varadharaja, D., Vengadesh, P.N., Thilagavati, B., Manikandarajan, T., Sampathkumar, P., & Balasubramanian, T. (2013). Diversity of phytoplankton in different stations from Muthupettai, South East Coast of India. *Journal of Marine Science: Research Development*, 3(3), 1-11. <https://doi.org/10.4172/2155-9910.1000128>
- Baker, P. D., & Fabbro, L. D. (2002). *A Guide to the Identification of Common Blue-Green Algae (Cyanoprokaryotes) in Australian Freshwaters* (2nd Ed.). CRCFE and MDBC Identification Guide No. 25, CRCFE, Australia.
- Bellinger, E. G., & Sigee, D. C. (2010). *Freshwater Algae Identification and Use as Bioindicators*. Antony Rowe, Ltd. Chippenham, 285.
- Bharathi, M. D., Sarma, V. V. S. S., Ramaneswari, K., & Venkataramana, V. (2018). Influence of river discharge on abundance and composition of phytoplankton in the western coastal Bay of Bengal during peak discharge period. *Marine Pollution Bulletin*, 133, 671-683. <https://doi.org/10.1016/j.marpolbul.2018.06.032>
- Bouhaddada, R., Nelieu, S., Nasri, H., Delarue, G., & Bouaicha, N. (2016). High diversity of microcystins in a *Microcystis* bloom from an Algerian lake. *Environmental Pollution*, 216, 836-844. <https://doi.org/10.1016/j.envpol.2016.06.055>
- Duffy, L. K., & Weber-Scannell, P. K. (2007). Effects of total dissolved solids on aquatic organisms: A review of literature and recommendation for Salmonid species. *American Journal of Environmental Sciences*, 3(1), 1-6.
- El Gammal, M. A. M., Nageeb, M., & Al-Sabeb, S. (2017). Phytoplankton abundance in relation to the quality of the coastal water-Arabian Gulf, Saudi Arabia. *Egyptian Journal of Aquatic Research*, 43(4), 275-282. <https://doi.org/10.1016/j.ejar.2017.10.004>
- Gao, X., & Song, J. (2005). Phytoplankton distributions and their relationship with the environment in the Changjiang Estuary, China. *Marine Pollution Bulletin*, 50(3), 327-335. <https://doi.org/10.1016/j.marpolbul.2004.11.004>
- Gharib, S. M., El-Sherif, Z. M., Abdel-Halim, A. M., & Radwan, A. A. (2011). Phytoplankton and environmental variables as a water quality indicator for the beaches at Matrouh, south-eastern Mediterranean Sea, Egypt: An assessment. *Oceanologia*, 53(3), 819-836. <https://doi.org/10.5697/oc.53-3.819>
- Green, R. H., & Vascotto, G. L. (1978). A method for the analysis of environmental factors controlling patterns of species composition in aquatic communities. *Water Research*, 12(8), 583-590. [https://doi.org/10.1016/0043-1354\(78\)90137-9](https://doi.org/10.1016/0043-1354(78)90137-9)

- Howmiller, R. P., & Sloey, W. E. (1969). A horizontal water sampler for investigation of stratified waters. *Limnology and Oceanography*, 14(2), 291-292. <https://doi.org/10.4319/lo.1969.14.2.0291>
- Hu, X., Zhang, R., Ye, J., Wu, X., Zhang, Y., & Wu, C. (2018). Monitoring and research of microcystins and environmental factors in a typical artificial freshwater aquaculture pond. *Environmental Science and Pollution Research*, 25(6), 5921-5933. <https://doi.org/10.1007/s11356-017-0956-4>
- Huertas, I. E., Rouco, M., Lopez-Rodas, V., & Costas, E. (2011). Warming will affect phytoplankton differently: Evidence through a mechanistic approach. *Proceedings of the Royal Society B: Biological Sciences*, 278, 3534-3543. <https://doi.org/10.1098/rspb.2011.0160>
- Inyang, I. A., Sunday, E. K., & Dan, U. M. (2016). Effect of hydroclimatic conditions on phytoplankton community at Epe Lagoon tributary, Southwest Nigeria. *Journal of Oceanography and Marine Science*, 7(2), 12-23. <https://doi.org/10.5897/joms2016.0129>
- Janse van Vuuren, S., Taylor, J., Gerber, A., & van Ginkel, C. (2006). Easy identification of the most common freshwater algae. A guide for the identification of microscopic algae in South African freshwaters. North-West University, Potchefstroom, South Africa.
- Jose, L., Kumar, C., Albert, S., Sastha, D., & Devaswom, T. (2011). Evaluation of pollution by Palmer' s Algal Pollution Index and physico-chemical analysis of water in four temple ponds of Mattancherry, Ernakulam, Kerala. *Nature Environment and Pollution Technology*, 10(3), 471-472.
- Kang, L., He, Y., Dai, L., He, Q., Ai, H., Yang, G., Liu, M., Jiang, W., & Li, H. (2019). Interactions between suspended particulate matter and algal cells contributed to the reconstruction of phytoplankton communities in turbulent waters. *Water Research*, 149, 251-262. <https://doi.org/10.1016/j.watres.2018.11.003>
- Lehtinen, S., Tamminen, T., Ptacnik, R., & Andersen, T. (2017). Phytoplankton species richness, evenness, and production in relation to nutrient availability and imbalance. *Limnology and Oceanography*, 62(4), 1393-1408. <https://doi.org/10.1002/lno.10506>
- Lobban, C. S., Chapman, D. J., & Kremer, B. P. (Eds.). (1988). *Experimental Phycology: a Laboratory Manual*. CUP Archive.
- Mixson, S. (2007). The effects of temperature on diatom species richness and diversity in a streams lab facility from the Maple River of Northern Michigan. <https://hdl.handle.net/2027.42/57313>
- Morris, E. K., Caruso, T., Buscot, F., Fischer, M., Hancock, C., Maier, T. S., Meiners, T., Muller,

- C., Obermaier, E., Prati, D., Socher, S. A., Sonnemann, I., Waschke, N., Wubet, T., Wurst, S., & Rillig, M. C. (2014). Choosing and using diversity indices: Insights for ecological applications from the German Biodiversity Exploratories. *Ecology and Evolution*, 4(18), 3514-3524. <https://doi.org/10.1002/ece3.1155>
- Muhammad Adlan, A. H., Wan Maznah, W. O., Khairun, Y., Chuah, C. C., Shahril, M. H., & Mohd Noh, A. (2012). Tropical marine phytoplankton assemblages and water quality characteristics associated with thermal discharge from a coastal power station. *Journal of Natural Sciences Research*, 2(10), 88-99.
- Naemah, F., & Norulaini, N. (2006). Identification of pollution sources within the Sungai Pinang River Basin. *Proceeding of the Malaysian Research Group International Conference*, 478-485. <https://doi.org/10.4155/fmc.09.157>
- Noyma, N. P., Silva, T. P., Chiarini-Garcia, H., Amado, A. M., Roland, F., & Melo, R. C. N. (2015). Potential effects of UV radiation on photosynthetic structures of the bloom-forming cyanobacterium *Cylindrospermopsis raciborskii* CYRF-01. *Frontiers in Microbiology*, 6(1202), 1-13. <https://doi.org/10.3389/fmicb.2015.01202>
- Nursuhayati, A. S., Yusoff, F. M., & Shariff, M. (2013). Spatial and temporal distribution of phytoplankton in Perak Estuary, Malaysia, during monsoon season. *Journal of Fisheries and Aquatic Science*, 8(4), 480-493. <https://doi.org/10.3923/jfas.2013.480.493>
- Nurul Salma, A., Fatimah, M. Y., & Mohamed, S. (2013). Effect of salinity and temperature on the growth of diatoms and green algae. *Journal of Fisheries and Aquatic Science*, 8(2), 397-404. <https://doi.org/10.3923/jfas.2013.397.404>
- Onyema, I. C. (2013). Phytoplankton bio-indicators of water quality situations in the Iyagbe Lagoon, South-Western Nigeria. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 4(3), 639-652.
- Palmer, C. M. (1969). A composite rating of algae tolerating organic pollution. *Journal of Phycology*, 5(1), 78-82. <https://doi.org/10.1111/j.1529-8817.1969.tb02581.x>
- Poniewozik, M., & Lenard, T. (2022). Phytoplankton composition and ecological status of lakes with cyanobacteria dominance. *Int J Environ Res Public Health*, 19(7), 3832. <https://doi.org/10.3390/ijerph19073832>
- Prasad, V. R., Srinivas, T. N. R., & Sarma, V. V. S. S. (2015). Influence of river discharge on abundance and dissemination of heterotrophic, indicator and pathogenic bacteria along the east coast of India. *Marine Pollution Bulletin*, 95(1), 115-125. <https://doi.org/10.1016/j.marpolbul.2015.04.032>

- Rasconi, S., Winter, K., & Kainz, M. J. (2017). Temperature increase and fluctuation induce phytoplankton biodiversity loss - Evidence from a multi-seasonal mesocosm experiment. *Ecology and Evolution*, 7(9), 2936-2946. <https://doi.org/10.1002/ece3.2889>
- Renaud, S. M., Thinh, L. V., Lambrinidis, G., & Parry, D. L. (2002). Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture*, 211(1-4), 195-214. [https://doi.org/10.1016/S0044-8486\(01\)00875-4](https://doi.org/10.1016/S0044-8486(01)00875-4)
- Sidik, M. J., Rashed-Un-Nabi, M., & Hoque, M. A. (2008). Distribution of phytoplankton community in relation to environmental parameters in cage culture area of Sepanggar Bay, Sabah, Malaysia. *Estuarine, Coastal and Shelf Science*, 80(2), 251-260. <https://doi.org/10.1016/j.ecss.2008.08.004>
- Singh, U. B., Ahluwalia, A. S., Sharma, C., Jindal, R., & Thakur, R. K. (2013). Planktonic indicators: A promising tool for monitoring water quality (early-warning signals). *Ecology, Environment and Conservation*, 19(3), 793-800.
- Soja-Wozniak, M., Darecki, M., Wojtasiewicz, B., & Bradtke, K. (2018). Laboratory measurements of remote sensing reflectance of selected phytoplankton species from the Baltic Sea. *Oceanologia*, 60(1), 86-96. <https://doi.org/10.1016/j.oceano.2017.08.001>
- Striebel, M., Schabhtt, S., Hodapp, D., Hingsamer, P., & Hillebrand, H. (2016). Phytoplankton responses to temperature increases are constrained by abiotic conditions and community composition. *Oecologia*, 182(3), 815-827. <https://doi.org/10.1007/s00442-016-3693-3>
- Sukumaran, M., Muthukumaravel, K., & Sivakami, R. (2013). Seasonal variations in physico-chemical characteristics of Agniar Estuary southeast coast of India. *Asia Pacific Journal of Research*, 2(8), 108-120. <http://apjor.com/files/1376844720.pdf>
- Telesh, I. V. (2004). Plankton of the Baltic estuarine ecosystems with emphasis on Neva Estuary: A review of present knowledge and research perspectives. *Marine Pollution Bulletin*, 49(3), 206-219. <https://doi.org/10.1016/j.marpolbul.2004.02.009>
- Thakur, R. K., Jindal, R., Singh, U. B., & Ahluwalia, A. S. (2013). Plankton diversity and water quality assessment of three freshwater lakes of Mandi (Himachal Pradesh, India) with special reference to planktonic indicators. *Environmental Monitoring and Assessment*, 185(10), 8355-8373. <https://doi.org/10.1007/s10661-013-3178-3>
- Vajravelu, M., Martin, Y., Ayyappan, S., & Mayakrishnan, M. (2018). Seasonal influence of physico-chemical parameters on phytoplankton diversity, community structure and abundance at Parangipettai coastal waters, Bay of Bengal, South East Coast of India. *Oceanologia*, 60(2), 114-127. <https://doi.org/10.1016/j.oceano.2017.08.003>

- Vinayachandran, P. N., Murty, V. S. N., & Ramesh Babu, V. (2002). Observations of barrier layer formation in the Bay of Bengal during summer monsoon. *Journal of Geophysical Research: Oceans*, 107(C12), SRF 19-1-SRF 19-9. <https://doi.org/10.1029/2001jc000831>
- Williams, O. J., Beckett, R. E., & Maxwell, D. L. (2016). Marine phytoplankton preservation with Lugol's: a comparison of solutions. *Journal of Applied Phycology*, 28(3), 1705-1712. <https://doi.org/10.1007/s10811-015-0704-4>
- Woelkerling, W. J., Kowal, R. R., & Gough, S. B. (1976). Sedgwick-rafter cell counts: A procedural analysis. *Hydrobiologia*, 48(2), 95–107. <https://doi.org/10.1007/BF00040161>
- Zargar, S., & Ghosh, T. K. (2006). Influence of cooling water discharges from Kaiga nuclear power plant on selected indices applied to plankton population of Kadra reservoir. *Journal of Environmental Biology*, 27(2), 191-198.
- Zhang, M., Strail, D., Chen, F., Shi, X., Yang, Z., Cai, Y., Yu, J., & Kong, F. (2017). Dynamics and drivers of phytoplankton richness and composition along productivity gradient. *Science of the Total Environment*, 625, 275-284. <https://doi.org/10.1016/j.scitotenv.2017.12.288>
- Zhao, C. S., Shao, N. F., Yang, S. T., Ren, H., Ge, Y. R., Zhang, Z.S., Feng, P., & Liu, W. L. (2019). Quantitative assessment of the effects of human activities on phytoplankton communities in lakes and reservoirs. *Science of the Total Environment*, 665, 213-225. <https://doi.org/10.1016/j.scitotenv.2019.02.117>