

CHAPTER 4

# *Material and Methods*

## Chapter 4

### MATERIAL AND METHODS

#### 4.1 Introduction

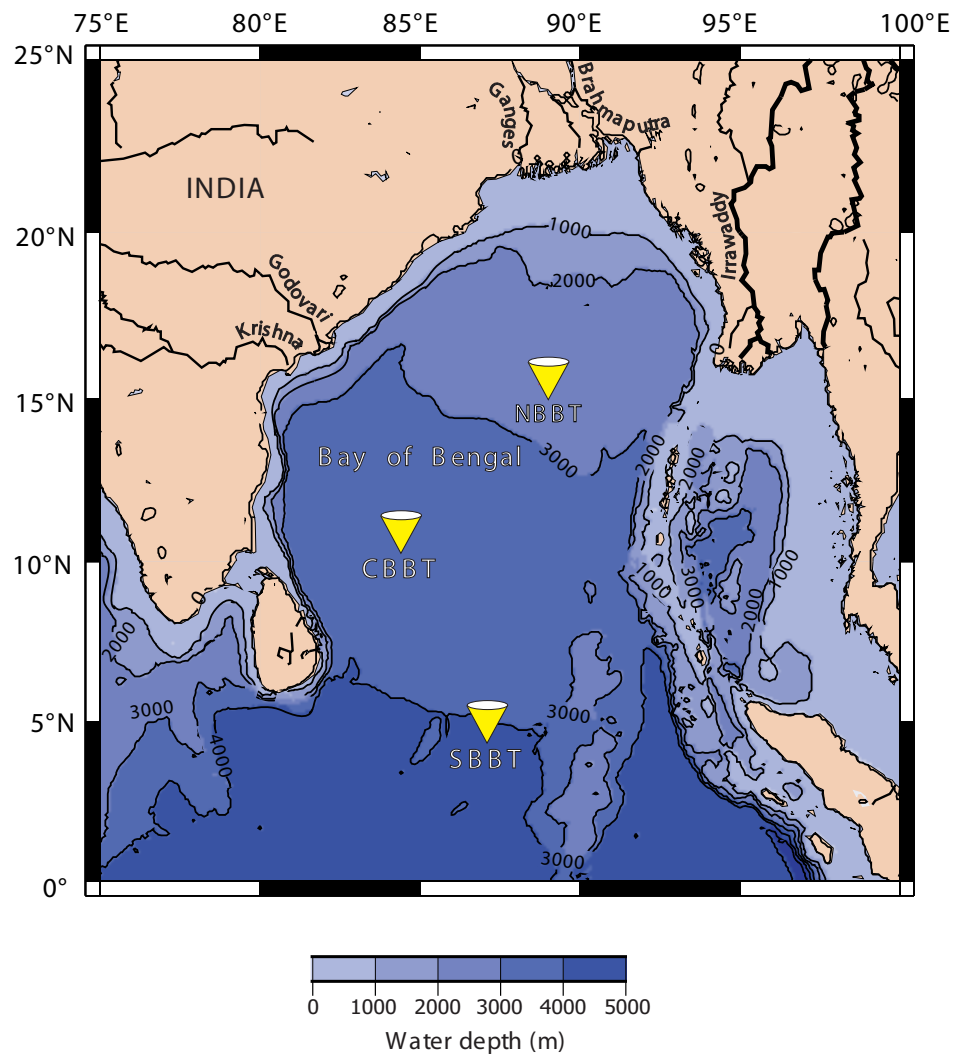
In order to carry out comprehensive study on temporal distributional pattern of coccolithophores and their implication on hydrography in the Bay of Bengal, three PARFLUX Mark VI time series sediment traps (Fig. 4.1) were deployed in the Bay of Bengal at three mooring sites located longitudinally at the northern Bay of Bengal (NBBT), central Bay of Bengal (CBBT) and southern Bay of Bengal (SBBT) regions (Fig. 4.2). Two traps at two different depths were deployed in each mooring (shallow and deep) (Fig. 4.3). The samples collected in these traps were analyzed for coccolithophores. Sediment trap locations and samples collection schedules are given in Tables 4.1 and 4.2. The mooring operations were carried out as part of the collaborative research program between the National Institute of Oceanography (India) and the University of Hamburg (Germany). These sediment trap experiments which were carried out continuously for over a period of 17 years have successfully demonstrated their ability to measure the fluxes of settling particulate material in the water column.

#### 4.2 Time-series sediment Traps

Time-series sediment traps are the most versatile sampling gears available today to capture the settling particles in the ocean. By measuring the coccolithophore flux using the time series sediment traps we can determine how the biocoenosis (living assemblage) is transformed into the thanatocoenosis (death, or sedimentary, assemblage) by studying the selective dissolution, horizontal transportation (advection) and grazing of coccolith during the sinking process. Thus, we can understand the importance of coccolithophores to the calcium carbonate cycle in the ocean (Fig. 1.2). With the advent of sediment trap technology, researchers are able to deploy anchored mooring or floating devices which are capable of trapping the settling particle from days to months duration in the open ocean. These



**Fig. 4.1.** Photograph of a sediment trap (PARFLUX Mark VI).



**Fig. 4.2.** Locations of sediment trap moorings (shown with yellow symbols) and bathymetry in the Bay of Bengal.

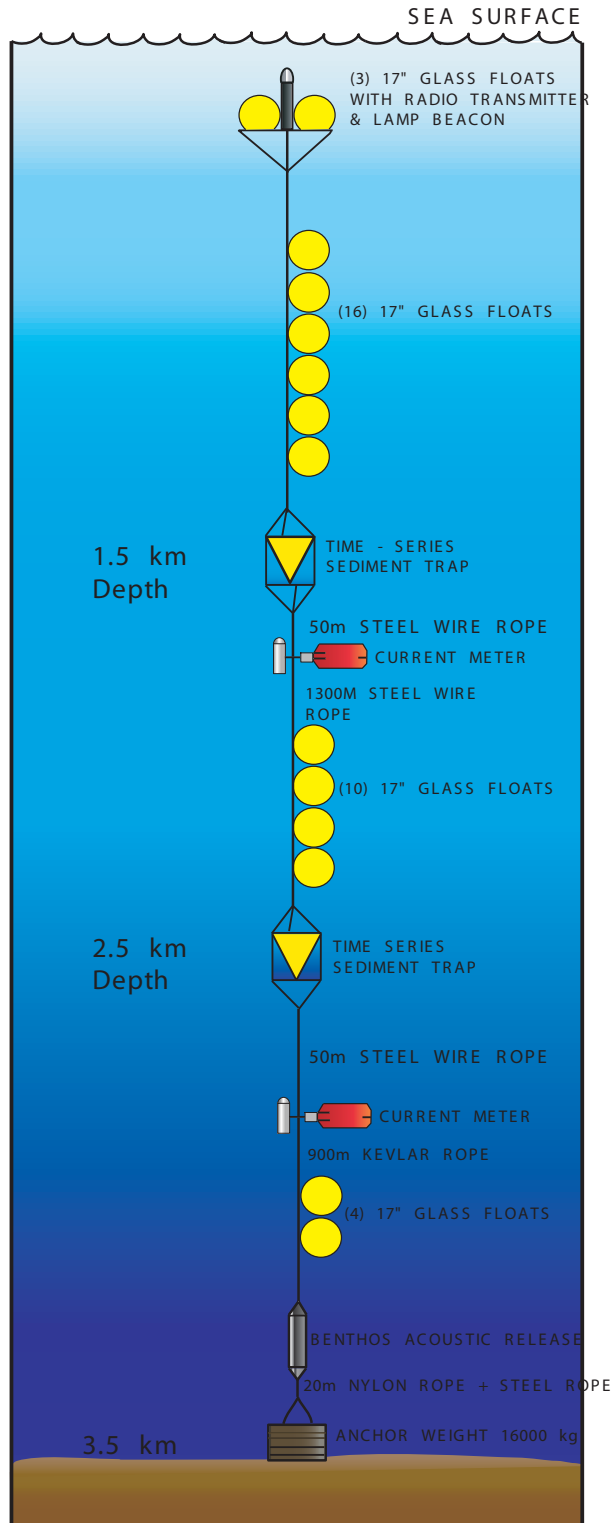


Fig. 4.3. Schematic diagram of a sediment trap mooring array.

**Table 4.1.** Mooring locations, water depths, sampling schedules and sediment trap depths.

Mooring	Position	Water depth (m)	Date of		Trap depth (m)
			start	end	
<b>NBBT</b>	Lat 15°44.62'N	2682	29-1-1992	6-12-1992	1156 (shallow)
	Long 88°58.06'E				2146 (Deep)
<b>CBBT</b>	Lat 11°01.66'N	3462	3-2-1992	16-12-1992	1588 (shallow)
	Long 84°26.21'E				2527 (Deep)
<b>SBBT</b>	Lat 5°00.33'N	3997	5-2-1992	16-12-1992	1518 (shallow)
	Long 87°03.23'E				3011 (Deep)

**Table 4.2.** Schedules of the sediment trap sample collection in the Bay of Bengal.

<b>Northern Bay of Bengal Trap (NBBT 06)</b> <b>(Shallow and Deep Trap)</b>				
<b>Stn. No.</b>	<b>Sampling period</b>		<b>Coccolith split Shallow trap (1156 m)</b>	<b>Coccolith split Deep trap (2146 m)</b>
	<b>Opening date</b>	<b>Closing date</b>		
1.	January 29, 1992	February 8, 1992	1/16	1/16
2.	February 8, 1992	March 5, 1992	1/16	1/16
3.	March 5, 1992	March 31, 1992	1/16	1/16
4.	March 31, 1992	April 26, 1992	1/16	1/16
5.	April 26, 1992	May 22, 1992	1/16	1/16
6.	May 22, 1992	June 17, 1992	1/16	1/16*
7.	June 17, 1992	July 13, 1992	1/16	1/16*
8.	July 13, 1992	August 8, 1992	1/16	1/16*
9.	August 8, 1992	September 3, 1992	1/16	1/16*
10.	September 3, 1992	September 29, 1992	1/16	1/16*
11.	September 29, 1992	October 25, 1992	1/16	1/16*
12.	October 25, 1992	November 20, 1992	1/16	1/16*
13.	November 20, 1992	December 16, 1992	1/16	1/16*

<b>Central Bay of Bengal Trap (CBBT 06)</b> <b>(Shallow and Deep Trap)</b>				
<b>Stn. No.</b>	<b>Sampling period</b>		<b>Coccolith split Shallow trap (1588 m)</b>	<b>Coccolith split Deep trap (2527 m)</b>
	<b>Opening date</b>	<b>Closing date</b>		
1.	February 3, 1992	February 8, 1992	1/16	1/16*
2.	February 8, 1992	March 5, 1992	1/16	1/16
3.	March 5, 1992	March 31, 1992	1/16	1/16
4.	March 31, 1992	April 26, 1992	1/16	1/16
5.	April 26, 1992	May 22, 1992	1/16	1/16
6.	May 22, 1992	June 17, 1992	1/16	1/16
7.	June 17, 1992	July 13, 1992	1/16	1/16
8.	July 13, 1992	August 8, 1992	1/16	1/16*
9.	August 8, 1992	September 3, 1992	1/16	1/16*
10.	September 3, 1992	September 29, 1992	1/16	1/16*
11.	September 29, 1992	October 25, 1992	1/16	1/16
12.	October 25, 1992	November 20, 1992	1/16	1/16
13.	November 20, 1992	December 16, 1992	1/16	1/16*

<b>Southern Bay of Bengal Trap (SBBT 06)</b> <b>(Shallow and Deep Trap)</b>				
<b>Stn. No.</b>	<b>Sampling period</b>		<b>Coccolith split Shallow trap (1518 m)</b>	<b>Coccolith split Deep trap (3011m)</b>
	<b>Opening date</b>	<b>Closing date</b>		
1.	February 5, 1992	February 8, 1992	1/16	1/16
2.	February 8, 1992	March 5, 1992	1/16	1/16
3	March 5, 1992	March 31, 1992	1/16	1/16
4.	March 31, 1992	April 26, 1992	1/16	1/16*
5.	April 26, 1992	May 22, 1992	1/16	1/16
6.	May 22, 1992	June 17, 1992	1/16	1/16
7.	June 17, 1992	July 13, 1992	1/16	1/16
8.	July 13, 1992	August 8, 1992	1/16	1/16*
9.	August 8, 1992	September 3, 1992	1/16	1/16*
10.	September 3, 1992	September 29, 1992	1/16	1/16*
11.	September 29, 1992	October 25, 1992	1/16	1/16*
12.	October 25, 1992	November 20, 1992	1/16	1/16*
13.	November 20, 1992	December 16, 1992	1/16	1/16*

\* Samples not available for coccolithophore analysis



devices are ideally designed to capture the settling particles continuously in water column. Therefore, sediment traps are immensely important in obtaining the vital information on the vertical transport of particle flux through the water column. The term “flux” denotes the rate of transfer of material with time from one reservoir to another, or one physical or chemical state to another i.e. water column to the trap. Flux estimates are essential pre requisites in modeling of biogeochemical processes, which allow to study the seasonal variations of particle fluxes. Biological processes that are taking place in the oceans contribute to the particle flux of the ocean, which also act as ‘carriers’ that scavenge fine particles from the water column and effectively transport them to the sea floor. The importance of biological and physico-chemical processes in aggregation and acceleration of sinking particles was demonstrated by Honjo (1982) and Deuser et al. (1983).

The sinking particles are comprised of both biogenic and non-biogenic material. The principal biogenic components of calcium carbonate are constituted by coccolithophores, foraminifera, pteropods, calcareous dinophytes etc. Similarly, biogenic components of silica include diatoms, radiolarians, silicoflagellates and organic matter a product of intense biological activity. Whereas, non-biogenic or lithogenic components are comprised of clay, quartz, volcanic fragments, cosmogenic particles etc.

Owing to the fact that the sediment trap data is of immense help in understanding the hydrography of the overlying waters, utmost care should be exercised in interpretation due to problems associated with trap efficiency. Besides, these settling particles on their journey from surface of water to deep sea are subjected to many oceanographic processes such as lateral transport (advection), re-suspension, carbonate dissolution, grazing etc.

The Particulate Flux Experiment was designed to measure total particulate flux to the sea-floor (Honjo, 1980). Sediment trap experiments are particularly important in obtaining information about the transport of material through the water column which facilitates in studying temporal variation of fluxes. Besides, they are designed to collect settling particles on hourly to yearly time scale. Whereas, plankton-tow and water bottle-casting studies

(snap shot samples) provide qualitative and quantitative information regarding the biology and ecology of coccolithophores, such as standing-stock estimates, geographic and vertical distribution of species and seasonal variation in abundance and composition. Similarly, surface-sediment studies provide information about the spatial distribution of calcareous nannoplankton in the overlying water, but they reveal very little about coccolith production and their vertical transportation, which forms the major part of sedimentary record. Therefore the study of sediment trap samples would be very useful in understanding the various biogeochemical processes operating in the ocean. Besides, we can also understand about dissolution and fragmentation that control the coccolith carbonate cycle.

Until late nineties, the sediment trap samplers were designed to collect a single sample relatively over longer periods. Studies by Deuser (1986) and Honjo (1990) have indicated a necessity to collect time series samples for shorter duration, which would enable to study temporal distribution of particulate flux. This gave a tremendous impetus to improve the technology and develop the desired sediment traps.

#### **4.3 Sediment trap techniques for collection of particle flux and analyses**

Sediment traps have been widely used for the collection of settling particulate matter in the ocean water column which can be deployed in the water column moored either to the seabed or to drifting surface buoys. This enables (a) to collect time-series samples hourly to yearly time scale (b) the estimation of the settling particulate fluxes of a wide variety of chemical and biological components and (c) the elucidation of the qualitative nature of these components. Thus temporal and spatial information can be obtained by using the sediment trap technique.

The particulate flux is defined as the quantity of flux settling through a given horizontal area in a given time.

$$\text{Flux} = \text{mg dry weight m}^{-2} \text{ d}^{-1}$$

A sediment trap provides an estimate of the particulate flux trapped during a specified period. The particulate matter, which settles to the sea floor can be broadly divided into two groups.

**a)** Suspended particles are discrete material of diverse nature and composition such as clay flakes, coccoliths, diatoms etc. Stokesian vertical settling velocity of such particle is insignificant and theoretically their residence time may be more than several hundred years (Honjo, 1986).

**b)** Macroscopic settling particles, which include aggregates, agglomerates, or flocculates of inorganic and organic matter that are loosely bonded together is commonly called as “marine snow”. These particles are composed of living and dead bacteria, phytoplankton, and zooplankton, fecal pellets and detritus, river-and wind-derived inorganic minerals, organic material (Honjo et al., 1982). These particles settle through the water column at high velocities due to density and arrive at the seafloor before being seriously altered by chemical or biochemical reactions (Honjo, 1986).

The settling particles tend to sink to the ocean floor by the force of gravity and are thus trapped in the horizontal openings of the trap without any biases regardless of the rate of settling particles are concerned. With the technological advancement in the development of sediment traps, various types of traps with different shapes, sizes, automation and operational mechanisms are produced which are being extensively used. Traps can be classified into different categories of shapes such as cylinders, funnels, cones, containers with bodies much wider than mouth and a basin like container having much more wider than their height (Gardner, 1980a).

It is believed that the cylinder shaped traps are the best suited ones for effective trapping of settling particles as unbiased samples under varying flow regimes (Gardner, 1980a, b). In case of funnel shaped traps, most of the settling particles are accumulated at the mouth, while in case of cone shaped traps, there is a possibility of loss of material due to adhesion to the sloping walls.

With the advent of microprocessor technology, the time series sediment traps are provided with a multiple sampler which can collect a maximum of 21 samples with a time ranging from hours to years as per requirement and at desired intervals. Therefore, considering the high cost factor of ship time this multiple sample cup device is much preferred over a single cup traps.

A brief description of various components of the sediment trap is given below (Fig. 4.4).

#### **4.4 Mechanical Description**

Time-series sediment trap comprises the following five major components:

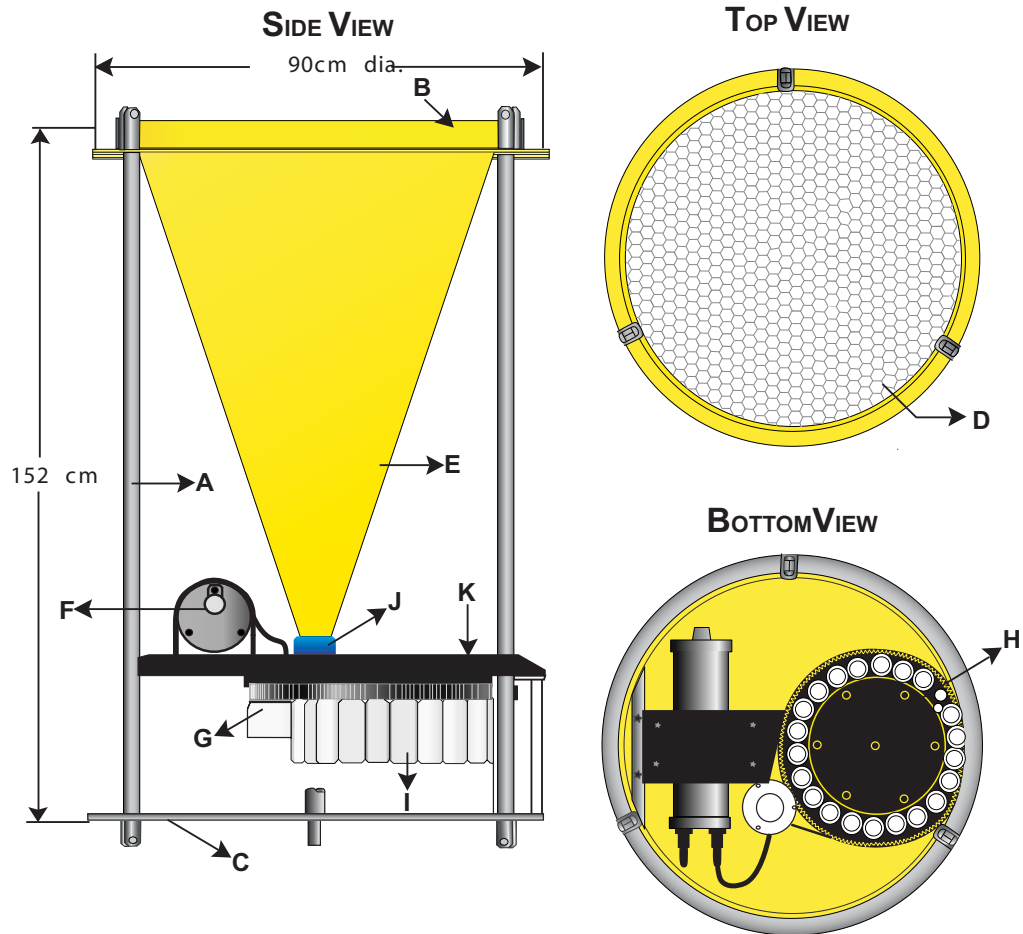
- a) Protective Titanium Frame
- b) Multi-celled Baffle With Funnel
- c) Rotator Plate Assembly
- d) Stepper Motor Housing
- e) Controller Housing

##### **a) Protective Titanium Frame (Fig. 4.4)**

It is a protecting frame made of a titanium weldment with six vertical tubes welded to a pair of cold-rolled rings (Fig. 4.4), which is housed with all components of the sediment trap. This is large enough to protect all the components from any unforeseen damage which may occur at the time of carrying out mooring operations (deployment and retrieval).

##### **b) Multi-Celled Baffle with Funnel (Fig. 4.4)**

The baffle is composed of approximately 368 honeycomb cells, each with a 2.5 cm diameter and 0.5 mm wall thickness. The baffle is inserted into the short polyethylene mounting cylinder at the top of the funnel and fastened using six nylon nuts and bolts. Since each baffle cell is narrower than the small aperture at the bottom of the funnel, large objects or animals can effectively be prevented from clogging the sediment trap.



- |                         |                           |
|-------------------------|---------------------------|
| A: Titanium frame       | G: Stepper motor housing  |
| B: 3 Upper mooring eyes | H: Rotator plate assembly |
| C: 3 Lower mooring eyes | I : Sample bottles        |
| D: Honeycomb baffle     | J: Funnel adapter         |
| E: Funnel               | K: Cross channel          |
| F: Controller housing   |                           |

**Fig. 4.4.** Schematic diagram of a sediment trap in three different views.

The sediment trap funnel is 152 cm long with an opening of 90 cm diameter at the mouth (Fig. 4.4), which tapers to 5 cm at the apex end. The trap is colored bright yellow for high visibility using inert organic pigments which do not contaminate the settling material. The bottom of the funnel is inserted into a cylindrical adaptor.

### **c) Rotator Plate Assembly (Fig. 4.4)**

The purpose of the rotator collector mechanism is to sequentially place each sampling bottle under the narrow aperture of the funnel while preserving the integrity and preventing the contamination of previously collected samples. This assembly consists of a funnel connecting to the adaptor, a fiberglass chassis, a stationary disk, and a rotary gear disk with 13 or 21 sample bottles depending on the type of trap such as Mark V or VI (Fig. 4.4). The Mark V or VI sediment trap is made of Delran (an engineering plastic) and is fastened to the chassis with stainless steel bolts. The stationary disk hole is aligned with the center of the sediment trap as well as with the center of funnel adaptor. The gear plate is located under the fixed oval-shaped plate with a hole larger than the number of sample bottles. The rotator assembly should be set at the extra hole during both the deployment and recovery operations in order to ensure the integrity of the adjacent sealed sample bottles. The holes are spaced equidistantly around the outer edge. Each one of these holes are automatically aligned with the hole on the fixed plate as the gear plate completes one rotation. All but one of the lower set of holes are threaded to fit screw-in sampling bottles. The diameter of each hole in the gear plate is 5% greater than the single hole in the fixed plate. This precaution is taken so as to ensure that the particles can settle into the sample bottles without any obstruction.

The circumference of the upper opening of holes on the sampler disc is fitted with a Teflon  $\text{O}$  spring-loaded gasket, which is C-shaped in profile and open to the outside. The upper and lower lips of the gasket are expanded by a continuous Hastelloy  $\text{O}$  (a highly corrosion resistant alloy with high yield

strength) circular spring, to positively seal the bottle contents from ambient water and air.

The controller housing is mounted on an epoxy fiberglass U-channel chassis which is coated by inert epoxy paint with stainless steel U-bolts covered by silicon rubber tubing. The drive motor housing is mounted directly on a corner of the stationary disk by stainless steel bolts and nylon spacers.

#### **d) Stepper Motor Housing (4.4)**

The drive motor housing consists of a high-torque electronic stepping motor, a reducing gear train, a micro switch/cam device and a titanium main drive shaft (Fig. 4.4). This motor interfaces between the electronic signals to mechanical systems on the sediment trap converting the electronic signals to mechanical energy in order to move the sample bottles. The micro switch in the motor housing detects mechanically the position of the rotary disk holes relative to the hole on the stationary disk. The precise position thus achieved in each advancement of sampling bottle is recorded in the microprocessor memory of the powerful computer ITC (Instrument Timer and Controller).

#### **e) Controller Housing (Fig. 4.4)**

The ITC and batteries are housed in a pressure-resistant chamber known as the controller housing (Fig. 4.4). Seven ampere-hours at 21V are available from an alkaline battery packed inside the chamber which is designed to resist static pressure up to a depth of 5,500 m deep.

### **4.5 Mooring operations of sediment traps**

#### **4.5.1 Deployment of sediment traps**

Prior to the deployment of traps, sampling cups are filled with sea water collected from the deep sea depth to which 35 g/l NaCl and 3.3 g/l HgCl<sub>2</sub> added for preservation and poisoning to prevent degradation by bacteria. The time series sediment traps are attached to the mooring line with PVC coated special bridles. Two traps in each mooring are deployed one each around 1000 – 1600 m (shallow) and ~ 2000 - 2500m (deep) depths from the surface to prevent the entry of migrating zooplankton and other small animals living in

the top 500 m of the ocean water. And, the deeper traps are always deployed at about 1000 m above the sea bottom so as to prevent re-suspension of sediment.

Glass floatation spheres used in the mooring lines provide the necessary buoyancy required for the mooring (Fig. 4.5a). The mooring remains stationary in upright position by means of anchor weights consisting of four railway wheels (Fig. 4.5a) having a total weight of about a ton.

Deployment begins with the lowering of the three sphere radio float attached to the 16 floats in water which is further connected with a nylon rope (Fig. 4.5b). This nylon rope is joined to the wire rope and connected to the sediment trap. The deployment operation of the sediment trap (shallow) is done with the help of cranes and jib boom to lower the traps. The wire rope and nylon rope are looped on to the winch and the wire rope is passed through sheaved pulleys. While the mooring operation is in progress, it is necessary that the ship drifts away from the mooring line to prevent possible entanglement with the propeller. The sediment trap is further connected to series of wire ropes and floats and then nylon rope through which the second trap (deep depth) is chained. This trap is further attached to wire ropes, connecting the floats to the acoustic release, which is hooked by railway wheels as anchor weights. After the anchor weights are detached, they begin to descend along with the entire array of mooring line. The sinking/descending rate is monitored by the Benthos transponder deck unit. The anchor drop position is noted down with the help of Global Positioning System (GPS) and the triangulation method is used in ascertaining the exact location.

#### **4.5.2 Retrieval of the sediment traps**

After reaching the anchor drop position the Acoustic Release is interrogated by the transducer of the Benthos deck unit from the ship and the slant ranges displayed on the deck unit are computed to obtain the horizontal range. This procedure is repeated until the ship reaches to a close vicinity of the mooring system. When the safe distance between the ship and mooring array is achieved, the Acoustic release is then activated to release from the



a)



b)



Fig. 4.5. a) Photograph showing Glass flotation spheres and anchor weights (Railway wheels).

b) Photograph showing the deployment of three sphere radio float attached to the glass floats.

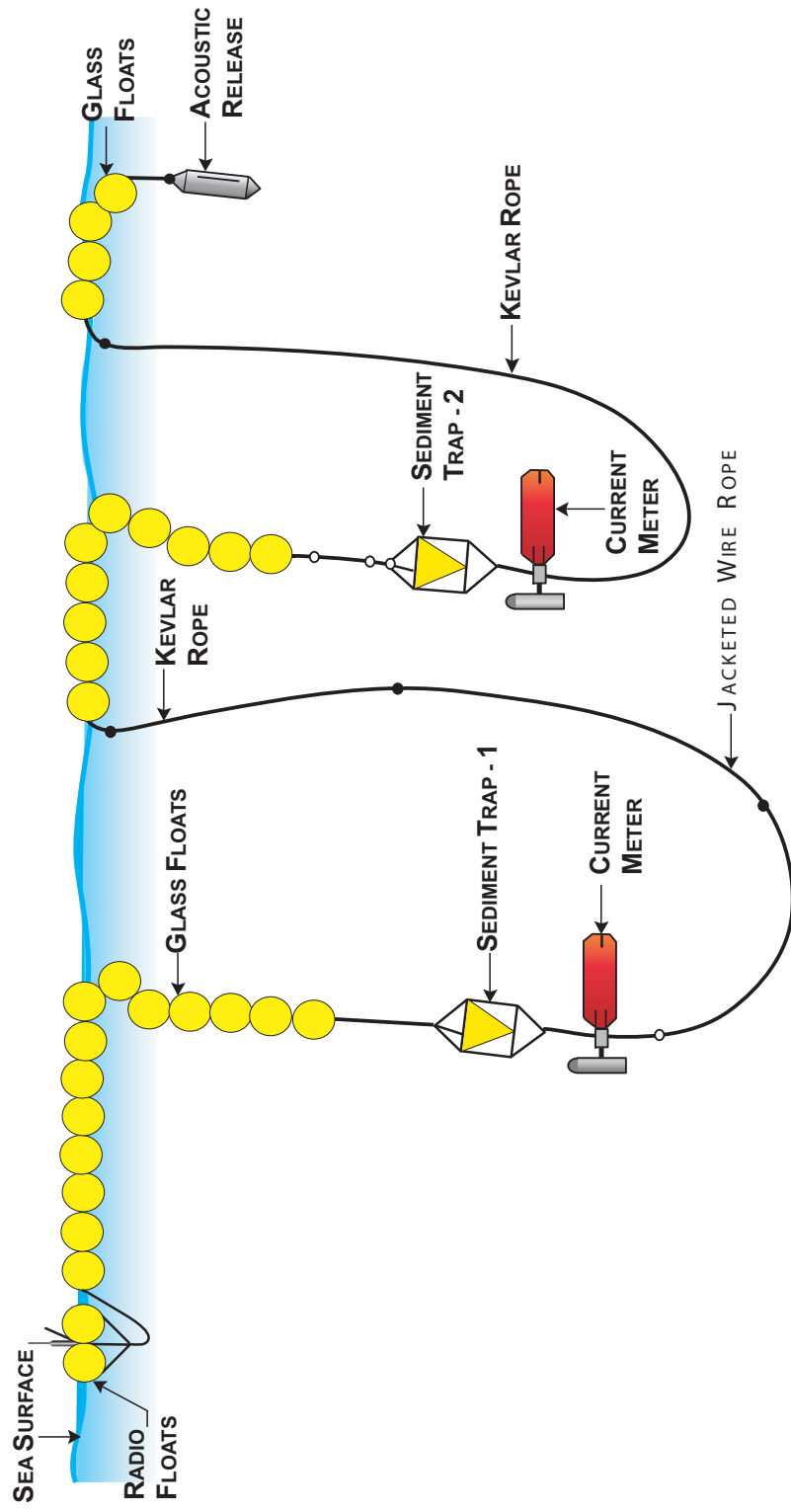
anchor weight. After ascertaining the release by the Deck unit, slant range readings are taken at regular intervals and accordingly the ascending rate is calculated (approx. 80 m per minute). The VHF picks up the signal pulse from the Radio beacon of the mooring float and the mooring system pops up to the surface within about 45 minutes (Fig. 4.6), then the ship is navigated and maneuvered to reach to the close vicinity of the radio floats (Fig. 4.7a) and then hooked to grapnels and mooring line (Fig. 4.7b & c). Thus, the retrieval of the entire array of mooring with traps are brought on to the main deck of the ship, washed sample bottles are removed and refrigerated immediately.

#### **4.6 Possible sources of error in the collection of settling particles**

The time-series sediment traps are the most versatile sampling gears to capture the settling particle in the ocean yet, we encounter with some possible errors. A variety of factors, reflecting both natural oceanographic processes and artifacts induced by the trap/mooring may result in incorrect estimation of Flux values. Because, these are dependent for some extent on the choice of trap/mooring design and hardware, deployment methods, sample treatments, analytical procedures and data interpretation. It is very difficult to explain the errors in sediment trap samples as they are subjected to several important sources of potential errors (Bloesch and Burns, 1980; Blomquist and Hakanson, 1981). Nevertheless, sediment traps are the only tools for directly collecting/trapping the rain of sinking particles in the ocean for meaningful interpretation of results. Some of the possible factors – affecting the settling of particles are highlighted in the following paragraphs.

##### **a) Hydrodynamic Effects**

One of the most difficult sources of error may be due to the interaction of trap structure with the flow of water and associated particles. Therefore, several studies have been carried out to clearly understand the nature of biases that may possibly occur due to strong currents (Gardner, 1980a, b), tilt of the trap (Gardner, 1985) and with respect to the current vector and the effect of trap geometry (size, shape, baffles etc.) on the quality and the quantity of material collected (Gardner, 1980b; Spencer, 1981). Recent studies have shown that trapping biases may occur in high energy



**Fig. 4.6.** Mooring diagram of the sediment trap for retrieval after the anchor weights are released.



Fig. 4.7. a) Photograph showing three sphere radio float with glass floatation spheres at the surface before retrieval.  
b) Photograph showing the retrieval of sediment trap array.  
c) Photograph showing the retrieval of sediment trap.

environments. Earlier studies have also indicated that the sediment traps can be best used with accuracy in bathypelagic and abyssal areas.

### **b) Swimmers**

Variety of organisms get trapped in sediment traps, especially the “swimmers” which are alive at the time of their entry into the trap and are not members of the community of sinking particle, hence should not be considered as particle flux. Often, these swimmers may pose a problem with regards to the true total flux by their consumption and/or defecation in the trap.

The intensity of swimmers is especially acute in the upper regions of the ocean, normally where the concentrations of migrating zooplankton are high and food sources are relatively dispersed. Therefore in the present study, traps were deployed well below the depth range of migrating zooplankton.

### **c) Preservatives**

One of the major problems encountered in the preservation of the sample collected by sediment traps is the degradation and decomposition of particulate matter (Knauer et al., 1984). However, this problem can be resolved by using poisons such as sodium azide, mercuric chloride which prevent bacterial degradation effectively. Besides, there are many mild preservatives such as formaldehyde, glutaraldehyde that minimize both biological and chemical degradation of biological materials. Most of these preservatives complicate the sample processing by interfering with some of the chemical analyses. However, it was found that mercuric chloride in minor proportion is the best preservative, which has been used in the present investigation.

## **4.7 Processing of sediment trap samples for coccolithophores**

### **4.7.1 Processing of samples in the laboratory**

After the retrieval of the sediment traps, the sample cups were removed with due care, volume of the sample noted and refrigerated immediately. The pH and conductivity of the decanted supernatant solutions

were measured. The particulate matter from each sample cup was poured onto a clean plastic tray and examined visually. The supernatant was decanted and the residue was wet sieved using a >1 mm sieve. The >1 mm fraction was filtered on a millipore filter and stored in a plastic box, this fraction was not included in the calculations of total fluxes (Honjo, 1982; Lee et al., 1988). The <1 mm fraction was split using a precision splitter into aliquots of 1/4 to 1/16. These samples are generally comprised of particulate matter, foraminiferal tests, coccoliths and pteropods. Samples from one of the aliquots are filtered onto pre weighed Millipore filter (0.4 µm), dried at 50°C and then scanned under SEM for coccolithophore analysis (Fig. 4.8).

#### **4.7.2 Sample preparation for coccolithophore analyses using the Scanning Electron Microscope (SEM)**

A small strip of the dried Millipore filter is cut and placed on a sample stub and coated with (Gold-Palladium alloy) at 100 A° in a sputter cotter. This gold-coated stub is scanned using JEOL JSM-5800 LV SEM for coccolithophore at 15 KV. Species composition and abundances are determined by identifying and counting the species (at least 300-500 specimens) of coccospheres and coccoliths on measured transects (1 mm) at a magnification of x3,500. Identification of coccolithophores was done following references of Winter et al. (1994), Nishida (1979) and Kleijne (1990, 1991, 1992, 1993).

#### **Fluxes of coccolith were calculated as follows**

$$Flx = \frac{F C S}{A D O}$$

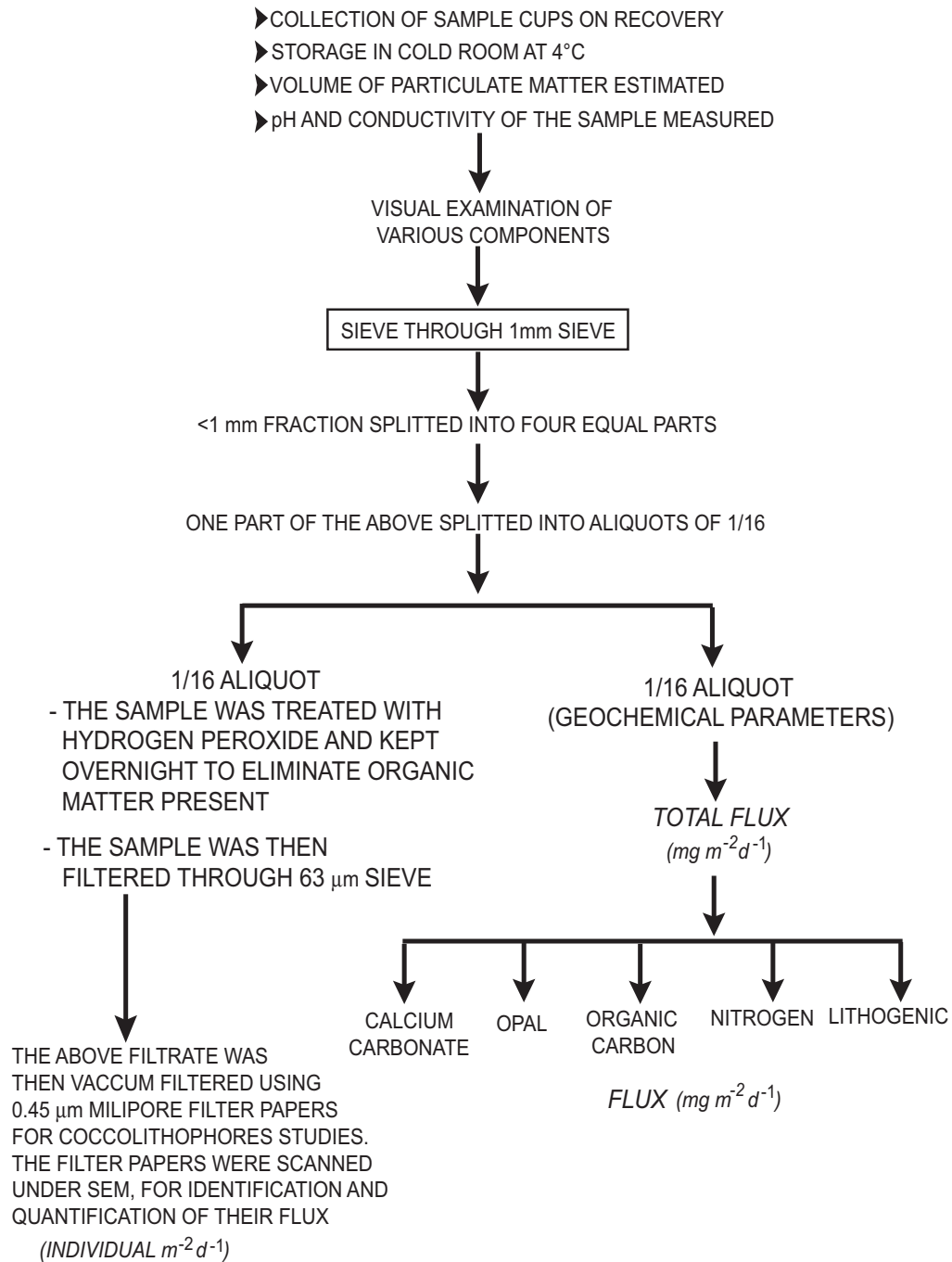
$Flx$  = coccolith flux (specimens m<sup>-2</sup>d<sup>-1</sup>)

$F$  = effective filtration area (mm<sup>2</sup>)

$C$  = number of coccolithophores

$S$  = split factor (reciprocal of split fraction)

$A$  = investigated filter area (mm<sup>2</sup>)



**Fig. 4.8.** Flow chart showing various stages for processing of sediment trap samples.

$D$  = length of sampling interval (d)

$O$  = opening area of the trap cone ( $m^2$ )

#### **4.7.3 Estimation of $CaCO_3$ contribution by coccolithophores**

The coccolith- $CaCO_3$  was estimated by multiplying the specific weight of the coccolith species by their corresponding fluxes following Broerse et al. (2000a). Similarly coccosphere- $CaCO_3$  is estimated by multiplying the coccosphere fluxes by the mean number of coccolith per coccosphere by the specific coccolith weight. For all other species an average weight was used (Table 4.3).

#### **4.8 Working of Scanning Electron Microscope (SEM)**

Owing to the fact that the living coccolithophores are very tiny unlike those found in sediment which are robust due to calcification, it is very essential to use the Scanning Electron Microscope (SEM). Therefore, the SEM (Fig. 4.9) was extensively used to carry out the coccolithophore analyses in the present study; hence it is felt necessary to give a brief description on the working of SEM.

The SEM is a microscope that uses electrons rather than light to form an image and is designed for examining the surfaces of solid objects. The specimen surface is scanned by an incident electron beam and the backscattered electron thus generated have information of the surface topography, physical and chemical properties of the specimen which is then focused to form an image in much the same way as TV (Fig. 4.10).

There are many advantages of SEM over light microscope.

- i) The SEM has a large depth of field which allows a large area of sample to focus at a given time.
- ii) The SEM produces images of high resolution which means that closely spaced features of an object can be examined at high magnification. Sample preparation is relatively simple.

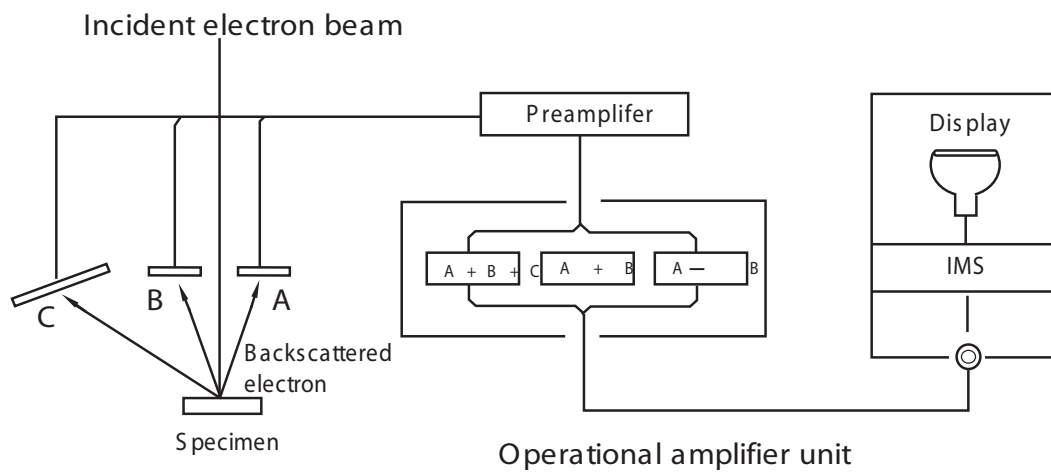


**Table 4.3.** Overview of coccolith masses and lengths of individual species and the mean number of coccoliths per coccosphere as determined by various researchers.

Species	Coccolith Mass (pg)	Coccolith Length (µm)	Reference	Coccoliths per Coccosphere	Reference
<i>G. oceanica</i>	18	5	Knappertsbusch and Brummer (1995)		
<i>E. huxleyi</i>	3.5	3.2	Knappertsbusch and Brummer (1995)		
<i>C. mediterranea</i>	12.1	4	Young and Ziveri (2000)		
<i>S. pulchra</i>	16	6	Knappertsbusch and Brummer (1995)		
<i>U. sibogae</i>	35	6	Young and Ziveri (2000)		
<i>U. irregularis</i>	23.86	5	Beaufort and Heussner (1999)		
<i>U. tenuis</i>	23.9	5	Beaufort and Heussner (1999)	24	Knappertsbusch (1993)
<i>H. carteri</i>	142.9	9.11	Beaufort and Heussner (1999)	31	Knappertsbusch (1993)
<i>C. leptopora</i>	74.1	7	Young and Ziveri (2000)	76	Knappertsbusch (1993)
<i>F. profunda</i>	1.3	2.5	Young and Ziveri (2000)		
<i>D. tubifera</i>	5.1	3	Young and Ziveri (2000)		
<i>R. clavigera</i>	67.5	10	Young and Ziveri (2000)		
<i>N. coccolithomorpha</i>	40.5	10	Young and Ziveri (2000)		
<i>Others</i>	8	5	Average value used in this study	20	Average value used in this study



**Fig. 4.9.** Photograph of the Scanning Electron Microscope (JEOL JSM-5800 LV).



**Fig. 4.10.** Flow diagram on the working of the Scanning Electron Microscope.

- iii) The combination of higher magnification, larger depth of focus, greater resolution can produce an image that is a true representation of the three-dimensional sample.
- iv) Usage of SEM enables to identify coccolithophores directly unlike in a light microscope where the identification is indirect through interference figure produced by calcite.

## **4.9 Outline of instrumental design**

### **1. Electron gun**

Essential requirements for the electron source of a SEM are high brightness, a small source area and high stability especially that of the velocity of emitted electrons. These requirements are met by several types of electron guns, which are basically composed of three electrodes.

### **2. Electron lens**

Electron lenses are used to de-magnify the crossover (electron source) formed in the electron gun and focus the electron probe to scan the specimen surface under the desired condition.

### **3. Secondary electron detector**

The secondary electron detector effectively collects secondary electrons (including some backscattered electron) emitted from an irradiated specimen surface, amplifies the detected electrons, and converts them into the video signal.

### **4. Vacuum system**

For the SEM operations, the column and sample must always be in vacuum in order to minimize the interference of extraneous particles with the electron beam. The vacuum in an SEM is very essential as the filament should not be surrounded by air which can quickly get burnt, like a light bulb. And also the column should be free of air as the electrons can collide with the gas molecules and never reach the sample. If the gas molecules react with the sample, different compounds could form and condense on the sample

which can then reduce the quality of the image. A vacuum condition is also necessary in the sample preparation for coating the samples with carbon or gold-palladium in the sputter coater.

#### **4.10 Function of a sputter coater**

A sputter coater (Fig. 4.11) is a special device to coat the sample with gold or carbon atoms. The main purpose of coating is to make non-metallic objects electrically conductive. The sample is placed in a small chamber, which is kept at vacuum to which the Argon gas introduced. An electric field removes an electron from the Argon atoms to make the atoms ions with a positive charge. The Argon ions are then attracted to a negatively charged gold foil acting like sand in a sandblaster, knocking gold atoms from the surface of the foil. These gold atoms then settle onto the surface of the sample, producing a gold coating and making it conductive.



Fig. 4.11. Photograph of the Sputter coater (SPI-Module).