ECOLOGY OF CILIATES IN THE KONDAKARLA AVA LAKE OF ANDHRA PRADESH

Jyothula. Rambabu*[1] Gollu Srinivasa Rao[2], Subrahmanyam.Ch[3], S. Krishnakanth [4] and G. Simhachalam[1]

1. Department of Zoology and Aquaculture , Acharya Nagarjuna University, Guntur , A.P. India
2. Lecturer in Zoology, SN Govt. Junior college, Chebrole, Guntur, A.P., India
3. Department of Zoology, Andhra University, Vishakhapatnam , A.P. India,
4. Lecturer in Zoology, SRR Govt. Junior college, Vijayawada, A.P., India.

ABSTRACT:

Protozoan's are one of the most common components in these man-made ecosystems and play an important role in the water purification process. Protozoans are responsible for improving the quality of the effluent maintenance and also to maintain the dispersed of bacterial populations by predation. Ciliates are well-known as water pollution indicators and the presence or absence of some ciliates can be related to particular environmental conditions. Increasing environmental population and continuous development of new chemicals and drugs has led to ever-growing concern about the potential effects of these compounds directly or indirectly on human health as concerns water pollution. Protozoan seems to be an excel tool to assess both toxicity and pollution. In the lake the current study is a focus on the reuse of pollution. So, findings of the study can help to build up a better understanding of reuse options for treated effluent and preparation of appropriate water resources management plants.

KEYWORDS: Protozoa, Ecosystems, Ciliates, Toxicity, & Pollution Indicators.

1. 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).

2. EXPERIMENTAL METHODS

Study Area:

Kondakarla Ava wetland, a natural freshwater lake (stretches between latitudes 17º35'30" and 17º 36'02" N and longitudes between 82º 59'27" and 83º 01'02" E) of Visakhapatnam district, Andhra Pradesh, India. It is the second largest natural fresh water lake in Andhra Pradesh, is 50 km south west of Visakhapatnam, a port city on the East Coast of India, which is the second largest city in the state of Andhra Pradesh. The Kondakarla Ava lake is a part of the Sarada riverine system and is classified as a perennial, warm, polymitic, euphotic, eutropic shallow fresh water lentic body. The wetland is named after a village, "Kondakarla", abutting the lake.

For the natives, the wetland is a livelihood source, while for the nearby towns and city people it is a getaway famous for avian diversity. During the British period, Kondakarla Ava Lake (wetland) was a famous tourist place and the Britishers used the place to hunt birds. The Raja (King) of Vizianagaram had built a rest house here, which is now the Zilla Parishad Bungalow.

Figure 1: Figure Showing geographical location of Kondakarla Ava Lake (Visakhapatnam District)

Figure 2:Figure of the Sampling Site, Kondakarla Ava Lake (Visakhapatnam district)

2. Material s and Methods:

Sample collection and implemented protocol for growing anaerobic ciliates

Kondakarla Ava, the second largest (fresh water) lake in Andhrapradesh was chosen for the present study. Water samples were collected from Kondakarla Ava lake by collecting water samples once in a month for the period of one year period 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 samples 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 *Spirostomum, Euplotes and Tetrahymena* . For each genus the important morphological features were observed for at least 15-20 cells.

In-vitro Culture:

Water samples were collected from the Kondakarla Ava 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 *Spirostomum, Euplotes* and *Tetrahymena* species were 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.

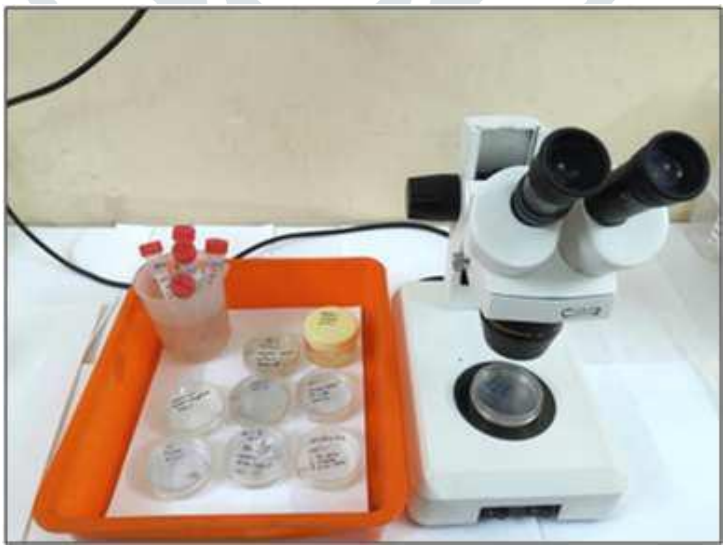

Figure 3: Ciliates Cells of Monocultures and Stereoscopic Microscope

3. RESULTS and DISCUSSION

Genus: *Spirostomum :*

Spirostomum Ehrenberg, 1838 are conspicuous ciliates protists that are easily recognized by their large sizes (500-1000 µm) and elongate bodies, being easily confounded with small helminthes. The name *Spirostomum* refers to the ability these ciliates have to contract in a spiral mode. This type of contraction is due to the presence of post-ciliary, sub-pellicular fibers that arise on the anterior end and spiral in a counter clockwise direction toward the posterior end of the body (ISHIDA *et al.* 1988).

Spirostomum sps. have also been used in studies of environmental impacts because they are considered good indicators of water quality (FOISSNER *et al.* 1987, BERGER *et al.* 1997, BERGER & FOISSNER 2003) and show sensitivity to certain toxic substances (e.g. heavy metals like nickel, copper, mercury, and zinc; the phenol Na-PCP) (MADONI *et al.* 1992, MADONI 2000). Live cells about 300 – 500 X 35 – 60 µm in width, dark brown in colour. *Spirostomum sps.* is a freshwater ciliate with a slightly tapering posterior end. Mouth (Peristome) occupies ½ of the body length. Single contractile vacuole round in shape, located at the posterior end. Homogeneous or heterogeneous Cortical granular rows, variable in number per stripe (2-5). CG stripes that run parallel to the main body axis. 15-20 stripes are noted. Moniliform macronucleus with 8-25 nodules, nodules length 5 – 10 µm, width 3.8 – 5.6 µm when stained by Feulgen reaction. Micronuclei variable in number up to 4 - 10, diameter 1.5 – 3 µm, near or overlapping the macronucleus. Cytoplasm yellow-brown in colour. *Spirostomum sps.* Moved by gliding slowly over the substrate or by swimming freely.

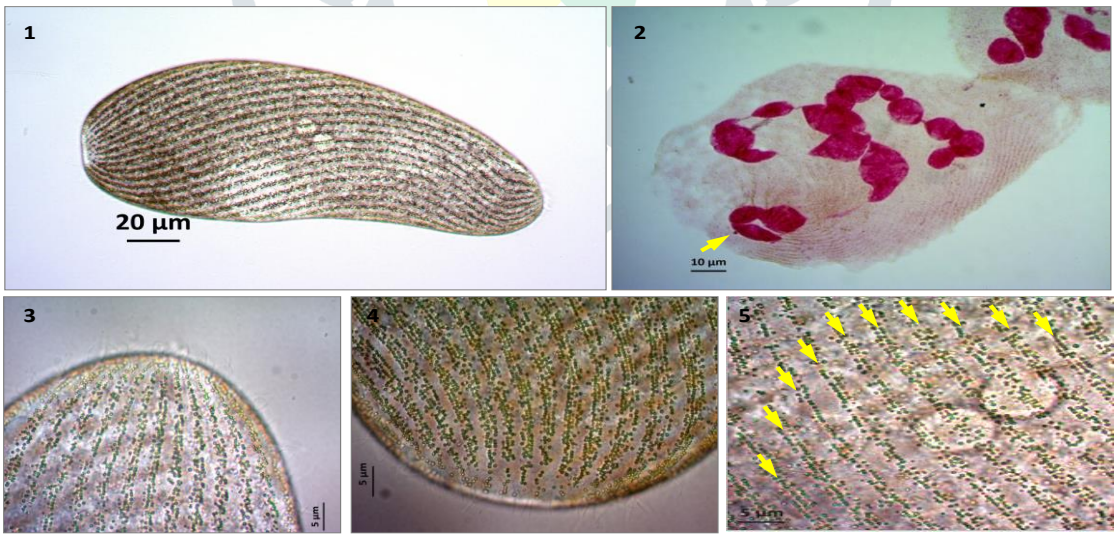

FIGURE:4

Fig.4.1.*Spirostomum sps.* in-vivo. Fig.4.2. Arrow marks the micronucleus, the macro nuclear beads of cells stained with Feulgen. Fig.4.3 – 4.4. Cortical granular stripes at the anterior and posterior region. Fig 4.5. Arrowheads mark the interkinetal cortical granules (CG) stripes consisting of several CG rows. Scale bars = 20 µm (1), Scale bars = 10 µm (2), Scale bars = 5 µm (3 – 5).

3.2 Genus : *Euplotes* :

Euplotes is a relatively large fresh-water Euplotes. Since the original description of *Euplotes aediculatus* by Pierson (1943), two authors have been described this species, but have identified it as Euplotes eurystomus. These include reports by Tuffrau (1960) and Carter (1972). Pierson (1943) clarified the taxonomic identification of this species and *Euplotes eurystomus*. Reference of the first description: Pierson, 1943. Size in vivo up to length 47 X 70 µm, body width 27 X 38µm.

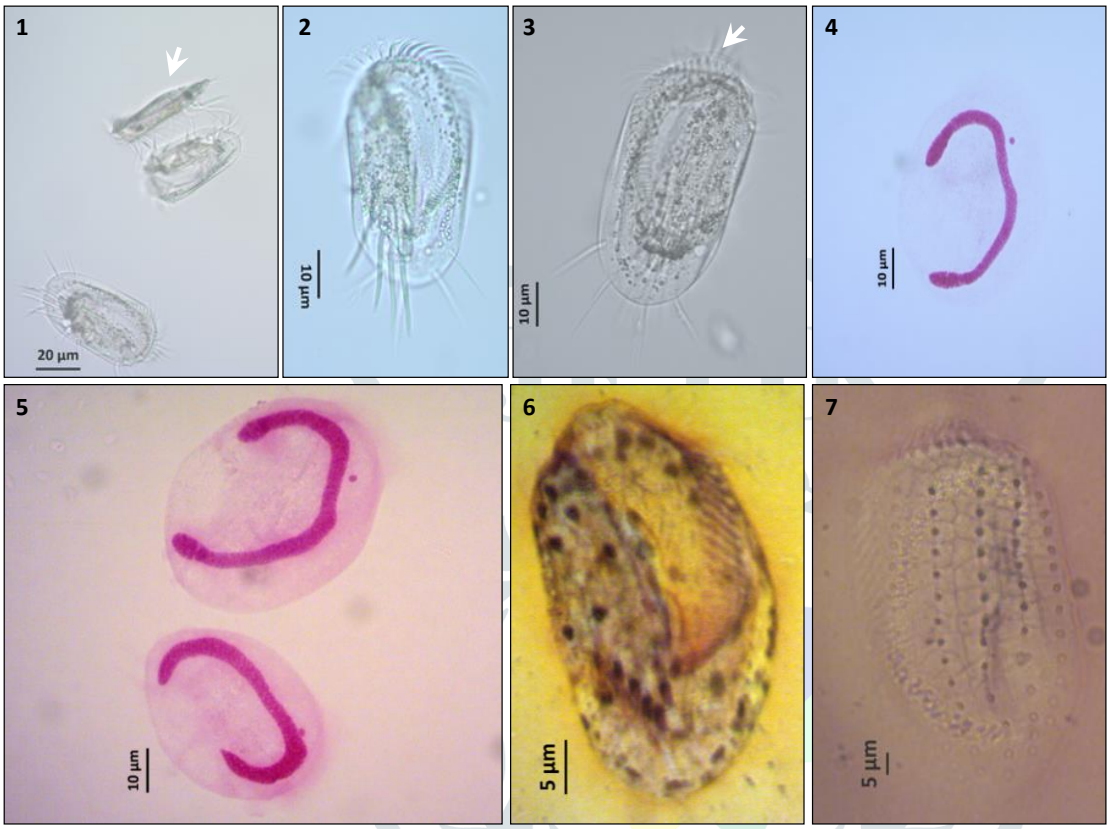

FIGURE.5

Fig.5.1. *Euplotes sps.* in-vivo: Arrow marks the lateral view of the cell; Fig.5.2. Ventral view; Fig.5.3. Arrow marks the collar at dorsal view; Fig.5.4. Feulgen stained 'C' shaped macronucleus and micronucleus; Fig.5.5. Feulgen stained 'C' shaped macronucleus and micronucleus of two cells; Fig. 5.6 –5. 7. Euplotes *sps.* Silver nitrate staining ventral view and dorsal view.

Euplotes *sps.* Scale bars 20 µm = (1). Scale bars 10 µm = (2, 3, 4, 5). Scale bars 5 µm = (6, 7). Body with an elongated ellipsoidal body. The anterior end is nearly straight with prominent shoulders along both right and left margins. The posterior end is rounded. The buccal cavity is widest anteriorly, extending in a deep triangular depression three fifths the length of the body. Adoral zone of membrane occupies ¾ of the body. Macronucleus "C" shaped and its length 70 – 80 µm. One micronucleus is nearly spherical and located in the upper right third of the cell, its diameter ranges from 1.5 – 2.2 µm when stained by Feulgen reaction. Caudal cirri length 15 – 18 µm. In wet-silver nitrate preparations, there are nine front ventral, five transverse, two left marginal, and two right caudal cirri.

Longitudinal interlineal argentophilic lines are equidistant between the kineties and are connected by transverse lines, forming a dorsal meshwork defined as the double silver line system (eurystomus type).

Large polygonal areas delimited by argentophilic lines describe the ventral meshwork. The contractile vacuole pore lies to the right of and posterior to the transverse cirri.

3.3 Genus: *Tetrahymena:*

Tetrahymena is a genus of small ciliates which were previously studied by the names of *Leucophyres* and *Glaucoma*. Most of the *Tetrahymena* species are cosmopolitan in distribution. They have eight ciliated membranous structures including four oral, one undulating and three adoral membranelles. Macronuclei of all the members of genus *Tetrahymena* are transcriptionally active (Simon *et al.*, 1979). *Tetrahymena* species rapidly grow in axenic medium and thus become a model organism for research in physiology and biochemistry (Ye AJ et. al 2002). *Tetrahymena* show closer genetic resemblance to human as compared to yeast model and share a higher degree of functional conservation to human genes (Ethuin P *et al.*, 1995). This is a valid point to use *Tetrahymena* instead of other organisms for ecotoxicological studies (Martin-Gonzalez *et al.*, 1999).

Tetrahymena, a free-living ciliate considered to be the most highly developed protozoan, because it possesses specialised organelles that perform each of the cell functions. Size in vivo 35 – 50 µm length, 15 – 25 µm width; pear-shaped microorganism can be found in almost all freshwater environments. The oral apparatus is located on the anterior end of the cell and can be easily seen as a small cavity in the ventral view of the cell. *Tetrahymena* have a bigger macronucleus and a small micronucleus. *Tetrahymena* have rows of cilia cover the surface of the cell which help them to swim rapidly in the medium. Cilia create currents so as to move the food particles into the mouth (cytostome). Single contractile vacuole located at the posterior region. Pure culture of *Tetrahymena* grows quickly to high density. *Tetrahymena* can be grown in a large quantity in the laboratory easily.

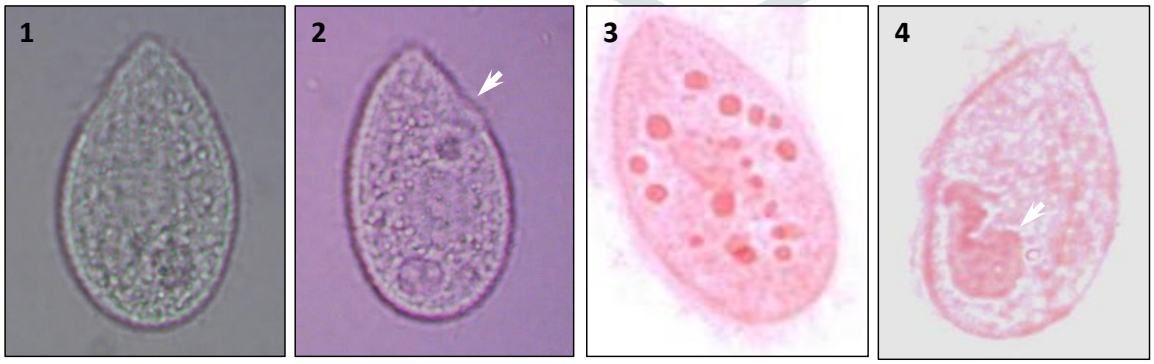

FIGURE:6

Fig.6.1. *Tetrahymena sps.* in-vivo; Fig. 6.2. Arrow (at anterior region) marks the oral apparatus (Cytostome) of a live cell; arrow (at posterior region) marks the contractile vacuole (CV); Fig.6.3. Haematoxylene stained (Dark pink in color) food vacuoles of *Tetrahymena sps*; Fig.6.4. Arrow shows the macronucleus of *Tetrahymena sps.*

4. 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.

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