Developing methodology for observation of plankton in Tapi River using Scanning Electron Microscopy

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Abstract

Scanning Electron Microscopy (SEM) is one of the techniques used to study inter alia the morphology of plankton. In this paper a simplified sample preparation technique using Critical Point Dryer (CPD) and Sputter Coater (SC) method, for SEM study of Plankton is proposed. The samples of fresh water planktons, including phytoplankton, zooplankton and diatoms of Tapi River in Gujarat have been studied. This technique can be applied to capture high resolution morphological images of natural blooming of plankton water samples. This method can be used to study the diversity of planktons at higher resolution, and provide useful information for understanding plankton morphology and ecology.

Key words: Plankton, Tapi River, Critical point dryer (CPD) method, Sputter coater (SC), Scanning Electron Microscopy (SEM).

Introduction

Plankton is a part of aquatic life, which is composed of tiny organisms living and drifting in the direction of water current. It acts as the main source of food for most fauna, both in lotic and lentic water ecosystems. Ecologically, plankton are one of the most important biotic components influencing all the functional aspects of an aquatic ecosystem, such as food chains, food webs, energy flow and cycling of mater. Due to their large density, shorter lifespan, drifting

nature, high species diversity and different tolerance to the stress, they are being used as indicator organisms for the physical, chemical and biological processes in the aquatic ecosystem. The abundance of plankton is an important biological tool for monitoring the ecology of any river system (Gunwant et al. 2014).

To understand the ecology of plankton, observation of natural plankton assemblies using Scanning Electron Microscopy (SEM) is one of the informative methods, because this technique helps to study the detailed morphology and the identification of nanoplankton at the species level (Keizo et al. 1993). Several authors have studied the pivotal role of SEM observations to study plankton in nature; however, reports of SEM observations using field samples remain limited (Kei et al. 2012). It is due to methodological difficulties in sample preparation of plankton for SEM. The purpose of the present study was to evolve the method for studying plankton by SEM in high resolution of Tapi Water River.

In this study, plankton water sample were collected from Tapi River, Gujarat-India. Tapi river water contains the higher amount of pollutants because of industrialization in nearby area. Pollution causes decreasing biodiversity and population of plankton and it is directly related to physico-chemical parameter of water (Monika Dubey et al. 2013) (Taruni Sarang et al.2017) and thereby it disturbs the biological processes in the aquatic ecosystem. In this study, we tried to tune the Critical Point Dryer (CPD) method and Sputter coater (SC) to obtain SEM images of highly conserved natural plankton assemblies in Tapi River. The resulting images confirm the effectiveness and impact of SEM observation.

Materials and Methods

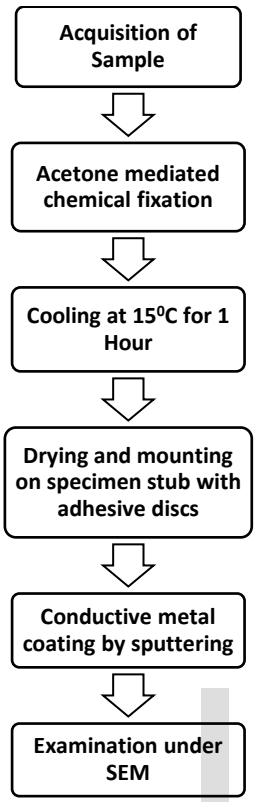


Fig.1 Diagram showing the steps in the preparation of Plankton water sample for SEM (Bozzola& De Russel, 1999).

Plankton samples were collected from river Tapi, Gujarat-India, by filtering surface water through plankton net (Mesh Size No: 25) and preserved in 100 ml of plastic bottles with 4% formalin. The successive steps for preparing the material for observation using scanning electron microscope are shown in Fig. 1.The first stage, acetone mediated chemicals fixation, causes the fixation of the material and increases its mechanical and thermal stability through cross binding proteins. The samples should be dried because samples have to be compatible with the vacuum in the microscope. Air drying is not recommended because of the surface tension force which causes contraction and shrinkage of the material surface. Preparations should, therefore, be dried under appropriate conditions, which are provided during chilling process by chiller device (E4800, Quorum Technologies) where temperature should be 15^{o}C for 60 minutes. The low

temperature by chiller converts gaseous carbon dioxide into liquid phase. Afterwards, the samples are subjected to carbon dioxide mediated critical point drying at constant temperature of 31.8^{o}C and pressure of 79 bar (1180 PSI). These conditions keep the carbon dioxide in gaseous state. Though prolonged heating is beneficial for drying, 30 minutes of heating incubation is sufficient to dry substances having thickness of less than 1mm. It must be taken into consideration that the carbon dioxide has a serious drawback as an intermediate fluid as it is not miscible with water. Therefore, water is replaced with acetone, which in turn mixes with water and liquid carbon dioxide. In this process the acetone is replaced by the intermediate fluid under high pressure to provide a complete exchange of fluid. During heating process the liquid reaches phase transition in which the liquid phase changes into vapor and samples are embedded in a dense vapor phase to protect it from surface tension force. Therefore, in order to maintain morphology of the substances, drying should be done under controlled environmental conditions, which are provided by CPD device (CPD E3000/E3100, Quorum Technologies) and ensures that the vapors will not go back to a liquid state. As heating is over, the vapors are gently released from the CPD device until atmospheric pressure is obtained. The vessel is slowly exhausted. The samples are mounted on stub holder using conductive double-sided carbon tape. All stub holders are then transferred to the sputter coater device (SC 7620, Quorum Technologies) as soon as possible. Sputtering is essential for non-conductive samples such as biological sample. Sputter coating is a layering of Gold-Palladium (20-80%) over the dried sample in order to make the surface conductive. Sputtering time is set for 60 seconds at 10mAcurrent and 10^{1} psi pressure. The samples are ready to be viewed under the SEM (EVO-18, Zeiss). The sample images are observed under Back Scattered Detector (BSD). This detector is used for imaging the hydrated

specimens in Extended Pressure (EP) mode with 100µm aperture, it will enable to study hydrated specimen in their native state.

Results and Discussion

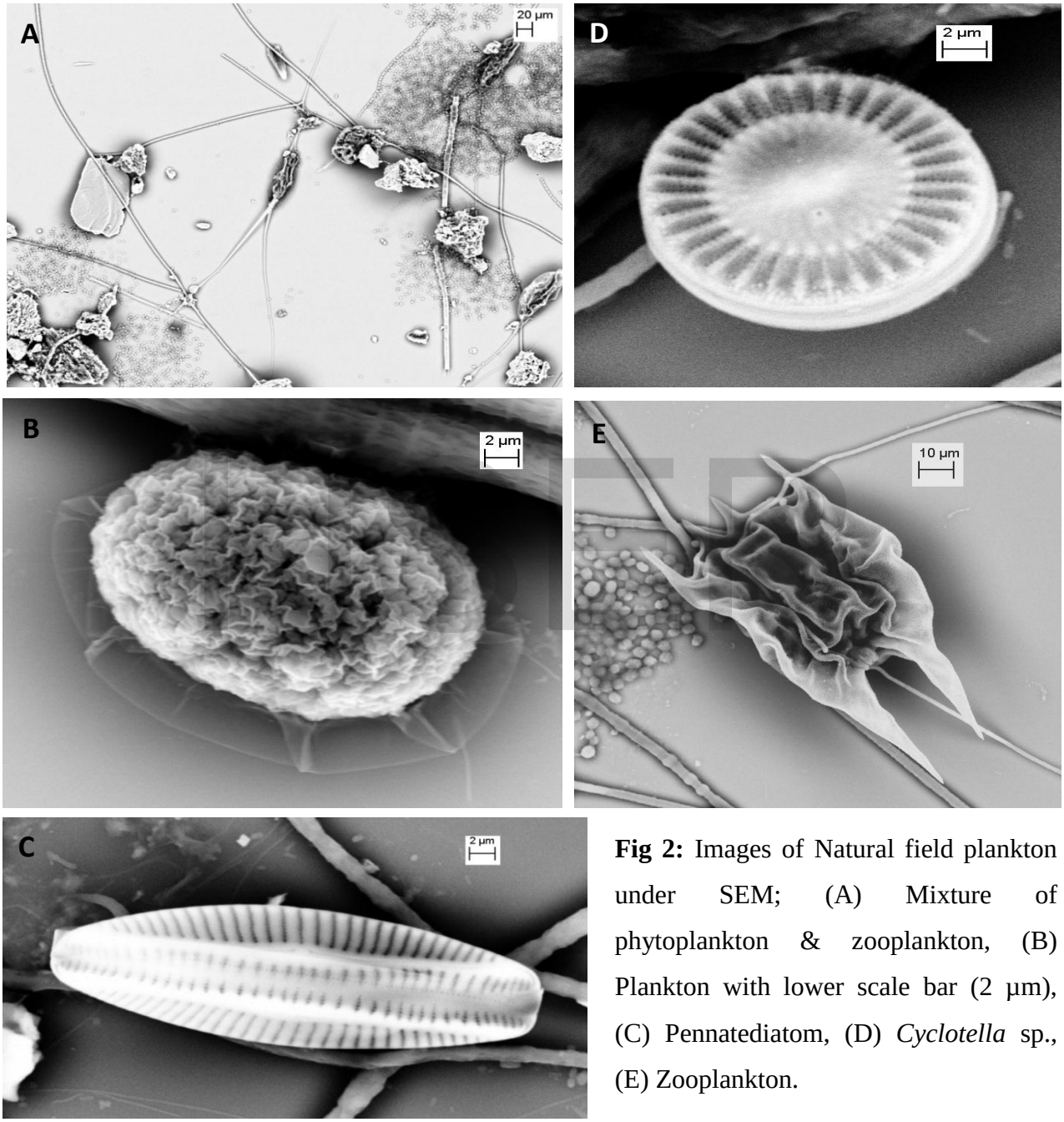
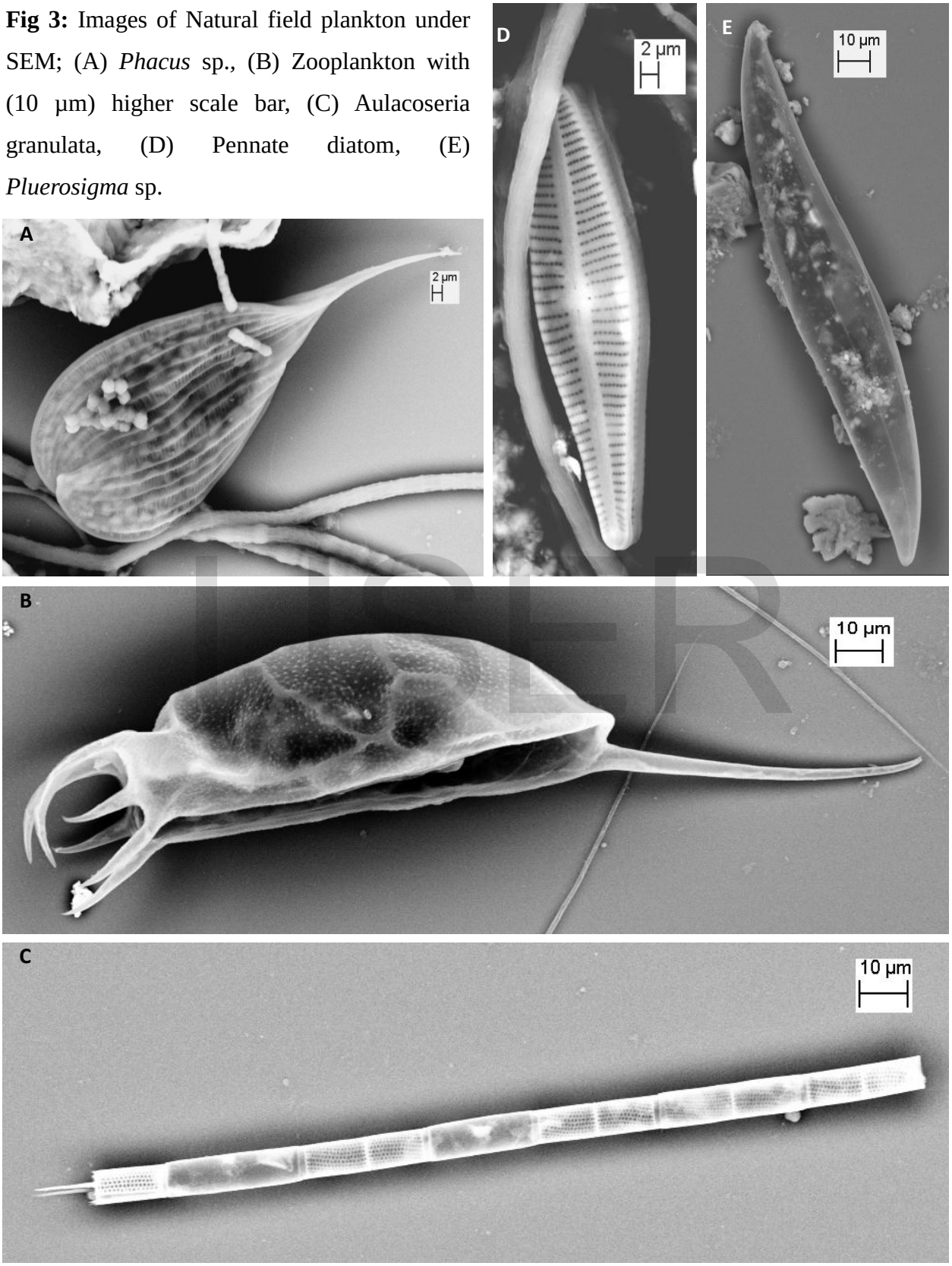


Fig 2: Images of Natural field plankton under SEM; (A) Mixture of phytoplankton & zooplankton, (B) Plankton with lower scale bar (2 µm), (C) Pennatediatom, (D) *Cyclotella* sp., (E) Zooplankton.

Fig 3: Images of Natural field plankton under SEM; (A) *Phacus* sp., (B) Zooplankton with (10 µm) higher scale bar, (C) Aulacoseria granulata, (D) Pennate diatom, (E) *Pluerosigma* sp.



Our studies show that the SEM method provides a lot of new information on plankton morphology in sample. The main advantage of the method of sample preparation is that the specimens are not distorted or spoiled and it gives us better image without any extra effort. It is simple and easy method for SEM study within a short period of time. There is no shrinkage and destruction of any region of the surface of sample (Saha et al, 2011).

Planktons are the base of aquatic food. So, to understand the aquatic ecosystem plankton study is very useful (Shakila & Natarajan 2012). Figure 2 and 3 vividly illustrates the presence of Phytoplankton, Zooplankton and diatoms in the fresh water samples. In this study, planktons are segregated into Phytoplankton, Zooplankton and Diatoms such as Navicula, Cyclotella, Brachious, Phacus, and Pluerosigma. The samples have high density of Plankton and diatoms. By using this method with CPD and SC system, the planktons were clearly observed (Figs. 2 and 3). In addition, the procedure does not require any special or expensive reagents; it is a simple and low cost technique. However, the SEM method also has some limitations. The main problem is dirt (e.g. algae, mud, debris, etc.), which often settles on the surface of external structures or which are uneven. Attempts to remove these contaminants do not always give satisfactory results, particularly on surfaces with high spatial diversity (Kownacki et al. 2015). These impurities, after being coated with gold, are more pronounced under SEM than the real structure. So, one way of cleaning the surface can be gentle, repeated washing of the sample.

Our results indicate that the SEM examination helps us to get to discover, investigate and understand new morphological details which will greatly facilitate the identification of species and help to study the ecological monitoring of Tapi River. To improve our understanding of the ecology of plankton and other micro-organisms in natural waters, development of SEM analysis using the CPD and SC method in this study might provide valuable and novel information.

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