CHAPTER-3

METHODOLOGY

3.1 INTRODUCTION

The role of microfossils is immense in geological sciences for resolving many issues that are related to evolution of the earth's crust. Apart from evolution of organisms, palaeogeographical, palaeoecological, palaeoclimatological and biostraigraphical used; many of them are index fossils and have globally a datum lines are controlled the finer divisions of the Geological Time Scale. Moreover, this group is heterogeneous in nature and comprises a large variety of compositional characters including both mineral and organic materials. Their first appearance and later diversification in the different types of continentals, marine and transitional environments, unbroken stratigraphic records, and some of the groups are common in different environments, it has lot of significance in solving the biochronological and bio-correlation problems of local, regional and inter continentals. Further, due to their omnipresent nature their occurrences are observed throughout geological columns and all types of sedimentary rocks. Palynofossils are handy tools for petroleum and coal exploration, their presence in terrestrial, deltaic and marine environments, widely used for correlation and dating the sedimentary succession. Their thermo-sensitivity nature shows remarkable change in colour of the fossils are used for maturation index for the sedimentary rocks that indicate the generation of the hydrocarbon.

Looking their wider applications in the geological sciences, investigator has tried to analyse this group for solving different issues of the intertrappean succession of the Saurashtra Peninsula. To generate the systematic data set for the geological succession, the sampling techniques are the backbone of the research and must be done with the utmost care and following the standard procedure. The investigator has selected the sites and planned for collecting the samples as per their requirement and also modified the planned with change in lithological characters. The next step involves analyses of the samples in the laboratory, very carefully and meticulously followed the procedures to separate them from the extraneous material, preparation of slides, photography and identification. These data set are used for

establishing the biostratigraphy and further used for interpreting the paleoenvironments, paleogeography and paleoclimate.

This chapter discusses the methodology and laboratory techniques that were employed in the investigation that play a significant role in achieving the objectives in the present study. It is divided into two broad sections, which are follows:

1. Field collection techniques and handling of the samples; and
2. Laboratory techniques (Fig. 3.1).

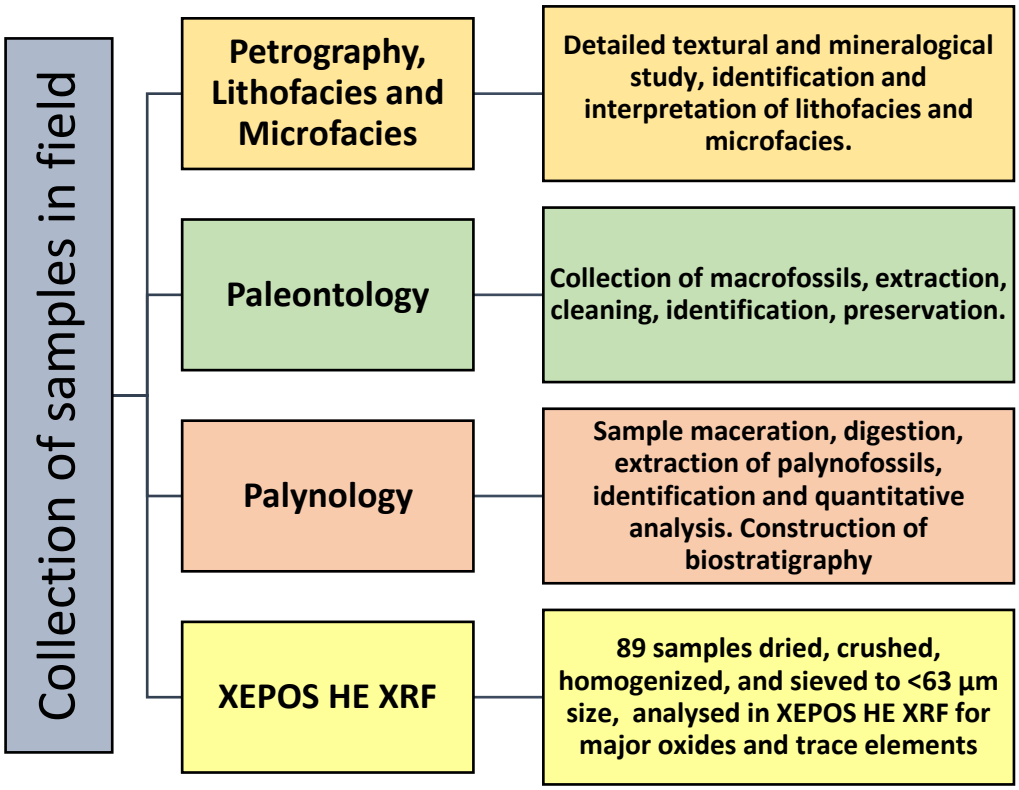

Fig. 3.1 Flowchart showing methodology that adopted for analysing the samples for palynofossils.

3.2 FIELD WORK AND SAMPLING

The Saurashtra Peninsula's exposes intra-, and intertrappean successions which are occur as patchy outcrops and mainly observed in river cut and road cut sections as well as in the freshy mined quarries. Prior to field investigation, some pre-field investigations in the form of review of literature, base map preparation, marking of point of interest's, localities on Google Earth Pro using the previous literatures were carried out. The field work was planned accordingly, and was carried out in December 2021, December 2022 and February 2023. Lateral continuity of the strata was observed, mapped and their upper and lower contacts are

marked. Detailed documentation of the bed geometry, lateral and vertical lithological variations, thickness of the strata, sedimentary structures and biological entities were observed and noted. Each section was measured, and a stratigraphic and systematic sampling plan was employed for collecting the samples. The exposures were scraped, removed the weathered surface and ensured that in each sample materials collected are fresh, unweathered, insitu and free from the scree materials. During the sample collection, precautions were also taken to avoid surface contamination and mixing. Depending on the thickness of the strata, lithological variations and exposed stratigraphic units, sampling plan was modified, i.e., in each case a systematic plan was employed but the vertical distance between the samples was adjusted to gather the maximum information about the exposed sequence. The samples were collected in polyethylene plastic bags, sample number, date and locality were written on the bags and the samples were again put in other bags to avoid contamination. Care was also taken that the samples of each locality were kept in separate larger plastic bags to avoid any types of confusion. Sampe details were marked on lithologs immediately in the field to ensure their position. More than 250 samples were collected and were brought to the laboratory for further studies. Macrofossils collected from various beds were recorded in the lithologs for position and specimens were carefully collected, labeled, wrapped in cotton, kept in sample bags, and brought to the Paleontology Laboratory of the Department of Geology.

3.3 LABORATORY TECHNIQUES

Various laboratory techniques are used to obtain the different types of the dataset to fulfill the requirement of the present investigation are described briefly in the subsequent sections.

3.3.1 PETROGRAPHY

The petrographic study of the clastic (sandstone and siltstone) and chemically formed sedimentary rocks (limestone and chert) are attempted for textural and mineral composition to understand the role of the depositional environment, provenance, and climate. The study includes the detailed examination of the textural properties of the rocks i.e., the relationship between the framework grains, matrix, and cement; mineral composition of the rocks and bioclasts identification. This study has helped in the identification of the rock types and microstructures of the chert and further analysing the detailed microfacies for the carbonate rocks. The thin sections were prepared following standard procedures and study them under

the petrographic microscope (Leica DM EP with MC120HD camera, 2.5x, 4x, 10x, and 40x objectives and 10x eye piece) and representative photographs were captured. The sections were was prepared in the Thin-section Laboratory, Department of Geology of The M. S. University of Baroda, Vadodara. Detailed petrography of the rocks, utility in facies analysis and their interpretation is discussed in Chapter 5.

3.3.2 XRF

The inorganic geochemical laboratory analysis was carried out to determine the chemical compounds of the rocks that support present investigation to interpreting the various paleoecological parameters. To get geochemical information, major, minor and trace elements were determined using an advanced Heavy Element energy dispersive X-ray fluorescence spectrometer.

Sample Preparation: The 89 systematic samples were selected from the different intertrappean localities that represent the entire successions were initially allowed to be oven dried at 50°C and were further crushed to a size of <63 μm. The crushed samples, were, then packed into the sample cups using a thin propylene film and were later, analysed in SPECTRO XEPOS HE XRF at the Institute of Seismological Research, Gandhinagar, India. The Geochemical Standard Reference Soil Sample JMS-1, JSd-1 (Geological Survey of Japan) and NCSDC-73310 along with Spectro provided standards were used to check the accuracy of the measurements. The precision of the analysis was better than 5% for the major elements and 10% for the trace elements (Das et al., 2017; Makwana et al., 2019).

Instrument: The instrument used was a SPECTRO XEPOS HE XRF ('HE' stands for 'Heavy Elements'). Commonly used XRF instruments are designed for rapid screening analyses and the quantification of many elements; they frequently determine heavy element concentrations only in screening quality, whereas XEPOS HE is an instrument that offers all the benefits of modern XRF and can reliably quantify even heavy elements in ppm ranges. It features a versatile excitation source. The instrument employs a 50-Watt end-window tube that is exceptionally steady. With up to eight polarization and secondary targets, exceptional sensitivity and accuracy is achieved for an ED-XRF device for analysing medium and heavy elements.

3.3.3 PALEONTOLOGY

For the macrofossils collection, many different methods are used/adapted depending on a particular situation. It is vital to remember that the specimens collected contain the greatest information. During their field excursion in the intertrappeans of the Saurashtra Peninsula, investigators made rigorous attempt to locate the fossiliferous horizons.

The fossils were recovered by digging/scrapping up to a few centimeters into the fossiliferous beds of intertrappean. Geological tools such as a hammer, various types of chisels, and a mallet were used to split and break rocks in order to extract fossils. A spade, flat bladed trowel, and stiff brushes were used to remove soft and unconsolidated sediments such as sands, muds, and clays. Larger samples were carefully excavated because a complete specimen was required for a thorough paleontological examination. Because it is often difficult to recover whole fossils in the field due to softness and fissility of the host rocks, which has resulted in the crumbling of the rocks and shells, the specimens were collected with the blocks or large chunk of the rocks and later extracted with due care in the laboratory. Investigator is also successful in extracting a few complete specimens in the field using hand tools removing the superfluous matrix, which was then taken to the Paleontological Laboratory, Department of Geology, The M. S. University of Baroda, Vadodara.

Cloth, cotton, cardboard boxes, and plastic boxes were utilised to protect and strengthen fossils. Scrapping of the extra materials was completed around the specimen margins and ensured that delicate and vulnerable portions were secured/intake. Every specimen and jacket were labelled with its field number. The collecting and locality notes, the specimen orientation, were written directly on the dry plastic jacket or recorded in the field notebook. At the site, photographs of the horizon with the embedded specimens were taken. Field descriptions were also written in a notebook, which served as a useful resource for specimen identification in the lab. The precise location and orientation of each fossil, as well as the position of each sediment layer in the stratigraphic succession, were recorded.

The macroinvertebrate fossils that were collected were washed and sorted from the rock matrix. They were rinsed and cleansed with water and dry for more appropriate investigation and confirmation of genera. The specimens were cleaned with a soft nylon brush, needle, and penknife, then thoroughly rinsed to remove extraneous sediments and dried; because of their delicate nature, extra precautions were taken. All fossil samples were wrapped with cotton and kept in well labelled transparent plastic bags and transformed into durable plastic boxes. They

were measured and photographed with scale in various orientations such as dorsal, ventral, and lateral, and their careful examination was done.

3.3.4 PALYNOLOGY

The palynology, science of organic wall microfossils has immense value in geological sciences and their applications are becoming wider and wider in the present-day scenario. These microfossils resist against the earth processes and least affected by the temperature and pressure have culminated their values, particularly in economic mineral exploration. The palynological studies, helps establish the relative ages of sedimentary rocks, reconstruct past climates, vegetation types, and environmental changes, identifies potential oil and gas reservoirs and helps to investigate and understand dynamics of past ecosystems, evolution of flora and fauna, and the impact of mass extinctions. A wide range of processing techniques are employed for the extraction of palynofossils which depend on lithology, amount of induration, types of organic matter and their richness.

Traditional techniques for the separation of palynofossils include the procedures of Erdtman (1952), Norem (1953, 1956), Staplin et al. (1960), Hughes et al. (1964), Tschudy and Scott, (1969), Higgins and Spinner (1969), Barss and Williams (1973), Faegri and Iversen (1975), Doher (1980), Batten and Morrison (1983), Herngreen (1983), Phipps and Playford (1984), Evitt (1984), Johnson and Fredlund (1985), Litwin and Traverse (1989), Aldridge (1990), and Poulsen, N.E. Gudmundsson et al. (1990). The most recent procedures include the works done by Grey (1999) and Green (2001). Based on the type of lithologies, standard techniques as elucidated by Bharadwaj (1962), Bharadwaj and Salujha (1964), Grey (1999) and Green (2001) were followed with the necessary alterations and modifications during work.

3.3.4.1 Extraction of Palynomorphs

The samples of the study area show a wide variation in the rock type and their mineral composition. It includes both clastic and chemically formed intertrappean sedimentary rocks, characterised by variety of rock types including the shales, limestones, marlite, siltstones, sandstones, mudstones, chert etc. It also depends on the nature of the rocks to obtain the palynofossils in good conditions with distinct identifiable morphological features. Difference in the rock types offers different methodologies to be employed to extract the organic walled microfossils from the samples. However, the standard extraction techniques proposed by Grey (1999) and Green (2001) and Traverse (2007) has been followed with required necessary alteration and modification for the maximum recovery of the palynofossils (Fig.3.2).

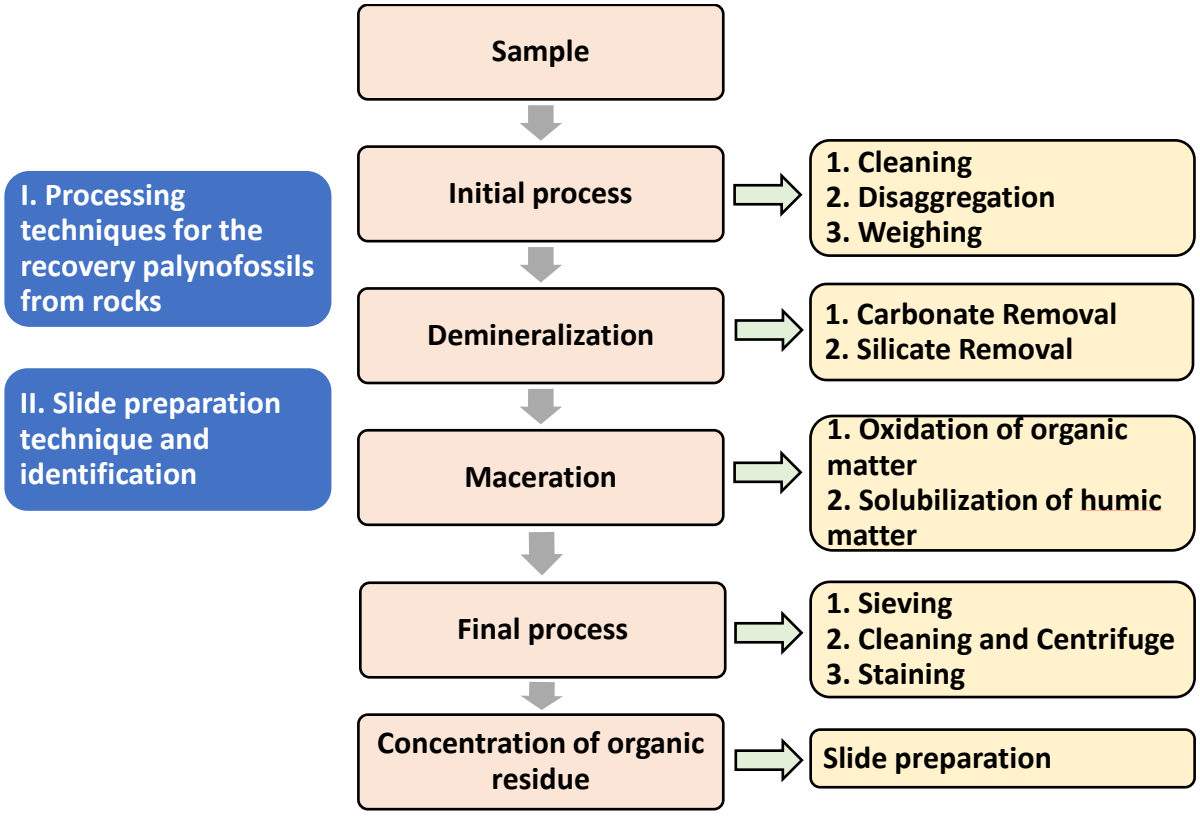

Fig. 3.2 Palynofossil extraction technique chart (after Grey (1965), Green (2001)).

3.3.4.2 Cleaning and Crushing

Samples were cleaned using distilled water after removing the loos particles and were oven dried. Following which they were crushed into pea sized fragments (<2 cm in diameter) using a mortar and pestle (Plate 3.1). Between each sample, the mortar and pestle were thoroughly cleaned to ensure least contamination. About 20 gram of sample was collected in 500 ml glass/PPE beakers for further treatment.

3.3.4.3 Hydrochloric Acid (HCI) Treatment

To remove the carbonates, like calcite and dolomite, calcium sulphates like gypsum and anhydrite, HCL treatment was given to the samples. Initially the samples were subjected to 10% HCl in a fume hood, until the reaction ceased. For carbonate dominating rock types 20-30% HCl was used. The samples were continuously agitated/stirred using glass/PPE rods. After 24-36 hours after the reaction ceased, the samples were washed using distilled water until it's become neutral (minimum 4-5 washes) (Plate 3.2), check by using pH indicating paper. The washing of the samples was done by settling technique where the acidic water in the beakers was diluted with distilled water and the supernatant was decanted after every 8-10 hours of settling.

Plate 3.1 a. Geochemistry lab showing fume chamber and working space for palynological processing of samples, b. Hotplate, c. Oven, d. Centrifuge, e. Mortar and Pestle and f. Weighing scale.

3.3.4.4 Hydrofluoric acid (HF) treatment

To remove silicates from the samples, 40% HF was used to disintegrate/digest the siliceous matrix of the rock samples. Depending on the amount of silica the treatment and digesting time highly varied for carbonates, the treatment lasted for 24 hours while for silica

rich cherts, the treatment lasted for 7-8 days. The samples were continuously agitated/stirred using PPE rods. Once the sample completely pulverised, they were washed by settling technique (6-8 hours) using distilled water until it's become neutral (minimum 4-5 washes) (Plate 3.2), checked by using pH indicating paper. (Note: Before HF treatment, the samples were carefully transferred to PPE beakers if not the case). The washing of the samples was done by settling technique where the acidic water in the beakers was diluted with distilled water and the supernatant was decanted after every 8-10 hours of settling.

3.3.4.5 Nitric acid (HNO_3) treatment

To dissolve and oxidise sulphide compounds like pyrites (FeS_2), marcasite (FeS_2) etc. and also remaining carbonate and sulphate compounds, HNO_3 treatment was given. HNO_3 being an efficient oxidant, oxidizes the organic materials in the samples. The samples were continuously agitated/stirred using PPE rods. Once the sample completely pulverised, they were washed by settling technique (6-8 hours) using distilled water until it's become neutral (minimum 4-5 washes) (Plate 3.2), check by using pH indicating paper.

3.3.4.6 Potassium hydroxide (KOH) Treatment:

To remove the humic matter from the samples 10% KOH was used for 5-10 minutes for effective reaction. The KOH worked for swelling the clay minerals which soften the matrix to the extent so it can wash away easily, and fossil grains become clearer with the finer details. Utmost care was taken during KOH treatment to prevent the damage to palynofossils. The samples were washed by centrifuging process (Plate 3.2).

3.3.4.7 Separation of organic matter - Ultrasonicator, sieving, and centrifuge:

After the KOH treatment the samples were sieved using 10-15micron sieve, in an ultrasonicator for 10-15 minutes. The collected sample in the sieve was washed using distilled water to remove the unwanted organic matter and was collected in beaker. The sample was poured in PPE vials for centrifuge process at 900 RPM for 15 minutes. The remaining material was stored in 15 ml PPE vials for further and emergency uses (Plate 3.2).

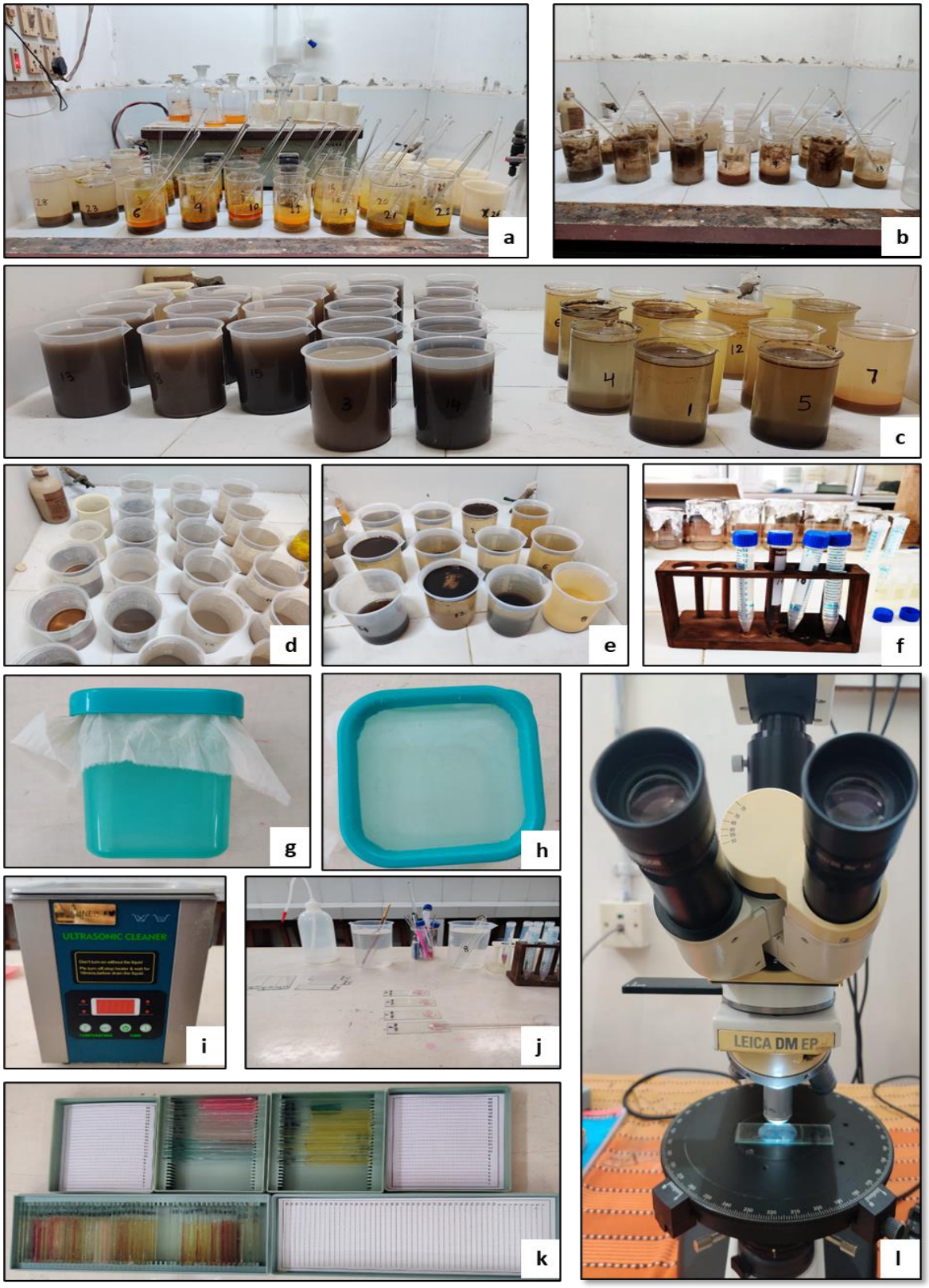

Plate 3.2 a and b. Sample demineralization in fume hood (HCl, HF and HNO_3 treatments), c. Sample washing, d and e. Sample settling, f. KOH treatment and washing by centrifuge, g and h. sieving using 10–15-micron mesh, i. Ultrasonicator, j. Slide preparation, smearing, staining, k. Cooked, permanent slides with coverslip, l. Leica DM EP microscope.

3.3.4.8 Preparation of slides

To prepare permanent slides, the concentrated organic matter at the bottom of the centrifuge vails was mixed thoroughly with polyvinyl alcohol and smeared on glass slides. Three slides were prepared for each sample with one stained slide by Saffranine O (0.1%). The slides were kept in remote and safe place to avoid contamination, and left out to dry for 24-48 hours depending on conditions. The cover glass was mounted thereafter on the clean slides using/ by smearing the Canada balsam. For permanency, the slides were cooked on a hot plate for 5-6 hours at 60°-70°C. Once properly cooked (Golden Brown), they were allowed to cool and cleaned by using xylene solution. The slides and the unused macerated materials are stored in the Micropalaeontology laboratory, Department of Geology, The M. S. University of Baroda (Plate 3.2).

3.3.4.9 Microscopic Examination

The slides were thoroughly examined for palynomorphs in transmitted light as well as under the crossed Nicol's. The high-resolution microscope (Leica DM EP with MC120HD camera, 40x objectives and 10x eye piece) (Plate 3.2) is used to get the clear and finer details of the fossils.

3.3.4.10 Qualitative and Quantitative Analysis

To find out comparative analysis of the frequency of palynofossils, composition of all specimens at random selection of slides with constant counting areas were recorded and noted. Quantitative analysis of spores and pollen grains is based on 200 counts. The frequency of fungal spores and fungal elements, pteridophytic spores, angiosperm pollen grains, algae, testate amoeba, dinoflagellates, phytoplanktons, phytoclasts, opisthokonta and amorphous organic matter is plotted for each formation according to their abundance. MS Excel was used for reliable point counts and plots.

3.3.4.11 Photography

Photographs are essential part of the research and art of photography need special techniques to employed by the skilled person. It is required to capture the finer details of the objects (palynofossils) that can be illustrated and systematically described and efficiently communicate to the other ends. Hence, the photography of the palynofossils is carried out in a such manner that the diagnostic features, a surface structures are enabled to helped in identified to them. The investigator has used the instrument is MC120HD camera fitted onto Leica DM EP (Plate 3.2).