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Free-Living Ciliates in the Kolleru Lake, Andhra Pradesh

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Abstract: *Water has curious and unusual properties, and plays an important role in living systems. Thus, "**No life without water**" is a common saying. Microorganisms get into natural waters from air, soil, sewage, organic wastes, dead plants and animals, etc. Thus almost any type of organisms may be found in water. For the present study water samples were collected regularly for a period of one year at random from the Kolleru Lake. Ciliates are well-known as water pollution indicators and the presence or absence of some ciliates can be interconnected to specific environmental surroundings. Growing environmental population and constant growth of new chemicals and drugs has led to ever-growing concern about the long-suffering effects of these compounds directly or indirectly on human health as concerns water pollution. Protozoan implies to be an excel tool to evaluate both toxicity and pollution. The present study is a great attention on the reuse of pollution. So, outcomes of the study can support to improve a better understanding of reuse alternatives for treated effluent and preparation of proper water resources management plants.*

Keywords: *Microorganisms, Kolleru Lake, Protozoan, Ecosystems, Ciliates, Toxicity, & Pollution Indicators.*

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I. Introduction

Freshwater Source

Freshwater lakes remain abundant economic, ecological and cultural significance, through billions of people depend upon directly on lakes for drinking water, food and their livelihood. Lakes have more complicated and delicate ecosystems than rivers, as they do not have a self-cleaning tendency. More than half the world's five million lakes and reservoirs face huge ecological threats that are endangering the global environment, experts have warned Chourey (2001).

India is no exception to the global scenario. Indian wetlands are not only the home of a wide variety of plants and animals but they also provide livelihood to thousands of communities with a wide spread of ecological services. Even though all these benefits from the wetlands, they have been mistreated and are habitually ignored. Wetlands suffer from over-exploitation, overuse of their resources, drainage, unconventional use and pollution. The Wildlife Institute of India's survey expose that at present, only 50% of India's wetlands remain. They are disappearing at a rate of 2% to 3% every year. The damage of one km^2 of wetlands in India will have much bigger impact than the damage of one km^2 of wetlands in low population areas of plentiful wetlands SACON, (2005a).

Lakes are responsible for humankind by numerous services: water used for the purpose of drinking, irrigation, fish, and dilution of pollutants (Postel and Carpenter, 1997). These facilities are decreased by mistreatment of lakes and their catchments lands. The objective of management should be to balance the usages of lakes with conservation methods to sustain ecosystem facilities over time, and keep the interests of the native sustenance communities. Focused research can provide understanding of lakes' ecological mechanisms that sustain ecosystem services; the reasons of dreadful conditions of lakes and their catchments, and can responsible for approaches technologies for lake restoration (Carpenter and Lathrop, 1999).

II. Experimental Methods:

STUDY AREA:

Kolleru Lake is the largest freshwater lake and is located in Andhra Pradesh. Kolleru is located between Krishna and Godavari delta and covers an area of 308 km². The lake helps as a natural flood-balancing reservoir for these two rivers. The lake is nourished directly by water from the seasonal Budameru and Tammileru streams, and is connected to the Krishna and Godavari systems by over 68 inflowing drains and channels. It provides as a habitat for migratory birds. It supports the livelihood of fishermen and riparian population in the region. The lake was notified as a wildlife sanctuary in November 1999 under India's Wild

Life (Protection) Act, 1972, and designated a wetland of international attention in November 2002 under the international Ramsar Convention. Thousands of fish tanks were excavated inside the wetland transforming the lake into a mere drain. Apart from this the farmers had transformed the land use pattern of the lake. This had a lot of impact in terms of pollution leading to even difficulty in getting drinking water for the local people. The total area of the lake converted to aquaculture ponds accounts for 99.73km2 in 2004 in comparison to 29.95km2 in 1967. The area under agricultural practice in the wetland also increased from 8.40 km2 in 1967 to 16.62km2 in 2004.

Figure 1: Map of India Showing Andhra Pradesh State and Kolleru Lake

Figure 2: Image of Sampling Station (Kolleru Lake)

III. Material s and Methods:

Sample collection and implemented protocol for growing anaerobic ciliates

Water samples were collected from Kolleru Lake by collecting water samples once in a month for the period of one year during the study seasons from January 2019 to December 2019. The sample was collected in the morning hours between 7.00 am to 8.00 am in pre-sterilized polypropylene bottles of one liter capacity. The collected water samples were preserved in the icebox and transported to the laboratory within 2 hours for further analysis and the samples were preserved at 22^{O} C -24^{O} C in the laboratory.

Live Cell Observations:

Cells were picked out from cultures with the help of a micropipette while observing them with the help of the stereo zoom microscope and transferred onto a clean slide. A thin film of Vaseline petroleum jelly was

applied on each edge of a cover slip. Keeping the cells in the minimal culture fluid, a cover slip was gently placed with the Vaseline-smeared edge down the face on them. The Vaseline film raises the cover slips just enough to provide sufficient space between the cover slip and the slide allowing the cells to remain functionally viable but arrest their movement. By this technique , live cells can be preserved for few hours, letting observation and for capturing images . Live cell observations were made using Axio Cam ERC 5s microscope.

Different types of ciliates isolated were preserved as clone cultures in their own living culture collection and examined while alive as well as on fixed material stained by Feulgen staining and impregnated with silver nitrate. I have kept successfully genus *Blepharisma*, *Frontonia* and *Stentor* . For each genus the important morphological features were observed for at least 15-20 cells.

In-vitro Culture:

Water samples were collected from the Kolleru Lake from the period January 2019 to December 2019. Identification of the freshwater ciliates isolated from the sample was done in-vivo under the Stereoscopic Microscope. Collected water sample were placed in Petri dishes and observed under a stereo zoom microscope in order to detect organisms belonging to the genus and divide them according to their main morphotype. The resulting populations were then maintained at 18 - 20° C in their original medium, periodically en riched with rice grains, modified Cerophyl medium [4] inoculated with *Roultella planticola* (Gamma proteo bacteria).

The monoclonal cultures were acquired by isolating single cells from the original populations. These cells were briefly washed for several times in sterile distilled water. Clonal cultures of *Blepharisma, Frontonia and Stentor* species was maintained in the laboratory at 22-24°C in a medium made of hay infusion, Cerophyl, Na_2HPO_4, and Stigma sterol and distilled water inoculated with *Roultella planticola* was added to the medium to promote the growth of bacteria which served as the primary food source for the ciliates. The green algae *Dunaliella tertiolecta* was employed as food for ciliates.

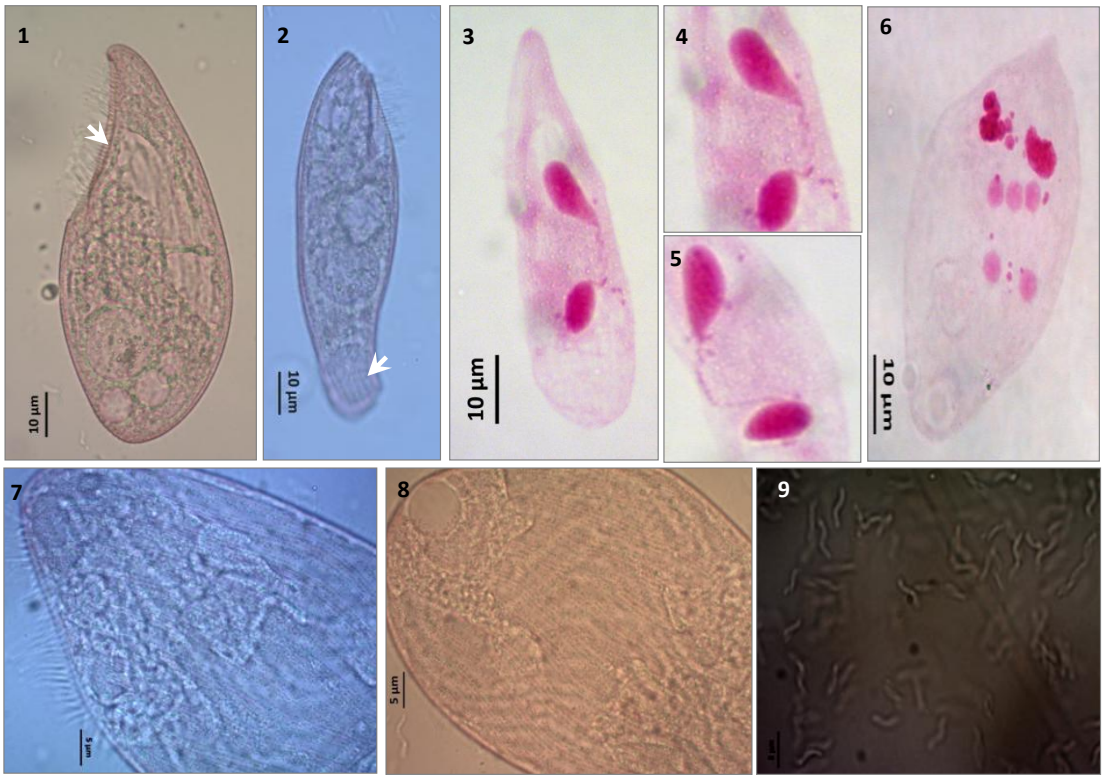
Morphological study was done for ciliate **c**ells which were picked from monoclonal culture were harvested from the culture medium and observed with an Axio Cam ERC 5s microscope equipped with a digital camera, Carl Zeiss. Length measures, on both living and fixed cells, were taken on collected pictures with the software magnification.

Figure 3: Ciliates from Monocultures and Stereoscopic Microscope

IV. Results

Genus: *Blepharisma* (Perty, 1852)

The genus *Blepharisma* created by Perty, has been the subject of a number of investigations since its first description in detail by Stein. Perty in 1849 gave the following diagnostic characters for *Blepharisma* : "Body flat, lancet-shaped, pointed posteriorly ending anteriorly in a short snout; the deeply incised region extending from the anterior end to about the middle of the body is provided with a row of long straight cilia arranged in parallel lines. 'Molecular rows' bearing extremely fine cilia, difficult to see are borne by the remainder of the body".



Morphology of *Blepharisma sps.* Fig.(1 – 2, 7 – 9) in vivo. Fig.1 Arrow marks the undulating membrane; Fig.2. Arrow shows the contractile vacuole (CV). Fig. 3 – 6 Feulgen stained cells; Fig.3 – 5 shows the macronucleus and 4 – 6 micronuclei; Fig. 6. Shows the macronucleus attaining the condensed condition; Fig.7. shows the cilia at peristome; Fig.8. Shows the cortical granule stripes at posterior end; Fig. 9. Shows the rod shaped bacteria eat by Blepharisma.

The taxonomy of the genus *Blepharisma* has undergone several taxonomical revisions starting with Kahl (1932) then Bhandary (1962), Hirshfield *et al.* (1965, 1973) and finally Repak *et al.* (1977). In India, Bhatia was the first to report the ciliate. He described the macronucleus of *Blepharisma* species as rounded, oval, bipartite or moniliform. Apart from Bhatia's reference three other Indian forms have been recently described. One of them was studied by Padmavathi and the other by Bhandary, Seshachar and Bhandary.

Blepharisma is a typical rose-colored ciliate, oviform shaped body. Live cells length range from 75 X 170 µm, width 35 X 48 µm. Macronucleus possesses two nodes and connected by a very thin strand; total length is 35 – 45 µm. Each macronuclear bead length 9 – 17 µm, width 4.5 – 7.5 µm. Distance between two macronuclear nodules 4 – 9.8 µm. 8 – 12 micronuclei, diameter ranges from 0.6 – 1.2 µm. During binary fission, condensation of macronucleus was noticed. (Fig.6) Single contractile vacuole, often quite large, located at the posterior end, diameter ranges from 11 - 18 µm. Peristome extended to 1/3 of the body.
Blepharisma possess an extensive series of membranelles on the left side of the oral groove, and a very prominent "undulating membrane" on the right side of the peristome, in the direction of the posterior. Somatic ciliature is uniform and generally arrange longitudinally. 10 cortical granular stripes, within each stripe 9 cortical granular rows are present. Swims to the left (anticlockwise direction). *Blepharisma* be able to reproduce by binary fission, dividing transversally. They as well engage in a type of sexual reproduction known as ciliate conjugation, a process during which genetic material can pass directly from one cell to the other.

Genus: *Frontonia :*

Frontonia ciliates are frequently found in aquatic (marine, brackish and freshwater) habitats (Al-Rasheid 1999; Carey 1992; Fan et al. 2011a, 2012; Long et al. 2008) and many species have been described using live observation and silver impregnation methods . *Frontonia species* have usually been distinguished from each other by the arrangement of the following characteristics: their body shape and size, the number and location of their contractile vacuoles, the morphology of their oral apparatus, and their general somatic ciliary pattern (Corliss 1979; Dragesco 1960; Dragesco & Dragesco-Kernéis 1986; Foissner 1994; Roque & de Puytorac 1972). Many Oligohymenophorea species are inadequately investigated in respect to current

taxonomic criteria; that is, they are poorly defined and described and, often, lack a statement of the type material (Burkovsky 1970; Fan et al. 2011b, 2011c; Long et al. 2005; Pan et al. 2011, 2013a, 2013b, 2013c; Petz et al. 1995; Roque & Puytorac 1972). *Frontonia* are large sized freshwater forms. *Frontonia are* typically not bacterivorous, but flourish on chrysophytes, cryptophytes, chlorophytes, diatoms and even testate amoebae (Dias & D' Agosto, 2006; Skogstad et al., 1987).

Size in-vivo about 200 – 380 µm in length, 110 – 130 µm width. Body shape long, ellipsoidal in outline. Slightly flattened at dorso-ventrally. Single, elongated-elliptical, macronucleus 35 – 55 X 20 – 35 µm, positioned at the center of the body. Micronucleus single/ two/ many? Closely associated with macronucleus, oval, about 2 – 2.8 µm diameter. One contractile vacuole without collecting canals located in the posterior of the body. Cytoplasm slightly greyish, brown colored food vacuoles with 20 – 28µm diameter, and 10 – 38µm green color globules. Crystal granules distributed randomly in cytoplasm. Extrusomes (trichocysts) long, spindle shaped (fusiform), about 4 – 8 µm densely arranged beneath the pellicle. Oral apparatus situated ¾ of the body. Locomotion frequently by gliding backward and forward on substrate or by swimming, moderately and swiftly while rotating clockwise about long body axis.

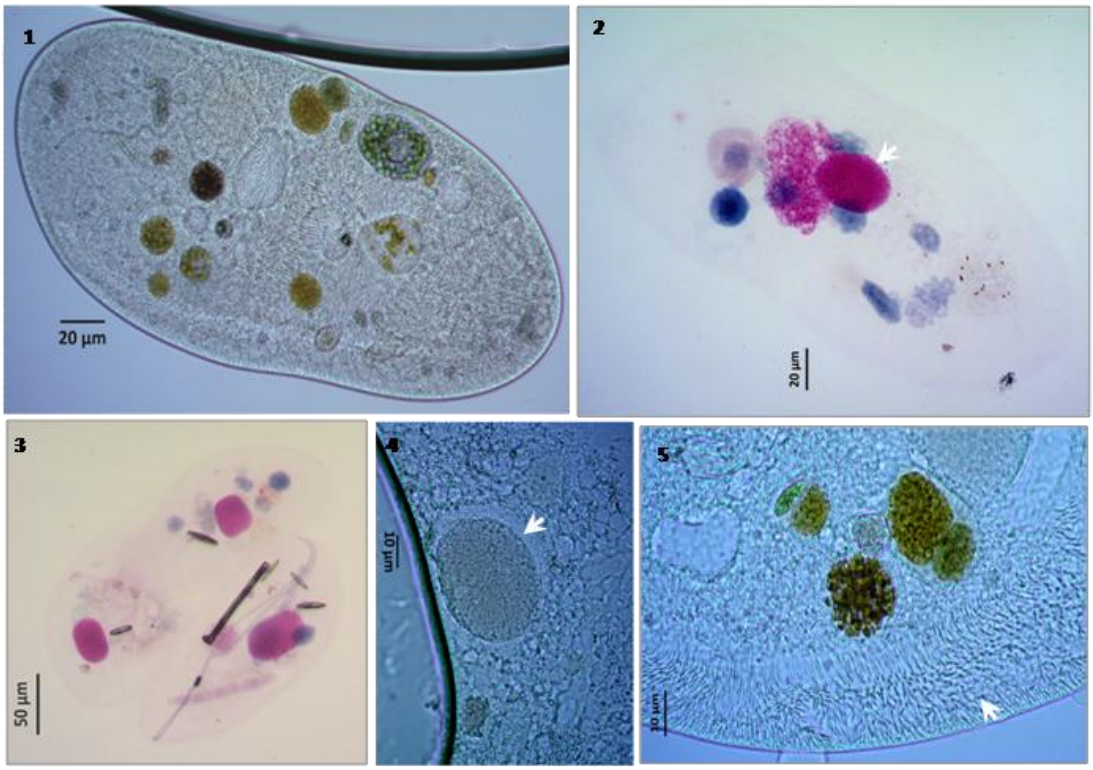


Fig.1. *Frontonia in*-vivo; Fig.2. Arrow marks the macronucleus of a Feulgen stained cell; Fig.3. Shows the macronucleus of three Feulgen stained cells; Fig.4. Arrow marks the macronucleus of live cell; Fig.5. Arrow marks the extrusomes (trichocysts). Scale bars = 50 µm (3). Scale bars = 20 µm (1, 2). Scale bars = 10 µm (4, 5).

Genus *Stentor* (Oken, 1815)

Abraham Trembley, who thought it was a type of hydra, first identified *Stentor* in 1744; but actually *Stentor* is a member of the Ciliate phylum in the class Heterotrichae. The name *Stentor* is a reference to its trumpet shape and the herald in Greek mythology known for having a loud voice. Faure-Fremiet *et al*. (1956) and Faure-Fremiet & Rouiller (1958) were the first to study the electron-microscope appearance of *Stentor*. *Stentor* is basically sessile and trumpet – shaped. *Stentor* is the "Majestic king of the ciliates" Tartar (1961). *Stentor* may be described as a carnivorous protozoan, its diet consisting mainly of small ciliates and flagellates, though bacteria may also be ingested and utilized. Previous investigations into energy intake and utilization in Protozoa have been devoted to bacterial or fungal feeding species (Heal, 1967a; Curds and Cockburn, 1971; Laybourn and Stewart, 1975).

Stentor is most famous for its amazing regenerative abilities. Even if a single cell is cut into multiple small fragments, each fragment will generate into a normal-looking cell. The fact that a single cell could rebuild its complex anatomy while displaying many of the same developmental processes as animal embryos, including axiation and induction, grabbed the attention of the leading embryologists around 1900, including Balbiani, F.R.

Lillie, and even Thomas Hunt Morgan. Following the lead of these early luminaries, Vance Tartar and Noël De Terra developed an astounding variety. References for species identification: Foissner et al. (1992), Kahl (1932). Size in vivo about 500 – 700 µm. Highly contractile, globular to pyriform shape, slenderly trumpet – shaped when elongated. Cells brown in color. Red to brownish colour cytoplasmic granules. Macronucleus vermiform to slightly nodular, Nucleus beads 11 – 16. One contractile vacuole present at the right side in the posterior position.

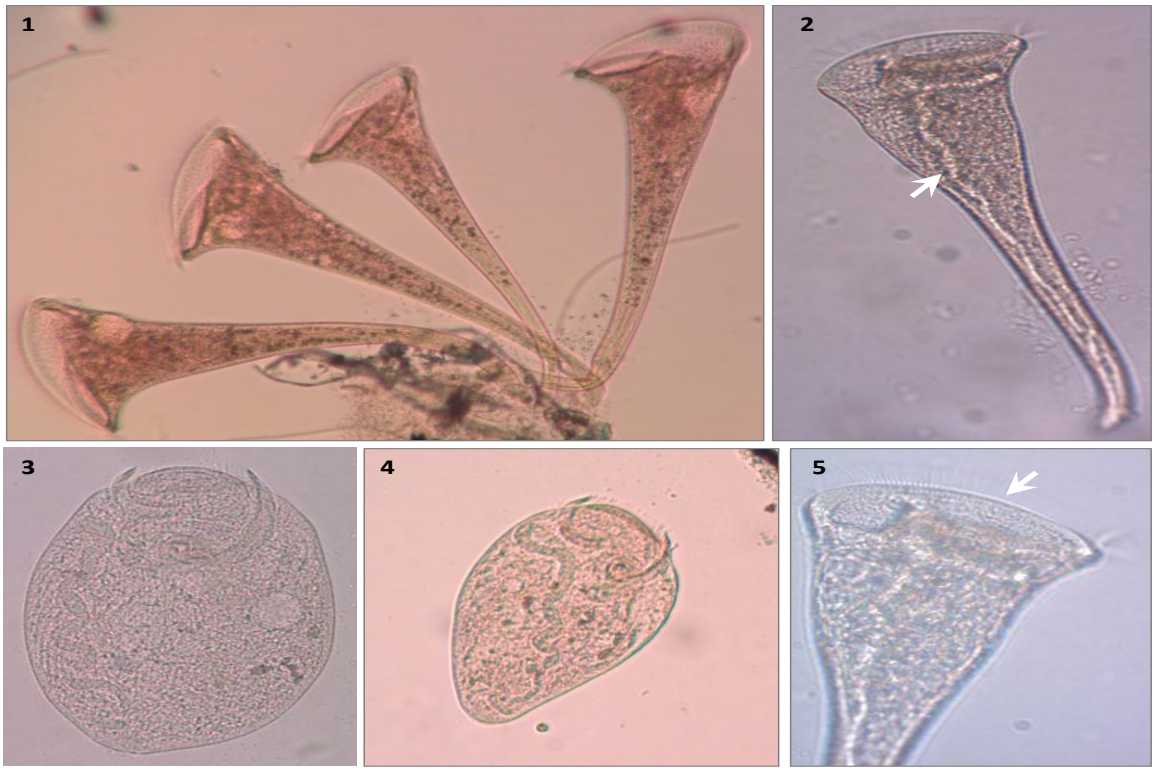


Fig. 1 – 5 *Stentor* in – vivo. Fig.1. *Stentor* (4cells) attached to the substratum;
Fig.2. Arrow head shows the macronucleus of a live cell; Fig.3 & 4 Contracted forms of *Stentor* Fig.5. Arrow marks the adoral zone of membranelles (AZM).
Stalk like posterior region of the body attached to the substratum, they are broadly to slenderly trumpet-shaped, and rotates any direction to feed. Filter feeding manner. When they swim on the surface they are contracted and form into turbinate to globular shape.

V. Conclusions

Protozoans have recognized to be an excellent tool for assessing the occurrence of the pollution and most of the ciliate species in lake be either primarily or solely bacterivores feeding on a good form of microorganism. Numerous scientific studies are created on the impact of various microorganism diets on the speed of procreation. Much abundant has been written on the ecological role that ciliates fulfil within the earth. More studies on this subject particularly aimed to collecting the data relating the effects of toxicants on this community [8].

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