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Scanning Electron Microscopic study on few Diatoms from KRP Dam, Tamil Nadu- India.

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ABSTRACT:

A study was carried out to examine the morphology of diatom species in Krishnagiri Reservoir, Tamil Nadu. The sediment samples were collected and transferred immediately in to zip lock bags to avoid the oxidation. Then the samples were stored at 5ºC in an ice box and carefully transferred to the laboratory. The sediment sample was treated with acid wash before detailed observation under the light and electron microscopes. All samples were identified up to species level based on Scanning Electron Microscope (SEM) observations. The SEM photographs provided fine morphological features of the species for easy identification. Qualitative aspects of the differences between LM (Light Microscope) and SEM approaches are discussed in the text.

Keywords:
Diatom, KRP Dam, SEM, Sediment diatom.

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INTRODUCTION

Diatoms are single celled microscopic algae that posses highly ornamented cell wall composed of glass silica (SiO_2) which provide variety of shapes from nano to micro-scale structures. They are free floating Planktonic or attached to a substrate, benthic forms (Werner, 1977). Diatoms are key components present nearly in all types of fresh and saline environments. All most all species are good indicators for a range of water quality variables because they have narrow optima and tolerances for many environmental variables (Van Dam *et al.,* 1994). In the last 20 years, numerous ecological studies were conducted regarding the use of diatoms as indicators of water ecosystem (Agbeti and Dickman :1989; Underwood *et al* ., 1998).

Diatoms are mostly identified based on the structure of their cell wall features. The diatom frustules have two valves namely epitheca (larger upper valve) and hypotheca (smaller lower valve) structures. Although their ecological importance is evident, the taxonomy of the genus is confused and has created much controversy (Round, 1981). Earlier many researchers worked on the diatom and their relationship with physico chemical parameters and nutrients absorption. With an increasing amount of information on details of the siliceous diatom cell wall, especially observed under electron microscope will be helpful in its easy identification. Additionally, SEM observations have a remarkable impact on diatom taxonomy rendering traditional identification methods insufficient for recognition of newly created taxa. The consequent lack of taxonomic resolution at the LM level may be leading to over estimation of geographical distributions, ranges of tolerance to environmental parameters and optimal conditions for growth. Qualitative aspects of the differences between LM and SEM micrographs are discussed in the present paper.

MATERIALS AND METHODS

Study Area

History and Morphometric of Krishnagiri reservoir

Krishnagiri dam is located in Krishnagiri Dharmapuri district of Tamil Nadu (**fig.1**) at the latitude of 12°28' north on the longitude of 78º11' east. The Krishnagiri dam was constructed across the Ponnaiyar River (also called as Ponnaiyar) near Periyamuttur village about 10 km from Krishnagiri town. The reservoir has two main canals, one on the left side called Left Main Canal (LMC) and other on the right side Right Main Canal (RMC) running almost parallel to the Ponnaiyar River.

The Ponnaiyar takes its source near Nandi durg in Karnataka of Chennakesava hills. It is known as Dhakshina Pinakini in Karnataka. It enters Tamil Nadu at places near Bagalur village in Hosur taluk. The river is called Ponnaiyar from this point of Tamil Nadu. It is located at the latitude of 12 28'N and longitude of 78 11'E. Irrigation projects are constructed across Ponnaiyar by Kelavarapalli reservoir project, Krishnagiri reservoir project and Sathanur reservoir project. The reservoir is being used for multipurpose utility such as irrigation, fishing and washing.

Sediment samples were collected by PVC corer to a depth of 20 cm. Samples were transferred immediately in to zip lock bags to avoid the oxidation of sediment samples and stored at 5ºC in an ice box and carefully transferred to the laboratory. All extractions were done in duplicate.

Preparation of diatoms:

Detailed diatom studies were done following hot HCl and $KMnO_4$ method (recommended technique of acid digestion) by Taylor *et al*. (2005). The benthic diatom samples subjected to Scanning Electron Microscopic observations. The SEM Photos were taken at CAS in Botany, University of Madras, Guindy campus, Chennai -25.

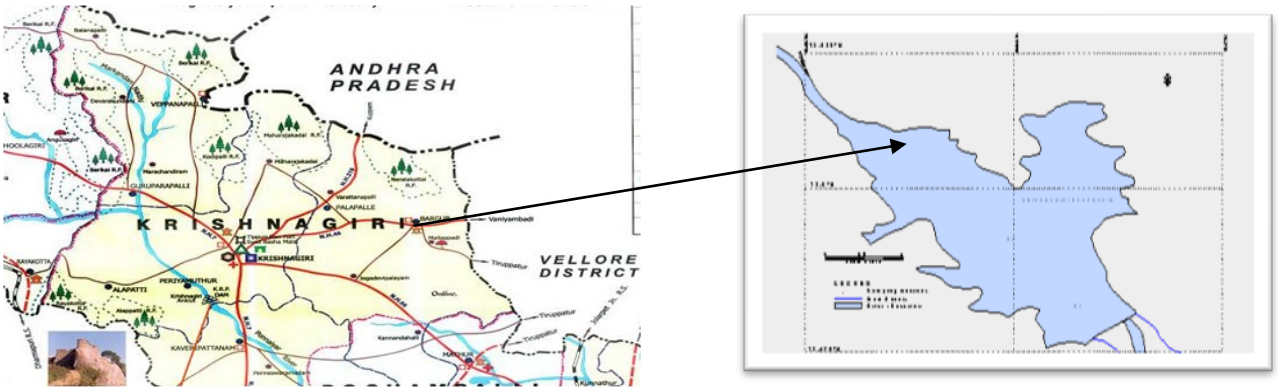

Fig.1 Map showing Krishnagiri Dam

Scanning Electron Microscopy (SEM)

The specimens were cleaned by adopting the same procedure as described earlier. Acid washed samples were placed onto a clean glass cover slip. Samples were left air dried overnight. The samples were coated using gold-platinum using a JEOL JFC -1600 Auto Fine Coater (JEOL, Tokyo, Japan). The samples were then examined under a JEOL JSM-6390 LA Analytical SEM (JEOL, Tokyo, Japan) and digital images were taken using the system.

RESULT

The topography of diatoms cells were observed in light microscope and as well as scanning electron microscope. The identified diatoms are *Cyclotella menghiniana, Gomphonema parvulum* and *Aulacoseira granulatae.*

Under the light microscope (**fig.2**) the cell wall of diatom looked drum-shaped one. Valves are circular with a tangential undulation in the central zone. With this information, identification of the genus is easy but up to species level, it is difficult to be concluded. Under the Scanning Electron Microscopic view (**fig.3**) the cell wall was drum shaped and the margin of the cell well defined and clearly differentiated from the central part of the valve face. The marginal zone had 6-10 radial striae measured at 10 µm, each composed of a single alveolus which was open on the inside of the valve. This gave the marginal zone a chambered appearance. A single marginal rimoportula was present. The central zone was structure less which are radially streaked. Hence with SEM image the Cyclotella is identified as *Cyclotella meneghiniana*.

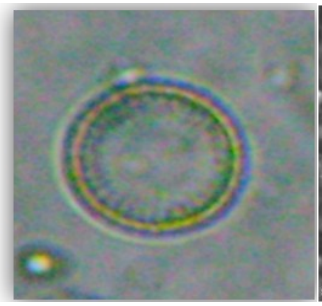

fig.2 LM view of *Cyclotella meneghiniana*

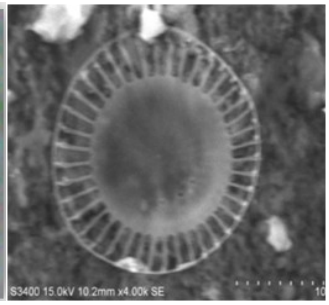

fig.3 SEM view of *Cyclotella meneghiniana*

SEM image of *Gomphonema parvulum* (**fig.4**) showed that, they were slightly asymmetrical to transapical axis, symmetrical to apical axis. Apices rounded raphe are slightly sinuous. A single stigma was present on one side of the central area. Striae were coarse and often visibly punctuate. But under light microscope only cell wall can be seen visible and other information are lacking in LM view (**fig. 5**).

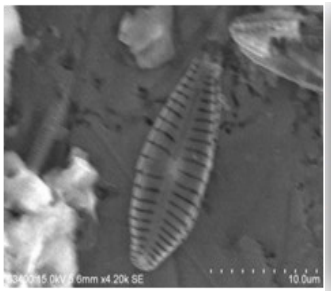

fig. 4 SEM view of *Gomphonema parvulum*

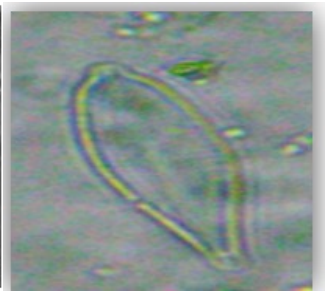

fig.4 LM view of *Gomphonema parvulum*

The SEM image of *Aulacoseira granulate* (**fig. 6**) showed valves which were 4-17 µm in diameter, with a mantle height of 4-20 µm. The mantle had straight sides and the valve face was flat. The mantle areolae are square. Linking spines are located at the end of pervalvar costa but it got damaged during the sediment process. Under LM (**fig. 7**) the valves and mantle are not clearly focused and at one end of pervalvar costa the spine is present.

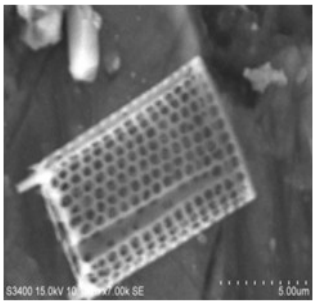

fig.6 SEM of *Aulacoseira granulate*

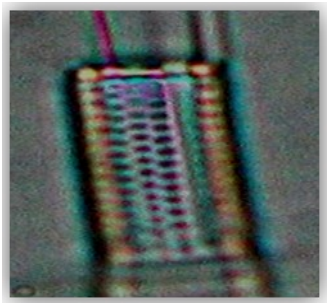

fig. 7 LM of *Aulacoseira granulate*

DISCUSSION:

Despite the high resolution offered by TEM, the introduction of scanning electron microscopical analysis had a more profound impact on diatom taxonomy (Masse et al., 2001). Phycologists appreciate the three dimensional structure of diatom valves and the highly characteristic architecture of the silica shell through SEM analysis (Gerloff and Helmcke ;1974) and Round and Crawfold (1990).

Fine morphological structures of three genus of diatom namely *Cyclotella meneghiniana, Gomphonema parvulum and Aulacoseira granulate* had been documented in this study. Fine tune of

morphological structure studies of other diatom species from various locations are currently underway so as to provide a comprehensive documentation of diatom species in KRP DAM. The epical valve and also the spines were very clear in the SEM images when compared to light microscopic images. At higher magnification the valve features were observed in even greater detail. Above all the SEM images were helpful to explore the unexplored fine structure of diatoms collected especially from sub and bottom sediment of aquatic ecosystems.

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