CHAPTER 3

METHODOLOGY

Any environmental monitoring program generally involves collection, initial processing and analysis of different types of samples in order to determine considerable diversity, concentration and complexity. Many sensitive procedures have been developed by various laboratories over the years to assess different parameters in environmental matrices. A variety of sophisticated instrumentation systems are employed that are amenable to detecting low level concentration in environmental samples. However, estimation of some key parameters requires proper treatment, digestion, pre-concentration, complex chemical and radiochemical separation, specific standards for calibration and purification of the samples, before proceeding with estimation.

3.1 SAMPLING PROTOCOL

3.1.1 Sampling Locations

In order to accomplish the objectives of the present study, water, air and sediment samples were collected from geographically fixed locations of the Hooghly estuary and the Sundarbans mangrove (Figures 3.1 and 3.2, respectively) based on their respective Latitude-Longitude (Tables 3.1) recorded using GARMIN GPS 12. Thirty sampling sites were selected (for surface water and bed sediment samples) from each study area based on the salinity gradient from freshwater sites to seaward sites. All the Hooghly and the Sundarbans sampling sites are named as H1 to H30 and S1 to S30,
respectively. Water samples were collected to quantify the possible spatial and seasonal variations of nutrients, particulate matters, dissolved gases (CO$_2$ and CH$_4$).

Apart from thirty mentioned sites, one extra site was selected (H31) from the Hooghly estuary to collect only surface water sample for LOICZ modeling purpose. Site H31 has been considered under the ocean box in LOICZ model.

Four depositional environments were chosen for sampling of cores, representative stations were selected by considering the type of human activities, vegetation type and the degree of terrestrial and marine influence. Among four sediment cores, three cores were collected from the Sundarbans mangrove (SC1, SC2, SC3) (Figure 3.2) and one collected from the Hooghly estuary (HC1) (Figure 3.1) during monsoon in order to get the broader picture of the interaction of various components and the sedimentation rate of that area.

3.1.2 Seasonal Sampling

The samplings in the Hooghly estuary and the Sundarbans mangrove were carried out seasonal basis in a year covering monsoon (18$^{th}$ - 29$^{th}$ June 2008), post monsoon (16$^{th}$ - 27$^{th}$ December 2008) and pre monsoon (1$^{st}$ - 12$^{th}$ May 2009).
Figure 3.1  Sampling sites in the Hooghly estuary
Table 3.1  Sampling sites of the Hooghly estuary and the Sundarbans mangrove with respective GPS positions

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Figure 3.2 Sampling sites in the Sundarbans Mangrove
3.2 FIELD SAMPLING TECHNIQUES

3.2.1 Sampling of Surface Water Samples

Water samples were collected from different sites at a depth of 1 m using a Niskin water sampler (1.2 L). The water samples were immediately transferred into the 250 ml relevant labeled Nalgene bottles by at least two sample volumes using a silicon rubber tube. All the sample bottles were pre-rinsed with dilute HCl (0.1N) and Milli-Q (Millipore Corp; conductivity ~10 µS cm\(^{-1}\), bacterial rejection >99%) water. Samples were deep frozen in dry ice (-40°C) immediately after collection, brought to the laboratory and stored in a deep freezer until they were taken for analysis.

3.2.2 Sampling of Sediment Cores

Four sediment cores were collected by inserting a core tube (PVC - Polyvinyl chloride tubes) with a 7 cm diameter into the sediment during low tide in June 2008 (monsoon). The corer was pushed manually as far down as possible. Cores were cut at 2 cm intervals, and the segments were transferred into airtight labeled polythene zip lock covers. All the collected samples were stored in a dry ice chest in the field and were immediately transferred to the laboratory in 4°C until further analyses.

3.2.3 Sampling of Bed Sediments

The bed sediments were collected in almost all locations where water samples were taken, during monsoon season using Van-Veen Sediment Grab sampler. Sediment samples were transferred to airtight zip lock covers with a small plastic scoop, marked and sealed immediately after collection and stored in a dry ice chest in the field. Ultimately, samples were brought to
laboratory and kept in cold room at a temperature of 4°C, until the further analyses.

3.2.4 **Sampling for Dissolved CO₂ and CH₄**

Surface water samples for the measurement of pCO₂ and dissolved CH₄ and Total Alkalinity analyses were collected from the water sampler via silicon tubing into 100 ml pre-rinsed and pre-combusted amber colour glass bottles. Samples were overfilled by at least three times the volume of the bottles to reduce sample contact with air. The collected samples were poisoned immediately with 0.1 ml of saturated HgCl₂ to cease the microbial activity, and the bottles were closed with rubber stoppers and crimped with an aluminum seal. Samples were stored in a sealed cool box at 4°C. All the samples were analyzed within 48 hours of sample collection.

3.2.5 **Physico-Chemical Parameters of Surface Water**

Hand-held water quality multi probe (HORIBA, DKK - TOA, WQC - 24) was used to measure physic-chemical variables of the surface waters at the field. Samples were transferred into a pre-rinsed container from the Niskin sampler. Water temperature, Dissolved oxygen (DO), Salinity, pH, Total dissolved solids (TDS), Conductivity, Turbidity and Specific gravity (σ) were measured *in-situ* using multi probe, which was allowed to stabilize for 30 seconds to attain sensor equilibration. Stated accuracies according to the manufacturer’s handbook are pH (± 0.05), DO (0.1 mg L⁻¹, ± 1 %), Salinity (± 0.1 %), TDS (± 2 g L⁻¹), Specific Gravity (± 0.1 σt), Temperature (± 0.25°C) and Turbidity (± 3 % mg L⁻¹). The probe was calibrated prior to the survey using the manufacturer recommended techniques. Probe measurements of DO were routinely cross-checked by collecting two samples in every five for
standard Winkler’s titration method (APHA 2005). The two methods gave satisfactory agreement: \( O_2 \) (probe) = 0.992 \( O_2 \) (Winkler) - 0.02 (\( r^2 = 0.959 \), \( n = 61 \)). Water current was measured using Current Meter (accuracy ± 2 % or ± 1 cm s\(^{-1}\)). A hand-held cup Anemometer (AM 4201; resolution ± 1 m s\(^{-1}\), accuracy ± 2 % + 1d) was used to measure the wind speed. Atmospheric temperature was measured by using a well graduated Thermometer. Secchi disc was used to measure transparency of the water column.

3.2.6 Sampling for Dissolved Organic Carbon (DOC) in Surface Water

A known quantity of surface water samples were collected for estimation of DOC. The water samples were immediately filtered in the field using pre-combusted (at 450°C for 4 hrs) cellulose nitrate filter paper (0.45 µm pore size), and the filtrate were transferred to pre-rinse and pre-combusted glass tubes of 12 ml capacity. In order to preserve the samples 2 - 3 drops of 10% ortho-phosphoric acid (made up to 10% Milli-Q water) were added to bring the pH down to 2. Care was taken to ensure the absence of air bubbles in the samples before sealing by screwing on the caps.

3.2.7 Measurement of CO\(_2\) and CH\(_4\) Emission using Floating Chamber

The quantification of CO\(_2\) and CH\(_4\) air - water fluxes from the Hooghly estuary and the Sundarbans mangrove were done indirectly using the model after Borges et al (2004b) and Wanninkhof (1992), respectively and also directly using free floating chambers (Frankignoulle 1988). A comparative study was done, in order to find the similarities or differences in flux estimations through direct method and flux calculated through established indirect method.
The principle of floating chamber is based on the detectable variation in the headspace mixing ratios of trace gases (CO₂ and CH₄) relative to the initial headspace concentration over time. Hence an increase in chamber headspace mixing ratio denotes the flux of CO₂ and CH₄ from the water column into the chamber headspace. Positive flux values refer that the environment is source of these gases and if the mixing ratio of the trace gases decrease over time, implies a flux from headspace into the water column which says environment is acting as a sink for these trace gases. Repeated measurements of headspace analysis (at intervals of 30 min) over time periods of 1 - 2 hrs along with the rate of fluxes were estimated. In the present investigation sampling for water - air gas exchange was done thrice in the Hooghly estuary and the Sundarbans mangrove surrounding waters, during the month of June 2008 (monsoon), December 2008 (post monsoon) and May 2009 (pre monsoon) using gas exchange floating chamber (Figure 3.3).

Figure 3.3  Floating chamber for measurement of air-sea fluxes of CO₂ and CH₄

The floating chamber made of acrylic, has an inner diameter of 30 cm, total height 46.5 cm, above water height of 25 cm and head space
volume of 19824 cm$^3$. The water level inside the chamber is up to a height of 21.5 cm, and it covers a water surface area of 707 cm$^2$. It has an air-filled car tyre tube and lead weights around the chamber to ensure stability in the water. The chamber is allowed to float freely in the estuarine and mangrove waters, and air samples were collected every half an hour for a period of 2 hours. The chamber has a small opening of about 3 cm diameter at the top with a one holed rubber cork, in which a needle is fixed. Through this needle, air sample inside the chamber were collected in sterilized labeled evacuated vacuutainer. The samples were brought back to the laboratory for analysis by using Gas Chromatography.

3.2.8 Collection of Air Samples

Atmospheric air samples were collected using gas-tight syringes, which were pre-flushed with the sample air were used. The gas samples were transferred to a vacuumed glass air sample collection tubes (Vacuutainer, Labco Exetainer, 12 ml) via sterile needle. In order to prevent over pressurization of the sample within the vacuutainer a venting needle was inserted. Both the needles were removed after filling the tube with sample air and the self-sealing butyl septum of the vacuutainer secure the integrity of air samples within.

3.3 LABORATORY ANALYSIS

3.3.1 Measurement of Suspended Particulate Matter

The suspended particulate matter (SPM) was determined by filtering a known volume of surface water samples using pre-weighed 1.2 µm GF/C (Whatman, 47 mm diameter) filter paper and using a vacuum filter unit. Then filtrates were used for further hydro-chemical analyses. Prior to filtration, the filter papers were oven dried (100°C for 12 hrs, for complete
moisture removal to get a constant weight) and weighed before and after filtration and the differences in the weight to the volume of water filtered were expressed as concentration of SPM in mg L\(^{-1}\).

### 3.3.2 Nutrient Analysis and Major Anion Analysis

Nutrients were analyzed by spectrophotometric methods. The dissolved Ammonium (NH\(_4^+\)) was analyzed spectrophotometrically (HITACHI, U2000) by following Indo-phenol blue method from Standard Manual for Sea Water Analysis by Grasshoff et al (1999). Nitrate (NO\(_3^-\)) and Nitrite (NO\(_2^-\)) was analyzed by using Cadmium Reduction and Di-azo methods, respectively. The phosphate (PO\(_4^{3-}\)) in the water sample was measured by Ascorbic Acid Reduction Method (Edwards et al 1965; Murphy and Riley 1962). Dissolved silicate (SiO\(_4^{4-}\)) was measured spectrophotometrically using ammonium molybdate (APHA 2005). Nutrient concentrations were expressed in µM L\(^{-1}\) unit. A relative error of accuracy was ± 2% for dissolved inorganic phosphorus (DIP), ± 3% for nitrate, ± 5.2% for ammonia, and ± 5% for silicate. The detection limits were 0.06 µM L\(^{-1}\) for DIP, 0.1 µM L\(^{-1}\) for dissolved inorganic nitrogen (DIN) and silicate.

### 3.3.3 Measurement of pCO\(_2\) and dissolved CH\(_4\)

#### 3.3.3.1 Gas Chromatography

Gas chromatography (GC) is a chromatographic technique that is used to separate a mixture of organic compounds into separate components. A gas chromatograph consists of a flowing mobile phase, an injection port, a separation column containing the stationary phase, a detector, and a data recording system. The organic compounds are separated due to differences in their partitioning behavior between the mobile gas phase and the stationary phase in the column. Usually volatile and thermally stable compounds are
used for GC analysis. If the compounds or molecules are in the gas or vapor phase at 400 - 450ºC or below, and they do not decompose at these temperatures, the compounds can probably be analyzed by GC.

The injection chamber is a heated cavity (100ºC) which serves to volatilize the compounds. The sample is injected by syringe needle into this chamber through a port which is covered by a rubber septum. Once inside, the sample becomes vaporized and is carried out of the chamber and onto the column by the carrier gas. The carrier gas or mobile phase in GC is an essential, but limiting facet in separations. The carrier gas must be chemically inert. Commonly used gases include Nitrogen, Helium, and Argon. The choice of carrier gas is often dependent upon the type of detector, which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities. Since the partitioning behavior is dependent on temperature, the separation column is usually contained in a thermostat-controlled oven. Most columns contain a liquid stationary phase on a solid support. Separation of low-molecular weight gases is accomplished with solid adsorbents.

The various components in the sample travel through the column at a rate determined by their physical properties, temperature and composition of the column. Effluent from the column enters a detector where the composition of the carrier gas stream is characterized through one of the several possible chemical or physical properties of molecules. An electronic signal is generated upon interaction of the eluted component with the detector. The size of the signal is recorded by a data system and is plotted against elapsed time to produce a chromatogram.

In the present study, for the measurement of CO₂ and CH₄, GC (HP- 6890 and HP - 5890, respectively) fitted with a Flame Ionization
Detector (FID) were used, which separates gases using a packed column. Their subsequent concentrations can be calculated using standards. Depending on the mixing ratio gases in the sample a POROPAK QS Column (4 m x 2 mm internal diameter, mesh range 80 - 100) separates the various components based on the charge / mass ratio. The column, injector and detector temperatures were 60ºC, 100ºC and 250ºC respectively. The carrier gas used was high pure Nitrogen, which flows through the GC at the rate of 15 ml min\(^{-1}\). Compounds are burned in hydrogen - air flame. Carbon containing compounds produce ions that are attracted to the collector. The number of ions hitting the collector is measured and a signal is generated. The detector is capable of detecting compounds with C - H bonds. In order to detect CO\(_2\) in the GC (HP - 6890), it is fitted with a Methaniser which converts CO\(_2\) to CH\(_4\) at high temperatures in the presence of hydrogen flow.

### 3.3.3.2 Non Dispersive Infra Red Analysis (NDIR)

The analyzer is a single beam, dual wavelength NDIR sensor (LiCOR, Li 840). The CO\(_2\) molecules can absorb infrared light, causing them to bend, stretch or twist. The amount of IR light absorbed is proportional to the concentration of CO\(_2\). The energy is converted to kinetic energy, causing the molecules to speed up and thus heat the gas. A familiar IR light source is an incandescent household bulb. Each molecule absorbs IR light at particular wavelengths representative of the types of bonds present. CO\(_2\) has a strong absorbance at 4.26 µm, which is unique and highly selective. The detector measures the absorption of the IR beam passing through the optical path. The ratio of sample and reference signals (at 3.95 µm) indicates the amount of light absorption by CO\(_2\), and thus, the gas concentration. The CO\(_2\) mole fraction in dry air (xCO\(_2\)) was measured continuously (every 1 min) using the NDIR. The NDIR can detect only gases that pass through the optical path. In order to measure the dissolved trace gas's principle of equilibration is adopted.
3.3.3.3 **Equilibration**

The principle of dissolved trace gas measurement is based on the equilibration process. The main objective behind equilibration is that at the end of it, the partial pressures of both the liquid and gaseous phases reach equilibrium. This is dependent on the initial partial pressures, volume of the two phases and the solubility of the gas. Different types of equilibration set up was proposed by various researchers, among them three are main; (I) the bubble type (either bubbling air or another gas of known concentration through the water phase), (II) the shower type (showering the water into the gas phase, there is a continuous flow of sea water through the system) and (III) the thin-film type. The common theme is to expose the well-mixed water phase with the well-mixed gas phase through a large surface area. In the present study, the bubbler type (Upstill Goddard et al 1996) and shower type (Frankingnoulle et al 2001) equilibrators were used, both of which are well established procedures gives accurate results with good precision.

3.3.3.4 **Determination of pCO$_2$ and dissolved CH$_4$ through Gas chromatography**

Measurement of partial pressures of pCO$_2$ in the water sample was done by a high precision single - phase equilibration GC, based on the method of Upstill-Goddard et al (1996). Prior to the analysis, system was purged with compressed air to remove all traces of previous gas sample. After temperature equilibration (25ºC), the glass stopper in the sample flask was removed and replaced with the equilibrator manifold, sealed and secured with a retainer clip (Quickfit KC19) (Figure 3.4) and then a fixed head space was created with compressed air of gas known CO$_2$ composition.
The gases in the sample were equilibrated with those in the head space by continuously re-circulating this head space through the liquid sample using a high delivery (> 1.5 L min⁻¹) diaphragm pump (Charles Austin) for 10 min (for equilibration to achieve the theoretical 99.9% efficiency). The equilibrated head space was injected into the column of a gas chromatograph (GC - HP 6890) fitted with a Methaniser (PCi TC - 06). The FID which detects hydrocarbons is unable to detect CO₂ in the sample that passes through it. Hence, a methaniser was used to convert the CO₂ in the sample to CH₄ at high temperature (250°C) in the presence of Hydrogen gas over a nickel catalyst. Already present gaseous CH₄ in the sample does not interfere with the analysis of CO₂ because these are separated in the GC column and hence have different retention times. Calibration was carried out using certified gas standards of 372, 1000 and 5000 ppmv of CO₂ in Nitrogen (Scott et al., 1996).
Speciality Gases). Accuracy of standards was typically ±2% (Upstill-Goddard et al 1996). Analytical precision of GC method was typically ±0.5% determined using repeated analyses (n = 30). For measurements of estuarine water column samples, a combined run involving analysis of ambient air, a standard gas and the water sample typically took 30 minutes. The concentration of the gas in the water sample was calculated from the mixing ratios of CO$_2$ obtained from the GC as follows:

\[
W_i = A_e + [(A_e - A_i) \times V_a / (V_w \beta_{teq})] 
\]

(3.1)

\[
P_{teq} = W_i (1013.25/P) 
\]

(3.2)

\[
C_{in\,situ} = P_{teq}\beta_{teq} 
\]

(3.3)

\[
P_{in\,situ} = P_{teq}\beta_{teq}/\beta_{in\,situ} 
\]

(3.4)

Where,

\(W_i\) = effective corrected mixing ratio of CO$_2$ in the sample; \(A_e\) = measured mixing ratio of CO$_2$ in the headspace; \(A_i\) = mixing ratio of the equilibrator gas with known composition; \(V_a\) = Volume of headspace; \(V_w\) = Volume of water samples; \(\beta_{teq}\) = Bunsen solubility (v/v atm$^{-1}$) at the equilibration temperature (25ºC ± 0.1ºC); \(P_{teq}\) = Gas partial pressure; \(P\) = System internal pressure (= atmospheric pressure in mbar); 1013.25 = standard atmospheric pressure (in mbar); \(C_{in\,situ}\) = in situ gas concentration; \(P_{in\,situ}\) = in situ partial pressure; \(\beta_{in\,situ}\) = Bunsen solubility at in situ temperature.

Bunsen solubility is the volume of dry gas absorbed by a unit volume of solution when the partial pressure is 1 atmosphere. \(V_a\) and \(V_w\) are consistent as the samples were collected in certified 100 ml sampling bottles and did not significantly vary from the mean of all the flasks. The in-situ gas concentration was obtained as µatm of CO$_2$. 

Measurement of dissolved CH$_4$ in the water sample was carried out using a high precision single-phase equilibration gas chromatography (GC-HP 5890) with FID. Calibration was carried out by using certified gas standards of 1.5, 2.5 and 10 ppmv of CH$_4$ (Scott Speciality Gases). Accuracy of standards was typically ± 2% (Upstill-Goddard et al 1996). Analytical precision of GC method was typically ± 0.4% determined using repeated analyses (n = 20). Air samples for the determination of CH$_4$ were injected directly into the GC and mixing ratios obtained using the FID without any equilibration.

**3.3.3.5 In-situ pCO$_2$ measurement with NDIR sensor (LiCOR)**

For continuous in-situ field measurements of pCO$_2$ a shower type equilibrator was used according to the design proposed by Frankingnouille et al (2001) with slight modifications (Figure 3.5). The time required for equilibration was reported at less than 2 min. The entire setup was mounted on a boat with inlets and outlets, and computer terminals were set for data acquisition. A Perspex cylinder (length of 80 cm and an inner diameter of 10 cm) served as the equilibrator which was filled with glass marbles for increasing the surface area for gas exchange. The sample water was pumped continuously from the top of the equilibrator at the rate of 3 L min$^{-1}$ using a submersible pump from a depth of ~1 m. The water was not allowed to stagnate and flows out from the equilibrator to waste. Simultaneously, a closed air circuit from the bottom of the equilibrator to the top of the tube ensured circulation of air (at the rate of 3 L min$^{-1}$) via Charles Austin membrane pump. The pumped air flows through a moisture trap which was a cylindrical tube filled with an upper layer of Magnesium perchlorate (Mg(ClO$_4$)$_2$) and bottom layer of silica gel. Then the air entered NDIR analyzer, LiCOR (Li 840). The pumps that pumped in sample water or those that circulate air were regulated by regulators as shown in (Figure 3.5).
In order to maintain atmospheric pressure within the system a thin tube (length 15 m and diameter 2 mm) runs from inside the equilibrator and was left open to the atmosphere outside the boat. The LiCOR was calibrated using series of high pure standards (Scott Speciality Gases: 372, 1000 and 5000 ppmv CO\textsubscript{2} in balance Nitrogen). The LiCOR measured pCO\textsubscript{2} from the head space after equilibration every second and the output was given as values averaged every minute. The values obtained from the LiCOR were used to calculate the pCO\textsubscript{2} in sample water as follows:

\[
pCO_2 \text{ in the equilibrator} = (P - VP(H_2O, s/w)) \times xCO_2
\]

\[
= pCO_2 \text{ in dry air} - xCO_2 \times VP(H_2O, s/w) \quad (3.5)
\]

Where,

\begin{align*}
P & = \text{atmospheric pressure} \\
VP(H_2O, s/w) & = \text{saturated water vapour pressure in the equilibrator according to the Weiss and Price (1980) formula} \\
xCO_2 & = \text{CO}_2 \text{ measured from the LiCOR}
\end{align*}
The equilibrium concentration depends on water temperature, salinity, ambient air pressure and atmospheric pCO$_2$ concentration (Weiss and Price 1980). The aqueous pCO$_2$ from the equilibrator was corrected to in-situ temperature via a temperature coefficient. Air samples for the determination of CO$_2$ were injected directly into the GC and mixing ratios obtained using the FID and methaniser without any equilibration.

### 3.3.3.6 Indirect Method for the measurement of pCO$_2$

pCO$_2$ measured by GC was cross-checked with indirect measurement of pCO$_2$ from pH and Total alkalinity (TAlk). The pH was measured using a combination electrode (Orion, Thermo) calibrated on the U.S. National Bureau of Standards scale (NBS scale) pH buffers (using phthalate ($C_8H_5KO_4$ 0.05 mol kg$^{-1}$, pH = 4.002 at 25°C) and phosphate ($KH_2PO_4$ 0.025 mol kg$^{-1}$ and $Na_2HPO_4$ 0.025 mol kg$^{-1}$, pH = 6.881 at 25°C) buffers (Bates 1973). TAlk was determined through the classical Gran (1952) electro-titration method with 25 ml of water sample and 0.1M HCl as titrant using a pH meter and Dosimat. Acid was added to the sample, the decrease in pH of the sample along with the acid added was noted down. The analysis was conducted at constant temperature of 25 ± 3°C using a water bath. The normality of HCl was calibrated against 0.1 N NaOH using Bromothymol blue indicator. The amount of HCl added was plotted against the decrease in pH of the sample, and the slope was calculated. Using the slope the Gran function was derived, and this was used to calculate TAlk of the sample. The reproducibility of TAlk was estimated to be ±5 µmol kg$^{-1}$ and the necessary corrections were made while computing the pCO$_2$.

Mangrove water pCO$_2$ and DIC were computed indirectly from the TAlk and pH calculations, using the carbonic acid dissociation constants given by Millero (2007), applicable for a salinity range of 1 - 35 and
temperature range 1 - 50°C. Estuarine water samples with low salinity (<1) were computed using the carbonic acid dissociation constants of Cai and Wang (1998), applicable to fresh and entire salinity (0 - 35) and temperature range of 0 - 35°C. The pH values on the NBS scale were first converted to pH \textit{in-situ} and then ultimately to sea water (pH$_{sws}$) and total scale (pH$_T$) respectively, prior to computation through the thermodynamic constants of Millero (2007) and Cai and Wang (1998).

For data reliability, the computed pCO$_2$ and DIC values were compared with the one obtained from an updated version of co2sys.xls (version 14) program developed by Pelletier et al (2007). In the original co2sys.exe, the dissociation constants of Cai and Wang (1998) and Millero et al (2006) were used. The differences observed between these two separate computations were found to be insignificant (3 - 4 µatm for pCO$_2$ and 1 - 2 µmol kg$^{-1}$ for DIC) which can be considered negligible due to the analytical limitations.

3.3.4 Estimation of Chlorophyll

For determination of Chlorophyll a (Chl$\alpha$), known volume of water was filtered through 47 mm Whatman GF/C filter paper (1.2 µm pore size), preserved by adding few drops of saturated MgCO$_3$ solution as a thin film on the filter paper and then extracted in 90% acetone for 12 hrs, and analyzed spectrophotometrically according to Lorenzen’s (1967) method, as described by Parsons et al (1984).

3.3.5 Measurement of Dissolved Organic Carbon (DOC)

Samples that were collected in glass tubes and preserved with 10% ortho phosphoric acid to maintain the pH between 2 and 3 were used for DOC. The
DOC in the water sample was measured using a TOC analyzer (Shimadzu TOC-VCPH) by following high temperature catalytic oxidation method in the chemical laboratory of Integrated Coastal and Marine Area Management Project Directorate (ICMAM - PD), Chennai. It is measured as non-purgable organic carbon (NPOC) after acidifying samples to pH less than 2 with ortho phosphoric acid. This converts the inorganic carbon to dissolved CO$_2$, which is stripped out of the solution during sparging with CO$_2$-free air, which removes inorganic carbon. Then the remaining OC of sample is drawn into a platinum catalyst chamber where at 680°C in an oxygen-rich atmosphere it is oxidized to CO$_2$. The concentration of CO$_2$ generated was measured with a NDIR detector as a direct correlation to TOC (Figure 3.6). The accuracy and precision of the DOC measurements were checked once in every five samples with Certified Reference Material (supplied by Dr. D. Hansell, University of Miami, USA; Batch 5 FS) and internal standards (10 and 20 mg L$^{-1}$) were prepared using potassium hydrogen phthalate (KHC$_8$H$_4$O$_4$). The accuracies were found to be within the ± 1% level.

![Figure 3.6 Box diagram of NPOC analysis](image)

3.3.6 Sediment Geochronology

Sediment accumulation rates were determined in four sediment cores from the Hooghly estuary and the Sundarbans mangrove. The sections
of the cores were cut into 2 cm intervals and processed for $^{210}\text{Pb}$ and $^{226}\text{Ra}$ determination in the laboratory using standard procedures.

### 3.3.6.1 Measurement of $^{210}\text{Pb}$ in sediments

Direct determination of $^{210}\text{Pb}$ by beta counting is difficult since $^{210}\text{Pb}$ has very low energy beta emission. Since its immediate daughter, $^{210}\text{Bi}$ has high-energy beta emission; the radiochemical determination of $^{210}\text{Pb}$ is usually accomplished indirectly by measuring beta activity of $^{210}\text{Bi}$. In about 30 days, $^{210}\text{Bi}$ attains equilibrium with $^{210}\text{Pb}$ by more than 98%.

The decay scheme of $^{210}\text{Pb}$ is as follows:

$$
\begin{align*}
^{210}\text{Pb} \rightarrow ^{210}\text{Bi} \rightarrow ^{210}\text{Po} \rightarrow ^{206}\text{Pb} (\text{stable})
\end{align*}
$$

The activity of $^{210}\text{Pb}$ was determined in dry sediment samples by allowing it to attain equilibrium with its immediate daughter $^{210}\text{Bi}$ and the emission of $^{210}\text{Bi}$ was measured by following the procedure adopted by Flynn (1968); Kannan (1983, 2004) as given below.

A known quantity (5 gm) of dried sediment sample was taken in Teflon bomb and wet ashed on a hot plate with a mixture of acids (HNO$_3$, HClO$_4$ and HF). HNO$_3$ was added to the digested sample and heated to dry to convert it into the nitric acid medium. The dried sample was dissolved in 100 ml of 4N HNO$_3$ and filtered. The supernatant was taken for the further analysis of $^{210}\text{Pb}$. To an aliquot of the acid filtrate (~25 ml), 5 mg Barium (Ba) and 200 mg Lead (Pb) carriers were added and Ra(Pb-Ba)SO$_4$ got precipitated. The precipitate was dissolved in 10% ammoniacal EDTA. Acetic acid was then added to co-precipitate radium isotopes along with Ba as Ra(Ba)SO$_4$ left PbSO$_4$ ($^{210}\text{Pb}$) in solution. Ra(Ba)SO$_4$ precipitate was
separated using a centrifuge and discarded. To the supernatant containing $^{210}$Pb (Pb EDTA complex) Bismuth carrier (20 mg) was added and was kept aside for 30 days to allow $^{210}$Pb to attain equilibrium with its daughter $^{210}$Bi ($t_{1/2}$: 5 days). On reaching equilibrium, 200 mg calcium carrier was added as a hold back carrier. $^{210}$Bi was precipitated as bismuth hydroxide [Bi(OH)$_3$] by drop wise addition of ammonium hydroxide solution and centrifuged. The Bi(OH)$_3$ precipitate was dissolved in 0.5N HNO$_3$ and Bismuth phosphate (BiPO$_4$) was precipitated using H$_3$PO$_4$. BiPO$_4$ precipitate was beta counted using the gas flow type low background beta Counter (Geiger Muller counter) (EC, Model K2700B). From the activity of $^{210}$Bi, concentration of $^{210}$Pb was back calculated (Kannan 2004).

The recoveries of radiochemical separation analyses were checked by analysis of spiked samples. Corrections were made for counter background, efficiency, blank recovery and decay wherever necessary. The detection limit established by this method was 15 mBq at 69% confidence level. $^{210}$Pb activity in the sediment samples was calculated as follows:

$$^{210}\text{Pb (mBq kg}^{-1}\text{)} \text{dry sediment :}$$

$$\frac{\text{SB - B}}{\text{t}_1 - \text{t}_2} = \frac{10^8}{\text{D}_{\text{Bi}} \times \text{E} \times \text{G} \times \text{Q} \times \text{R}_{\text{Bi}}}$$

(3.7)

where, $\text{SB} =$ Sample + Background counts; $\text{B} =$ Background counts; $\text{t}_1 =$ Sample + Background counting duration (seconds); $\text{t}_2 =$ Background counting duration (seconds); $\text{D}_{\text{Bi}} =$ $^{210}$Bi decay factor ($e^{-\lambda t}$), $\lambda$ is Decay constant of $^{210}$Bi i.e., 0.0058 hr$^{-1}$; $\text{t} =$ The time duration in hours between the time of its separation from $^{210}$Pb (i.e., After Bi(OH)$_3$ precipitation) upto the mid time beta counting of $^{210}$Bi; $\text{E} =$ Percent efficiency of the low background beta counter; $\text{G} =$ $^{210}$Bi build up factor from $^{210}$Pb i.e., $1 - e^{-\theta}$, $\theta$ is build up time in hours; $\text{Q} =$ Quantity of dry sediment sample taken for analysis (grams); $\text{R}_{\text{Bi}} =$ Chemical recovery factor of Bismuth carrier.
3.3.6.2 Measurement of $^{226}$Ra in sediments

Determination of $^{226}$Ra activity in sediment samples was carried out using radon emanation technique (Ku and Lin 1976; Iyengar 1990). This method is very specific for the determination of $^{226}$Ra and interference from $^{223}$Ra and $^{224}$Ra are eliminated since they decay due to their short half-lives. The sediment samples were first digested with 8N HNO$_3$, with occasional addition of hydrogen peroxide. The sample was completely evaporated to dryness. Finally, the residue was dissolved in 4N HNO$_3$ and filtered using Whatman 42 filter paper, and the acid filtrate was made up to a known volume. Approximately, 50 ml of the acid filtrate was transferred to the radon bubbler (Figure 3.7). The bubbler was connected to a vacuum pump, and the air was bubbled through the sample to scrub any residual $^{222}$Rn. The sample in the bubbler was kept aside for about 15 days to allow $^{222}$Rn in the sample to attain secular equilibrium with its parent $^{226}$Ra.

$$^{226}\text{Ra} \xrightarrow{\alpha 4.78 \text{ MeV}} \frac{T_{1/2}}{1624 \text{ y}} \xrightarrow{\text{222 Rn}} \frac{\alpha 5.49 \text{ MeV}}{T_{1/2} \ 3.8 \text{ days}} \xrightarrow{218 \text{ Po}}$$

After attainment of secular equilibrium, the bubbler was connected to an evacuated background counted scintillation cell to de-emanate radon from the bubbler to the scintillation cell (Figure 3.8). The scintillation cell was stored for about 3 to 4 hours to allow the radon daughters to attain equilibrium with radon in the scintillation. At the end of the storage period, the scintillation cell was connected to a photomultiplier assembly and counted for alpha activity in an Alpha counting system (EC, RCS 4027A) for a period of 1000 seconds. $^{226}$Ra content in the sample was derived using appropriate
Figure 3.7 Radon Bubbler

Figure 3.8 Scintillation Cell Assembly
buildup and decay factors for $^{222}$Rn. From the measured counts, $^{226}$Ra activity in the sediment samples (mBq kg$^{-1}$) in dry sediment was calculated as follows:

$$^{226}\text{Ra (mBq kg}^{-1}\text{)} \text{ dry sediment:}$$

$$\frac{69.67 \times S}{B_1 \times B_2 \times D_{Rn} \times E_{sc} \times Q} \quad (3.9)$$

where,

$S = \text{Sample counts after subtracting the background counts};$  
$B_1 = \text{Radon build up factor in the radon bubbler i.e. } 1-e^{-\lambda \theta};$  
$\lambda$ is the decay constant of Rn = 1.258 x 10$^{-4}$ min$^{-1}$;  
$\theta$ is build up time in bubbler in minutes;  
$B_2 = \text{Radon build up factor in the scintillation cell i.e. } 1-e^{-\lambda \theta};$  
$\theta$ is build up time (minutes) in the scintillation cell during counting time of T minutes;  
$D_{Rn} = \text{Rn decay factor i.e., } e^{-\lambda t};$  
$t$ is time duration (minutes) between collection of Rn and the mid time of counting;  
$E_{sc} = \text{Percent efficiency of the scintillation cell};$  
$Q = \text{Quantity of the dried sediment sample taken for analysis (gram).}$

A standard $^{226}$Ra solution (1.15Bq) received from USA National Bureau of Standards (NBS), was used for calibration of the emanometric method. An average overall efficiency of about 80% was achieved using this standard.

### 3.3.7 Measurement of Organic Carbon in Sediment

Organic Carbon (OC) content in sediment was determined following a wet oxidation method described by Gaudette et al (1974). 1 gm of dried soil sample was taken into a 500 ml conical flask. 10 ml of 1N potassium dichromate solution along with 20 ml silver sulphate ($\text{Ag}_2\text{SO}_4$)
solution (1.25 gm Ag\textsubscript{2}SO\textsubscript{4} dissolved in 100 ml conc. H\textsubscript{2}SO\textsubscript{4}) was added into it and allowed to stand for 30 min for digestion. Mixture was diluted to 200 ml with distilled water. After dilution, 10ml of ortho-phosphoric acid was added into it. Mixture was then titrated against Mohr's salt solution (393.13 gm ferrous ammonium sulphate dissolved in 50 ml conc. H\textsubscript{2}SO\textsubscript{4} and volume was made up to 1L) in presence of 1ml diphenyl amine indicator until violet colour of the mixture changed into brilliant green at the end point and following equation was used for OC.

\[
\% \text{ of Organic Carbon} = \frac{(V_1 - V_2)}{W} \times 0.003 \times 100 \tag{3.10}
\]

where, \(V_1\) = Volume of Mohr's salt required to titrate; \(V_2\) = Volume of Mohr's salt required to titrate 10ml potassium di-chromate as blank; \(W\) = Weight of the sediment sample taken.

\[
\% \text{ of organic matter} = \% \text{ of organic carbon} \times 1.724 \tag{3.11}
\]

3.3.8 Grain Size Analysis

Granulometric analysis was carried out for bed sediment samples using standard sieve and pipette techniques after organic matter removal with H\textsubscript{2}O\textsubscript{2} (Folk 1974). This technique relies on the fact that in a dilute suspension, particle settles through a column of water at velocities, which are dependent upon their particle size. As particles decrease in sizes, they become increasingly cohesive as surface ionic charges grow in relative significance (McManus 1988). To determine the size of the flocculated sediments, it is necessary to introduce a dispersing agent (Sodium hexa-metaphosphate, is the commonest dispersing agent used). 50 grams of organic matter free (treated with H\textsubscript{2}O\textsubscript{2}) sediments were used for pipette analysis. A pinch of Sodium hexa-metaphosphate (dispersing agent to separate clay particles from silt) was added to the sample and was washed through 230 ASTM sieve until clear
solution passed through. The washing was collected carefully in a 1000 ml measuring cylinder and made up the volume with de-ionized water and stirred manually. As soon as the agitation was stopped, after exactly 123 minutes, a 20 cm$^3$ pipette was inserted to a depth of 10 cm in solution and from that level; the sample was withdrawn with uniform suction. The pipette out sample was transferred into a pre-weighed beaker, and oven dried at 90ºC. From final weight, Clay % was calculated. The sand particles (>63µm) on the sieve were transferred into a pre-weighed beaker and dried accordingly. From final weight Sand % and Silt % were calculated.

3.3.9 Determination of CaCO$_3$ in Sediments

CaCO$_3$ in bed sediment samples was determined by Gravimetric method following the standard procedure of Loring and Rantala (1992). The sediment samples were placed in a pre-weighed stoppered flask and treated with HCl. By adding excess HCl to CaCO$_3$, a certain volume of CO$_2$ was evolved, while an equal volume of air was expelled. The loss of weight due to the escape of air expelled by the evolved CO$_2$ was determined. Weights were determined instead of volumes. CaCO$_3$ content was estimated using below mentioned calculation:

\[
\% \text{ CaCO}_3 = \frac{P}{Q} \times 0.100 / \text{weight of dry sediment} \times 100
\]

(3.12)

where, $P$ = loss of weight in grams.

3.3.10 Determination of Trace metals in Sediments

In the present study, to determine the total trace metal concentration of sediment samples, complete digestion procedure was adopted described by Loring and Rantala (1992). The sediment samples (core samples and bed sediments) were oven dried at 60ºC for a period of 48 hours and then powdered using quartz motor and pestle. The fine sediment powder was then
passed through a 100 micron mesh-size sieve and collected sediment was subjected to complete acid digestion for trace metal analysis.

0.5 g of the powdered sediment sample was transferred to a Teflon beaker which was placed on the hot plate at 50 - 60°C and 2 ml of hydrofluoric acid (HF) was added whereby silica volatilizes as silicon tetrafluoride. This was followed by addition of 2 ml perchloric acid (HClO₄) and 5 ml concentrated HNO₃ to the sample to eliminate the organic matter. The complete digestion was confirmed by repeating the acidification until a clear solution was obtained and then the residue was diluted with adding Milli-Q water and allowed to evaporate for some more time. After evaporation of acid residues, it was dissolved in 0.1 N HCl. The dissolved solution was filtered through a filter paper (Whatman No. 42) into a 50 ml volumetric flask after repeatedly rinsing the Teflon beaker with 0.1 N HCl. The solution was made up to 50 ml and mixed well. The aliquots were then transferred to acid washed polypropylene bottles. After calibration with suitable EMerck elemental standards, samples were analyzed in Flame AAS (Perkin Elmer AA 800) equipped with deuterium background corrector for estimation of Fe, Zn, Co, Ni, Pb, Cu, Mn and Cr.

The accuracy and precision of the analytical methodology were assessed by triplicate analyses of certified reference materials, Sediment T (NOAA/7) along with analytical grade (MERCK, Germany) chemical standards for each element. Good to excellent recoveries ranging from 93 to 100% were obtained for the studied metals (Zn = 98%; Cu = 95%; Cr = 99%; Ni = 99%; Co = 93%; Fe = 99%; Mn = 98%; Pb = 100%) indicated an overall good accuracy of the analyses. The concentrations of trace metals were calculated as follows:
Metal concentration in = \frac{AAS reading \times Volume of sample (50 ml)}{X D mg kg^{-1}/ppm/µg g^{-1}} \\
\text{Weight of the sediment taken in grams}

(3.13)

Where, D = Dilution factor

3.4 SOFTWARE USED

Microsoft Word and Excel (MS Office version 2007) were used for writing thesis and data processing. Adobe Photoshop (version 7.0.1), Microsoft Paint, Corel DRAW (Graphics Suite X5) were used to edit and prepare different Figures. Graphical representations of data were done in OriginPro (version 7.0). All geochemical maps were constructed using Geographical Information System (GIS) software (ArcMap 9.3.1) to examine the spatial distributions of different parameters in the surface sediments. Multivariate statistical analyses [(eg. ANOVA, Pearson's correlation analysis, principal component analytical (PCA))] were performed using Statistical Program for Social Sciences (SPSS version 13.0) and MINITAB (version 13.0).