MATERIAL AND METHODS

1. STUDY AREA
    - 1.1. Location
    - 1.2. Site description
        - 1.2.1. Upstream
        - 1.2.2. Midstream
        - 1.2.3. Downstream

2. ABIOTIC STUDY
    - 2.1. Habitat survey and sampling
    - 2.2. Water analysis
    - 2.3. Sediment sampling and processing
        - 2.3.1. Scooping
        - 2.3.2. Pipe coring
        - 2.3.3. Sediment block study
    - 2.4. Sediment analysis
        - 2.4.1. Sediment composition
        - 2.4.2. Bulk density
        - 2.4.3. Water holding capacity
        - 2.4.4. Organic matter

3. BIOTIC STUDY
    - 3.1. Animal collection and preservation
        - 3.1.1. Crabs
        - 3.1.2. Molluscs
    - 3.2. Taxonomic identification
    - 3.3. Quantitative faunal studies
    - 3.4. Burrow casting
    - 3.5. Radiological studies
    - 3.6. Behavioural studies
    - 3.7. Avifaunal studies
    - 3.8. Photographic and videographic documentation

1. STUDY AREA

1.1. Location

Mahi River (583 km long and 34,842 km in drainage surface) is one of the major rivers of Gujarat (Fig. 1). It originates from the Mahi Kanta hills in the Vindhyachal range of Madhya Pradesh. The Mahi River basin spreads over Rajasthan, Madhya Pradesh and Gujarat states. The present climate in the Mahi river basin is sub-humid to semi arid with warm wet summers and cool dry winters. The mean annual rainfall at the upper reach and the lower reach are 850 mm and 600 mm respectively (Shridhar, 2009). The estuarine stretch of Mahi river extends up to 50 km upstream (Vasad - 22° 26 'N and 73° 04 'E) and opens into Gulf of Khambhat at Kamboi (22° 12 N and 72° 36 °E) (Plate1a).

1.2. Site Description

The estuarine stretch of Mahi River was taken into consideration for present study. The geomorphology and hydrodynamics of the Gulf of Khambhat makes the estuary very specific in terms of sedimentology as well as water quality. The hydrodynamic cause high sediment input on the banks as well as erosion of the opposite bank subsequently forming geologically important exposed sections as well as well developed ravines. For convenience of the study, the entire estuary was divided into 3 zones; Upstream, Midstream and Downstream based on salinity, total dissolved solids as well as area division. In general, the study area comprises of various geomorphic features, such as total freshwater region, freshwater and marine mixing zone, lagoon, small uplands, sand flats/dunes, tidal mudflats, ridge and runnel systems and fine sandy low intertidal areas.

A total of 14 sites were considered for present studies of which 10 sites were located on the south bank and 4 sites were located on the north bank of Mahi estuary (Fig. 2). The section wise distribution was; 4 sites in upstream, 5 sites in midstream and 5 sites in downstream. Since all the sites were not approachable throughout the year, particularly during monsoon, Fajalpur from upstream, Dabka from midstream and Sarod and Kamboi from downstream were considered as representative sites and visited frequently for studies.

1.2.1. Upstream sites

Upstream was represented by 4 sites viz., Fajalpur, Rayka, Singhrot and Jaspur on the south bank. Upstream site Vasad (north bank)/Fajalpur was the uppermost reach which mostly remained freshwater section and was influenced by tidal water only during the highest high tide. During the study period a wear was constructed by government down to the Vasad near Umeta/Singhrot to prevent the salinity ingress in freshwater and groundwater in adjacent areas (Plate 1d). This resulted in blocking the ingress of tidal water thus increasing the water logging at Vasad/Fajalpur. Mahi River is an important pilgrim place having few temples and places of religious interest on its bank and attracts religious visitors on special occasions. Fajalpur, at upstream being easily approachable from highway and being religiously active, remains busy with pilgrims and gets crowded on special religiously important days. Moreover, small scale local fishing activity is carried out at the site. The adjacent banks on upstream have good open sections which are geologically important.

1.2.2. Midstream sites

Down the line Mujpur, Dabka, Mohammadpura and Chokari on the south bank and Gambhira on the north bank of Mahi River formed the midstream sites. Amongst all, Dabka was selected as a frequent study site due to its specific midstream location and easy approachability while others were visited intermittently. Topographically midstream shows a meandering of the river thus specifically showing erosional and depositional sites (Plate 1b). Midstream sites suffer regular tidal effect twice a day (Plate 1c). Few of the midstream sites show deposition of coarse sand. Dabka and Mohammadpura were the sites of high river meandering which was reflected by the sand dunes on the depositional site of Dabka. The erosional side of river banks at Dabka, Mujpur, and Jaspur shows prominent cut faces of varying height showing paleogeological features in terms of sediment depositions. Most of the midstream sites other than Mohammadpura does not show exposed intertidal/bank area during low tide. Mohammadpura on the other hand shows good exposed intertidal area during low tide. Moreover, Mahi estuary in midstream area shows many small uplands/islands in the riverine channel which diverge the channel at few points.

1.2.3. Downstream sites

Downstream area of the Mahi estuary was represented by sites viz. Badalpur, Dhuvaran and Khambhat on the north bank and Sarod, Nahar and Kamboi on the south bank of Mahi River. Kamboi, located at the Mouth of the estuary that shows high marine influence, was selected as a prime site for the study. Kamboi represents a broad estuarine mouth opening into the gulf of Khambhat and represent tidal mudflats (Plate 2a). Kamboi shows distinctive estuarine intertidal area with a high tide line and low tide line. The upper intertidal area is represented by elevated mudflat while the lower intertidal area is a flat plain with fine sandy composition. The two areas are separated by a runnel system and small tidal pools (Plate 2b-e).

2. ABIOTIC STUDY

2.1. Habitat Survey and Sampling

Study area was initially surveyed for site selection based on long term approachability of an area. The survey also analysed the important topographic features which directly or indirectly affect the animal distribution and can be taken into consideration during the entire study period. Few of the sites were not feasible for water sample collection but were considered for faunal sampling for the overall estuarine benthic diversity study. Preliminary habitat mapping was done using the topographic features.

2.2. Water Analysis

Water samples were collected from study sites (upstream, midstream and downstream) using 1 lit. polypropylene bottles. Looking to the approachability of an area, samples were taken from bank of the river (knee deep water) or from the main channel using boat whenever possible. In case of downstream sampling (i.e. kamboi), samples were taken from main channel during low tide and direct incoming tidal water during high tide timings. Sampling from very shallow and churning water was usually avoided as can give altering turbidity level than the routine.

Vasad (Upstream), Dabka (Midstream) and Kamboi (Downstream) were kept as fixed sites for sample collection while samples from other sites along the estuary were also taken whenever visited. Finally, the sites were pooled to 3 zones viz. upstream, midstream and downstream based on which salinity zone they lie. Salinity was measured using handheld refractometer (IRMA) with range of 1-100 ppt. Upstream samples with salinity less than 1ppt were subjected to titrimatric method using $AgNO_3$. Samples were brought to laboratory on the same day and were analyzed for various parameters using standard methods (APHA);

2.3. Sediment Sampling and Processing

Sediment samples were collected from different sites, from aquatic bed material and different sedimentological features within the site from upstream, midstream and downstream sites. Different methods of sediment collection were employed based on type of analysis and study components. Coring provides undisturbed subsurface sedimentary inspections and sedimentary structure within. It is also an effective tool to look into details of the subsurface oxic and anoxic layer and animal penetration.

2.3.1. Scooping

This method was employed for superficial (upper 5-7 cm) sediment layer. The sediments were scooped using a shovel after removing upper 1-2 cm layer of sediment. The sediments were taken in plastic zip bag and carried to the laboratory for further analysis. The sample to be used for animal isolation was preserved in 4 - 6% formalin.

2.3.2. Pipe Coring

PVC pipe coring was used to sample the subsurface sediments for a profile study. For the purpose, PVC pipe of 37cm (15 inch) length and 75mm diameter with 1 - 2mm wall thickness was used for coring. Core was taken at selected sites of upstream, midstream and downstream locations (Plate 3a & b). The core was placed straight (right angle) on the sediments and pressed into the bed by hand as much as possible and later by a strokes with wooden piece. After the core was filled by sediments to full extent, a PVC cap was put on the exposed end of core and the pipe was pulled from the sediments with twisting motion. At many instances, due to high compactness of the surrounding sediments the area adjacent to the core was dug out to trace the end of the core. The core was carefully taken out and was sealed on the other end casing with the PVC cap. In case of sampling for microbial studies, pipe core was immediately sealed with wax after capping and covered with adhesive tape to avoid air contamination. Finally, the core was carried to the laboratory for further analysis. The core was later on divided and cut into 3 divisions/depths 0-5 inch, 6-10 inch and 11-15 inch.

2.3.3. Sediment block

Hard substratum (partially calcritized sediments) in the tidal pools is biogenically important for few of the benthic species. Also they are being sculptured by various animals and serves as a good protection to the dwelling animals from the external environment and disturbances. To study their internal modifications as a whole, the sediment block was taken and carried to the laboratory by wrapping in paper/cloth in order to minimize the travel damage to the block (Plate 3c).

2.4. Sediment Analysis

After bringing to laboratory the samples were air dried and powdered in order to break the clumps and sediment aggregation. These samples were later used for physical and chemical analysis other than pH.

2.4.1. Sediment composition

Sediment sample were dried, powdered and treated with hydrogen peroxide washed with distilled water and supernatant was drained twice to remove the hardness due to salts which causes clumping of the grains. Later on sodium fluoride was added which serves as antiflocculent and avoids clumping of grains. Mechanical dry sieving method was employed as described by Folk (1957). Sample was taken after coning and quartering so as to minimize biasness. As differential analysis of sediment composition into silt and clay does not make a significant difference with reference to animal distribution, silt/clay were taken as a whole. Sieves with standard ASTM no.

150 (0.106mm), 200 (0.075mm) and 250 (0.063mm) were used looking to the nature of the sediments to find the sand and silt/clay composition. At some instances wet sieving method was also employed with same sieves in case sample contained high silt and clay.

2.4.2. Bulk density

Bulk density is the measurement of the sediments compactness in a given particular volume. Tapping method was used to define the bulk density modified from method adopted by Blake and Hartge (1986). Volume of the flask was measured accurately. After drying, the flask was filled to rim with the powdered sediments and tapped for fixed number by counting (100 taps). Tapping was done to allow the proper settlement of the grains and sediment sample was added further if required.

$$\textbf{Bulk Density} = \frac{\textbf{A-B}}{\textbf{Volume of Flask}}\ \textbf{gm/cm}^3$$

Where: A = Total weight (Flask +sediments) gm

B = Weight of Flask (gm)

2.4.3. Water holding capacity

Specific amount of measured dry sediment sample was taken in the funnel lined with Whatmann no. 4 filter paper. The filter paper was saturated with water so that it does not soak additional water. Specific amount of water was added gradually and allowed to drain slowly in the flask. The sample was allowed to stand for 3-4 hours so as to allow drainage of water. The amount of water retained in the flask was measured and final calculation was made for percentage water retention by the sample.

2.4.4. Organic matter

Sediment samples were air dried and finely powdered. If needed the sediment were made free from calcretes and pebbles. A required amount of sample was taken from the bulk after a enough mixing taking care of avoiding any biasness. The organic matter analysis was done by acid digestion of samples following titrimetric method commonly known as Walkly black method (1934).

3. BIOTIC STUDY

3.1. Animal Collection and Preservation

Benthic animals were collected on field and carried to the laboratory for further preservation. The macrofauna like crabs, mollusks, polychetes were collected, narcotized with either menthol crystals or adding freshwater in case of crabs to avoid shrinking of appendages. Later on they were preserved in 4-8% formalin. Polychetes were mostly preserved in 70% alcohol.

3.1.1. Crabs

The crabs were either handpicked or were forced to come out of their burrows by pouring menthol crystals or some alcohol in the burrows. In some cases the burrows were dug out and crab was collected.

3.1.2. Mollusca

The mollusca specimens were collected merely by handpicking from all the regions as well as sediment sieving specially in case of downstream mudflats. The collected shells were brought to the laboratory and separated for live and dead specimens. Shells were cleaned using dilute HCl in order to remove the deposited debris and retain the original colour pattern of the shells.

The sediment samples were preserved in formalin, stained with rose Bengal and sieved through 150 ASTM mesh sieve to collect the benthos.

3.1.3. Foraminiferan sampling

Foraminiferans are the important tools for paleoenvironment assessment and prediction. During the course of study sediment samples for foraminiferan analysis were collected to in different ways depending on the site of collection. A core of nearly 1.5m was taken from downstream Kamboi mudflat for depthwise analysis. Regular cores of 36cm were also used for the purpose. In midstream site where coring was not possible, a vertical trench was made on the exposed bank by removing superficial eroded sediment. In case of upstream, in order to obtain a marine clay traces (having foraminifera), a trench was dug which showed different layers of sediments which were collected for analysis. (Plate 5).

3.2. Taxonomic Identification

In case of crabs, the detailed identification to species level was done using the morphometry of animal. Important identification marks like appendages, carapace structure, pleopod structure, 3rd maxilliped, chelepeds etc. were studied by separating different parts and drawing them to get the exact picture (Ajmal khan et al., 2005, Ng et al., 2008; Sakai, 1937; NIO database). Molluscs were identified using standard key (Subbarao, 1989; Apte, 1998). As invertebrate identification is complex and needs preciseness to deal at species level, the national and international experts were consulted, sent specimen (to national experts only) or photographic documents even the concern of international experts was taken (by photographic correspondence) to make the identification more authenticated.

3.3. Quantitative Faunal Studies (Transects, Quadrates and Biomass)

In order to reveal the brachyuran crab distribution on the estuarine intertidal area, transect sampling method was employed. A total 16 line transects, extending up to 175 m perpendicular to the shore line (surf zone to lower intertidal area) and covering all specified regions, were laid, spreading nearly 5 km of the length of the estuary. A burrow count technique involving counting of open burrows falling on transects line was employed to analyse the density and distribution pattern, keeping in mind both efficiency and reliability of the method as discussed by Butler and Bird (2007). The transect line was divided into 35 units, in which each unit represented 5 m length of transect and the burrow counts for every 5 m (=1 Unit) were considered to demarcate easily the different zones and correlate burrow counts with linear distance and estuarine zoning. All crab burrow openings falling on the transect line and within 5 cm in its vicinity on both the sides were counted (Plate 3e).

Quadrate sampling (0.25 X 0.25 m) was done for each zone randomly and amplified to density per sq. m (Plate 3f). Multiple quadrates were taken from each zone to study the distribution pattern, as well as to minimize the error and support the transect data.

Biomass samples were taken by digging 25 cubic cm of the sediments (Plate 3d). The sediments were sieved and after their separation the wet weight was recorded. In case of highly silty clayey samples where sieving was not possible, animals were separated by manual separation with continuous draining water on it.

3.4. Burrow Casting

Species specific burrow architecture pattern exists in different macrofaunal groups. While X-ray gives a single dimensional view to subsurface burrow structure, burrow casting helps to reveal the actual subsurface 3 dimensional architecture of burrow. Burrows of crabs and mudskippers were casted in present study. Initially, paraffin wax (congealing point 58-60°C) was used. Wax was melted using gas stove on field itself and was poured into the burrow after forcing the animal out of the burrow. The wax was allowed to stand for hardening and was later on traced out by digging the adjacent area sediments taking care that the cast is not damaged (Plate 4b). As the area was muddy and water logged, wax did not consolidate easily. Moreover, wax casts are more brittle and fragile and were damaged easily while carrying to laboratory and handling. To overcome this problem, industrial resin was used as a good option (Griffis and Suchanek, 1991). A clear cast epoxy resin was used and poured in burrows. Resin was allowed to stand and harden and was dug out finely to get a burrow model (Plate 4a). Resin casts were usually much stronger and sturdy for transportation and handling. The casts were cleaned with water, wrapped in a cotton/ cloth or paper and were carried to the laboratory.

After reaching laboratory, the casts of different species were subjected to morphometric studies like burrow cast length, diameter at different levels (opening, trunk diameter, neck diameter, resting chamber diameter etc.), number of bending points, number of resting chambers etc. in order to get the detailed subsurface structure of the burrow.

3.5. Radiological Studies

In instances to check the subsurface biogenic structures, radiological tools were used. Pipe cores after sealing at the ends were carefully taken to the radiological laboratory. Digital X-ray of pipe cores were taken to see the inner burrow structure other fine structures. In case of finer biogenic structures produced by smaller benthos like amphipods, isopods and few polychetes, resin or wax casting was not possible and moreover, X-ray did not give substantial results. In such cases, sediments blocks were directly taken to the radiological laboratory and were studied using Computed Tomography Scan (CT Scan). The result generates a 3D view and was even able to slice the sediment block at required levels to see the complex burrow structure virtually.

3.6. Behavioural Studies

Cryptic activities of benthos on exposed intertidal area can be well studied by their behavioral displays. The track and trail marks, burrowing patterns, feeding and burrowing expels by crabs and other benthic forms are some of the indirect evidence which mark the presence of the species in the ecosystem and provides clues to habitat interactions. Track and trail marks of different species were visually and photographically documented and standardized. The burrowing pattern of different crabs, mudskipper and few arthropods were documented with their marking specificity and dimensions. The feeding range and pattern of crab species was studied by taking a scaled photograph and measuring the area covered by crab using Bersoft Image measure software (Plate 4e). The feeding and burrowing pellets were weighed using portable electronic balance (Digital balance with weighing range min. 0.1g – 200g) (Plate 4d). Burrow orientation of selected crab species was studied by placing a stick in the burrow and noting the orientation using magnetic compass (Plate 4f).

3.7. Avifaunal Studies

Birds are one of the important predators in superior most trophic level of estuarine food chain. Avifaunal diversity was observed during field visits in the vicinity using various basic field techniques of avifaunal studies. The observations were carried out during early morning or evening hours or throughout the day during different seasons depending upon the species diversity and their status with reference to occurrence in the area and migration etc.

3.8. Photographic and Videographic Documentation

Many of the species are opportunistic and swift to be collected. Also, their preservation may, in due course, cause loss of original colour and pattern or some morphometric distortions particularly in case of soft bodied animals. This in turn can cause difficulty in their identification later on. Keeping in mind these aspects, photographic documentation of animals and habitat features was done in field using Canon S5IS digital camera. Further, the collected specimens were thoroughly cleaned and appropriately photographed in the laboratory. In case of crab morphometry, photographs of various body parts of the different species were taken using macro/super macro zoom function. Behavioural displays of crabs were documented by taking video recording in field using Sony Handycam without disturbing the animal.

The recording from distance was done continuously for five minutes and following a break of ten minutes rerecorded for further 5 minutes. Such several bouts of activities were recorded for at least 20 different individuals of a species during monthly visits. This was repeated during different seasons.

Figure 3 represents the flow chart of the work done and the adopted methodology with its respective justification as well as interrelationship. It clearly divides methods adopted for biotic and abiotic study.

Table 1. Locations of different sampling sites along the Mahi river estuary. Sites marked with * represents regular sites visited frequently.

Site No.	Site Name	Site category	Latitude	Longitude
1	Rayka	Upstream	22°27' 01.03 N	73°65' 21.59 E
2	Vasad		22°26' 28.33 N	73°04' 23.17 E
3	Fajalpur*		22°26' 16.80 N	73°04' 34.34 E
4	Jaspur		22°18' 31.00 N	73°02' 40.23 E
5	Mujpur	Midstream	22°15' 20.69 N	72°59' 13.75 E
6	Gambhira		22°15' 54.85 N	72°58' 18.00 E
7	Dabka*		22°15' 01.16 N	72°57' 29.67 E
8	Mohammadpura		22°17' 22.20 N	72°56' 21.61 E
9	Chokari		22°13' 13.15 N	72°52' 21.16 E
10	Badalpur	Downstream	22°15' 27.79 N	72°47' 54.89 E
11	Dhuvaran		22°13' 55.18 N	72°45' 39.94 E
12	Sarod*		22°11' 05.71 N	72°42' 53.40 E
13	Nahar		22°11' 36.55 N	72°41' 29.00 E
14	Kamboi*		22°12' 59.13 N	72°36' 55.47 E

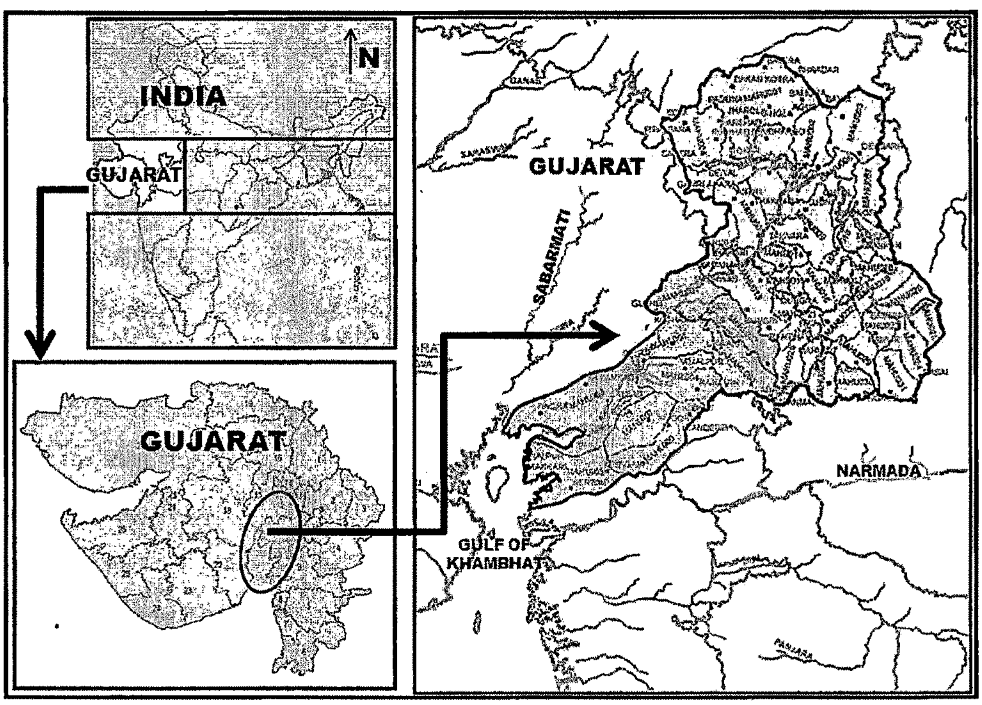

Fig. 1: Map showing location of Mahi River with reference to Gujarat and India.

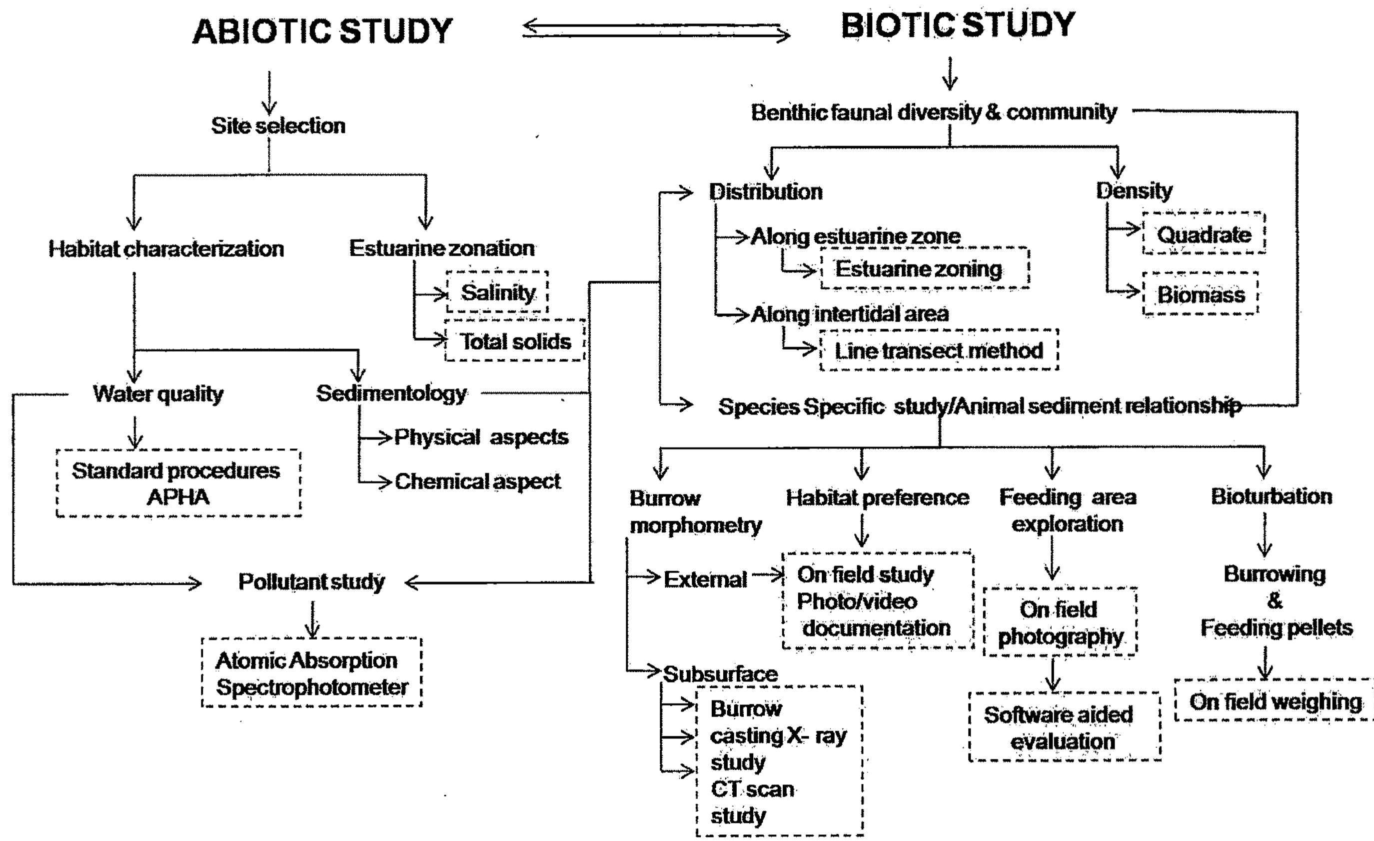

Fig. 3: Flow chart showing the diagrammatic representation of entire study and the important methodology applied. Text in [ ] represents the methodology applied to carry out the respective work.

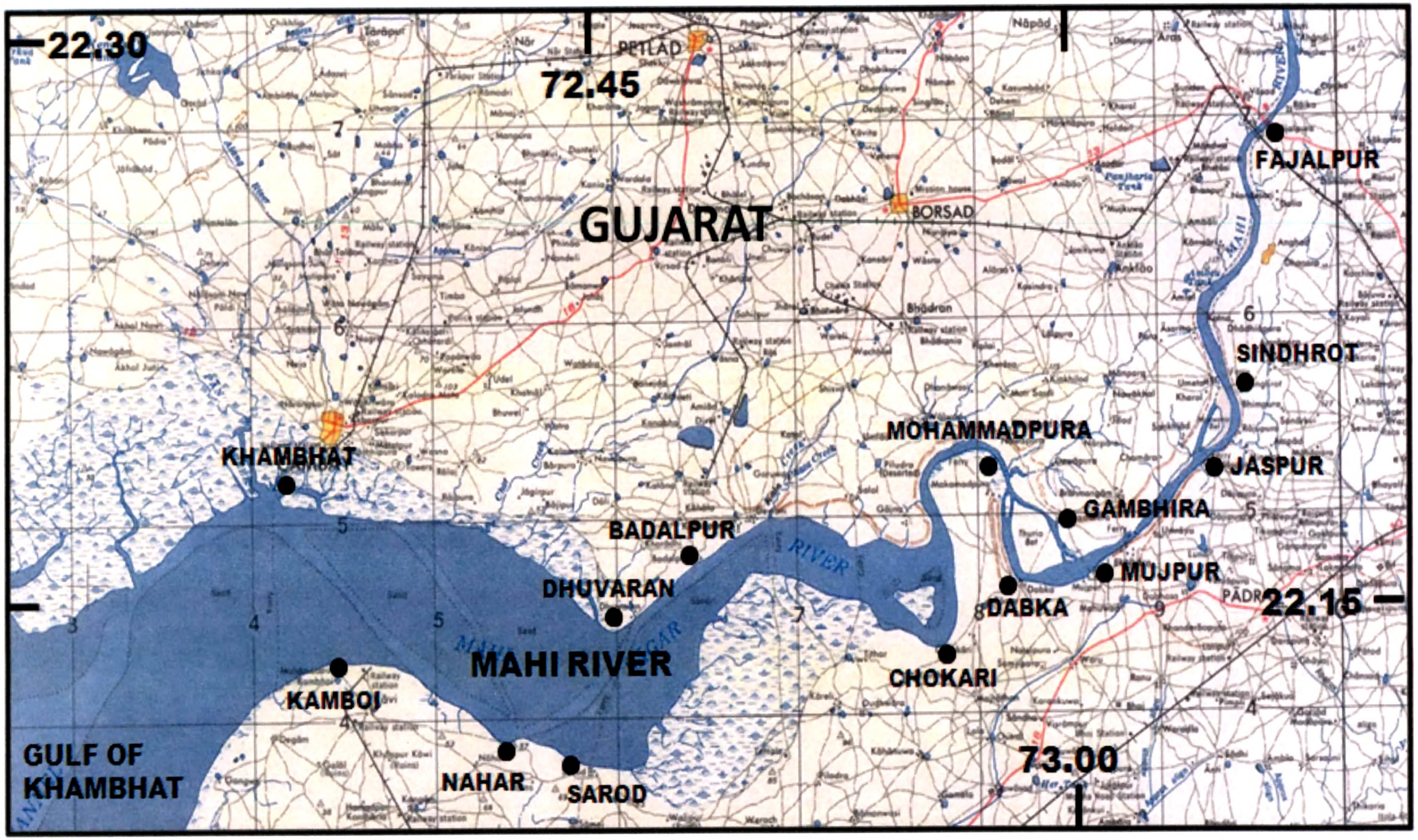

Fig. 2: Map showing locations of the study sites.

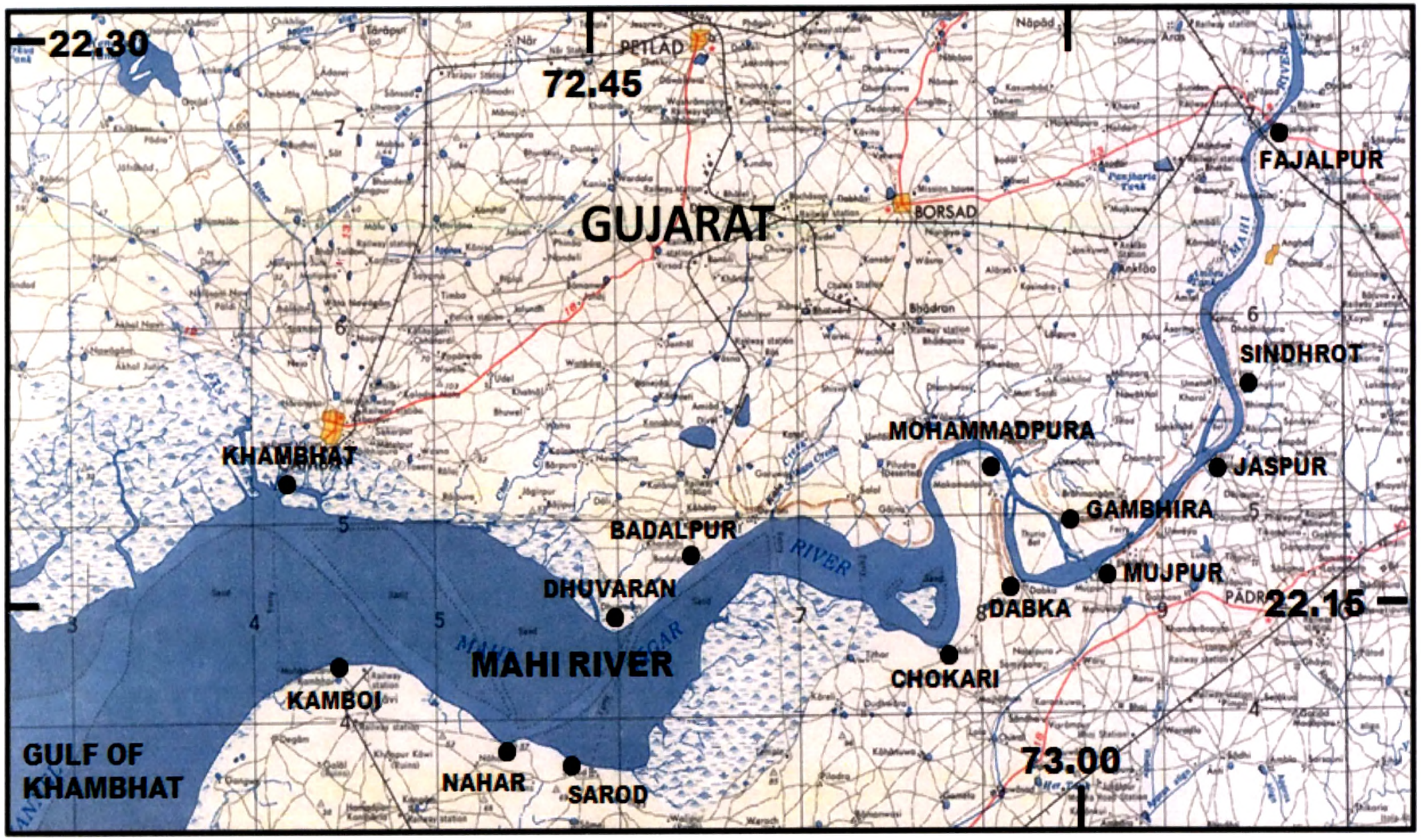

Fig. 2: Map showing locations of the study sites.

Plate 1

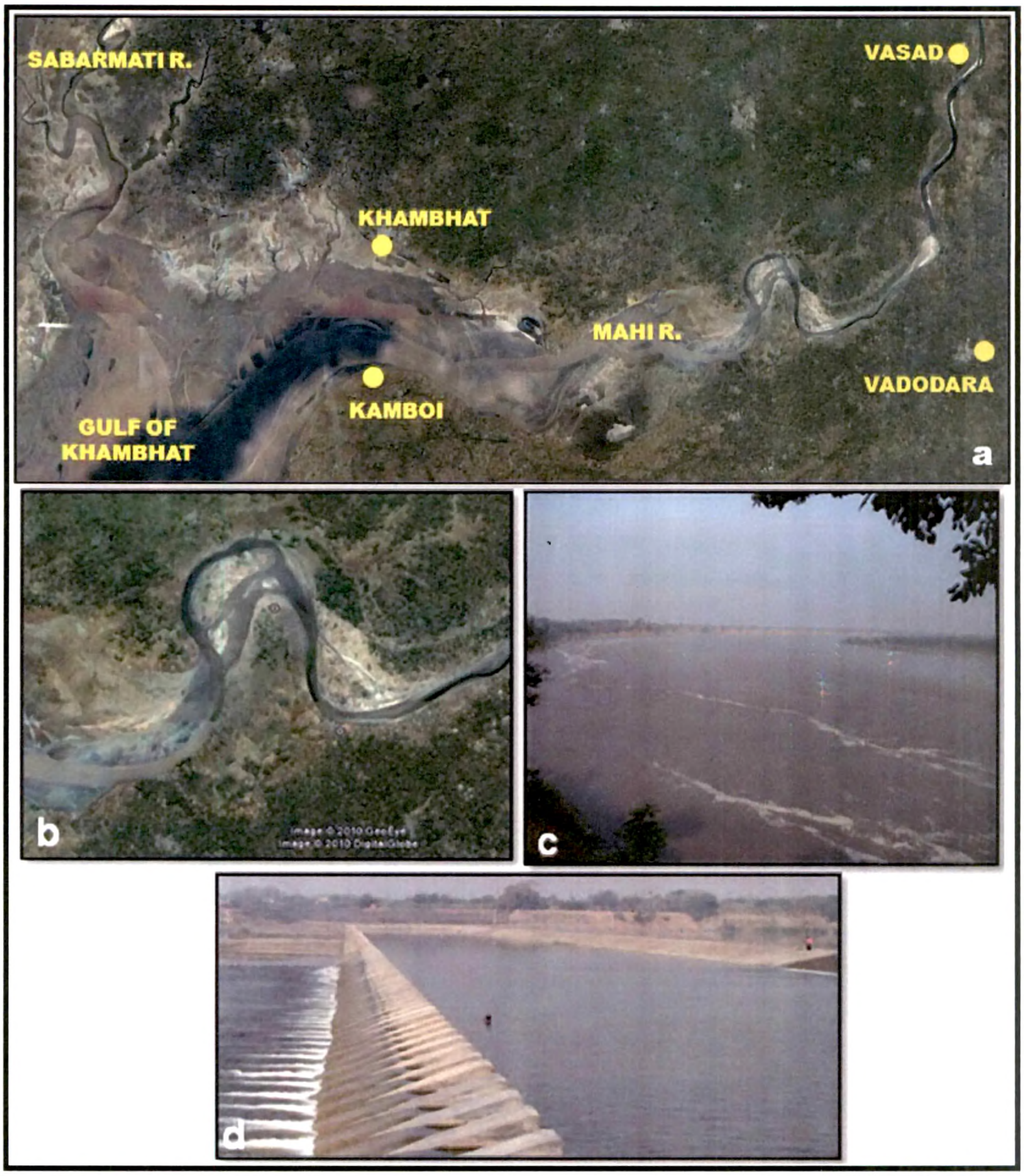

Plate 1: a) Spatial image taken from Google earth showing entire study area, Mahi river estuary. b) Sharp meandering of estuary at midstream. c) Meandering of river at midstream and tidal inflow. d) Wear constructed at Sindhrot (Upstream site) to prevent marine inflow and storage of fresh water.

Plate 2

Plate 2: a) Satellite image of mouth of Mahi estuary showing its expansion at downstream sites. b & c) Kamboi site status during low tide and high tide respectively. d) Upper intertidal mudflat of Kamboi. e) Lower intertidal area at Kamboi.

Plate 3

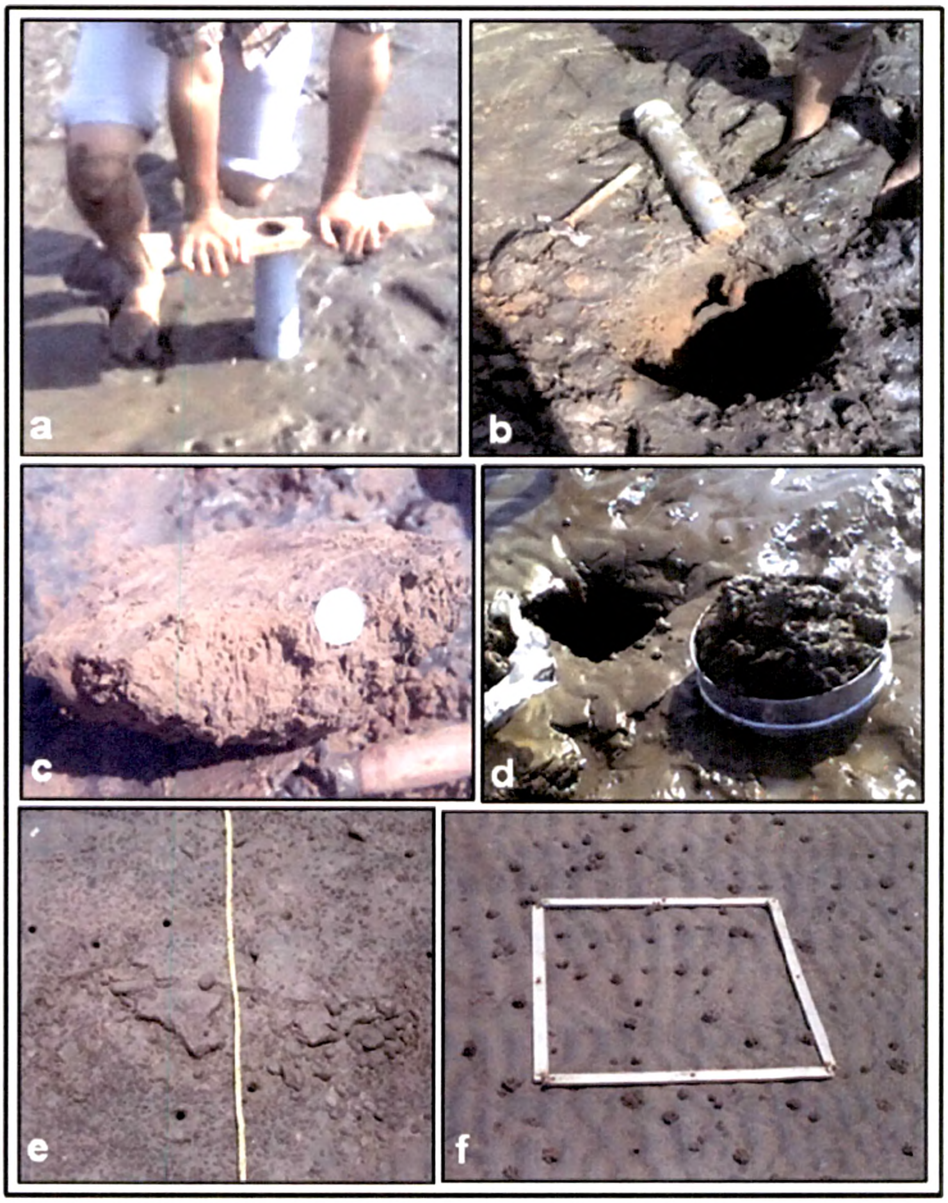

Plate 3: a & b) Pipe coring method for sediment profile studies. c) Sediment block for microhabitat and burrow architecture study. d) Sediment sampling of 25 cu m area for biomass estimations. e) Line transect method for counting crab burrow openings. f) Quadrate sampling for counting crab burrow openings.

Plate 4

Plate 4: a) On field Resin casts of crab burrows within the sediments. b) Wax cast of crab burrow. c) Tagging of burrows under observation using colour coded flags. d) On field measurement of bioturbated sediments using digital electronic weighing balance. e) Feeding exploration area calculation for crab after on field standardization of the technique. f) Burrow orientation study using magnetic compass for direction confirmation.

Plate 5

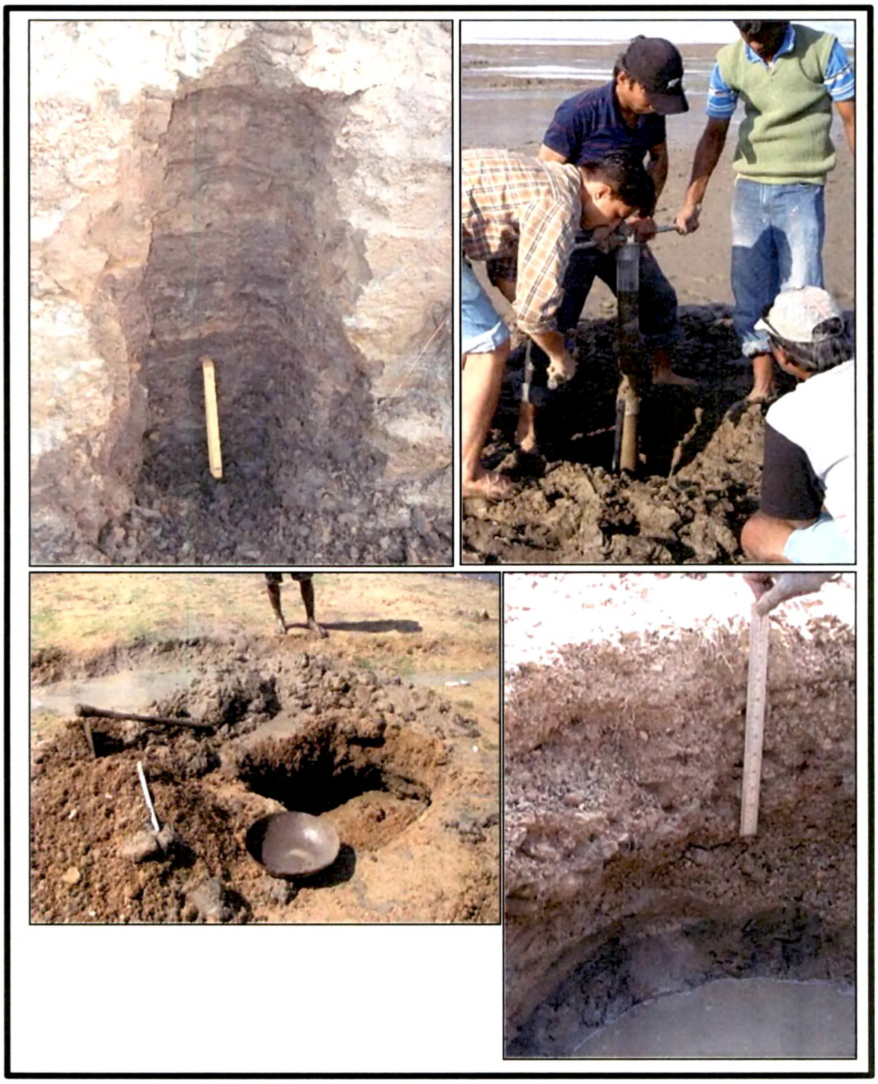

Plate 5: Sampling for Foraminifera studies from the cliffs and estuarine mud flat regions. Lower half images shows trenching at upstream site for foraminiferan traces evidencing marine influence.