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ECOLOGY OF CILIATES IN SEWAGE WASTEWATER TREATMENT PLANT APPUGHAR, VISAKHAPATNAM URBAN

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ABSTRACT

Biological wastewater treatment is a process of increasing importance in a world with an ever-increasing human population. Wastewater treatment facilities are designed to maintain the highest density and activity levels of those microorganisms like ciliates, flagellates i.e. Carry out the various purification processes. Protozoa are one of the most common components in these man-made ecosystems and play an important role in the wastewater purification process. Protozoa are responsible for improving the quality of the effluent maintaining the density of dispersed bacterial populations by predation. Ciliates are well-known water pollution indicators of pollution when their presence or absence can be related to particular environmental conditions. Increasing environmental pupation 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. The present study was undertaken indemnified ciliates in the sewage treatment plant in Appughar STP. The current study is a great focus on the reuse of sewage. 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: STP (Sewage Treatment Plant), Protozoa, Ciliates, Flagellates & Pollution Indicators

1. INTRODUCTION

1.1 Freshwater Source

Water is essential forever for life. Water is a precious commodity. Most of the earth water is sea water. The measure of crisp water on earth is limited, and its quality is under steady weight. About 2.5% of the water is fresh water that does not contain significant levels of dissolved minerals or salt and two third of that is frozen in ice caps and glaciers. In total, only 0.01% of the total water of the planet is accessible for consumption [20]. Water safety and quality are fundamental to human development and well-being. Safe and promptly accessible water is vital for public health whether or not it's used for drinking, domestic utilize, nourishment generation or recreational purposes. The objective is followed with the indicator of "securely maintained drinking water" – drinking water from an enhanced water source that is situated on premises, accessible when required, and free from fecal and need chemical contamination [20].

Sewage treatment is that the method of removing contaminants from effluent, primarily from the unit waste product. Physical, chemical, and biological processes are used to take away contaminants and turn out treated effluent (or treated effluent) that's safer for the setting. A by-product of waste product treatment is usually a semi-solid waste or slurry, known as sewage sludge. The biological reactors in Wastewater Treatment Plants (WWTP) are artificial ecosystems projected to achieve the development of bio aggregates (flocs and biofilms) in which stable communities of the organism,

mainly bacteria, and protozoa, are responsible for the removal of organic and inorganic pollutants [15].

2. EXPERIMENTAL METHODS

2.1. Study Area: GVMC Sewage Treatment Plant (STP), Appughar

Appughar Sewage Treatment Plant (STP) is in the Visakhapatnam city limits is situated between the M.V.P. Colony, Thenneti Park, and basin of Bay of Bengal towards roadway to Kailasagiri Hills. Some residential colonies are developed as Jalaripeta area nearby the STP. Appughar Sewage Treatment Plant (STP) is lying between the longitudes 83°20'41.3"E and 83^0 34’48" E and latitudes between 17^0 44’32.2" N and 17^0 74’25" N. It is located in Bay of Bengal Beach, Appughar, Sector 9, JalariPeta, Visakhapatnam city limits in Visakhapatnam District of Andhra Pradesh State, India. Appughar STP has 25 MLD capacity. The GVMC constructed WWTP here in order to propose for proper utilization of the Sewage and UGD water from 40110 households. These Wastewater treatment plant is planned and built with an intend to oversee wastewater in order to minimize and/or expel organic matter, solids, nutrients, disease-causing organisms, pollutants, and different toxins, before it re-enters a water body. The concentrations were assessed from two sewage treatment units. This STP supplies treated water to some Industries.

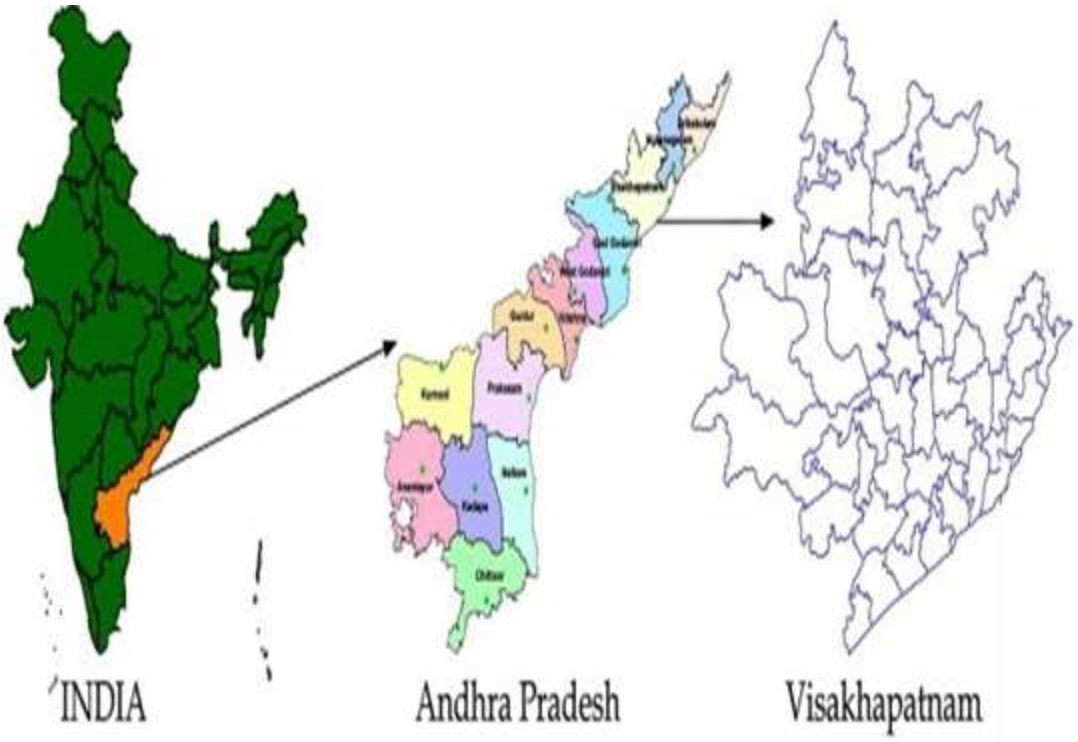

Figure 1: Map of India Showing Andhra Pradesh State and Visakhapatnam District

Figure 2: Site Layout Map Image of Sampling Station, Appughar STP (Sewage Treatment Plant)

2.2 Material s and Methods

2.2.1 Sampling Activated Sludge Wwtp

The microbial community of activated sludge plants develops on suspended flocs or freely in the mixed liquor. These flocs have abiotic – both organic and inorganic – and biotic components comprising bacteria, fungi and other organisms such as protozoa or small invertebrates. To sample all groups of ciliates in these artificial ecosystems, both flocs and mixed liquor are sampled at the same time submerging a flask in the aeration tank where the biological treatment takes place. Once the sample is taken, it should be kept with mechanical agitation or aeration using an aquarium air pump to kept flocs aerated and to avoid settling of suspended flocs.

2.2.2 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 coverslip. Keeping the cells in the minimal culture fluid, the coverslip was gently placed with the Vaseline-smeared edge down the face on them. The Vaseline film raises the coverslips just enough to provide sufficient space between the coverslip and the slide allowing the cells to remain functionally viable but arrest their movement. By this method, live cells can be kept for up to several hours, allowing observation and image capturing. Live cell observations were made using Axio Cam ERC 5s microscope.

Different types of ciliates isolated were kept as clone cultures in the own living culture collection and investigated while alive as well as on fixed material stained by Feulgen reaction and impregnated with silver nitrate. I have successfully kept in my collection the following species: *Metopus, Colpoda, and Zoothamnium.* For each species the main morphological characteristics were observed for at least 10-15 cells.

2.2.3 In-Vitro Culture

Water samples were collected from the Appughar sewage treatment plant (STP) from February 2016 to January 2017. Identification of the freshwater ciliates isolated from the sample was done in-vivo under the stereoscopic microscope. Aliquots of sediment and water from each 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 enriched with rice grains, modified Cerophyl medium [4] inoculated with *Roultellaplanticola* (Gammaproteobacteria).

The monoclonal cultures were obtained by isolating single cells from the original populations. These cells were briefly washed several times in sterile distilled water. Clonal cultures *Metopus, Colpoda, and Zoothamnium*species was maintained in the laboratory at 22-23°C in a medium made of hay infusion, Cerophyl, Na_2HPO_4, and Stigmasterol and distilled water inoculated with *Roultellaplanticola* was added to the medium to promote the growth of bacteria which served as the primary food source for the ciliates. The green algae *Dunaliellatertiolecta* was employed as food for ciliates.

Morphological study was done for ciliate **c**ells from monoclonal culture were harvested from the culture medium and observed with an AxioCam 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.

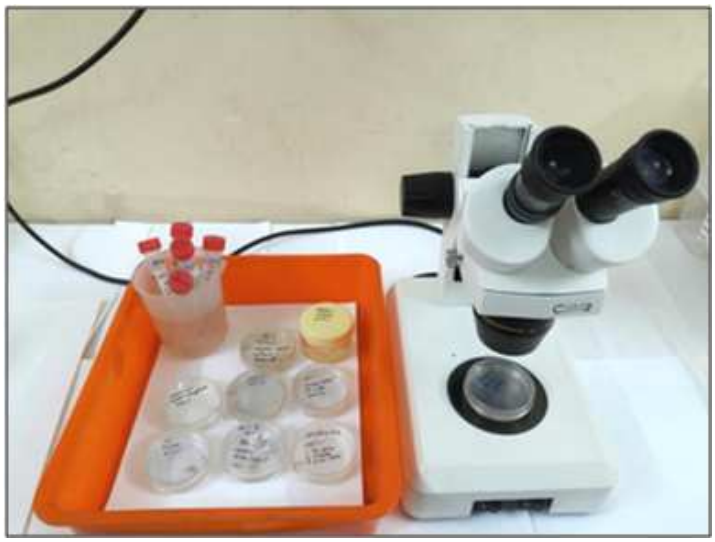

Figure 3: Ciliates Cells of Monocultures and Stereoscopic Microscope

The present study of the identification of ciliates in effluent treatment plants is vital to keep up the standard of the biological effluent treatment system. Ciliates create population control of bacterium, square measure agents of organic matter biodegradation and influence the agglutination of the bacterium in biological flocs, liable for separation of solid and liquid components of the waste material. The WWTP environments are necessary for the invention of little-known or maybe new ciliate species. The aim of this study is to identify the ciliates present in selected three wastewater treatment Plants (STP) situated in Visakhapatnam Urban, Visakhapatnam.

Sampling station is Appughar, GVMC sewage treatment plant, Andhra Pradesh, India. Samples of raw sewage water with sediments were collected from aeration basin during the period from February 2016 to January 2017in the period of two years. Samples were examined and ciliate cultures were established at Advanced Marine Biological Laboratory, Andhra University. Specimens were isolated under the stereomicroscope and studied in and then after protargol-impregnation and scanning electron microscopy.

The current study was designed on the taxonomy of ciliated protozoa (Phylum: Ciliophora) starting from classical methods and up to the Genes level. The main view was to identify the cultivable ciliates species from Wastewater Treatment Plants/sewage plants located in Visakhapatnam Urban, Visakhapatnam. Most of the samples were obtained from the GVMC sewage treatment plant, Appughar. Which is the main wastewater treatment plant in Visakhapatnam Urban.

3. RESULTS

3.1 Genus metopusclaparede & lachmann, 1858

These ciliate Genius belong to the genus Metopus Claparède & Lachmann, 1858, FamilyMetopidaeKahl, 1927 [10], Order Armophorida Jankowski, 1964; Subphylum: Intramacronucleata; Phylum Ciliophora (Doflein, 1901).

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Metopus is a genus of anaerobic organisms from the family of *Metopidae*[10]. Body elongate, ovoid; anterior left kineties curved (Fig.4). Right paroralkineties formed of several kineties [5,18]. Distinct frontal lobe overhangs the oral area. Members of this genus of metopid ciliates are 50 to 300 microns long. The cell is not flattened but is asymmetrical as the anterior region is twisted to the left (Fig. 5). The oral aperture is located equatorially or sub equatorially [18]. An adoral zone of membranelles arises from the oral aperture and runs obliquely towards the left anterior end of the cell, following under the twisted anterior end. The cell is covered with somatic kineties; those on the ventral surface extend from the posterior to the adoral zone of membranelles; those on the dorsal surface extend to the anterior end and over the top of the spiraled anterior end [5, 18]. There is a series of closely aligned kineties, to the right and above the adoral zone of membranelles, that produce an anterior mane of cilia [16]. A single ovoid macronucleus is typically situated in the middle of the cell. A single large contractile vacuole occurs in the posterior of the cell. Some species may be colored or have prominent bacterial symbionts. Common, found in benthic, freshwater, marine; soil, anoxic and saprobic sites. Metopus species are anaerobes and Nyctotherus is a common symbiont in the gut of amphibians [10-11, 23]

Figure 4: *Metopus*

Figure 5: Live Cell of *Metopus*

3.2 Genus : *colpoda*o.f. muller, 1773

These ciliate belong to the genus *Colpoda O.F. Muller*, 1773, Family *Glaucomidae*, Order *Tetrahymenida*; Class *Oligohymenophorea*; *Phylum Ciliophora (Doflein*, 1901).*Colpoda* is a genus of ciliates within the class Colpodea, order Tetrahymenida, and family *Glaucomidae*, *Colpoda* are clearly kidney-shaped (kidney-shaped) and are powerfully gibbose on one facet, biconcave on the opposite (Fig. 6). The concave side typically appears like a bite was taken out of it. Though they're not as well-known because the paramecia, they're usually the primary protozoa to seem in fodder infusions, particularly once the sample doesn't return from an existing mature supply of standing water.

*Colpoda*usually found in wet soil and as results of their capability to promptly enter defensive growths can abundant of the time be found in dried up tests of soil and vegetation and to boot briefly traditional pools, as an example, tree openings. They need likewise been found within the biological process organs of various creatures and might be refined from their fecal matter [17]. *Colpoda* have been discovered occupying the surface of plants and seems to overwhelm the microfauna there. Some varieties of *Colpoda* are found within the carnivorous plant Sarraceniapurpurea, notwithstanding the closeness of proteolytic enzyme abdomen regarding enzymes within the fluid. *Colpoda* excessively has behavior to be found in wealth wherever enlarged levels of microscopic organisms readiness to improved nourishment source. In business chicken homes, for example, they were by all accounts ubiquitous however the species discovered dissent usually beginning with one place then onto consecutive, proposing that these populaces speak to neighborhood soil and oceanic populaces change position into the new habitat [3].

In addition to inhabiting a well appear of microclimates, *Colpoda* usually found nearly all over around the world where there is stagnant water or wet soil, even where these conditions unit of measurement only temporary. One of *Colpoda* species were discovered in Brazilian floodplains in 2003 [19]. *Colpoda* irregulars have been found among the high desert region of Southwest province. *Colpoda* everywhere found among the arctic where hotter temperatures and longer summers cause larger density and species diversity (fig. 6). Not only is that the genus widespread, but there also square measure several species that have nearly world distribution, and, indeed, it has been prompt this might be true of all species, a proven fact which will rather be borne out by the higher investigation. Although *Colpoda* is not typically found among the marine surroundings, a there unit of measurement some ways that they'll travel from one continent to a special.for example, cysts can become lodged among the feather of migratory birds, turning into dislodged a full heap or even thousands of miles away. Also, as a result of cysts unit of measurement thus small and light-weight, they'll be swept back by air currents into the lofty atmosphere, then come back down on another continent [14].

3.2.1 Reproduction

Colpoda normally divides in cysts, from that 2 - 8 cells emerge, four being the foremost common range. This produces genetically identical ciliates. The speed at that such copy happens and the way it's laid low with varied environmental conditions has been the topic of an excellent deal of methods and principles of investigation. On rare occasions, *Colpoda* are determined to divide into four cells while not reproduce a cyst wall. It’s been advised that cyst less copy was the conventional mode of produce again for *Colpoda*below optimum conditions which the formation of cysts was a reaction to adverse environmental conditions. However, the information gained by a few years of culturing *Colpodain* fodder infusions has shown that this mode of reproduction remains rare despite what would appear to be ideal environmental conditions [7]. Like several protists, Colpoda may also reproduce by conjugation. This involves 2 *Colpoda* joining at the oral groove and exchanging DNA, then later dividing, redistributing the DNA of the two original*Colpoda*to

provide various genetically distinct offspring.

Figure 6: *Colpoda*oval Cell

Figure 7: Trophozoiteof*Colpoda* spp

Figure I) *Colpoda* is an oval cell with an obvious small cytostome about midway down the cell. The cytostome has rows of small membranelles.

Figure II)Trophozoite of *Colpoda* spp. (Optical microscopy 40X)

***3.3 Zoothamnium*boryde St. Vincent, 1826**

According to the current taxonomic status of this species is belongs to Class: Oligohymenophora de Puytorac 1974; Order: Peritrichida Stein, 1859; Suborder: SessilinaKahl, 1933; Family: Vorticellidae Ehrenberg, 1838; Genus: *Zoothamnium*Bory de St. Vincent 1826, *Zoothamnium* was at first classified as a member of the family Vorticellidae by Ehrenberg in 1838 [2, 6]. it absolutely was later reclassified to the family Zoothaminiidae, newfamily elicited by Sommer, in 1951 [2, 6]. The distinctive ability of the central stalk to accept a zig-zag pattern created the classification a necessity [2, 6]. *Zoothamnium* is a sessile "Peritrich," that means it's a ciliate jar shape protozoan that's immobile in nature. The genus contains over 7 species. Differentiation between species will usually be troublesome because of the robust similarities in type and performance [12]. The foremost generally cited species arefollows: The species may be easily found in fresh, salt and/or marine waters between 5°C and 25°C [13], generally 0 – 8 meters deep (Fig. 8). They thrive in areas of high suspended solids as they're detritus and anaerobic microbe eater.

It is available in eutrophic waters and on the coastal regions bordering the Atlantic Ocean. They generally type a dependent relationship with a good form of animals, though some are also habitat as attaching to aquatic plants and inanimate substrates [6]. Any solid substrate will give a base for growth. Copepods with chitin covering are the most species affected [13]. The ciliate may attach to the gills and reduce the ability to pass oxygen to body tissues (Figure 8). This may result in hypoxia due to mechanical blockage and failure to thrive. This attachment may cause Black Gill Disease or Surface Fouling Diseases [9]. *Zoothamnium* has the potential to reduce reproductive abilities of the host with high prevalence. No histological damage occurs due to attachment but if colonization becomes too great the host animal may die. The ciliate could attach to the gills and cut back the ability to pass Oxygen 8 to body tissues (Fig. I). This could lead

to driving because of Block gill in infection [9]. This attachment could cause Black Gill infection Or Surface Fouling Diseases. *Zoothamnium* has the potential to increase propagative capability of the host with high prevalence. No cytological destruction happens attachment, however, if formation becomes too significant the host animal could die.

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3.3.1 Morphology

Ciliates that type branching colonies (Figure 9). The colonies will colonies in size from many zooids to 100 zooids based on the species [6]. Bodies wrestle a cone-shaped to nearly spherical shape and are hooked up posterior with a stalk to the most stalk. The stalk enables for multiple ciliates enable into contact with a feed enchanter once detected, or move removed from a possible hazard as necessary. The anterior finishes of the ciliates embed in circular rows of cilia referred to as the corporeal girdle [6]. The main stalk that connects all of the ciliates branches consists of a contracted spasmoneme, permitting stalk to contact as one functioning unit [6].

When arrangement the colony will decree its size by folding during as one unit in a zigzag fashion [6]. The protozoa will show polymorphism, or multiple physical patterns in a similar cell. Those on the stalks cycloidal formed like rods, however those within the region of the ciliate oral structure of the zooids cycloidal formed like little spheres totally 3 different patterns the individual ciliate cells cycloidal appeared at the ends of the stalk, that cycloidal distinct in each morphology and function:

The large macro zooids, the adult developed cell, size varies significantly (20–150 μm) and might remodel into swarmer cells and leave the colony. These cells are extant massive and spherical and hooked up at many random locations throughout the branching colony (Fig. 9). The micro zooids can be extant little cells specialist for feeding, that the colony (Fig. 9) will by consumption of their dependent microorganism and alternative organic particles. These cells are extant smaller and oval formed and have on each branch of the stalk in multiple activities [13]. At the terminal ends of the colony have been specialized zooids that may elongate and facilitate the agamogenesis of the colony.

Figure 8: Shows *Zoothamnium*

Figure 9: Shows *Zoothamnium* Colony

4. CONCLUSIONS

Protozoa have proven to be an excellent tool for assessing the occurrence of the pollution during wastewater biological treatment. Along with it is a role in the control of pollution itself through the grazing of dispersed bacteria and maintains of the healthy tropic web in those artificial ecosystems. Most of the Most of the ciliate species in WWTP be either primarily or solely bacterivores feeding on a good form of microorganism, that embraces Moraxella [8]. Many 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 fulfill within the earth. More studies on this subject particularly aimed to collecting the data comparing the effects of toxicants on this community. Further research is needed to establish whether treatment options could render the sewages into pathogen-free biosolids which can be safely disposed into the environment [8].

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