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# Free living protozoans as bioindicators in Vembanad lake, Kerala, India, an important Ramsar site

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## Abstract
The use of the free living protozoan communities has benefited in perfectly characterizing and monitoring the prevailing environmental conditions of aquatic habitats that are typically found at marginal freshwater regions. A particular community of organism may be useful as an environmental indicator due to many reasons. Some may have sensitivity to low levels of anthropogenic contaminants, yet some others may tolerate and survive in the hardy and extreme conditions, and others also may react quickly to change in environment. Thus they tend to become a unique biotic tool to understand the ecological status of an aquatic habitat. Vembanad Lake and its adjacent kol lands has acclaimed international recognition as a Ramsar site. This lake is a biological niche of a multitude of organisms and it is intricately woven with the lives of the resident communities of its banks. The lake has also been facing severe environmental crisis during the last 3 decades due to anthropogenic influences.
Presently, 19 species of free-living protozoans have been identified and characterized from this lake. A total of 15 testacid rhizopods belonging to 2 orders, 6 families and 9 genera were recorded. And the ciliates of 3 orders, 3 families were recorded. Among the testaceous rhizopods 1species from Arcellidae family, 5 from Centropyxidae, 1 species from Nebelidae, 6 from Difflugidae belonging to the Class Lobosea and 2 species from the Class Filosea belonging to Cyphoderiidae and Euglyphidae families were identified. Some of these freeliving forms have given certain insights of the prevailing ecological conditions of this lake thus acting as perfect Bioindicators. *Euglypha tuberculata* reported in the present study is a species of wide tolerance and survives in diverse habitats. Similarly *Cryptodifflugia oviformis* which was reported for the first time in India in this study prefers dryer environments. Due to its small size, this species mainly feeds on bacteria and yeasts, their high abundance explains active decomposition process in the area. The diversity of the free-living ciliates in the study area included species belonging to 3 genera namely Euplotes, Tachysoma and Coleps and they were pollution indicators possessing the property of heavy metal uptake. The water quality analysis and heavy metal analysis also proved the waters of the lake polluted with heavy metal concentrations. Thus the present study draws our attention to the possibility of using these dominant ciliate species for bioremediation of aquatic pollutants in this lake. Thus these freshwater free living protozoans serve as good bioindicators reflecting the natural ecological conditions prevailing in the Vembanad Lake. They can also be effective bioremediation tools that can be applied to solve the heavy metal pollution crisis of the lake.

**Keywords:** Marine ornamental fish, Parasites, Caligus, Aquaculture, Fish diseases.

## 1. Introduction
Lakes hold nearly 90% of the liquid surface freshwater on earth and are major regulators in global carbon, nitrogen and phosphorus cycles. They are important reservoirs for freshwater, excellent purifiers of the terrestrial wastes and zones for aquifer recharge that provide critical habitat to a number of fauna and flora (Laurie Duker and Lisa Borre, 2001) [1]. The Vembanad lake in Kerala, India fosters high biological diversity and has been providing hydrological and ecological services thus supporting livelihoods of a huge rural population.
It is a designated Ramsar site since 2002. The Lake which is spread across in three districts of Kerala – Ernakulam, Kottayam and Alappuzha – has a total surface area of 36,500 ha. It is a complex system of backwaters, marshes, lagoons, mangrove forests, reclaimed land and an intricate network of natural and manmade canals. Unique cultural traditions, water centered social institutions and lifestyles have evolved around the wetlands over time. The evolutionary history of this lake shows that this lake plays an important role in the ecology and economy of the south-west coast of India. The lake is presently undergoing severe environmental crisis due to the unregulated anthropogenic activities that are disrupting the natural balance of this rich

wetland system. Among the lacustrine ecosystems of India, Vembanad Lake has attracted most of the naturalists, limnologists and scientists and consequently has been subjected to intense biological research. The salinity gradient of the lake supports an assemblage of diversified fauna in accordance to their tolerance to saline environments.

Protozoans are common predators on bacteria and fungi (Hausmann *et al.,* 2003) [2], having the role of nutrient cycles (Mitchell *et al*., 2008) [3]. Protozoans feed on and regulate the abundance of most types of aquatic microbes, and they are an integral part of all aquatic microbial food webs. They also have a long history of use as indicators. By nature these occur in large numbers in a very limited sample volume and many biological indicators commonly used in monitoring and impact assessment studies are organisms like molluscs, polychaetes, bacteria that are logistically difficult to collect and expensive to analyse. The free-living protozoans in chief include rhizopods and ciliates that have a wider distribution in freshwater environments. Perfect characterization and monitoring of all aquatic environments typically found in marginal marine settings is possible with the use of these minute organisms. Though minute in size and apparently insignificant, they take on a wondrous variety of form and structure. Because of their small size, they are sometimes rated as least important, but they play a useful and important role in the biosphere. But their overall role may be considered as mixed as some are useful, others harmful and few others border lined.

The useful forms of the protozoans constitute important links in the food web, are employed very well in biological and medical research, act as indicators of pollution and petroleum deposits and they are above all good natural enemies of harmful bacteria, aiding in soil fertility. Many of the protozoans are harmful as they cause dreaded diseases in man and other organisms and interfere with the production of nitrate, thereby reducing soil fertility. They are considered to be the first animals to evolve and thus have special place in the evolutionary history of animals. Many scientists have been attracted towards protozoa during the last two decades and due to availability of fine tools and technology many facts about them have been revealed. This accelerated interest in these creatures has had a great bearing on their classification, which has undergone important changes in the present decade. The group protozoa have now been raised to the level of sub kingdom under the kingdom Protista.

## 2. Methods

Vembanad Lake lies 0.6-2.2 m below mean sea level (MSL) along the south-west coast of India (9035’N 76025’E of the Arabian Sea). The lake has a freshwater dominant southern zone and a salt-water dominant northern zone, both separated by a bund at Thanneermukkom where the lake has its minimum width. Since the entire Cochin estuary is a part of the Vembanad-Kol wetland system, the pollutants from the industrial area of Cochin are transferring towards the fresh water region of the Vembanad Lake (Harikumar *et al*., 2007) [4].

The collection of the free living protozoan’s was from Karaparambil site (figure 1) located in banks of Vembanad Lake. The collection was done periodically during June, July and August 2012. The water samples were collected during the early morning hours using 63 µm mesh sized plankton net. Water samples were brought to the laboratory in wide mouthed plastic bottles, their lids were removed and they were kept open in a place where adequate light is available which promotes the increase in the number of protozoans occurring in those samples. Rice bran was given as feed for these protozoans. Then the samples were thoroughly examined under the microscope from time to time. The free living ciliates were observed in both 10x and 40x magnification. Then different species were observed and photographs were taken and identified. Physical, chemical and biological parameters were analysed. Nutrients like nitrate, nitrite, sulphate, phosphate were analyzed in the water samples. These parameters were analysed by adopting relevant methods from APHA (1998) [5]. The air and water temperature readings were taken using an accurate centigrade thermometer. Dissolved oxygen content was analyzed according to classic Winkler’s method. Then the nutrients like nitrate, phosphate, nitrite, sulphate and chloride were analysed and their concentrations in the sample were determined using the method of APHA (1998).

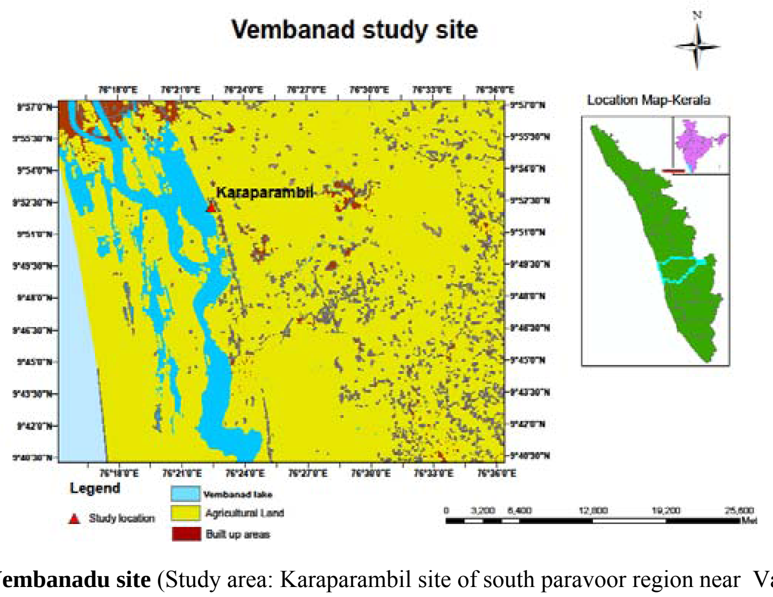


**Fig 1: Vembanadu site** (Study area: Karaparambil site of south paravoor region near Vaikom)

### 2.1 Heavy Metal Analysis

The digestion of water samples were done according to the EPA vigorous digestion method described by Gregg (1989) [6] and after digestion using conc. $HNO_3$, HCl and NaOH, the water samples were subjected to heavy metal analysis. A total of six metals were determined in the pre-treated water samples using Atomic absorption spectrophotometry as described by Gregg (1989). These include Cadmium, Chromium, Zinc, Lead, Nickel and Manganese.

**Fig 2: Station 1**: An area which was densely covered with the water hyacinth and at midst of anthropogenic activities

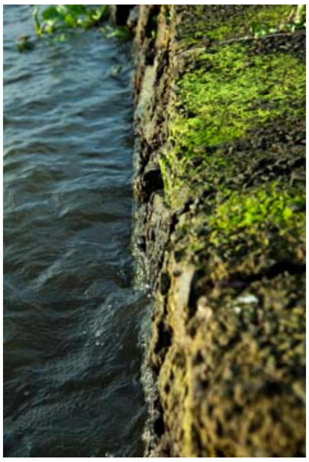

**Fig 3: Station 2:** An area where mosses grow densely attached to the boulders and the stone embankments

**Fig 4: Station 3:** A mangrove rich area

## 3. Results

In the present study 15 species of rhizopods and 3 species of ciliates have been recorded and among these two species of rhizopods from Vembanad lake *viz*., *Difflugia binucleata* (fig

5) and *Cryptodifflugia oviformis* (fig 6) have been recorded for the first time from India. All the species are first reports from this important water body. The occurrence of different species like *Difflugia*, *Arcella*, *Euplotes*, *Coleps* and *Tachysoma* clearly indicates the polluted nature of the Vembanad lake. The correlation of the results of water quality parameters including heavy metals from Vembanad lake with the occurrence of different rhizopods and ciliates confirm the polluted nature of the water body in certain study sites.

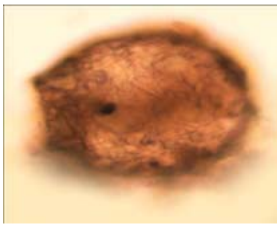

**Fig 5:** *Difflugia binucleata*

**Fig 6:** *Cryptodifflugia oviformis*

### I. On Site Parameters

**Table 1:** On site parameters

| Sl. No. | Parameters | Mean Value |
|---|---|---|
| 1. | Temperature($^0$C) | |
| | Air | 32 |
| | Water | 29 |
| 2. | Colour | Colourless |
| 3. | Odour | Unobjectionable |
| 4. | Taste | Tasteless |
| 5. | Foam/ Froth | Nil |

### II. Lab Parameters

**Table 2:** Water quality parameters (Lab parameters)

| Sl. No. | Parameters | Mean Value(Mg/L) | Standard Deviation |
|---|---|---|---|
| 1 | Do | 4.06 | ±1.427 |
| 2 | Bod | 1.5 | ±0.022 |
| 3 | Alkalinity | 125 | ±1.63 |
| 4 | Acidity | 25 | ±1.63 |
| 5 | Total Hardness | 44 | ±1.87 |
| 6 | $Ca^{2+}$ HARDNESS | 8.016 | ±0.2 |
| 7 | $Mg^{2+}$HARDNESS | 5.847 | ±0.083 |
| 8 | Chloride | 127.8 | ±0.081 |
| | **Nutrients** | | |
| 9 | Nitrate | 0.0315 | ±0.1 |
| 10 | Sulphate | 5.22 | ±0.026 |
| 11 | Nitrite | 0.160 | ±0.01 |
| 12 | Phosphate | 0.271 | ±0.01 |

(Heavy Metal Analysis)

### III. Heavy Metal Analysis

| Heavy Metals | Concentration (µg/L) |
|---|---|
| Cadmium | 1.012 |
| Chromium | 1.032 |
| Lead | 1.54 |
| Nickel | 0.42 |
| Zinc | 0.260 |
| Manganese | 0.739 |

**Table 3:** Heavy Metal Concentrations

| Sl. No | Systematic Position |
|---|---|
| | Phylum: Sarcomastigophora |
| | Class: Lobosea |
| | Order: Testacealobosa |
| | Family: Arcellidae |
| 1. | *Arcella discoides* Ehrenberg, 1843 |
| 2. | *Arcella hemispherica* perty 1852 |
| | Family: Centropyxidae |
| 3. | *Lesqueresia spiralis* (Ehrenberg, 1840) |
| 4. | *Centropyxis aculeata* (Ehrenberg, 1841) |
| 5. | *Centropyxis ecornis* (Ehrenberg, 1841) |
| 6. | *Centropyxis spinosa* (Cash and Hopkin son, 1905) |
| 7. | *Plagiopyxis declivis* Bonnet and Thomas, 1955 |
| | Family Difflugidae |
| 8. | *Difflugia corona* Wallich 1864 |
| 9. | *Difflugia globulosa* Dujardin, 1837 |
| 10. | *Difflugia binucleata* Penard, 1902 |
| 11. | *Difflugia lithophila* (Penard, 1902) |
| 12. | *Difflugia lobostoma* Leidy,1879 |
| 13. | *Cryptodifflugia oviformis* Penard, 1902 |
| | Family Nebelidae |
| 14. | *Nebela collaris* (Ehrenberg,1848) |
| | Class: Filosea |
| | Order: Testaceafilosa |
| | Family: Euglyphidae |
| 15. | *Euglypha tuberculata* Dujardin, 1841 |
| | Phylum Ciliophora |
| | Class Prostomatea |
| | Order Prorodontida |
| | Family Colepidae |
| 16. | *Coleps hirtus* Muller, 1786 |
| | Class Polyhymenophorea |
| | Order Hypotricida |
| | Family Euplotidae |
| 17. | *Euplotes sp.* |
| | Class Spirotrichea |
| | Order Sporadotrichida |
| | Family Oxytrichidae |
| 18. | *Tachysoma spp.* |

## 4. Discussion

An area which is unexplored is where science brings out wonderous facts to light, thus freeliving forms of protozoans in two wetlands were studied. The present study yielded useful results regarding the testate amoebae/ rhizopods and the ciliates that are highly involved in the pollution control of wetland ecosystems. A total of 15 testacid rhizopods belonging to 2 orders, 6 families and 10 genera were recorded during the study and the ciliates of 3 orders and 3 families were recorded. The present work is the first detailed study on the free-living protozoans of Vembanad lake although Zoological Survey of India had conducted a detailed survey of the fauna of Vembanad Lake during 2002 -2003 and the present work has yielded two new records of testate amoebae

*Difflugia binucleata* and *Cryptodifflugia oviformis* for the first time from India. The study also concentrates on the ability of protozoans in bio monitoring and pollution control. The physicochemical parameters and heavy metal content of Vembanad Lake strongly suggests the high rate of domestic and industrial pollution. Despite the important role in the food chain of wetland ecosystems, studies on the free-living protozoan fauna from various wetlands in India are meagre. Importance of protozoa as bioindicators of pollution and environmental bio monitoring has been recognized since long particularly in water purification plants and activated sludge processes (Kelkwitz and Marson, 1908) [7].

A total of 1567 species of free living protozoa have been recorded from India including the estuarine, marine and moss dwelling forms (Bindu, 2010) [8]. About 37 species of foraminiferans and 1 species of arcellinid rhizopod belonging to the phylum Sarcomastigophora have so far been recorded from Vembanad lake (Antony, 1975). From the present study certain Testaceous amoebae of paleontological significance were identified. The *Euglypha tuberculata* (fig 7) in the present study, which according to Dujardin (1841) [9] is a species of wide tolerance and survives in diverse habitats. Similarly *Cryptodifflugia oviformis* which was reported for the first time in India in this study prefers dryer environments. Due to its small size, this species mainly feeds on bacteria and yeasts, their high abundance explains active decomposition process in the area. The short generation time and wide distribution of testate amoebae makes these Rhizopods good indicators for the monitoring of environmental change (Chiverrell, 2001; Charman *et al*., 2004) [10]. Generally Ciliates are found in the polluted waters containing less than 10 µgm/mL concentrations of toxic metal ions (Shakoori *et al*., 2004) [11] and present results of heavy metal content supports this finding in Vembanad lake. Ciliates have a most significant role in water purification system. They regulate the bacterial population and control the BOD levels (Curds, 1973) [12], control pathogenic and faecal bacteria, release mucous substances to facilitate flake formation and successive sedimentation. It is quite evident that *Euplotes spp.* (fig 8 ) and *Tachysoma* spp. (fig 9) reported in the present study from Vembanad lake possessing heavy metal uptake properties and can be used in bioremediation of industrial wastewater. *Euplotes mutabilis*, a ciliated protozoan isolated from heavy metals laden industrial wastewater has been shown to tolerate multiple heavy metals, thus suggesting its significance in bioremediation of industrial effluents. The metal removal efficiency of *Euplotes mutabilis* is greater than 80% in metal contaminated waste waters (Rehman *et al*., 2006, 2008) [13, 14] and these ciliates are excellent and convenient bioindicators for evaluating the toxicity of waste waters polluted by heavy metals (Madoni and Romeo, 2006) [15]. The long-term survival of protozoa in media containing relatively high concentration of heavy metal ions show that these organisms have evolved strategies to tolerate, resist or detoxicate organic substances and heavy metals (Haq *et al*., 2000) [16]. The presence of the ciliates *Euplotes* and *Tachysoma* species in the Vembanad lake strongly indicates that the heavy metal contamination could be tackled and the possible bioremediation of the water of Vembanad Lake by applying these indicator species in pollution control is possible in the near future. The entire complex of purification plants can make use of these protozoans in order to reduce the pollutant level in Vembanad Lake.

**Fig 7:** *Euglypha tuberculata*

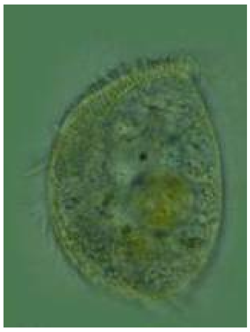

**Fig 8:** *Euplotes spp.*

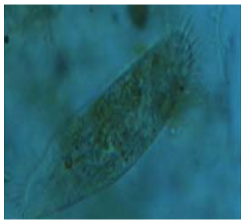

**Fig 9:** *Tachysoma spp.*

## 5. Conclusion
The free-living protozoans are effective bioindicators that are found in almost all fresh water bodies where they multiply in large numbers and thus are adapting to the changes in an aquatic body. With them the status of a lake's environment can be detected in a much cost efficient manner. They can be monitored well and understanding the ecological processes with the help of these microscopic cousins is very helpful in safeguarding the diseases. Since they are efficient in heavy metal uptake, can be used as tools of bioremediation of aquatic pollutants.

## 6. Acknowledgement
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