CHAPTER-3

MATERIAL AND METHODS

The areas selected for the present paleontological research are located in Dhar and Jhabua districts of Madhya Pradesh. The most important outcrop of invertebrate fossils was noticed in and around Bagh of tehsil Kukshi and Zirabad of tehsil Manawar district Dhar and some parts of the district Jhabua. The areas of main focus for the study were Rampura, Badkeshwer, Bagh, Khandla, Padalya, Zirabad, Chirakhan, Karondiya and Chakrud of Dhar district, and Pipldehla, Rajla, Phata and Ranapur of Jhabua district of Madhya Pradesh.

Many groups of invertebrate fossils were collected viz. Cephalopods, Gastropods, Bivalves, Brachiopods and Echinoids. Beside these, fragments of bones, egg shell and nesting sites of dinosaurs were also explored. The verification of collected and identified specimens was done at “Geological Survey of India” CHQ, Curatorial division, Kolkata, West Bengal, “Agharkar Research Institute” Pune, Maharashtra and “Centre of Advanced Studies in Geology” Punjab University, Chandigarh. Classification of macro invertebrate fossil fauna has been studied and illustrated as given in different volumes of the book ‘Treatise on invertebrate palaeontology’ (Moore, 1959-1978). The geologic history of different group of fossils in the present work is followed in accordance to “A text book of geology” Mukerjee (1997).

The present investigations have been carried out both in the field and in the laboratory by adopting the conventional as well as advanced techniques. The methodology can be grouped into two categories:

A. Field investigation
B. Laboratory investigation

Field investigation:

For collecting fossils, preparation was done in advance for more productive and safe visit (Fig.2.3A-B, Fig. 2.4A-B, Fig. 2.5 A-C, Fig. 2.6A-C and Fig. 2.7A-B). The procedure included –
Locating fossils:

Localization of different fossiliferous areas of district Jhabua and Dhar has been done by land records and accordingly survey was planned. Fossils are generally found in sedimentary rocks with differential strata representing a succession of deposited material. These rocks may possess fine, medium, and coarse grain materials. Although the fossil occurred in all grain types but most of the fossils have been found in the fine grained materials.

In appearance, the fossils were of different colors due to the presence of surrounding rocks made up of different mineral contents. They have varying shape and texture. Stratigraphical and geological studies of the fossiliferous rocks of this area have been done by previous worker. Invertebrate fossils were collected by digging the fossiliferous areas of Bagh Beds up to the depth of two to three meters while dinosaur eggs, egg shell fragments and nesting sites were observed in Lameta formation. Fossils were discovered by careful searching. Utmost care has been taken so that the accompanying fossils belonging to the same group or the other cannot be neglected. During the collection of fossils, caps, glasses, boots, and protective gloves were used as safety measures. Magnifying lens was used for the detailed study and identification.

Excavations:

Some geological tools like hammer, variety of chisels and mallet were used to split and break rocks for excavating fossils. For soft sediments and unconsolidated deposits such as sand, slits and clays, a spade, flat bladed trowel and stiff brushes were used. Excavation of larger samples was performed very carefully as complete specimen was needed for thorough paleontological study and it was often constrained by time, money, and man power. It is often difficult to extract complete fossils in the field, so the specimens were extracted with some of the surrounding matrix as a block. The extra matrix was removed by using hand tools and transported to the lab. The process of digging and carrying these blocks to the destination has not changed much in the past 100 years. If excavation and transport had not been done with care, it could cause serious damage to the valuable specimens.
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**Sieving:**

Sieves in a variety of mesh sizes were used to separate fossils from sand and gravels. Utmost care has been taken as it is a rough technique, which could destroy fragile ones.

**Jacketing:**

Cloth, cotton, card board or plastic boxes and aluminum foils were frequently used to give protection and additional strength to fossils. Occasionally, large fragile specimens may need to be protected with a jacket of plaster of Paris or latex.

**Trenching:**

After the edges of the specimens have been outlined and fragile areas secured, trenching work was done around the entire specimens. Beginning several inches away from the bone, earth was removed from below, where the bottom of the specimens was thought to be.

**Labeling:**

Every specimen and jacket was accompanied by its field number. The collection and locality notes, information about jacket, approach to its removal in the lab, the orientation of the specimens etc. was written directly on the dry plastic jacket or recorded in the field note book.

**Photography and field notes:**

Photographs of the locality with the embedded specimens have been taken at the site. Despite photographs, field notes were also written in note book, which formed invaluable resource for identification of specimens in the lab. The exact location and the orientation of each fossil and position of each sediment layer in the stratigraphical sequence have also been noted.

**Transporting:**

The collected and properly labeled samples were transported to the lab carefully in strong sample bags for laboratory investigations.
Laboratory investigation:
In the laboratory, the jackets were removed. The collected macro invertebrate fossils were washed and separated from the rock matrix. For more appropriate study and confirmation of genera, they were washed and cleaned with water or dilute HCl or 10% solution of soda or potash as per requirement. Some fossils might disintegrate rapidly, once they have been removed from their matrix, so they were varnished with a solution of amyl acetate and rosin, then with acetone and celluloid. All the fossils samples were preserved in well labeled transparent plastic bags. For detailed study, they were measured and photographed with scale in various postures viz. dorsal, ventral and lateral etc. The scale in the specimen photographs show their dimensions in mm. They were then carried to different institutes for verification of their identity (Fig. 2.8B).

Methodology for Dinosaur eggshell study
Dinosaur egg, egg shell fragments, and nesting sites have been found to be firmly embedded in the Lameta limestone. Excavations of these eggs have been done by using hammer and chisel. Precautions were taken to avoid any damage to the eggs. During the investigation, dinosaur eggshell fragments and their nesting sites have also been observed confined to Lameta formation. The detailed study of dinosaur eggs regarding taxonomic confirmation has been done by thin sectioning of eggshells using light microscopy and scanning electron microscopy.

Scanning electron microscopy:
For SEM study, small chips of egg shells were removed from the eggs. The chips were found to be attached to the matrix of rock on its interior surface. A manageable size of piece (5-15 mm) from the shell was broken and the matrix was removed as much as possible, without damaging the shell above. The samples used were freshly broken pieces from the intact eggshell. Before mounting, the egg shell fragments, they were ultrasonically cleaned with distilled water for about 4-5 second. This process removed the matrix or calcareous material of the eggshell fragments.
The samples were then mounted on aluminum stubs with the help of double sided sticky tape. For connecting the shell material to holder, silver paste was used which provided an electrical conduction between the shell material and the sample holder. The paste was then allowed to dry.

The mounted specimens were coated with a thin film (~20nm) of gold palladium on eggshell fragments. This coating ensures electron emission of a sufficient number of secondary electrons for imaging. The samples were then studied by JEOL JSM-9640 (Scanning electron microscope) to observe radial and outer morphostructural features (Fig. 2.8A).

Light microscopy:

For taxonomic study of dinosaur eggs, examination of mineralogy and their surrounding matrix and structure of crystals have been observed through thin sections (about 30 microns) prepared by precision, sectioning, impregnating, grinding, mounting, trimming, polishing the specimens and finally examining them under Leica S8 APO and Leitz light microscope. Techniques began by piecing of small chips of approximately 2 cm with the help of micropetrographic saw. Sections approximately 3 mm thick were prepared. Encapsulation and impregnation of samples was done with epoxy based rosin to fill the voids, pores, and cracks, to preserve original microstructures and for better handling of subsequent sampling steps. Epoxy can be used at room temperature or at a temperature up to 60°C and can be cured within nine hours. By lowering the viscosities of epoxy by slight warming, fasten curing which varies from a few minutes to several hours depending on the type of epoxy used and the ambient temperature.

Different types of dyes are sometimes mixed with epoxy to highlight cracks, voids and pore spaces in sample. Proper sectioning help to obtain a smaller specimen from parent material. For large sections, abrasive wheel or diamond bonded machine was used while for small sectioning, precision saw was used. The section sample should be thoroughly cleaned with water, acetone and alcohol to remove debris.

Grinding and lapping, removed deformation, surface irregularities and saw marks and provided a smooth flat surface. Grinding was done by using finer, grained, abrasives (carborandum powder) in water, solvent or oil based carrier on a horizontal rotary grinding/lapping. The abrasive water mixture was scooped from a container.
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with the fingers on to the rotating Lap for rough grinding of chip. The Lap must be sufficiently charged with abrasive during the grinding. The consistency of abrasive was maintained, neither too thin and nor too thick. The grinding was done continuously until the section became slightly translucent at the edges. Polishing has been done by using 100, 400, or 800 mash carborandum powder. It minimized all fine surface irregularities left over during grinding operation and provided mirror like appearance with sharp edges and good differentiations. Mounting the section on petrographic slides has been done by Canada balsam. Now the slides were kept on hot plate to expel bubbles from the slides. The extra epoxy has been removed by a razor blade. The cover slips were placed over the material with care. Thus, petrographic slides have been prepared.

The saw marks were abraded away by sandpaper up to 1200 grit. Canada balsam was used for embedding the egg shell fragments. The sample were thinned to around 100-120 µm, so as to become almost transparent and can be studied under a polarizing microscope. The observation under plane polarized light and crossed nicols revealed the shape of shell units composing the eggshells, the shape and size of the pore canals, growth lines, and herringbone patterns.