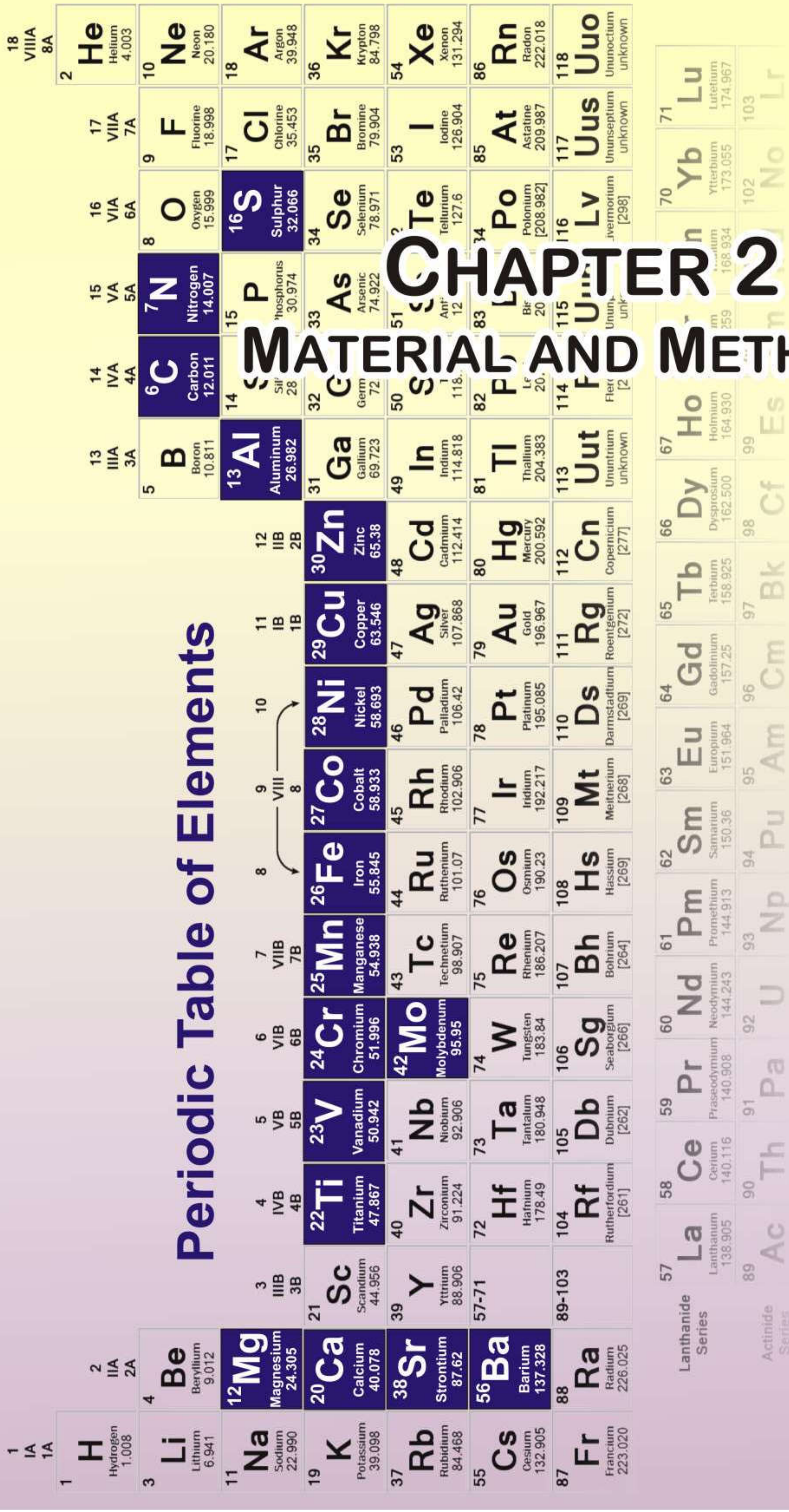
CHAPTER 2

MATERIAL AND METHODS



2.1 Sample collection

Reconstruction of Holocene climatic history can be achieved by using various archives. One of the important archives used for such studies are the mudflats. The rate of sedimentation in mudflats are slow and it records the climatic fluctuation that persisted during the deposition of the sediments. The studies of Holocene climatic variability using the sediment cores raised from the mudflats involved collection of the core from pristine and undisturbed location, determination of the chronologies, selection and precise measurements of appropriate proxies. The main objective of the present study is reconstructing high-resolution Holocene records of climatic variability along the southern Saurashtra coast. Towards this, sediment cores from the mudflats of the southern Saurashtra coast viz. Diu and Rohisa were raised. The following sections elaborate the sample collection, chronology estimation and various proxies used for the past climatic reconstruction.

Two cores of 70 cm and 60 cm with the help of hand operated PVC corer were collected from partially active mudflat of Diu (DV) and active mudflat of Rohisa (RH). The sediment core RH is nearly 28 km away from DV. Samples were also collected from a pit in relict mudflat of Vasoj Village (VV). Surface sample from the active mudflat of Diu (DM) was also collected (Fig. 2–1).

Both the sediment cores collected from the active mudflats (DV and RH) were subsampled at 2 cm interval using a thin perspex knife after the extraction. For the relict mudflat (VV) a pit was dug and samples were collected at 10 cm intervals. The subsamples were packed in cleaned ziplock bags and stored for further processing.

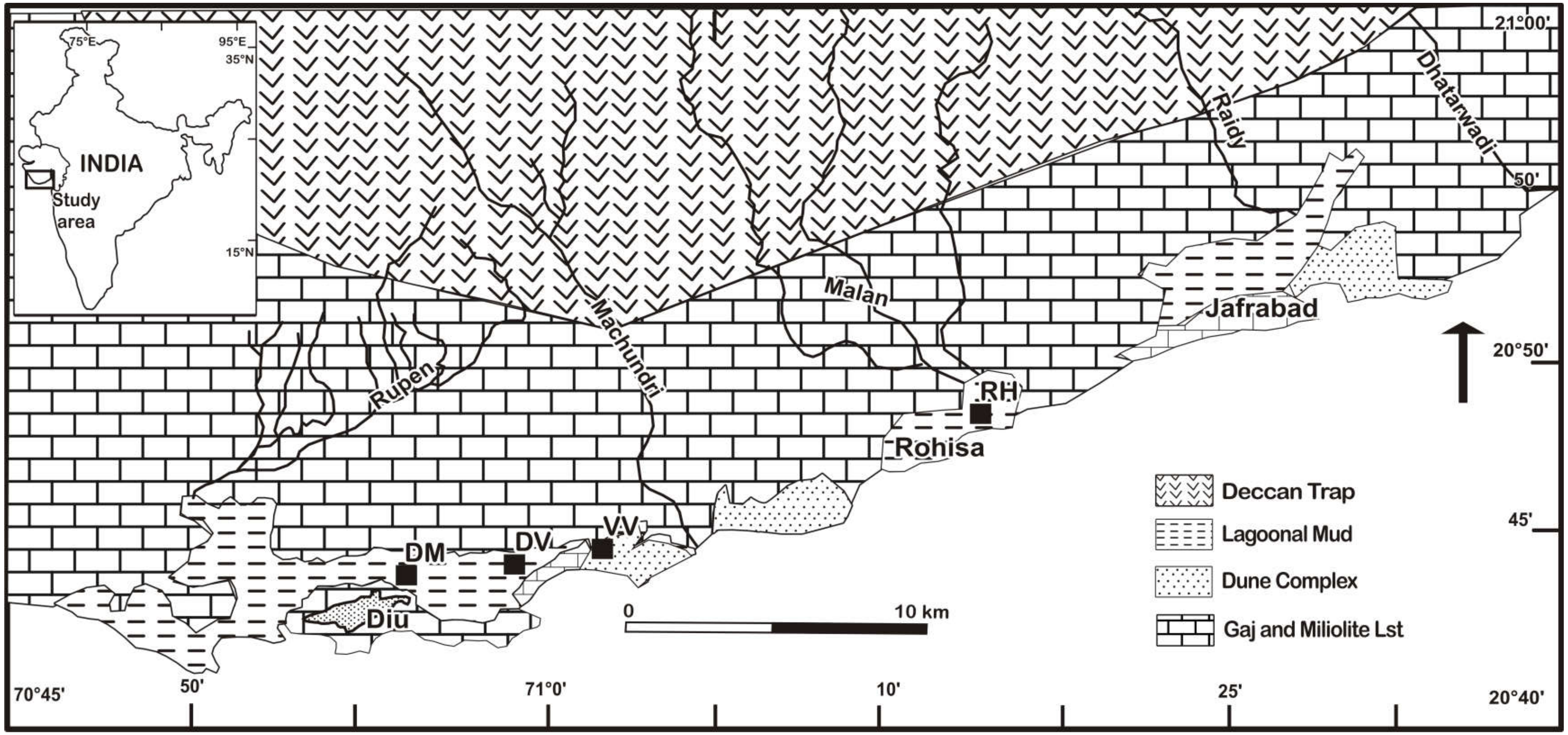


Figure 2-1. Sample collection sites along the southern Saurashtra coast wherein VV represents samples of relict mudflat, RH and DV represents the core raised from the active and partially active mudflat of Rohisa and Diu while DM represents the surface sample collected from the active mudflat of Diu (Image modified after Pant and Juyal, 1993).

2.2 Chronology– Radiocarbon dating

The chronologies of the sediment cores analysed as part of this work have been obtained using Radiocarbon dating method (Libby, 1955). In the present study, samples were analysed for obtaining radiocarbon dates using two methods, Conventional radiocarbon dating technique using Liquid Scintillation Counter and the Accelerator Mass Spectrometer (AMS) dating technique.

Figure 2-2. Field images for sample collection from (a–b) Rohisa active mudflat (RH); (c–d) Diu partially active mudflat (DV) and (e–f) Vasoj relict mudflat (VV).

2.2.1 Conventional radiocarbon dating technique

The conventional radiocarbon method includes conversion of organic/inorganic carbon of the sample into benzene following which beta counting is carried out with the help of liquid scintillator counter.

I. Benzene (C_6H_6)synthesis

The benzene was synthesized from the *Turitella* shells and organic carbon rich sediment using a TASK benzene synthesizer following method mentioned by Noakes et al. (1965) and Gupta and Polach (1985). The carbon dioxide (CO_2) is produced in two ways: by acid hydrolysis for inorganic carbon and by dry combustion in oxygen environment for organic carbon rich samples. The CO_2 produced is then converted into Lithium carbide (Li_2C_2) which is further hydrolysed to produce acetylene (C_2H_2). The final product C_6H_6, was produced through the polymerization of acetylene in presence of alumina coated vanadium as catalyst. The C_6H_6 was transferred into 7 ml glass scintillator vial and scintillator cocktail [42 g/l PPO (diphenyloxazole) + 0.7 g/l POPOP (2, 2'–p-phenylene-bis-5 phenyl oxazole)] was added. The sample cocktail was mixed and counted for ^{14}C activity (Chakraborty, 1993; Bhushan et al., 1994).

II. Radiocarbon counting

The C_6H_6 samples with scintillator cocktail was counted in Packard Tri-Carb Liquid Scintillator Analyzer, model 2250CA. it operate in two modes normal mode and low level count mode (LLCM). The β–particle of ^{14}C atom has a maximum energy of 156 keV and tritium has energy ranging between 0–18.6 keV. If the samples are contaminated with tritium, its interference can be minimized during ^{14}C counting by selecting an energy window for ^{14}C counting where the tritium interference would be minimal along with high efficiency and low background. With the usage of LLCM, the optimized region selected for ^{14}C in counting was 11–98 keV (Chakraborty, 1993; Bhushan et al., 1994).

From the relict mudflat section, the *Turritella* shells and organic rich sediment were collected separately (at a depth of ~50 cm and 95 cm respectively) for assigning the age of the section. For the conventional radiocarbon dating of organic carbon, samples were pre-treated with 10 % HCl to remove carbonate. In the case of *Turritella* shells, ~20 g of shells were cleaned with 1N HCl for nearly 1 minute to remove any surficial coating, followed by rinsing with 30 % H_2O_2 for nearly 10 minutes in ultrasonic bath to remove any foreign organic debris. Residual organic rich fractions from the sediment were dry combusted under vacuum with oxygen flow, whereas, the cleaned *Turritella* shells were dried and powdered and reacted with 40 % Orthophosphoric acid to liberate carbon dioxide, which was further converted into benzene following standard procedures. The radiocarbon activity was assayed using Liquid Scintillation Analyser (Packard TRI-CARB-2250CA) (Bhushan et al., 1994; Bhushan et al., 2000). The detail of the samples dated with conventional method is given in Table 2–1.

2.2.2 Accelerator Mass Spectrometer (AMS) dating technique

The fundamental difference between conventional radiocarbon dating i.e. Liquid scintillation method and AMS technique is that in the former decay of the atom is counted whereas, in the latter the atoms themselves are counted. This allows AMS technique to measure even small samples, which aids in improving the accuracy relative to the bulk method by the absence of various contaminants that are usually present in the bulk samples. The conventional radiocarbon dating method is time consuming which requires nearly two days just for the preparation of benzene and nearly three days for beta counting in Liquid scintillator. Unlike conventional method, AMS takes less than an hour for the analysis and more samples can be analysed at the same time (Agnihotri, 2001).

The AMS differs from the conventional mass spectrometry in the energies to which the ions are accelerated. In mass spectrometers, the energies are in thousands of eV (keV) whereas in the AMS they are in millions of eV (MeV). The practical consequence of having higher energies is that ambiguities in the identification of the atomic and molecular ions with the same mass (isobaric effects) are removed. Thus we can measure isotopic ratio for specific elements to a level of 1 in 10^{15} (Agnihotri, 2001).

In the inorganic fraction of the sediment, the mudflats surrounded by biogenic carbonate rocks (miliolites) dated to ~178 ka (Baskaran et al., 1987) are capable of getting contaminated with dead carbonate due to miliolite contribution. Therefore, for the age estimation, the organic carbon fraction is most appropriate fraction for dating the sediment.

For AMS radiocarbon dating, about 1 mg of either organic or inorganic carbon fraction is required. Therefore, on the basis of organic carbon content, appropriate amount of decarbonated sediment samples were used for obtaining AMS dates. The AMS dating was carried out by the Accelerator Mass Spectrometer at NSF Arizona AMS Facility, University of Arizona, USA in collaboration with Prof. A.J.T. Jull (Linick et al., 1986; Jull et al., 1989; Somayajulu et al., 1999). The details of the dates obtained for samples analysed are given in Table no. 2–1.

Table 2-1. Carbon-14 ages and calibrated ages of the samples for the cores VV, DV and RH collected from the Southern Saurashtra Coast.

Sr. No.	Sample Id	Sample Detail	Depth (cm)	Lab Code	C-14 Age (yr BP)	Calibrated Age (cal yr BP)	C-14 Dating Method
				RELICT MUDFLAT			
1.	VV 20–30	Organic carbon	20−30	B10116	2650±50	2165 ±100	AMS
2.	VV 40–50	Turritella Shells	50	PRLCH:863	3190± 95	2990±125	Conventional
3.	VV 90–100	Organic carbon	90−100	PRLCH:888	4680 ± 60	4710±110	Conventional
				ACTIVE MUDFLATS			
4.	DV 40-42	Organic carbon	40-42	B10110	2976± 41	2815.5±50	AMS
5.	DV 68-70	Organic carbon	68-70	B10111	3952± 42	4039±62	AMS
6.	RH 38-40	Organic carbon	38-40	B10118	1864± 43	1442±23	AMS
7.	RH 60-62	Organic carbon	60-62	B10115	2273± 39	1907±3	AMS

2.2.3 Reservoir age correction (Δ R)

The marine organism derive carbon with low $^{14}C/^{12}C$ compared to atmosphere (due to the mixing of deeper C-14 depleted water) from the seawater (Reservoir) which results in older ^{14}C ages for marine fossils compared to the contemporary terrestrial carbon samples. Therefore, on a regional scale, reservoir age correction is required for the necessary calibration of the ^{14}C ages of the marine samples (Stuiver et al., 1986; Stuiver et al., 1998). The 'ΔR' is estimated from the difference between the regional marine ^{14}C age and the global model marine ^{14}C age at any given time (Stuiver and Braziunas, 1993; Dutta et al., 2001). Therefore, ΔR is the value that has to be added to the global mean reservoir age (400 yrs) to obtain the age correction needed for the local reservoir effect. In the present study, the radiocarbon ages have been calibrated to calendar ages using the calibration program "Calib 7 (Mixed N Hemisphere)" (Stuiver et al., 1998). The errors reported for the radiocarbon dates of the samples are one standard deviation (1σ). The reservoir age correction (ΔR values) value for the samples from the Saurashtra is taken as 165 ± 57 yr BP (Dutta et al., 2001; Southon et al., 2002) and has been incorporated in all the C-14 calibrated dates.

2.3 Paleoclimatic proxies

2.3.1 Total organic carbon (TOC) and Total Nitrogen (TN)

TOC can be used as a proxy for the organic matter flux provided the organic carbon is not diagenetically altered (Meyers, 1997). In areas where the sedimentation rate is relatively constant the variability in TOC can be used as indicator for the temporal changes in organic matter flux (Tyson, 1995; Ku et al., 2005). Carbon/Nitrogen ratio is a reliable proxy in identifying the sources of terrestrial and marine organic carbon. Organic material received by the coastal sediments can be either from autochthonous or allochthonous sources. Total organic carbon (TOC) and carbon to nitrogen (TOC/TN) ratio in tidal flat/estuarine sediment is governed by the indigenous plants and organic material transported by the rivers or by the tidal current (Sampei et al., 1997; Allen et al., 2007). Similarly, the TOC/TN ratio can be used as a sensitive indicator to differentiate the contribution from the marine versus terrestrial source. Terrestrial vegetation normally has relatively high TOC/TN ratios i.e. >12 (Prahl et al., 1980) as it is predominantly composed of lignin and cellulose, which are nitrogen poor. C3 vascular plant material has TOC/TN ratios of around 12 and over (Tyson, 1995), whereas terrestrial C4 grasses typically have TOC/TN ratios of above 30 (Meyers, 1994). Lower plants such as phytoplankton and bacteria contains proteins and nucleic acids which are rich in organic nitrogen that lowers the TOC/TN ratios (Blackburn, 1983). Studies have shown that bacteria and algae typically have low

TOC/TN ratios of 4–6 and <10, respectively (Meyers, 1994; Tyson, 1995). The TOC/TN values of plant can be highly variable in a small area because of the large fluctuations in the nitrogen content of plants.

2.3.2 Stable Isotopes- $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$

The stable isotopes have been widely used for the paleoclimatic reconstructions. Generally, the isotopic anomalies are expressed in the form of ratios between less abundant (heavier) atoms with respect to naturally more abundant (lighter) atoms. Further, they are expressed using Delta (δ) notation. Therefore, the isotopic anomalies of carbon, nitrogen and sulphur are expressed as $\delta^{13}C$, $\delta^{15}N$ and δ^{34} S respectively and the 'δ' notation is defined as

$$\delta = [(R_{\text{sample}} / R_{\text{STD}}) - 1] \times 1000$$

Where $R = {}^{13}C/{}^{12}C$, ${}^{15}N/{}^{14}N$ and ${}^{34}S/{}^{32}S$

'δ' Values are general expressed in per thousand or per mil (‰). The standards (STD) used for Carbon, Nitrogen and Sulphur are Vienna-Peedee Belemnite, atmospheric Nitrogen and vienna-cation diablo troilite respectively.

I. Carbon Isotopes ($\delta^{13}C$)

The carbon has three isotopes ^{12}C, ^{13}C and ^{14}C with abundance of 98.89 %, 1.11 % and $\sim 10^{-12}$ respectively. The TOC/TN is usually measured along with the $\delta^{13}C$ which also helps in discerning the source of the carbon deposited in the site (Lamb et al., 2006). The $\delta^{13}C$ values for the terrestrial material ranges between -26‰ to -28‰ (Emerson and Hedges, 1988) while marine material ranges between -19‰ to -22‰ (Fontugne and Jouanneau, 1987). Nevertheless, the organic material pertaining values between these limits is ascertained as a mixture of terrestrial and marine organic matter

(Gearing, 1988). Riverine algae are usually more depleted than terrestrial organic matter as they assimilate carbon with low δ^{13}C values (Middelburg and Herman, 2007).

II. Nitrogen Isotopes (δ^{15}N)

Nitrogen has two isotopes viz. ^{14}N and ^{15}N with abundances of 99.64% and 0.36% respectively. The nitrogen isotopes can be used to distinguish algae and land plant source of organic matter. The δ^{15}N for dissolved nitrate ranges between 7 to 10 ‰ whereas for atmospheric nitrogen is about 0 ‰ (Peters et al., 1978; Meyers, 1997). Usually the marine organic matter has the δ^{15}N value of ranging between 3–12 ‰ with the mean of 5–7 ‰ as derived from phytoplankton that utilises the dissolved nitrate whose δ^{15}N ranges between 7 to 10 ‰ (Peters et al., 1978; Meyers, 1997; Brandes and Devol, 2002; Lamb et al., 2006; Gao et al., 2012). The δ^{15}N for the atmospheric nitrogen is 0 ‰ hence the organic matter derived from nitrogen fixing land plants has δ^{15}N values around zero, whereas plants using only mineral N from soil (NO_3^- and NH_4^+) usually have positive values. Generally, river suspension has lower values compared to oceanic values but are variable probably due to the contributions from forest and soil nitrogen as terrestrial plant ecosystems have low δ^{15}N (Maksymowska et al., 2000; Gaye-Haake et al., 2005).

III. Sulphur Isotopes (δ^{34}S)

Sulphur comprises of four isotopes ^{32}S, ^{33}S, ^{34}S, and ^{36}S with an abundance of 95.02%, 0.75%, 4.21% and 0.02% respectively. The sulphur isotopes are used to ascertain the source of sulphur and sufficient focus has been given to the dissimilate sulphate reduction and the formation of pyrite in marine environments (Berner, 1970; Jørgensen, 1977, 1990; Canfield et al., 2000). The oceanic sulphate isotope composition is uniform with a value of 20 ‰ (Thode, 1970; Rees et al., 1978; Peterson and Howarth, 1987; Strauss, 1997; Strauss, 2003; Bottrell and Newton, 2006).

The $\delta^{34}S$ for the continental vegetation ranges between 2-32 ‰ whereas the dissolved sulphate of continental precipitation ranges from 1 to 7 ‰ (Petersen and Howarth 1987). The $\delta^{34}S$ for the lacustrine sediments primarily depends upon the aqueous sulphate in lake water derived from atmospheric or lithospheric sources (Mayer and Schwark, 1999). Therefore, lacustrine organic $\delta^{34}S$ values are normally found to be in the range between the isotopic composition of SO_4^{2-} and that of pyrite (Urban, 1994). Low $\delta^{34}S$ values of organic material may be attributed to times of great eutrophication (Urban et al., 1999).

2.3.3 Major and Trace elements

I. Detrital proxy

Al_2O_3, MgO, FeO and TiO_2 are the major component of aluminosilicate phases and hence they are tagged as terrestrial proxy (Agnihotri et al., 2003; Kemp and Izumi, 2014). When normalized with aluminium, their ratio can be used to infer the detrital contribution of the element to the sediment (Tripathy et al., 2014) further it also helps in distinguishing the change in the chemical composition of the sediment which are not caused by the dilution of $CaCO_3$ (Luckge et al., 2001). The depth profiles of element normalised Al_2O_3 are independent of dilution caused by increased detrital material (Agnihotri et al., 2003). Therefore, to reconstruct the paleoclimatic changes various element are normalised by Aluminium and used as paleoclimatic proxy.

II. Productivity Proxy

Till date numerous proxies have been used to decipher the past productivity variations. $CaCO_3$ has also been extensively used to decipher the paleoproductivity patterns (Nath, 2007). Nevertheless, the dissolution and preservation of the $CaCO_3$ depends on various factors (Nath, 2007) which may cause the underestimation of the $CaCO_3$. Nevertheless, other proxies that serves as productivity indicators are CaO/Al_2O_3, Sr/Al_2O_3 and Ba/Al_2O_3 (Goldberg and Arrhenius, 1958; Dymond et al., 1992; Reichart et al., 1997; Agnihotri et al., 2002). Geologically, the study area comprises of miliolitic limestone and its contribution may reflect in the calcareous productivity proxy. Therefore, in order to overcome the discrepancy, TiO_2/ CaO has been estimated which serves as proxy for detrital carbonate contribution and chemical weathering (Tripathy et al., 2014).

Organic carbon also serves as productivity indicator however, it is also prone to degradation therefore, copper and nickel (normalised by aluminium) serves as organic carbon productivity due to the fact that Cu and Ni are associated with organic carbon by organometallic complexes (Tribovillard et al., 2006). Further, Ba/Al_2O_3 and Cu/Al_2O_3 indicates organic carbon productivity (Dymond et al., 1992).

III. Weathering Proxy

The chemical weathering is an crucial feature of global hydrogeochemical cycle of elements (Warrier and Shankar, 2009). The chemical weathering is mainly controlled by climate in the source region (Zhao and Zheng, 2015), while temperature and precipitation governs its intensity (Kump et al., 2000; Gislason et al., 2009). Simultaneous warming in the climate with increased chemical weathering has previously been documented (Burton and Vance, 2000; Ravizza et al., 2001; Cohen et al., 2004). Warm and humid conditions increases the elemental ratios such as Mg/Al and Ti/Al which in turn indicates the intensity of chemical weathering (Warrier and Shankar, 2009).

Table 2-2. Geochemical parameters used as climate proxy in the present study

Sr. No.	Geochemical Parameter	Paleoclimatic proxy
1.	Al_2O_3, MgO, FeO and TiO_2	Detrital
2.	$CaCO_3$, CaO/Al_2O_3, Sr/Al_2O_3, Ba/Al_2O_3, TOC, Cu/Al_2O_3, Zn/Al_2O_3	Productivity
3.	MgO/Al_2O_3 and Ti/Al_2O_3	Weathering
4.	V/Al_2O_3, Cr/Al_2O_3, Mo/Al_2O_3, Co/Al_2O_3	Redox
5.	TOC/TN>12 and $\delta^{13}C$ -26 to -28 ‰	Terrestrial TOC
6.	TOC/TN<10 and $\delta^{13}C$ -19 to -22 ‰	Aquatic TOC
7.	TOC/TN > 30	C4 Grasses
8.	TOC/TN = 4–6	Bacteria
9.	TOC/TN <10	Algae
10.	$\delta^{15}N$: +7– + 10 ‰	Dissolved nitrate
11.	$\delta^{15}N$: 0‰	Atmospheric nitrogen
12.	$\delta^{15}N$: 3–12 ‰	Marine organic matter

2.3.4 Pollen as proxy

Local vegetation is directly influenced by climatic, geographical and geological changes (Kumaran et al., 2005). In view of this, palynological study viz. the pattern and the relative concentration of the spores and pollens can be used to ascertain the paleoecological condition. Together with other proxies the pollen data can be used to ascertaining the relative changes in sea levels (Ellison, 1989; Kumaran et al., 2005). The mangrove forest grows in the coastal region where it is bathed by brackish or sea water (Caratini et al., 1994). The pollens and spores of mangroves (Thanikaimoni, 1987) and Pteridophyte spores (Caratini et al., 1994) are reliable markers of intense humidity. In the marine sediments, the dinoflagellate cysts may provide information about paleoenvironmental features viz. depth and distance from the coastline (Wall et al., 1977).

2.4 Measurement techniques

2.4.1 $CaCO_3$, TOC and TN measurement

Total carbon and nitrogen in the mudflat sediments were measured using the NC analyzer (FISONS model NA 1500) and Calcium carbonate ($CaCO_3$) was determined using Coulometer (UIC Coulometer; 5012) at Physical Research Laboratory, Ahmadabad.

Total carbon and nitrogen content in the samples were measured using CN analyser (*Fisions Model NA1500*). In order to estimate the organic carbon, the inorganic carbon is removed by decarbonation of the sediment sample with 0.1N HCl. Then the samples were dried and ~5–20 mg of samples was packed in tin capsules which are further released into a combustion chamber at 1020°C. The evolved CO_2 and nitrogen oxides are then allowed to pass through a reduction chamber which is maintained at a temperature of 650°C that contains metallic copper. After further purification the gases are passed through a gas chromatograph. The gases enter a thermal conductivity detector, which generate electric signals proportional to the concentrations of the gases present. The CN analyser calibration was done using Low Organic Soil Sample (LOSS), Batch no. 647582814 as a reference material containing 1.65 and 0.14 % carbon and nitrogen respectively (Bhushan et al., 2001).

The inorganic carbon in the form of Calcium carbonate was ascertained by coulometer (*UIC Coulometer, Model 5012*). About 15–25 mg of sediment was treated with 5 ml 40% orthophosphoric acid in a test-tube at a temperature ~70°C maintained in a closed system. The CO_2 free air (stripped by passing it through a 50% KOH solution) was used as carrier gas for flushing CO_2 from the system. The CO_2 liberate was flushed by the carrier gas and dried by passing it though a column of activated silica gel. The dried CO_2 thus obtained was then passed through the coulometer titration cell (with Pt cathode and Ag anode) containing monoethanolamine solution and an indicator. Pure and dried Na_2CO_3 was used as a standard for calibration. The precision of $CaCO_3$ measurement by coulometric analysis is less than 1% (Bhushan et al., 2001).

2.4.2 Major and Trace elements

Samples were dried (at 80°C), crushed and homogenised with the help of agate mortar in order to avoid any metallic contamination. Nearly 0.3 gm of bulk, dried crushed sediment samples were subjected to closed digestion (in Thermo Microwave digestion system) by treating them with concentrated acids (HCl, HF and HNO_3) using standard protocol at PRL, Ahmadabad. Open digestion was performed to remove all the remaining silica or organic particles. The dissolved solutions of the sediments were taken in 2 % HNO_3 medium and the volume was made to 50 ml (Singh, 1999; Dalai et al., 2004; Das and Krishnaswami, 2007). All the reagents used in the present study were of analytical grade and the contamination from the time of sampling to analysis were minimised by taking due care. The digested samples were analysed for major elements (Al, Fe, Ca and Mg) and trace elements (Ti, Sr, Ba, Zn, Cu, Co, Pb, Cr, Mn and Ni) by aspirating the sample solutions in the ICP-AES (JobinYvon 38S) and ICP-MS (Thermo-X series2) respectively. The reproducibility of the instrument was taken care by carrying out duplicate analysis for the elements analysed. The accuracy of analytical procedure was accessed using certified reference standard NOVA. NOVA is deep sea clay sediment collected from N. Pacific at water depth of 5351 m (Amin et al., 1972) and MAG which is a fine grained gray-brown clayey mud with low carbonate content, from the Wilkinson Basin of the Gulf of Maine (Abbey, 1983; Gladney and Roelandts, 1987 ; Govindaraju, 1994).

2.4.3 Pollen Analysis

In order to carry out the palynological investigations, 10 gm of sediment sample was treated with 10 % KOH solution and 40 % HF to deflocculate the pollen/spores and to dissolve the silica content of the sediments. Thereafter, the standard pollen analytical technique of acetolysis using acetolysing mixture (9:1 of acetic anhydride and concentrated sulphuric acid) was employed (Erdtman, 1943). The samples were prepared with 50 % glycerin and examined under a light microscope (BX-61). The palynomorphs were identified using pollen atlases (Thanikaimoni, 1987; Nayar, 1990) and reference slides of Birbal Sahani Institute of Palaeobotany, Lucknow, India. 300 pollen grains from each sample were counted and pollen diagram was prepared based on relative pollen frequencies of the different taxa. The identified taxa were categorised into four groups viz., mangroves, midland taxa, ubiquitous and fresh water. Although, pteridophytic, fungal spores, dinoflagellate cysts and foraminiferal linings were also observed however, they are not included in the pollen sum.

2.4.4 Nitrogen, Carbon and Sulphur Isotopes

The isotopic measurements for the carbon, nitrogen and sulphur were carried out at CSIR–National Physical Laboratory, New Delhi with the help of isotope ratio mass spectrometer (Isoprime 100, Isoprime UK®) coupled with an elemental analyser (Pyrocube, Elementar®). In order to measure the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, the sediment samples were decarbonated in 0.1N HCl in order to remove the carbonate fraction. Nearly 20 mg of the decarbonated samples were packed in the tin cups before introducing to ES's auto sampler. The packed tin cups were dropped into the combustion tube which was maintained at a temperature of 1120°C of Pyrocube EA (Agnihotri et al., 2015). The combustion tube was filled with tungsten oxide padded with corundum balls and quartz wool which acts as catalyst for the CNS mode of the instrument. The flash combustion takes place in the presence of high purity oxygen gas. The evolved gases were allowed to pass through the reduction tube which was filled with metallic copper (for the reduction of oxides of nitrogen) and maintained at 850°C. Sample N_2 is passed through directly to open split orifice of IRMS, while CO_2 and SO_2 are held on two special CO_2 and SO_2 columns normally kept at temperatures 20 to 25°C (room temperature) and 55°C respectively. When N_2 analysis is over, CO_2 is allowed to be desorbed from CO_2 column by raising the temperature to 240°C; similarly after CO_2 analysis, SO_2 is allowed to be desorbed from SO_2 column by raising the temperature to 220°C (Agnihotri et al., 2015).

Table 2-3. Comparison of average concentration of various elements with that of the reported values

MAG				**NOVA**		
Elements (%)	**Reported**	**Present Study**	**No. of readings**	**Reported (Agnihotri, 2001)**	**Present Study**	**No. of readings**
Al	8.21±0.16	7.9±0.7	17	8.4±0.29	8.2±0.4	13
Ca	0.978±0.071	1.02±0.1	13	–	–	–
Fe	4.76±0.42	5.0±0.4	14	5.41±0.23	5.6±0.4	14
Mg	1.8±0.06	1.8±0.2	17	2.34±0.09	2.3±0.2	8
Elements (ppm)						
Ti	4494±420	4379±261	20	3810±245	3790±226	17
Cr	97±8	96±10	18	63±4	73±8	20
Mn	759±68	726±31	19	7192±366	7039±376	13
Co	20±1.6	21±3	20	104±7	105±8	19
Ni	53±8	47±3	13	206±15	206±22	16
Cu	30±3	31±6	17	392±20	389±16	9
Zn	130±6	137±9	11	152±10	159±17	11
Sr	150±15	145±16	28	182±6	180±12	17
Ba	480±41	471±34	24	1635±52	1629±64	9
V	140±6	137±8	22	147±10	137±7	14
Mo	1.6	1.3±0.2	5	22±2	22±2	6

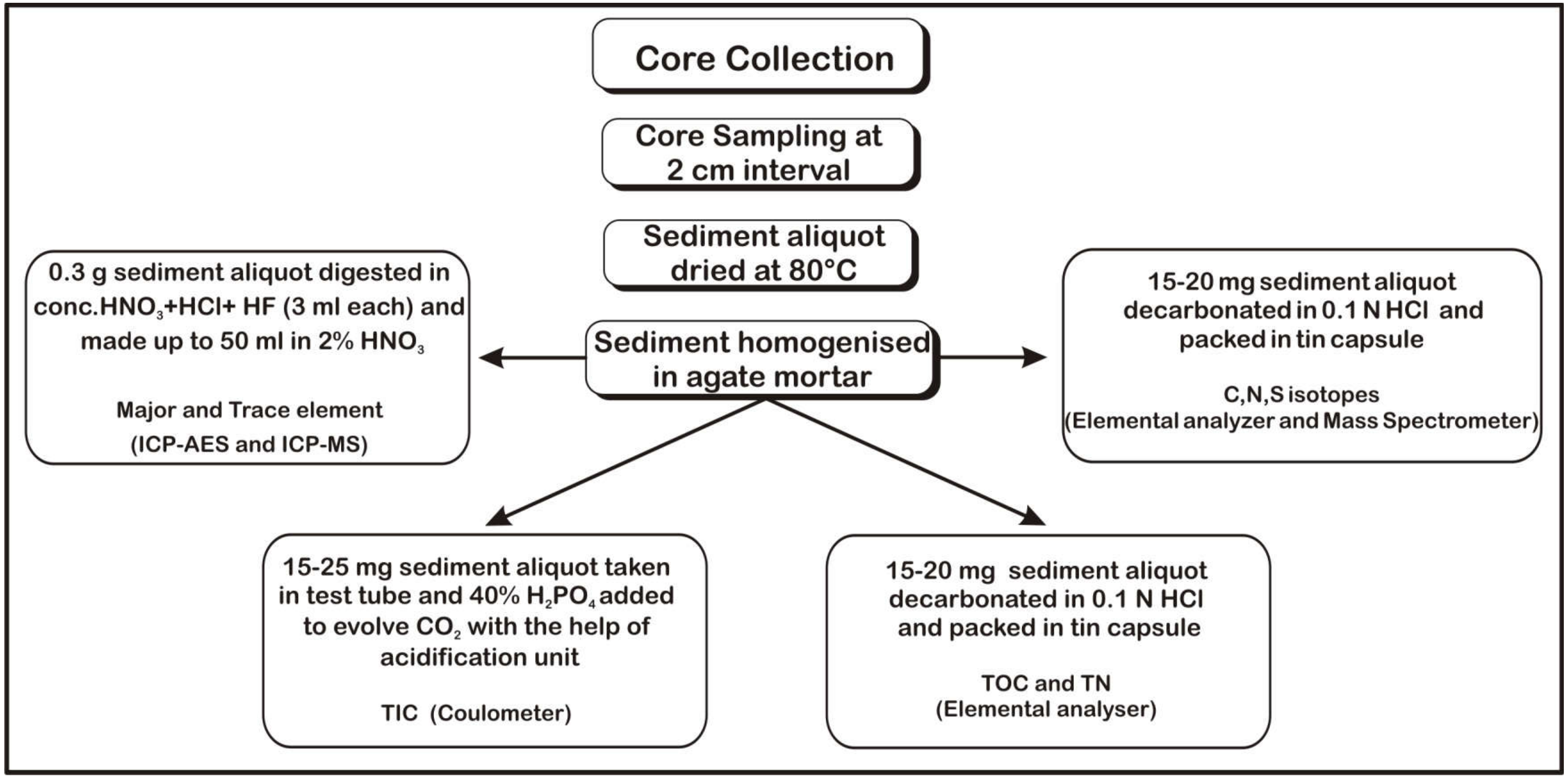


Figure 2-3.Sample processing protocol followed for the measurement of various parameters.