

# Environmental influence on the euglenoid species diversity and their abundance in Museum Lake, Thiruvananthapuram, India

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**Twenty-three species belonging to the class Euglenophyceae were recorded from the Museum Lake, Thiruvananthapuram, India. A canonical correspondence analysis (CCA) between environmental variables and the dominant euglenophytes indicated interaction with certain physical and chemical parameters, in particular rainfall, electrical conductivity and phosphate versus their distribution in the Museum Lake. The dominant species were *Euglena deses*, *Trachelomonas hispida* and *Trachelomonas volvocina*. The CCA results indicate interaction between a variety of environmental parameters and species abundance. A closer examination suggests some external factor (e.g. evaporation) affecting water quality rather than the environment changing the algal biomass. With low rainfall the phosphate concentration increased from 1.5 to 6.9 mg P/l (likely as a result of anoxic conditions in the muddy bottom) and simultaneously nitrate was (partly) taken up by these dominant algae, as a result of which N : P ratios decreased from 4.5 to 0.7. The present study shows that the dominant euglenoid species co-occur in relatively hard water with high phosphorus concentration, and can be considered as useful bio-indicators in assessing the health and extent of deterioration of a lake ecosystem.**

**Keywords:** Canonical correspondence analysis, environmental variables, euglenoid species, lake ecosystem.

EUGLENOPHYTES are microscopic, planktonic microorganisms that contribute to the primary production of an aquatic ecosystem. These groups of phytoplankton are studied mainly for their ecological importance as indicators of water pollution. Most of them are mixotrophic<sup>1</sup>, i.e. switching between photosynthesis and carbon utilization (dissolved and particulate). Hence these freshwater flagellates are considered as good indicators of organic pollution<sup>2,3</sup>. In India various taxonomic studies have been carried out on the diversity of euglenoids<sup>4-15</sup>. However, only a few of them deal with their relationship to pollu-

tion levels<sup>16-20</sup>. The present work aims at highlighting the abundance and distribution of the (dominant) euglenoids in a small lake – Museum Lake, Thiruvananthapuram, Kerala, India, that receives inputs of organic matter (bat faeces, litter) from its surroundings. The relationships between euglenoid abundance in the lake and a number of environmental variables are studied throughout the seasons. The question is whether the euglenoids present are suitable as indicators of (organic) pollution.

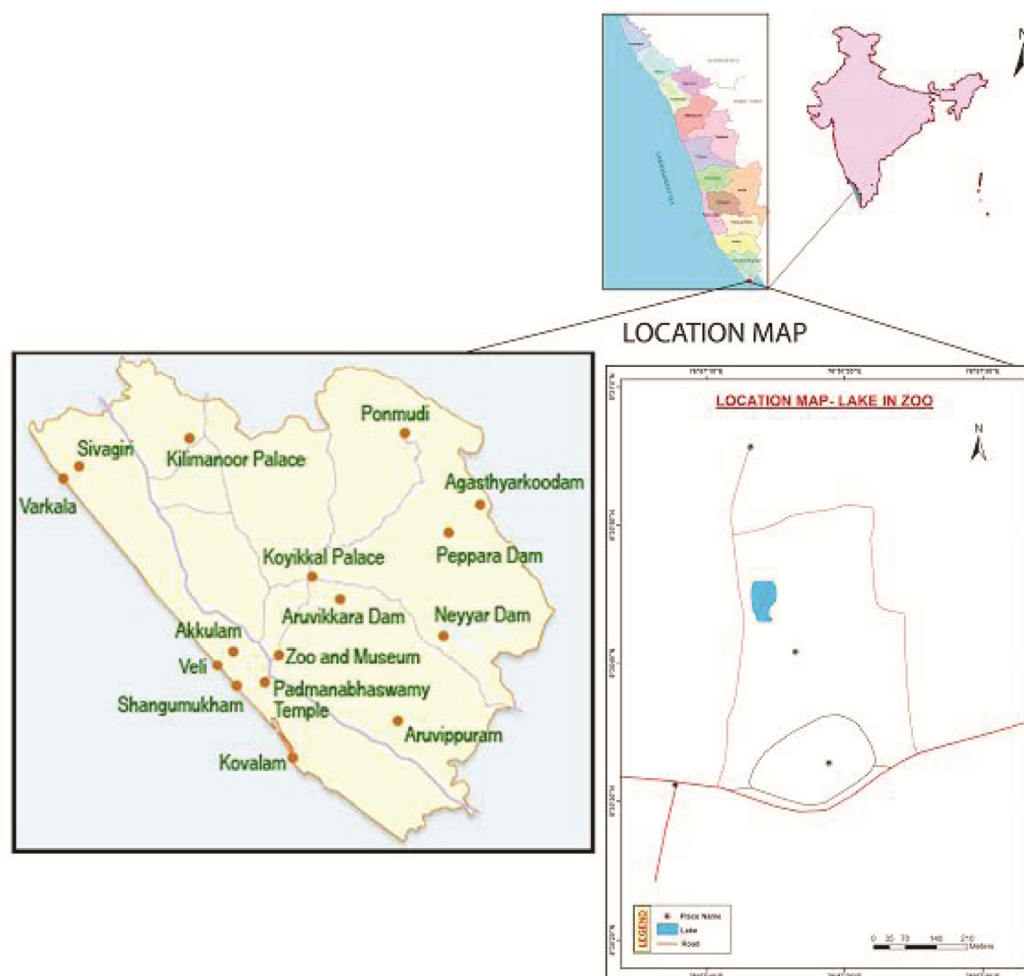
## Materials and methods

The Thiruvananthapuram Museum and Zoo is one of the oldest of its kind in India located at the heart of the city (08°30'N, 076°57'E). The present study was carried out in the small, expansive Museum Lake inside the zoo, which extends over an area of ca. 9000 m<sup>2</sup> and maximum depth of 6 m. Figure 1 shows the location map of the study area. It is a natural waterbody that maintains its water level even in the scorching summers. The waterbody is oriented in a north–south direction. The southern part is completely covered by floating macrophytes such as *Pistia* sp. and *Lemna* sp. A large bat population inhabiting the surrounding trees, as well as indigenous and exotic bird species in the trees of a small island in the lake, enrich it (and its bottom) with their faeces. In order to study seasonal variations in euglenoid cell numbers with variations of environmental parameters, samples were collected for two consecutive years. Monthly data were divided into three seasons (Box 1).

## Phytoplankton analyses

Water samples were collected from the lake in clean plastic bottles. Samples were taken at random at mid-depth in the water column and pooled. Samples were preserved by adding 1 ml/l 4% formalin solution. A Sedgwick Rafter cell was used to count the micro-algae in concentrated samples; species density/abundance was given in cells/ml. For identification, photomicrographs of the algae were

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**Figure 1.** Location map of the Museum Lake, Thiruvananthapuram, India.

**Box 1.** Monthly data divided into three seasons

Year	2013	2014
Pre-monsoon (February–May)	PreM1	PreM2
Monsoon (June–September)	Mon1	Mon2
Post-monsoon (October–January)	PoM1	PoM2

taken using a camera attached to compound microscope (Leica DM500). The taxonomic works of Philipose<sup>8–10</sup>, Woloski and Hindák<sup>21</sup>, Ciugulea and Triemer<sup>22</sup> and Triemer and Zakrýs<sup>23</sup> as well as on-line websites like Phycokey and Algaebase were consulted for identification of the euglenoids.

#### Environmental parameters

With a portable pH meter and a centigrade thermometer, the *in situ* pH and water temperature (°C) were measured. Rainfall data (mm/season) was obtained from India Meteorological Department, Thiruvananthapuram. Electrical conductivity (EC,  $\mu\text{S}/\text{cm}$ ) and total dissolved solids

(TDS, ppm) were measured with a digital TDS/conductivity meter (MK-509). Dissolved oxygen concentration (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total alkalinity (TA), total hardness (TH), magnesium ( $\text{Mg}^{2+}$ ), calcium ( $\text{Ca}^{2+}$ ), sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), nitrate ( $\text{NO}_3^-$ -N), nitrite ( $\text{NO}_2^-$ -N), soluble reactive phosphate ( $\text{PO}_4$ -P) and silicate (Si) were analysed using standard methods<sup>24–26</sup>.

#### Statistics

The euglenoid composition and abundance in relation to environmental parameters (physical and chemical data) were derived from canonical correspondence analysis (CCA) using Paleontological Statistics Version 3.10 (PAST).

#### Results and discussion

Tables 1 and 2 give the mean values plus their variations (mean  $\pm$  SD) in hydrographic parameters for the study

## RESEARCH ARTICLES

**Table 1.** Mean and variation (mean  $\pm$  SD) of hydrological parameters in the Museum Lake, Thiruvananthapuram for year 1 (2013–14)

Parameters	Units	Pre-monsoon (PreM1)	Monsoon (Mon1)	Post-monsoon (PoM1)
Rainfall	mm/season	65	141	243
Water temperature	°C	23.7 $\pm$ 1.5	23.25 $\pm$ 0.5	23 $\pm$ 0.82
pH	–	6.9 $\pm$ 0.42	6.8 $\pm$ 0.34	6.9 $\pm$ 0.52
Dissolved oxygen (DO)	mg O <sub>2</sub> /l	11.29 $\pm$ 10.98	8.28 $\pm$ 4.02	8.08 $\pm$ 5.11
Biological oxygen demand (BOD)	mg O <sub>2</sub> /l	10.07 $\pm$ 10.11	8.29 $\pm$ 4.29	6.00 $\pm$ 5.45
Chemical oxygen demand (COD)	mg/l	43.60 $\pm$ 16.30	30.08 $\pm$ 9.67	43.35 $\pm$ 10.35
Total alkalinity	mg CaCO <sub>3</sub> /l	21.56 $\pm$ 2.99	16.63 $\pm$ 3.38	17.35 $\pm$ 1.27
Nitrate–nitrogen	mg N/l	1.73 $\pm$ 1.55	3.99 $\pm$ 1.54	2.60 $\pm$ 0.07
Nitrite–nitrogen	mg N/l	0.39 $\pm$ 0.30	0.90 $\pm$ 0.21	0.22 $\pm$ 0.18
Calcium	mg Ca/l	13.4 $\pm$ 5.95	18.65 $\pm$ 3.59	17.23 $\pm$ 3.05
Total hardness	mg CaCO <sub>3</sub> /l	69.44 $\pm$ 26.76	62.59 $\pm$ 8.22	63.25 $\pm$ 30.54
Magnesium	mg Mg/l	13.30 $\pm$ 5.30	14.11 $\pm$ 5.88	11.03 $\pm$ 6.77
Phosphate	mg P/l	6.87 $\pm$ 3.22	3.46 $\pm$ 2.12	1.43 $\pm$ 0.91
Silicate	mg Si/l	5.35 $\pm$ 5.19	8.06 $\pm$ 3.35	9.72 $\pm$ 3.00
Sodium	mg Na/l	4.88 $\pm$ 2.17	4.94 $\pm$ 1.22	5.13 $\pm$ 1.05
Potassium	mg K/l	6.16 $\pm$ 4.48	12.24 $\pm$ 10.81	11.68 $\pm$ 11.48
Total dissolved solids (TDS)	ppm	76.30 $\pm$ 7.35	77.72 $\pm$ 3.47	76.41 $\pm$ 10.46
Conductivity	$\mu$ S/cm	156.20 $\pm$ 6.28	147.10 $\pm$ 8.16	153.88 $\pm$ 6.05

**Table 2.** Mean and variation (mean  $\pm$  SD) of hydrological parameters in the Museum Lake for year 2 (2014–15)

Parameters	Units	Pre-monsoon (PreM2)	Monsoon (Mon2)	Post-monsoon (PoM2)
Rainfall	mm/season	268	101	140
Water temperature	°C	24 $\pm$ 0.96	23 $\pm$ 0.82	23 $\pm$ 0.82
pH	–	7.05 $\pm$ 0.58	6.8 $\pm$ 0.32	6.6 $\pm$ 0.2
DO	mg O <sub>2</sub> /l	6.10 $\pm$ 3.33	7.2 $\pm$ 3.71	4.88 $\pm$ 2.06
BOD	mg O <sub>2</sub> /l	5.31 $\pm$ 3.50	6.79 $\pm$ 5.1	3.48 $\pm$ 0.66
COD	mg/l	38.47 $\pm$ 15.32	36.09 $\pm$ 10.44	45.87 $\pm$ 6.20
Total alkalinity	mg CaCO <sub>3</sub> /l	15.18 $\pm$ 7.65	17.19 $\pm$ 2.67	16.69 $\pm$ 0.90
Nitrate–nitrogen	mg N/l	4.10 $\pm$ 4.70	3.03 $\pm$ 0.77	2.88 $\pm$ 2.37
Nitrite–nitrogen	mg N/l	0.38 $\pm$ 0.36	0.58 $\pm$ 0.24	0.14 $\pm$ 0.02
Calcium	mg Ca/l	13.38 $\pm$ 4.61	19.54 $\pm$ 5.78	13.79 $\pm$ 1.91
Total hardness	mg CaCO <sub>3</sub> /l	30.48 $\pm$ 12.59	62.41 $\pm$ 12.69	61.23 $\pm$ 0.54
Magnesium	mg Mg/l	4.52 $\pm$ 3.62	10.45 $\pm$ 4.45	11.32 $\pm$ 3.59
Phosphate	mg P/l	2.79 $\pm$ 1.13	3.67 $\pm$ 1.91	1.66 $\pm$ 0.53
Silicate	mg Si/l	8.31 $\pm$ 5.43	6.5 $\pm$ 2.55	6.27 $\pm$ 2.75
Sodium	mg Na/l	4.17 $\pm$ 1.68	8.3 $\pm$ 2.56	6.31 $\pm$ 0.54
Potassium	mg K/l	4.82 $\pm$ 5.43	3.56 $\pm$ 0.85	13.32 $\pm$ 9.60
TDS	ppm	75.73 $\pm$ 7.03	72.01 $\pm$ 6.65	71.55 $\pm$ 3.56
Conductivity	$\mu$ S/cm	160.68 $\pm$ 13.12	137.67 $\pm$ 8.35	144.79 $\pm$ 16.43

period. These were all used as independent variables in the CCA.

Twenty-three Euglenophyceae species (of the genera *Euglena*, *Lepocinclis*, *Phacus* and *Trachelomonas*) were recorded from the Museum Lake (Table 3), among which *Euglenaria clepsydroides* was newly recorded in India<sup>3</sup>. Table 4 gives the average cell number in the two-year sampling period; *Euglena deses*, *Trachelomonas volvocina* and *Trachelomonas hispida* were the dominant species. Euglenoid cell numbers were highest during PreM1 and lowest during Mon2. Figure 2 gives the seasonal development of the three dominant euglenoids. *E. deses* showed a decreasing pattern during Mon1 and later increased during the PreM2. *Phacus acuminatus*, *P. orbicularis*, *P. rotunda* and *P. skujajae* were observed in all the

study seasons. They are generally abundant during the summer period<sup>27,28</sup>. *Trachelomonas cylindrica* and *T. naviculiformis* were observed only during PreM2.

CCA was performed to determine the ecological relationship between 23 euglenoid species and 18 environmental variables. Table 3 provides the list of euglenoids with species code used in CCA.

In the present study, percentage of variance and eigen values of each study season (Figure 3) in axis 1 are higher than the axis 2. Similar observations are reported by Liu *et al.*<sup>29</sup>, Sharma *et al.*<sup>30</sup>. The effect of multiple sets of environmental variables on the variability of plankton (euglenoid) species is clearly evident from the tri-plot obtained by CCA method for the two separate study periods. The CCA drawn (Figure 3) between all the

**Table 3.** List of euglenoids from the Museum Lake for canonical correspondence analysis with species code

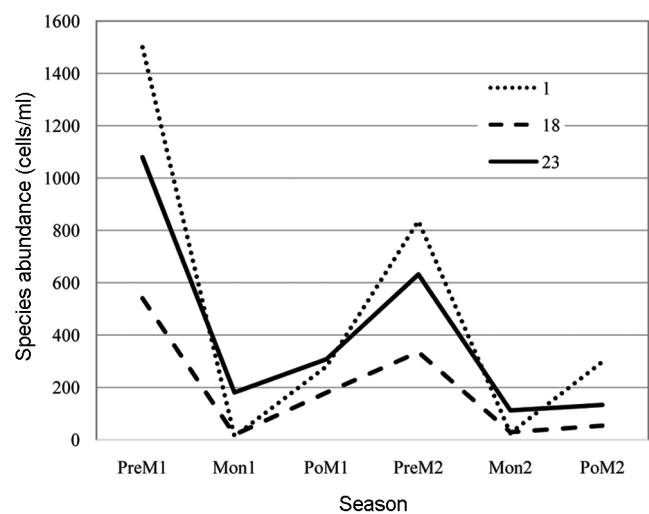
Euglenoid species	Species code	Euglenoid species	Species code
<i>Euglena deses</i> Ehrenberg	1	<i>Phacus skujae</i> Skvortzov	13
<i>Euglenaria clepsydroides</i> B. Zakrys	2	<i>Trachelomonas caudata</i> (Ehrenberg) F. Stein	14
<i>Lepocinclis fusiformis</i> (H. J. Carter) Lemmermann	3	<i>Trachelomonas conica</i> Playfair	15
<i>Lepocinclis horridus</i> Pochmann	4	<i>Trachelomonas conica</i> var. <i>richmondiae</i> Playfair	16
<i>Lepocinclis ovum</i> (Ehrenb.) Lemm. var. <i>bütschlii</i> Conr.	5	<i>Trachelomonas cylindrica</i> Ehrenberg	17
<i>Lepocinclis ovum</i> var. <i>gracilicauda</i> Deflandre	6	<i>Trachelomonas hispida</i> (Perty) F. Stein	18
<i>Phacus acuminatus</i> Stokes	7	<i>Trachelomonas naviculiformis</i> Deflandre	19
<i>Phacus granum</i> Drezepolski	8	<i>Trachelomonas playfairii</i> Deflandre	20
<i>Phacus orbicularis</i> K. Hübner	9	<i>Trachelomonas raciborskii</i> Woloszyńska	21
<i>Phacus longicauda</i> var. <i>rotunda</i> (Pochmann) Huber-Pestalozzi	10	<i>Trachelomonas superba</i> Svirenko	22
<i>Phacus pleuronectus</i> (O. F. Müller) Nitzsch ex Dujardin	11	<i>Trachelomonas volvocina</i> (Ehrenberg) Ehrenberg	23
<i>Phacus rotunda</i> (Pochmann) Zakrys and M. Lukomska	12		

**Table 4.** Average abundance during the study period (two years). The underlined leading species (1, 18, 23) are further discussed and plotted separately in Figure 1 for seasonal variations

Euglenoid species	Cells/ml
1. <i>Euglena deses</i>	<u>492</u>
2. <i>Euglenaria clepsydroides</i>	5
3. <i>Lepocinclis fusiformis</i>	67
4. <i>Lepocinclis horridus</i>	18
5. <i>Lepocinclis ovum</i> var. <i>bütschlii</i>	16
6. <i>Lepocinclis ovum</i> var. <i>gracilicauda</i>	6
7. <i>Phacus acuminatus</i>	30
8. <i>Phacus granum</i>	7
9. <i>Phacus orbicularis</i>	38
10. <i>Phacus longicauda</i> var. <i>rotunda</i>	21
11. <i>Phacus pleuronectus</i>	61
12. <i>Phacus rotunda</i>	47
13. <i>Phacus skujae</i>	79
14. <i>Trachelomonas caudata</i>	59
15. <i>Trachelomonas conica</i>	13
16. <i>Trachelomonas conica</i> var. <i>richmondiae</i>	6
17. <i>Trachelomonas cylindrica</i>	3
18. <i>Trachelomonas hispida</i>	<u>193</u>
19. <i>Trachelomonas naviculiformis</i>	2
20. <i>Trachelomonas playfairii</i>	74
21. <i>Trachelomonas raciborskii</i>	14
22. <i>Trachelomonas superba</i>	31
23. <i>Trachelomonas volvocina</i>	<u>408</u>

environmental variables and euglenoid species. This obtained an Eigen value for axis 1 (0.180) that explained 52.64% of correlation whereas, axis 2 (0.113) that explained 33% of correlation between the studied variables and species. The arrow length in the CCA tri-plot shows a positive or negative correlation with one or more variable<sup>29-31</sup>. Figure 3 shows that the seasonal variation in cell number of the dominant species has a relationship with some of the hydrographical parameters like rainfall (-), conductivity (+), phosphate (+) and nitrate (-) in the Museum Lake.

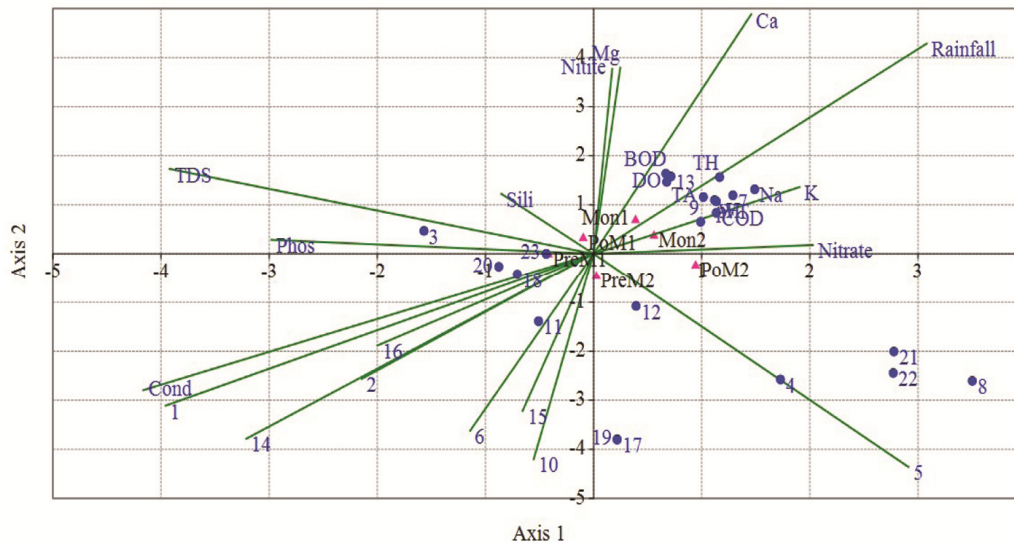
In order to understand the nature of these relationships, it has to be established whether these are direct interactions, indirect causalities or just coincidences. Although

**Figure 2.** Seasonal diversity of the three leading euglenoid species in the Museum Lake for the two-year study period: 1 = *Euglena deses*; 18 = *Trachelomonas hispida*; 23 = *Trachelomonas volvocina*.

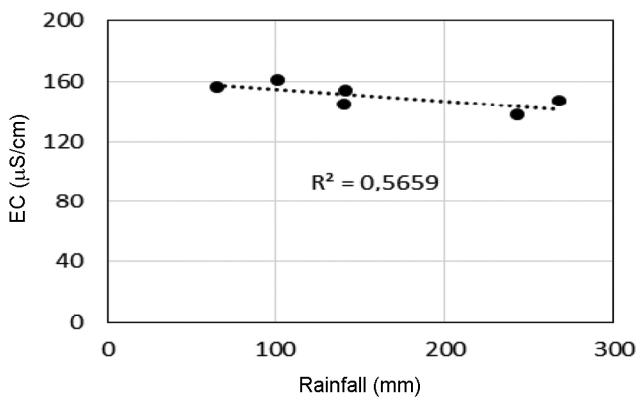
the environmental parameters have been used as independent input variables, some of these variables are influenced by others.

The correlation between EC and rainfall that is represented in the CCA triplot is explained in Figure 4. Here, rainfall rate is taken as an independent variable for the Museum Lake. From the graph, there was a visible correlation between rainfall and electrical conductivity. As a result of rain, the cold rainwater will dilute (overturn) the water column and thus it acidifies the lake water.

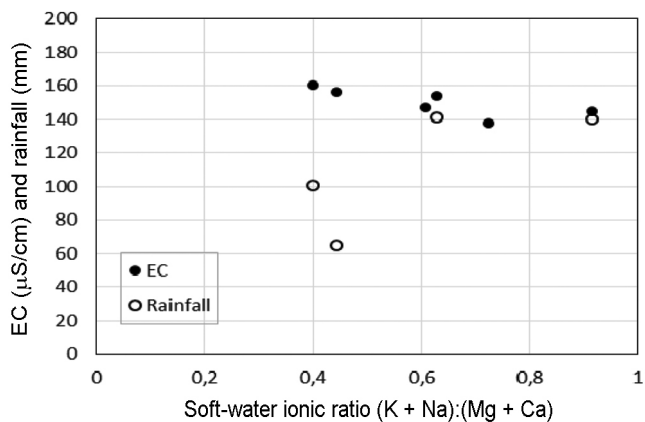
In the case of the Museum Lake (that maintains its water level) it is likely that ions are transported via the lake bottom, mainly by carrying in bottom minerals like calcium and magnesium that contributes to the water harness. This explains that the water conducts an electric current better as the water contains more divalent ions such as calcium and magnesium shown by a low soft-water ionic ratio (Figure 5). This means that rainfall did



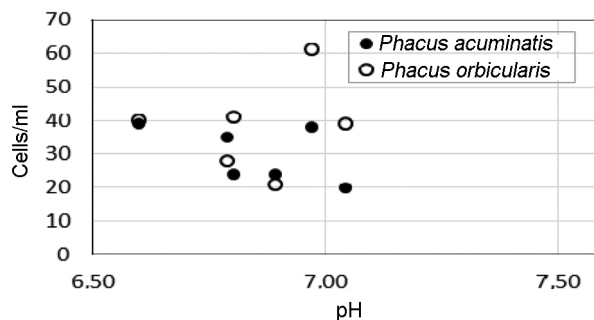
**Figure 3.** Canonical correspondence analysis (CCA) tri-plot showing the relation between euglenoid species (23) and environmental variables (18) in the Museum Lake, for the two-year study period. Dots represent the species (code numbers); arrow length indicates relative importance (correlation strength) towards those parameters; arrow direction indicates a positive or negative (opposite) relationship with each variables and species.



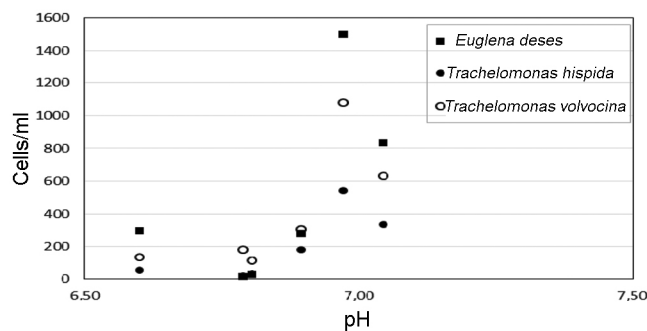
**Figure 4.** Electrical conductivity (EC) in the lake water versus amount of rainfall in the area.



**Figure 5.** EC of the lake water versus soft-water ratio expressed as the sum of the major monovalent cations divided by the sum of the major divalent cations.

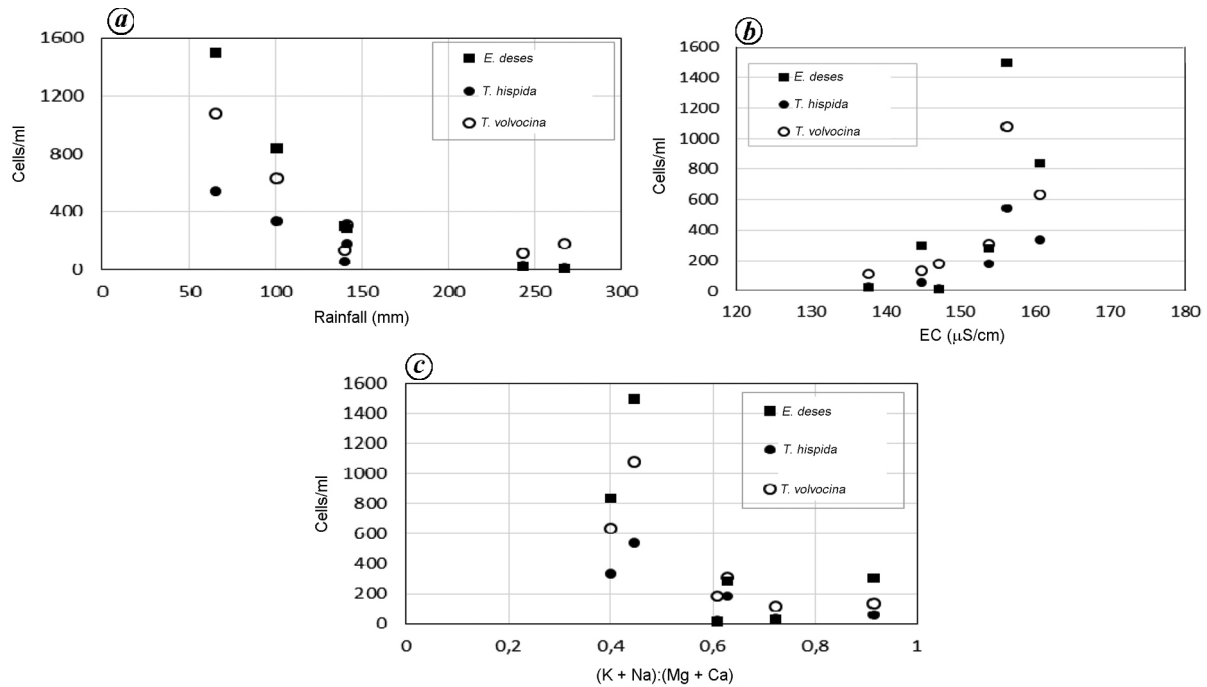


**Figure 6.** Relationships of two *Phacus* spp. with pH as indicated in the CCA plot.

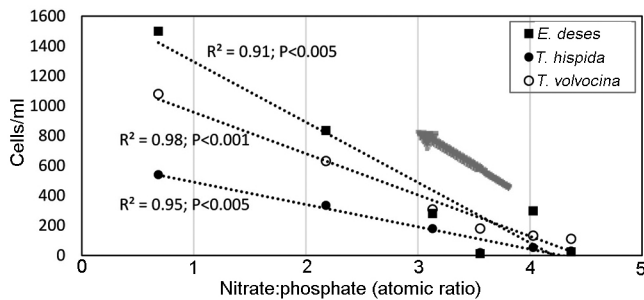


**Figure 7.** Cell number of dominant euglenoid species versus pH in the Museum Lake.

not help in softening the lake water. More rainfall is accompanied by more advection of groundwater and run-off from the surrounding land. It demonstrates how some ‘independent’ CCA input variables depend on each other. Cold rainwater may also overturn the water column.



**Figure 8.** Trends of cell numbers of the three dominant euglenoids versus (a) rainfall; (b) EC, and (c) ratio of monovalent and divalent major ions.



**Figure 9.** Cell number of dominant euglenoids versus inorganic N : P ratio in the lake water. Arrow indicates that as biomass of the dominant species increase the nitrate concentration decreases.

Rainwater may slightly acidify the lake, but in the case of the Museum Lake there was no significant influence of rainfall or EC on pH, which varied slightly between 6.5 and 7.6, viz. neutral to slightly acidic. The CCA tri-plot suggests that growth of both *P. acuminatus* and *P. orbicularis* is promoted by a higher pH; however Figure 6 shows no significant effect.

From the CCA tri-plot (Figure 3) pH is pointing oppositely to species nos 1, 18 and 23 which are dominant in the lake. This indicates that at lower pH, *E. deses*, *T. hispida* and *T. volvocina* would show higher abundance. This is contradicted by directly plotting the specific cell numbers versus pH (Figure 7); cell density increased with pH or at high micro-algal densities pH increased as a result of  $\text{CO}_2$  uptake. Usually, at high pH (8.5–10), there will be a limitation of inorganic carbon in algal cells and

increased photorespiration resulting in poor growth. Here the average pH is below 7.1.

A pH below 8.0 is considered as suitable for growth of euglenoids<sup>32,33</sup>. Venketeswarlu<sup>34</sup> found that in River Moosi an average pH of 7.7 resulted in 5.1% increase in euglenoids, whereas at pH 8.2 the euglenoid density decreased by 1.93%.

*E. deses*, *T. hispida* and *T. volvocina* were the dominant euglenoids observed in samples of the Museum Lake. They showed increased growth during the pre-monsoon and post-monsoon seasons. All three showed a negative trend with rainfall (Figure 8a), conductivity (Figure 8b) and with  $(\text{K}^+ + \text{Na}^+)$  relative to  $(\text{Mg}^{2+} + \text{Ca}^{2+})$ , i.e. the soft-water ratio (Figure 8c). This might be understood as 'dominant euglenoids are thriving in dry periods in relatively hard water'. The CCA tri-plot suggests that *P. pleuronectus* is negatively influenced by calcium (during PreM1, the  $\text{Ca}^{2+}$  concentration was 13.4 mg/l and cell number was 183 cells/ml). The CCA plot also suggests that  $\text{Ca}^{2+}$  suppressed the growth of *Lepocinclis ovum* var. *gracilicauda* (almost absent in this season); however, its low biomass may as well be due to unsuccessful competition with the dominant plankton species, not necessarily being a result of high  $\text{Ca}^{2+}$  concentration. Kim and Boo<sup>35</sup> and Rahman *et al.*<sup>33</sup> reported a bimodal pattern in the distribution of euglenoids in waterbodies, with maximum growth during pre-monsoon and post monsoon seasons.

*T. volvocina* was related to nitrate (–) and to phosphate (+) as indicated in the CCA tri-plot. An XY plot of the

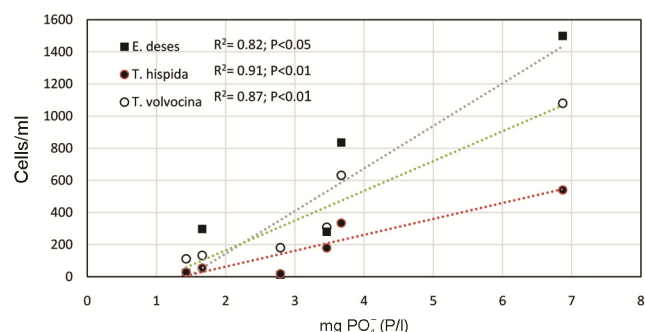
abundance (cells/ml) of *E. deses*, *T. volvocina* and *T. hispida* versus nitrate concentration showed that the species had the highest cell numbers (pre-monsoon 2013) at the lowest  $\text{NO}_3^-$  concentration (1.73 mg N/l), but nitrogen was never depleted. Nitrate/phosphate ratios were so low that phosphorus would never be the growth-limiting nutrient in the Museum Lake. Plots of cell abundances versus N : P ratio in the water show remarkably strong correlation (Figure 9).

If there was only a proportional decrease of phosphate with nitrate due to uptake by algae (atomic ratio N : P = 16 : 1), a slight decrease in phosphate would have been observed as the algal abundance decreased. However, exactly the opposite trend occurred; there was a considerable increase of inorganic phosphate parallel with algal cell numbers (Figure 10). As a consequence of nitrate uptake and phosphate input, N : P ratio in the water decreased from 4.4 to 0.7 (pre-monsoon till monsoon). Successful growth of certain euglenoids is influenced by reduction of flow and accumulation of organic matter<sup>36</sup>. Growth of *T. volvocina* was promoted by higher concentrations of nitrate and phosphate released after decomposition of organic waste<sup>36-38</sup>.

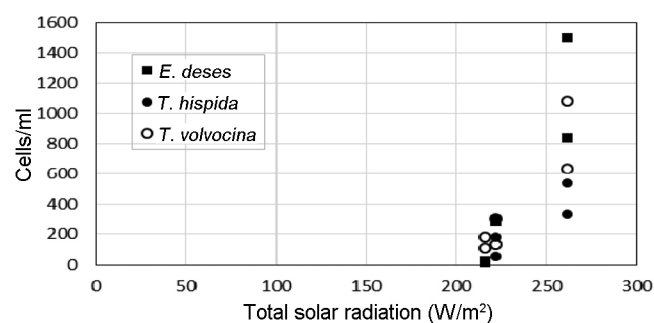
Most of the euglenoids utilize inorganic nitrogen and phosphate in the presence of sunlight (photosynthesis). However, overnight they are mixotrophic, ingesting organic particles (phagocytosis) or absorbing dissolved organic compounds. There is no interaction (CCA) between the leading species nos 1, 18 and 23 with either COD or BOD. So there is no justification that these species indicate 'organic pollution'. Moreover, BOD values lower than 10 mg  $\text{O}_2/\text{l}$  are largely dark oxygen respiration rates of micro-algal populations, rather than oxygen demand of heterotrophic (aerobic) microbes degrading organic pollution. The fact that BOD and phosphate ( $n = 6$ ;  $R^2 = 0.75$ ;  $P < 0.05$ ) were positively correlated does not imply that a high oxygen demand is related to phosphorus mineralization. It is more likely that an increase of phosphate in the water is a (combined) result of (a) input of P from the surrounding area (although unlikely in the dry pre-monsoon of 2013) and (b) release of P from the lake sediments under anaerobic conditions in the pore water but evidence for this cannot be provided in the present study. It can be concluded that the relative abundance of the leading species is positively correlated with phosphate in the lake water.

From the above it is clear that 'independent' (abiotic) environmental parameters used in CCA often depend on other variables. Likewise, biotic parameters (cell numbers) are not only dependent on 'environmental parameters'; biota interact with each other either via food webs (e.g. algae eaten by herbivores) or via competition. In CCA, *Lepocinclis horridus* and *L. ovum* var. *butschlii* seem to be negatively influenced by dissolved silicate, but there is no logical explanation for the same. However, they were more or less co-developing with *P. rotunda*;

there is no other explanation except that these three species are within the same niche. The number of *Euglenaria clepsydroides* from the Museum Lake was negatively influenced by rainfall, but also by *Trachelomonas caudata* (for unknown reasons). The CCA tri-plot shows fluctuations in cell number of *Phacus granum*, *Trachelomonas cylindrica*, *T. naviculiformis*, *T. raciborskii* and *T. superba* that were not influenced by any of the (measured) environmental variables. This may be due to selective grazing by 'herbivores'<sup>39,40</sup>. The present results are consistent with those of Zafar<sup>5</sup>, Barone and Flores<sup>41</sup>, Kim and Boo<sup>35,42</sup>. Euglenoids are cosmopolitans distributed over a wide range of environment especially in eutrophic waterbodies<sup>43</sup>. They respond to sudden changes in environmental conditions and therefore euglenoids are considered to be excellent bio-indicators. Because of their nutritional needs and their position at the base of the aquatic food web, phytoplankton indicators provide relatively unique information concerning ecosystem condition compared with commonly used animal indicators. However, information on unique indicators has to be clear in order to be useful. Three leading species were associated with a relatively dry season and hard phosphate-rich water, but in fact 'sunlight' may have also played a role. During pre-monsoon ( $261 \text{ W/m}^2$ ) solar radiation at the earth's surface is on average 20% higher than during monsoon ( $216 \text{ W/m}^2$ ) and post-monsoon ( $222 \text{ W/m}^2$ ). The



**Figure 10.** Plot of cell number of the three dominant euglenoids versus the inorganic phosphate concentration.



**Figure 11.** Cell number of the three dominant species versus 22-year average of seasonal (diurnal) solar radiation in Kerala (Source: NASA Atmospheric Science Data Center, USA).

seasonal pattern of cell numbers as in Figure 2 followed the daily dosage of solar energy and it is clear from Figure 11.

It is impossible to determine the effective water-column photosynthesis irradiance (400–700 nm) that these algae received (both during seasonal and diurnal cycle), since this irradiance depends on water transparency, light quality (absorption spectra), lake-water mixing (wind, thermal convection) and for (motile) euglenoids in particular, on the velocity of vertical phototaxis<sup>44</sup>. Considering this complexity, ‘light’ was not included as an independent parameter in the CCA. Still, periodic changes in solar radiation may interfere with the statistical analyses. In fact, a dry period with more sunlight may have favoured these dominant euglenoids, whereas inorganic phosphate was introduced in the system via external inputs or bottom processes. Phosphate was not a factor stimulating algal growth in this case, since it was available in excess. Here, ‘phosphate’ is considered to be a parameter indicating ‘pollution/eutrophication’; *E. deses*, *T. hispida* and *T. volvocina* are considered to be ‘guide’ species providing useful warning signals of deteriorating conditions in disturbed ecosystems. Among the pollution-tolerant algae<sup>45</sup>, *E. deses*, *P. pleuronectus* and *T. volvocina* also occur in the Museum Lake.

## Conclusion

This study summarizes the diversity and seasonal distribution of 23 euglenoid algae and the most influential environmental variables in the Museum Lake. It is clear that some of the independent environmental input variables in the CCA in fact influence each other. Some relationships between ‘abundance of algae’ and ‘environmental parameter’ cannot be explained without a closer look at direct causality between a number of species and some abiotic factor which was not studied in the present research. Further analyses were done with three leading euglenoids that may serve as potential bio-indicators. Species showed marked seasonal variation having higher density during summer season and lower density during rainy season. *E. deses*, *T. hispida* and *T. volvocina* occur during dry pre-monsoon season, have an affinity for relatively hard but slightly acidic waters, and also have an affinity for phosphate-rich water, which is in agreement with the observations of Rahman *et al.*<sup>46</sup>. They respond to changes in water quality (eutrophic state) of the lake. This study further confirms the importance of phytoplankton in bio-monitoring and also the importance of the still unexplored euglenoids from Kerala.

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