Chapter 4
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Methodology

4.1 Sampling

The collection of samples for palynological studies is done by various methods depending upon the requirement of study. For the present investigations located samples from both, open cast mines and drill cores were obtained. Bore cores are ideal material for palynological analyses because they provide fresh samples without any chance of contamination or mixing.

Samples from 4 bore cores and 3 open cast mines were collected and analysed for miofloristic studies. Each sample after collection in the field was labelled indicating its exact location using GPS (Garmin 12), stratigraphic position and geological details of the area. The samples were then packed in polythene and cloth bag to avoid contamination. There after the samples were brought to the lab where they were subjected to laboratory processing for palynological and SEM studies.

4.2 Preparation of Palynological slides

The procedures followed here to macerate the samples abide to the methodology suggested by Mathur (1964)

- About 50 gms of the sample was taken and is dipped in alcohol and burnt to remove the surface atmospheric contamination.
- The sample is crushed to pea-nut size, sometimes the sample is powdered in an iron mortar and pestle and the crushed sample is transferred to a 500 ml polypropylene beaker

- 10% HCl is added drop by drop to check the presence of carbonates if present, treat till effervescence ceases. Thorough stirring with plastic rods is essential to maintain uniform chemical reaction. The mixture is left to react for about 8 hours.

- After 8 hours distilled water is added and kept for about 3-5 hours for settling.

- After complete removal of carbonates, the residue is treated with 40% Hydroflouric acid for removal of silicates. The acid is added with constant stirring with a plastic / copper rod as glass rods will be dissolved in HF. The mixture is left in a fuming chamber for 24-48 hours for the complete removal of silicates

- After settling the supernatant is decanted and the residue is washed thoroughly with distilled water to remove the traces of acid from the residue.

- To the rinsed residue, Nitric acid is added which is an oxidizing reagent. The mixture is kept in the fume chamber for 3-6 days so that the humic matter present in the residue is oxidized completely.

- After treating the residue with Nitric acid, it is washed thoroughly with distilled water and is swirled in a watch glass where in the
centripetal force drags all the denser material to the center of the watch glass and the spores and pollen float on the surface.

- The floating spores and pollen in the watch glass are carefully decanted into a sieve with 5μ nylon mesh and is washed with a jet of water and transferred to small beakers.

- A drop of the sieved material is observed under the microscope for checking the extant of oxidation of humic matter and organic debris when treated with Nitric acid. If the material is not oxidized satisfactorily two to three drops of 10% KOH is added to the material and kept for 3-5 minutes and washed under a jet of water. Oxidation can be controlled either by leaving the mixture in Nitric acid for longer period or by careful treating of the sieved material by adding 10% KOH. There is a possibility of over bleaching of the palynoforms if the KOH treatment is not done carefully.

- After oxidization, the mixture is put into tapering end centrifuging test tubes and centrifuged at 800 rpm for 30 minutes.

- The supernatant is decanted and 2-3 drops of Polyvinyl alcohol is added to the test tube with residue and mixed thoroughly with gentle tapping using a glass rod.

- The mixture is then smeared on to a cover slip and dried in the oven. The dried cover slip is then mounted on to a glass slide using Canada balsam as adhesive and thus the slides were prepared for further palynological observation.
• Thus prepared slides were observed under phase contrast binocular microscope (Leitz Biomed) to document the palynomorphs.

• Digital photography of the observed palynomorphs was done under x1000 magnification in oil immersion.

• All the magnification and size character of the recovered palynoforms are well illustrated and mentioned in the explanation of plates.

4.3 Sample preparation for S.E.M studies

Scanning Electron Microscopic studies helps in examination of ornamental, textural and structural features of palynoforms with high resolution and depth of field, which in turn is helpful in the identification of characters to be used in taxonomic classification and used in unraveling the Palaeoenvironmental depositional studies.

The sample for S.E.M studies is obtained from the last stage of Maceration process where in the sample residue is treated with 10% KOH. The residue is centrifuged and the supernatant is decanted. 10% alcohol is added to the centrifuged residue and kept for 24 hours. Gradually the addition of alcohol with increase in concentration is done for every 4 hours. Finally absolute alcohol is used to store the material. Glass slide is cut according to the size of the S.E.M stage and the sample is smeared on to the glass slide and exposed to air for the alcohol to
evaporate. When the sample was dried it was kept in a sputtering machine for coating. The sample is coated with a suitable conductive material to make the non-conductive material into a conductive one. For coating gold / Palladium alloy is generally used which is supposed to be the best. The Scanning Electron Microscope photography was done in Jeol Scanning Electron Microscope 5800, carried out in the National Institute of Oceanography, Goa, India.

4.4 Cross Verification for Lab contamination

The samples collected from field and processed in the lab were prone to be contaminated by modern day spores and pollen while processing in the lab. To avoid and to identify the creeping of contaminated spores and pollen, sampling of aerospora was done by soil, water and also Cob web sampling from the localities in and around the lab so as to eliminate the probable modern day palynomorphs. These sampling in the vicinity of lab was done seasonally to get maximum varieties of contaminant spores and pollen. It will enable to eliminate any intrusion of contamination.

4.5 Repository

The samples thus collected from four Boreholes and two open cast mines have been documented and all the field details are catalogued with
respect to individual samples and have been deposited in the Palynological lab.

The palynological slides, aerospora slides and Transmitted Light photographs and Scanning Electron Microscopic photographs are deposited in the repository of Palynology Lab, Department of Studies in Geology, University of Mysore, Manasagangothri, Mysore.