CHAPTER-2
TECHNIQUES

2.1 - INTRODUCTION

Neoichnology lays a foundation for consideration of fossil lebensspuren as well as making palaeontological information gained from present-day traces useful without comparing fossil examples necessarily being considered (e.g. Seilacher, 1951). The chapter deals with techniques used during the study of the animal-sediment relationships. One of the prime and important approaches in studying animal-sediment relationship is 'Natural History Approach' which involves in field observation of the burrowing organisms. This is one of the most availing approaches in studying animal-sediment relationship. It has limited scope for its observation in the accessible zone or in an aquarium. A second approach, which was also followed, was similar to the first but relates functional morphology and trophic groups of the animal to their burrows. A variety of experimental approaches have also been made to study behavioral activities of mobile invertebrates, ranging from simple observation to the use of sophisticated equipment. This study provides valuable information regarding hydrodynamic conditions, when bioturbational structures replaces or alters physical sedimentary structures.

2.2 GRAIN SIZE ANALYSIS

For studying animal-sediment relationships, which includes analysis of the substrates and textural characteristics of the sediments the granulometric methods were used. Sufficient amounts of materials were collected systematically for surfacial sediments by grid patterns and by coring for subsurface sediments. Results of particle size analysis should be shown graphically as percentage per size class and features such as bimodality of sediments. Summary statistics e.g. Mean grain size, Sorting coefficient, Skewness and % of silt, sand and gravel should also be calculated. Sediment trigons were constructed based on the quantity of silt, sand and gravel from which samples can be classified, for example, sand, muddy sand or muddy gravel, etc.

The mechanical analysis was carried out for the textural study of the different geomorphic units of the Mandvi coast. Approximately, 400gm of the sample was initially washed with water and oven dried at 100^0C for over night. Then with the help of the coning and quartering of the oven dried samples, subsequently 100 gm sample

was taken. For sieving ASTM sieves of mesh size 18, 35, 60, 100, 120, 170, 230 and Pan were used and then sieved with the help of sieve shaker for 10 minutes. Then the samples retained on each sieve were weighed, calculated and values were plotted on the probability sheets. The calculations were made according to Folk (1966).

2.3 RELIEF PEELS

In order to envisaged the information regarding the hydrodynamic conditions and bedform association of the sediments of the intertidal zone, relief peels were taken during the low tide condition and subsequently trenching were made.

A relief peel consists of a thin, vertical layer of an unconsolidated material removed from the surface to downward ~50 cm. This was obtained by vertical cut and smoothening the face and applying an adhesive with cotton cloth (for increasing the strength of the slabs), when dry, it pulled off. This peel have undisturbed sedimentary property. Bouma (1964) suggested many methods, but considering the Indian climate and setting an araldite peel method was most useful. Araldite peels exaggerate the natural relief to obtain three-dimensional relief of the unconsolidated sedimentary structures.

First trenching of the selected site was done and then small amount of Quick-fix mixed with acetone was sprayed with the help of local garden spray in order to stabilize the sediments, then the paste of araldite was made thin by adding acetone to enable the material to penetrate between the sediment pores. Then thin cotton was applied, after this, the site was allowed to dry for nearly 4-6 hrs. After drying of the peels it was washed with soft spray of water for removal of loose sediments. The peels were soon photographed for further analysis.

2.4 CORING

The undisturbed subsurface sediment sampling is important for studying the different structure thickness of the layers and distribution of grain populations. Coring allow an undisturbed sample to be taken and easy inspection of the sedimentary structures. This may be important, where alternate layers of silt and coarse sandy material characterize the substratum. In order to obtain information on other features

such as the depth of the anoxic layer, etc, different small cores were used.

2.4.1 PVC Tube Cores

PVC tube cores were used to sample the subsurface sediment profile to a maximum depth of 50cm for all the sites. Cores were subsequently analyzed for grain size and X-ray radiograph for the detailed sedimentological and biological studies. To obtain cores 50cm long and 50mm diameter with 1mm thick PVC pipes were used. One end of the tube was sharpened using a file to aid its penetration in the sediment and two PVC caps fitted on each end, to seal off the core tube. The corers were used to take samples in the intertidal sediments and more successful where the sand size fractions are continue or thick, but very coarse material impeded the coring by obstructing the lower end of the tube. The sharpened end of the tube was placed gently on the sediment surface and then pushed into the seabed by hand as far as possible. A small mallet was then used to tap the corer to its full extent into the sediments. Care was taken to ensure the corer entered the sediment at right angles to the surface. A PVC cap was put on to the exposed end of the corer and the corer then pulled from the sediment with a twisting motion. A second cap was put on the other end of the corer as soon as it was dug out from the sediment. Thus, the retained undisturbed cores were taken up for laboratory study.

2.4.2 Box Cores

The box cores were used for lower intertidal substratum for obtaining the undisturbed sediment samples. These cores were taken in a similar fashion to that used for upper/middle intertidal zone, but care was taken that draining out of water may not disturb the sediments. The slightly modified version of the Senckenberg boxes were used to take cores, which reveal the internal structures of the undisturbed sediment and are therefore ideal for taking continuos samples of sedimentary structures and their surroundings. They are also useful in the preparation of sediment peels using polyester resin. The Senckenberg box (Bouma 1964) consists of two parts that slide together to enclose the sediment. The box core size used in present study is of 40cm x 30cm x 8cm in dimension, made from 1mm thick galvanized metal sheet to prevent corrosion. The

bottom edges of both parts were sharpened for easy penetration and also it prevented excessive disturbance of the sediments.

2.4.3 Plate Cores

This type of core was specially developed for getting good X-radiograph of the internal structures of bedform and biogenic structures. The corer is made up of plexi-sheet with dimensions of 15x12x1 cm. First a pit was made of more than 20 cm deep in the sediments and the other side of the pit was made wide, the section was leveled with a small trowel then plate was placed vertical and pushed into the section. After pushing, the slice was cut with the help of the trowel, and placed horizontally on the surface. Now with the help of thin wire the sediment slice was leveled up-to the height of the corer i.e. 1 cm. If the sediments were muddy then bunch of fine steel wires were used to level, care must be taken as not to draw any abnormality along with the wire, so as to avoid any sort of lines on the surface, this slice along with the corer was then warped in aluminum foil to save the moisture (Wetzel, A *personal Communication*), and avoid excessive drying and baking that disturbed the structures.

Another method employed for studying the subsurface burrows, is that a same size plate rim was made flat from one end and with the help of small pit the plate was pushed slowly, horizontally into the sediment then surrounding material was removed with the help of trowel, and plates were lifted carefully to avoid any sort of disturbances.

2.4.4 Other Corers

Small pocket corers were also used for quick sediment sampling. They comprised of two different size of cylindrical canisters having dimension of 48mm x 40mm and 35mm x 30mm. These were pushed into the sediment, twisted out and lids were replaced tightly. This method was quick and cheap and the canisters were easy to carry and handle in field. The sediment samples could also be transported easily back to the lab for drying in the samplers.

2.5 X-RAY RADIOGRAPHY

Soon after the cores were sealed, it was taken to the Maska village, where the X-ray radiography facility was available, the radio-photography was done on medical X-ray unit. Generally the plate cores with thickness of upto 3-cm yielded good results the cores were photographed, by exposing them on different settings depending upon the thickness of the cores.

2.6 BURROW CAST

Burrow casts were made to understand the 3-dimensional nature of the burrow systems. The burrows were marked, then hot molten bee-wax was poured from the burrow opening. The molten wax solidified and casted in shape of the burrow and later surrounding sediments were removed with the help of shovel and trowel. These burrow casts were warped in cotton cloth and paper to prevent it from breaking. This method was used in unconsolidated sediments of the beach and bars. Different methods are available to take the cast in consolidated substrates. The bee-wax was used because of its inflexible nature and unlike normal wax it dose not melt very fast even if exposed to sunlight.

2.7 COLLECTING ANIMALS

The collection of the animals was done according to the need and the method was fabricated accordingly. Taking deep spadeful of sand by digging, and transfering the sands into a plastic tub containing small quantity of sea water, the sediment were shifted slowly from one side of the tub to another side. If polychaetes were found they were picked with small forceps and transfer into bottle containing normal seawater. Sometimes, polychaete worms were collected by shifting sand through fingers, while water was slowly poured over the sand to clean the worms. These worms were picked by forceps and collected in to the bottle. For collecting small crustaceans, chloroform was poured through the burrow openings of the crabs and collected either by hand or with the help of forceps. For bigger crabs, local method was employed in catching the crabs either by hand or by big forceps and sometimes help was taken from local fishermen.

2.8 PRESERVING ANIMALS

Animals are needed to be properly preserved for their detailed identification. Standard methods are employed as given by Anonymous: Instructor for collectors (Invertebrate animal other than insect) 1954 British Museum Natural History, London. and Wagstaffe and Fiddler; The preservation of the Natural history specimen, Volume 1, published by Witherby LTD, London.

2.8.1 Polychaetes

Spadeful of sand was taken and was shifted with fingers for the solitary polychaetes. Then these worms were placed in a bottle for narcotizing. If the worm was found solitary then 70% alcohol was added drop by drop until the specimen no longer responded to touching. The tubicolous forms may be made to leave their tube by sprinkling crystals of methanol on the surface and leaving it overnight. Once flaccid, transfer the specimen from narcotizing fluid to any glass plate, then with the assistance of camel brush, arrange the parts of the worm. In polychaetes with a eversible proboscis, this may be permanently made to be extended by pressing with the injure just behind the worms head. Then add forma-alcohol and leave it for 20 minutes to kill, then transfer it to another place for permanent fixure.

2.8.2 Crustaceans

The narcotizing process is also same, but sometimes it can be varied, by placing the animal into the seawater and keeping it in the freezer. Once chilled add fresh water and again freeze it and when the specimen become unconscious, add either alcohol or 10% formalin. If the specimen has to be saved for longer time then add boiling formalin and preserve it. But since conserving in field requires special modifications, kill the specimen after narcotizing with methanol crystal and preserve it. The specimen could be prepared for transportation by wetting large piece of cotton into alcohol or formalin and warping it around the crab. This is possible only when the specimen are in the solution for nearly a week.

2.9 PHYSICO-CHEMICAL ANALYSIS OF THE WATER AND SUBSTRATE

2.9.1 Temperature

The parameter of the temperature is important for its effect on the chemical and biological environment of the habitat in which organism dwell. It is also important in determining of the various other parameters such as pH, conductivity, and saturation levels of other chemicals and gases, etc. Keeping all the factors into consideration, temperatures of the water and below lying sediment was taken by thermometer. Temperatures were determined for surface temperature of the water and sediment. The ambient temperatures were measured in the low tide conditions. The areas were selected based on the necessities and a well-calibrated mercury thermometer was inserted and readings were taken.

2.9.2 Dissolved Oxygen

Dissolved oxygen is one of the most important parameter in ecological consideration. It reflects the physical and biological processes prevailing in the water. Its presence is essential to maintain the higher forms of biological life in water. To calculate this, *Wrinkler's iodometric method* was followed (for details refer Trivedy and Goel 1984).

Principle:- The manganese sulphate reacts with the alkali (KOH or NAOH) to form a white precipitate of manganese hydroxide which in the presence of oxygen gets oxidized to a brown colour compound. In the strong acid medium mandanic ions are reduced by iodide ions that are converted to iodine equivalent to the original concentration of oxygen in the sample. The iodine can be titrated against thiosulphate using starch as an indicator.

Reagents: (a) Sodium thiosulphate, 0.025N (b) Alkaline potassium iodide solution (100 gm of KOH + 50gm of KI in 200 ml of boiled distilled water. (c) Manganese sulphate solution

(d) Starch Solution (e) Sulphuric acid (sp. gr. 1.84)

(1) First fill the glass stopered bottle with known volume, avoiding any kind of

bubbling. (2) Pour 1ml each of $MnSo_4$ and alkaline KI solution, and a precipitate will appear. (3) Shake the bottle by inverting it and add conc. H_2So_4 and shake well to dissolve the precipitate. (4) Titrate the content against the sodium thiosulphate using starch as an indicator. At the end point initial dark blue colour changes to colourless.

Calculations: When whole part is titrated.

$$\text{Dissolved Oxygen, mg/l} = \frac{(\text{ml x N}) \text{ of titrant x } 8 \text{ x } 1000}{V_1 - V}$$

When only a part of the content is titrated:

$$\text{Dissolved Oxygen, mg/l} = \frac{(\text{ml x N}) \text{ of titrant x } 8 \text{ x } 1000}{V_2 \frac{[V_1 - V]}{V_1}}$$

Where V_1 = Volume of sample bottle after placing the stopper. V_2 = Volume of the part of the content titrated. V = Volume of $MnSo_4$ and KI added. In Oceanography, the unit of ml/l is preferred over mg/l. It can be obtained by dividing the value in mg/l by 1.43.

Free Carbon dioxide: Free co2 can be determined by titrating the sample using a strong alkali (such as carbonate free NaoH) to pH 8.3. At this pH all the free co2 is converted into bicarbonates.

Reagents: (a) Sodium hydroxide, 0.05 N (b) Phenolphthalein indicator. (1) First take 100 ml of sample in a conical flask and add few drops of phenolphthalein indicator. (2) If the colour turns pink, free co2 is absent. If the sample remains colourless titrate it against 0.05N NaoH. At the end point a pink colour appears.

Calculation:- $$\text{Free } co_2\text{,mg/l} = \frac{(\text{ml} * \text{N}) \text{ of NaoH} * 1000 * 44}{\text{ml sample}}$$

2.10 PHOTOGRAPHIC DOCUMENTATION

Photographic documentation is the most important technique for studying the animal behavior. Since animals are very active, it becomes difficult to constantly monitor them, either in the field or in an aquarium. The Cameras used were Nikkormat with Micro lens of Nikkormat, 1:35, 55mm lens along with Tele-lens of power 200m and 300m (Nikon) and Ashai Pentax with Normal lens (35mm). The Tele lens was used for studying the crustacean behavior from a distance. Generally 200-300m tele is sufficient and provides enough coverage so as not to disturb the natural activity of the crustaceans, as they are very active and sensitive. Micro-lens was used in documenting the behavioral activity of polychaetes.