Materials & Methods:

4.1. Selection of the Study Site:-

Study site was choosed based on initial pilot survey to collect the molluscs species from different spots at Digha coastal belt (Fig. – 1 & 2). Study points were so located as to produce a significant and accurate understanding of the existing hydro-biological characteristics as the molluscs specimen is benthic in nature.

Inter tidal belts at Digha coast were selected for the detailed study of molluscs availability. But for detail study of hydro-biological parameters three contrasting sampling station viz. study site – I (Digha Mohana, noted as A – I), study site – II (New Digha Ghat, noted as A – II) and study site – III (Udaypur Ghat, noted as A – III) at Digha with Bay of Bengal, West Bengal-Odisha coast, India had been selected. The seasonal hydro-biological study is done covering three seasons i.e. Monsoon, Post monsoon and Pre monsoon.

4.2. Physiographic of the study site:-

The coastal belt of Midnapore (East) district of West Bengal, India extends longitude of 87^0 5′ E to 88^0 5′ E and latitude of 20^0 30′ N to 22^0 2′ N (Fig. – 1). In the present work, three contrasting study sites viz. study site – I (A – I), study site – II (A – II) and study site – III (A – III) located along the intertidal belts of an ecotone at the confluence of Dubda water basin estuary with Bay of Bengal, West Bengal - Odisha coast, India have been selected.

The history of old and new Digha both is not so old. In 18th century, the Digha village under Birkul Parganas under the British rules was a health resort for the British in India. It was considered as a most popular weekend beach resort in our then the Bengal. In the present day, about 40 lakhs or more tourists visit Digha every year. There are about 1757 or more lodges built up for tourists night stay. Hotel business is a most popular business at Digha and a major percentage of local people take it as their occupation. Digha beach is situated close to the Gangetic mouth on the East coast of India facing the Bay of Bengal at latitude of 21° 36′ 30′′ N and longitude of 87° 30′ E. It is under Purba Medinipur district (Fig. – 1). There is a

concrete road from Sea hawk ghat to Paschim Gadadharpur. The sea beach is one side of the road and on the other side, there is forest of casuarina and Keya trees. Here, the sea is quite shallow with very little wave action on the beach and an extensive area about 250 m of the intertidal zone is exposed during low tides. The beach slope in shore is very low up to the low water mark. The shore was subjected to considerable erosion in the recent past and the bank is presently protected with the construction of a sloping sea wall. The climate of study area observed by Chatterjiee and Mitra. 2003, is presented in Table – 1.

Table -1: Climatic conditions of Digha coast.

Parameters	Limit
Annual rainfall	1000 mm to 1300 mm
Atmospheric temperature	16^0C to 35.5^0C
Relative humidity	50% in December and 78% in July
Wind flow (Average)	30 Km / hour
Tidal amplitude (Average)	2 meters

Geographically Digha is situated at the junction of West Bengal - Odisha coast, India, bordering the northern fringe of Bay of Bengal. The intertidal and supra-tidal areas of this 1.5 km long siliciclastic coast is characterized by east- west oriented shore parallel to five linear sedimentary facies- i) an intertidal sandy beach, ii) a sandy barrier bar, iii) a supra-tidal marsh, iv) a number of intertidal mud flats and v) a mature dune field. Being contiguous part to the sandy beach of Dubda water basin, Purba Medinipur, West Bengal in the East and the confluence of non perennial Subarnarekha River and the Bay of Bengal in the West, the intertidal mud flat of Digha, the confluence of Dubda water basin and Subarnarekha estuary with Bay of Bengal is comprised of sand, mud and mixed fancies grading from one to the other. These meso tidal tropical oceanic coast experiences semidiurnal tides with slight diurnal asymmetry and three major seasons are distinctly found based on two meteorological parameters - temperature and rainfall. The pre monsoon (March - May) is characterized by highest temperature, monsoon (June - August) with considerable rainfall and moderate temperature while post monsoon (September – February) is characterized by scanty rainfall and lowest temperature. A tract of mangrove swamp is situated around the study sites and extensive salt marsh tract extends on the more saline flats of eastward tidal basin.

Tide is an important coastal process. The intertidal zone represents the part of the continental shelf which remains above water at the low tide and under water at high tide. The intertidal zone can further be divided into three different sub-zones; high tidal level (HTL), mid tidal level (MTL) and low tidal level (LTL). HTL is the uppermost part of the intertidal zone, the extent of which is determined by the difference in between the two tidal marks - the highest tidal level during maximum spring tide of the year and the highest tidal level during the maximum neap tide of the year. The LTL, represent the lower most part of the intertidal zone, the extent of which is also determined by the difference in between two tidal marks, the lowest tidal level during maximum spring tide of the year and the lowest tidal level during the maximum neap tide of the year. The MTL therefore enjoys the maximum area of any intertidal zone ranging from upper most border of the LTL to lower most border of HTL.

There are so many varieties of molluscs species inhabit at Digha and its surrounding coastal areas. The coastal line is straight & large inter-tidal zone. The beach is flat & compact. The beach is made up of sand mixed with variable proportions of silt & which makes it very compact. Digha has potential coastal line of about 10 km, which offers scope for more effective exploitation of marine fishery resources. There are 8 number of different ghats (spots) studies at Digha coast (Fig. – 110). The ghats (spots) and their nature at Digha are in Table – 11.

4.3. Duration of the study period:-

During 4 years and 7 months study period (55 months or 238 weeks or 1673 days) from January – 2013 to July– 2017, It is conducted the study of

- Collection of molluscs specimen from study areas and their identification.
- It is analysed the seasonal physico-chemical parameters of water and soil specially Salinity, Dissolved Oxygen, pH, Turbidity, Temperature etc in laboratory.
- Surveyed in coastal villages at Digha to know about the edible molluscs species among the available marine molluscs at Digha coast.

- Calculate the size and weight variation of available edible marine molluscs at Digha coast to take 10 numbers of species of different size and weight of each molluscs specimen. During this calculation, it is included maximum and minimum size and weight groups of edible molluscs available at Digha coast.

- Proximate composition analysis was a part of my research work. It is analysed the proximate compositions like moisture, ash, carbohydrate, crude protein, crude fat and minerals of most popular 4 edible cephalopods available at Digha coast from CIFT, Visakhapatnam Research Center, A.P. in the month of July, 2017.

4.4. Procedure for biological samplings:-

4.4.1. Collection of molluscs specimen:-

The inter-tidal part of study spots are saturated with luxuriant estuarine flora and fauna. Several molluscs species apparently have distinct preferences for microhabitats such as mussel clamps or sea grass patches on inter-tidal soft bottoms. All the benthic molluscs under study were collected by three ways like

The gastropods are collected by hand picking from reef areas and from burrow under soft mud using a spade.

The bivalves are collected by hand picking from reef areas and from inter tidal zone scattered here and there on the sandy beach.

The cephalopods are collected from inshore water by drag net operation.

For weight and size class assessment, random sampling of ten numbers of edible marine molluscs of each species was under taken.

4.4.2. Preservation of Specimen:-

After collection of molluscs species, they were washed in fresh water for cleaning. The cleaned species are preserved by using following solution followed by Digha Marine Aquarium and Regional Centre, Zoological Survey of India –

70% alcohol + 3% formalin + 27 % distilled water.

4.5. Analysis of physico-chemical parameters:-

Monthly samplings of soil and interstitial water done from different tidal levels of three study sites for estimating the different physico-chemical parameters of soil and interstitial water by following standard methods (Strickland and Parsons, 1968; Grasshoff, 1983 and APHA, 2005) with the help of Water Quality Checker (TOA), Model No- WQC22A, Japan).

4.5.1. Analysis of interstitial water sample:-

4.5.1.1. Temperature (^{0}C):

The temperatures of interstitial water from three tidal levels in each study site were measured with a mercury thermometer having 0.1^{0}C graduation.

4.5.1.2. pH:

pH of interstitial water was measured using portable pH meter as well as by using automatic water quality checker (TOA, model no-WQC22A, Japan).

4.5.1.3. Salinity (ppt):

The Mohr - Knudsen Method is the most convenient and widely accepted procedure for salinity estimation. The chlorinity in terms of precipitable halides (Cl^-, Br^-, I^-) are determined from a 50 ml volume of water sample by titrating with standard Silver Nitrate ($AgNO_3$) solution using Potassium Chromate (K_2CrO_4) as indicator. The silver nitrate solution is

Standardized periodically against a 50 ml aliquot of standard sea water sample having known chlorinity of 19.375 × 1.03. Standard sea water samples were produced from Institute of Oceanographic science, Wormby, Surrey, UK. Finally salinity of the sample is obtained from the given formula after necessary correction from the Knudsen Chart.

$$\text{Chlorinity} = \frac{\text{Volume of } AgNO_3 \text{ used} \times \text{Strength of } AgNO_3 \times 35.5}{\text{Volume of test water sample}}$$

$$\text{Salinity (ppt)} = (1.80655 \times \text{Chlorinity}) + 0.03$$

4.5.1.4. Dissolved Oxygen:

Dissolved Oxygen concentration was estimated by winkler's idometric method as described by Trivedy and Goel (1984). The samples were taken in glass stoppered 500ml bottles, were filled with Winkler reagents at the collection sites (2 ml $MnSo_4$ + 2 ml Alkaline KI). The bottles were transported to the laboratory in dark wooden boxes. In laboratory, 2 ml of concentrated H_2SO_4 was added and shaken well to dissolve the precipitate. From the acidified aliquot 50 ml was pipette out into a 100 ml conical flask and then it was titrated against sodium thiosulphate solution (0.02 N) using starch as indicator to change initial dark blue to colorless. The concentration of dissolved oxygen was calculated using formula given below –

$$\text{DO (ppm)} = \frac{(\text{ml} \times \text{N}) \text{ of titrant} \times 8 \times 1000}{\text{Volume of test water sample}}$$

4.5.1.5. Turbidity (NTU):

Turbidity of interstitial water was measured with the help of automatic water quality checker (TOA, model no- WQC22A, Japan).

4.5.2. Analysis of Soil:-

Metallic spade or shovel was employed for the collection of soil samples from low tide levels (LTL), mid tide level (MTL) and high tide level of three study sites (A - I, A - II and A - III) and were transported to the laboratory in air tight polythene bags. The samples were sun dried, powdered and sieved through a standard sieve (BSS NO. 40, pore size 0.42 mm.). The following parameters were estimated:

4.5.2.1. Temperature (^{0}C):

The temperatures of soil from three tidal levels in each study sites were measured with a mercury thermometer having 0.1^{0}C graduation.

4.5.2.2. pH:

The pH of soil sample was measured with the help of electronic pH meter.

4.5.2.3. Salinity (ppt):-

A soil suspension of fresh sample in distilled water at the ratio of 1:5 was prepared and the suspension was stirred mechanically for one hour and filtered through No. 42 filter paper. Then salinity of that filtrate was measured by following the standard method as mentioned by Strickland and Parsons, 1968

4.6. Analysis of bio-chemical/proximate composition:-

Proximate composition is the composition of major nutrients. It is the content of Water (Moisture), Carbohydrate, Crude Protein, Crude Fat, Vitamins, Ash and Minerals.

4.6.1. Moisture:

Weigh accurately 5 g of prepare sample in a pre-dried silica dish, keep the dish with sample in an air-oven at 100^{0}C ± 1^{0}C for 6 hours. Cool the dried material in a dessicator and weigh.

$$\text{Moisture \% by weight} = \frac{\text{Loss of weight in gram} \times 100}{\text{Weight of sample in gram}}$$

4.6.2. Ash:

Weigh accurately 5 g of minced sample in a previously heated, cooled and weighed silica dish and ignite it with a maker burner for about 1 hour. Complete the ignition by keeping in a muffle furnace at $500^0C \pm 5^0C$ until whitish grey ash results. Cool in a dessicator and weigh

$$\text{Ash (\%)} = \frac{\text{Weight of residue in gram} \times 100}{\text{Weight of sample in gram}}$$

4.6.3. Crude Protein:

Take 0.5 g of the well minced powdered dry sample into a Kjeldal flask of 100 ml capacity. Add a few glass beads and a pinch of digestion mixture ($CuSO_4$ and K_2SO_4) and 10 ml of AR Sulphuric Acid (H_2SO_4). Digest over a burner till solution turns colorless. $CuSO_4$ is a catalyst. K_2SO_4 elevates the boiling point of H_2SO_4 from 324^0C to 400^0C.

To the digested acid cooled solution in the digestion flask add distilled water in small quantities with shaking and cooling till the addition of water does not generated heat. Transfer quantitatively into a 100 ml standard flask and make up the volume. Transfer with a pipette 5 ml of the made up solution to the reaction chamber of the micro-Kjeldal distillation apparatus. Rinse down with distilled water. Add two drops of phenolpthalein indicator and 40% Sodium hydroxide (NaOH) till the indicator changes to a pink colour. Distill for four minutes and absorb the ammonia (NH_3) liberated in 2% boric acid (10 ml) containing a drop of Tashiro's indicator and determine the amount the ammonia by titrating with N/50 H_2SO_4.

$$\text{Crude Protein (\%)} = \frac{14/50 \times \text{Volume of N/50 } H_2SO_4 \times 100 \times 100 \times 6.25}{5 \times \text{Weight of sample in gram} \times 1000}$$

4.6.4. Crude Fat:

Fat content of moisture free sample is determined by extracting the extracting the fat with a suitable solvent (Petroleum ether) using a soxhlet apparatus. Weigh about ¾ of the thimble (10 g) of the sample into a thimble. Place extractor thimble in the soxhlet extractor with an attached receiving flask previously weighed. Pour the solvent washing into the thimble through a glass funnel. Connect the extractor and the receiving flask to the Soxhlet condenser. Circulate cold water through the condenser.The flask is heated using a boiling water bath. Extraction is continued till the solvent in the extractor becomes clear. After completing the extraction remove the flask, dry over a water bath maintained at $100^0C \pm 1^0C$.

$$\text{Crude Fat (\%)} = \frac{\text{Weight of fat in gram} \times 100}{\text{Weight of sample}}$$

4.6.5. Carbohydrate

weighed 100 mg of the sample into a boiling tube, hydrolyzed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence cease made up the volume to 100 ml and centrifuged, collected the supernatant and take 0.2 to 1.0 ml for analysis. It is prepared the standards by taking 0.2-1.0 ml of the working standards. 1.0 ml of water serves as a blank made up the volume to 1.0 ml in all the tubes with distilled water, then added 4.0 ml of enthrone reagent, heated for eight minutes in a boiling water bath, cooled rapidly and read the green to dark green colour at 630 nm.

Calculation

A standard graph was drawn by taking the concentration of carbohydrate on X axis and spectrophotometer reading on Y axis. From the graph the concentration of carbohydrate in the sample was calculated.

4.6.6. Minerals

1 gram sample was placed in flat bottomed platinum dishes and treated with 15 ml of 70% perchloric acid and 10 ml of 47% hydrofluoric acid. The sample was evaporated to near dryness using overhead heat and a stream of air directed over the surface of the platinum dish. The overhead heating gently reduced spattering and the air stream saved time. Some samples required 2 to 3 evaporations for complete solution and if high in Ca, a final evaporation with perchloric acid only to ensure complete removal of fluoride. Finally the sample was transferred to a 100 ml volumetric flask and diluted to the mark. Aliquots of this same 100 ml portion were diluted to volumes, so that the final Na or K concentrations were about 15 ppm and the Ca concentration was about 30 ppm. Dilution to these optimum concentrations often requires a preliminary dilution and rough reading on the flame photometer.

Calculation –

Draw calibration curves for Na and K on a sheet of millimeter-paper. Use concentrations as abscissa and instrument readouts as ordinate values. Mind the units. Find concentration of Na and K ions in test solution from calibration curves.

Fig. - 1: Map showing study sites located at Digha coast

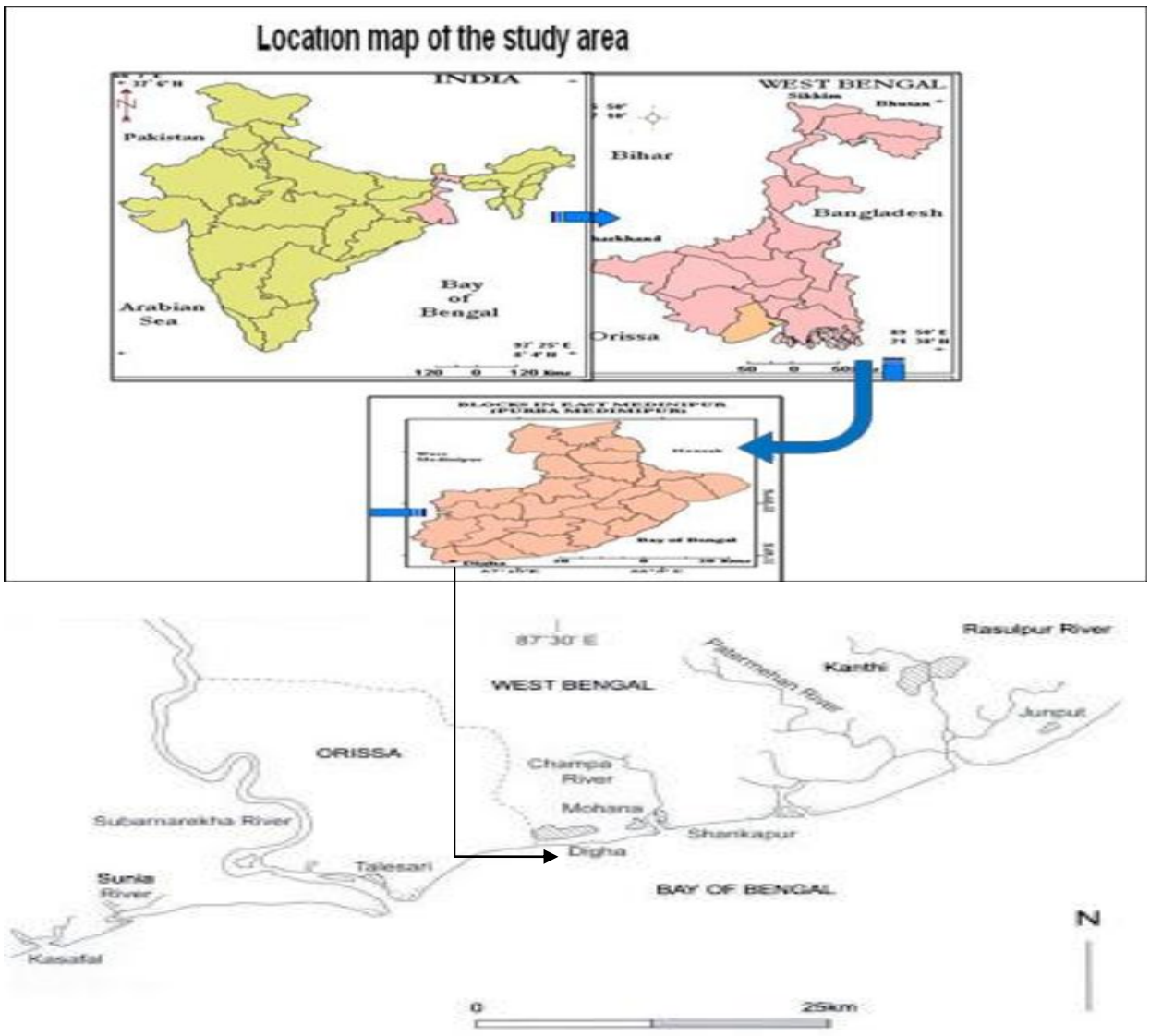
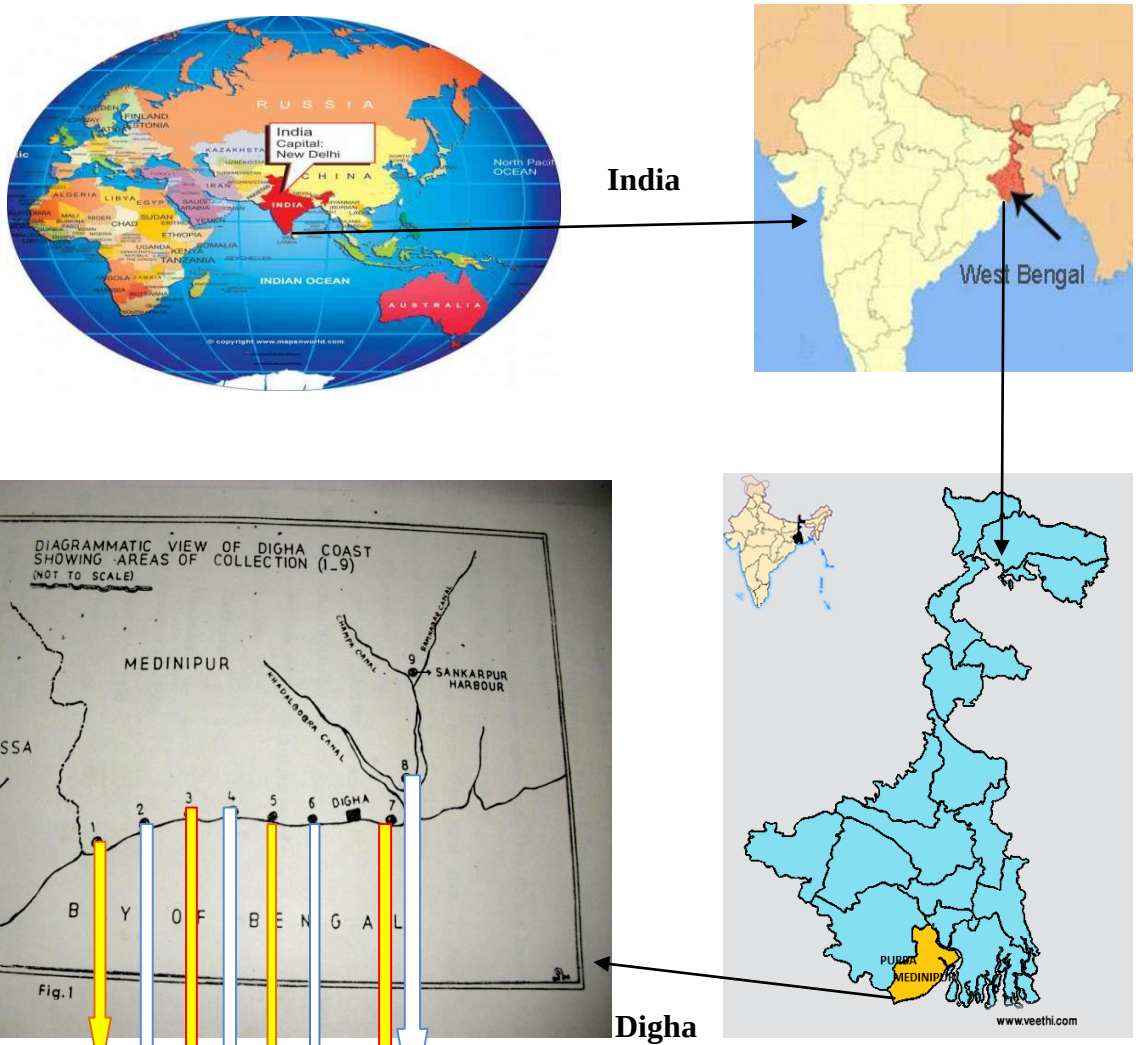


Fig. – 2: Diagrammatic view (a) of Digha coast showing areas of collection



Photographs of Molluscs Taken in Industries:

Fig. – 3: Industry View

Fig. – 4: Industry View

Fig. – 5: Industry View

Fig. – 6: Industry View

Fig. – 7: Work in progress in industry

Fig. – 8: Work in progress in industry

Fig. – 9: Work in progress in industry

Fig. – 10: Work in progress in industry

Fig. – 11: A heap of mollusks shell dust

Fig. – 12: Sieve for dust separation

Fig. – 13: Ice block & crushing machine

Photographs of Institutions:

Fig. – 14: Office of the Fishermen & Fish Traders Association, Digha Mohana

Fig. – 15: Laboratory for water and Soil testing at Naihati, W.B.

Fig. – 16: Laboratory for bio-chemical analysis at CIFT, Vizag