CHAPTER II

MATERIALS AND METHODS

MATERIALS AND METHODS

2.0 Study area

2.0.1 Location and site description

The study was carried out in the shoolpaneshwar wild life sanctuary (SWS) falling in latitude 21°29N-21°52'N and longitude 73°29'E-73°54'E in the hilly ranges of Narmada district of central Gujarat, India (Figure 1). SWS lies on left bank of the Narmada river/Sardar sarovar reservoir (Figure 2). The SWS is an extension of old Dumkhal sloth bear sanctuary (Sabnis and Amin, 1992). The area of SWS on record is 607 sq.kms. There are 68 sq.kms of revenue department lands within the area, bringing up the total area of the sanctuary to 675 sq.kms. The area is characterized by steep hilly terrain with hills ranging in height between 400 and 882 metres. The area is highly important because it forms the catchment area of Karjan and the Sardar sarovar reservoir. Sabnis and Amin (1992) classified the shoolpaneshwar sanctuary into 8 different eco-grade systems according to disturbance and/or degradation level. The first three grades (referred as relatively better ecosystems) occupy 329 sq.kms and the rest (referred as deteriorating ecosystems) account for about 346 sq.kms. The thick vegetative cover of the sanctuary mainly comprised of teak, bamboos and mixed floras (Figure 3), they also provide shelter and habitat to a variety of life forms. The sanctuary was covered with herbaceous vegetation during monsoon season and barren in summer season (Figure 3). Few ethnic tribes are staying in the sanctuary for many decades. They are profoundly dependent on the forest cover / land for sustenance. Parts of the forest land are converted as cultivable land for better living. Forest department does regular plantation as per the working plan. Human interference on the natural landscape is happening for the past 75-100 years.

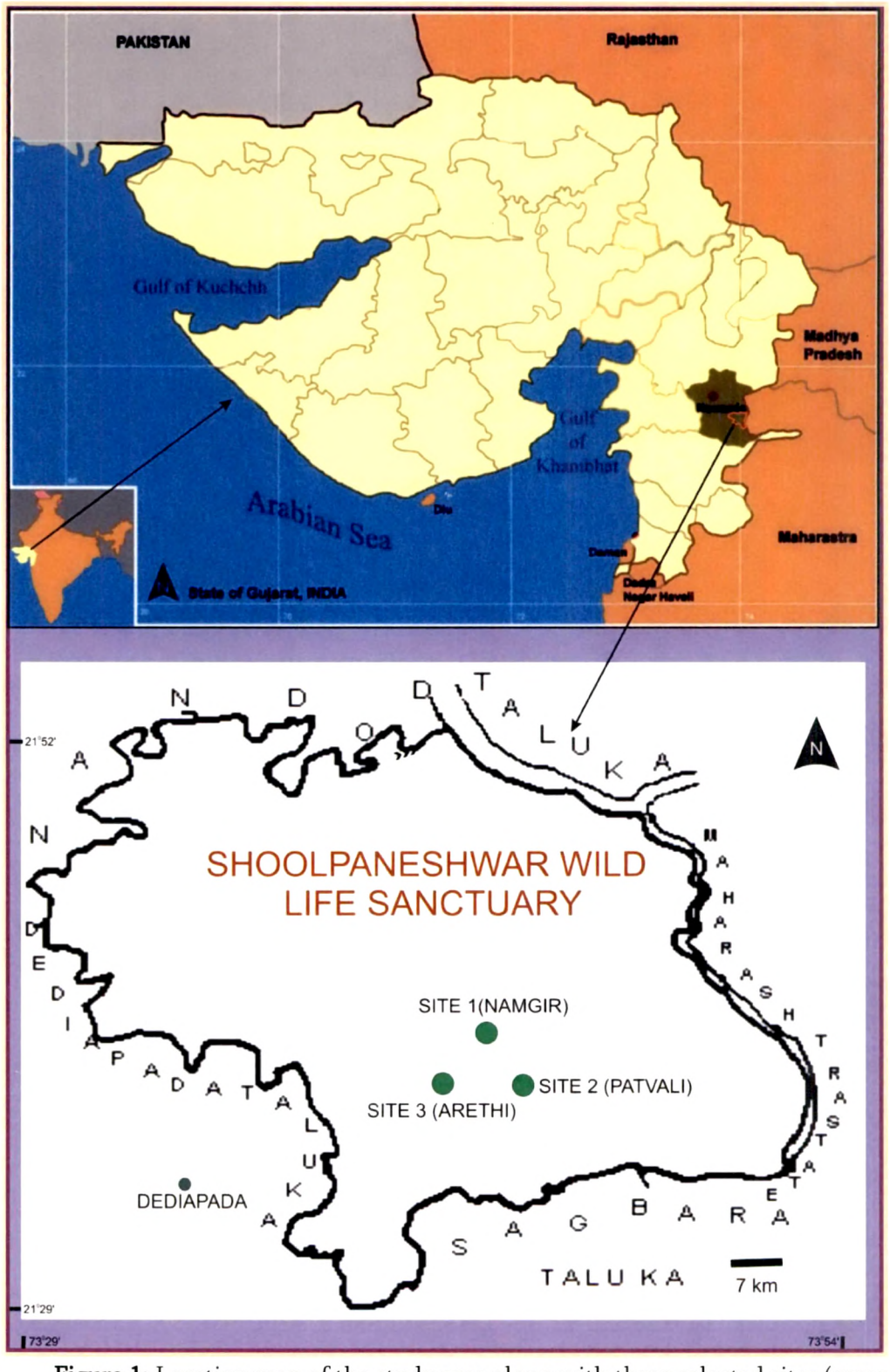


Figure 1: Location map of the study area along with three selected sites (green circles) in the Gujarat shoolpaneshwar sanctuary.

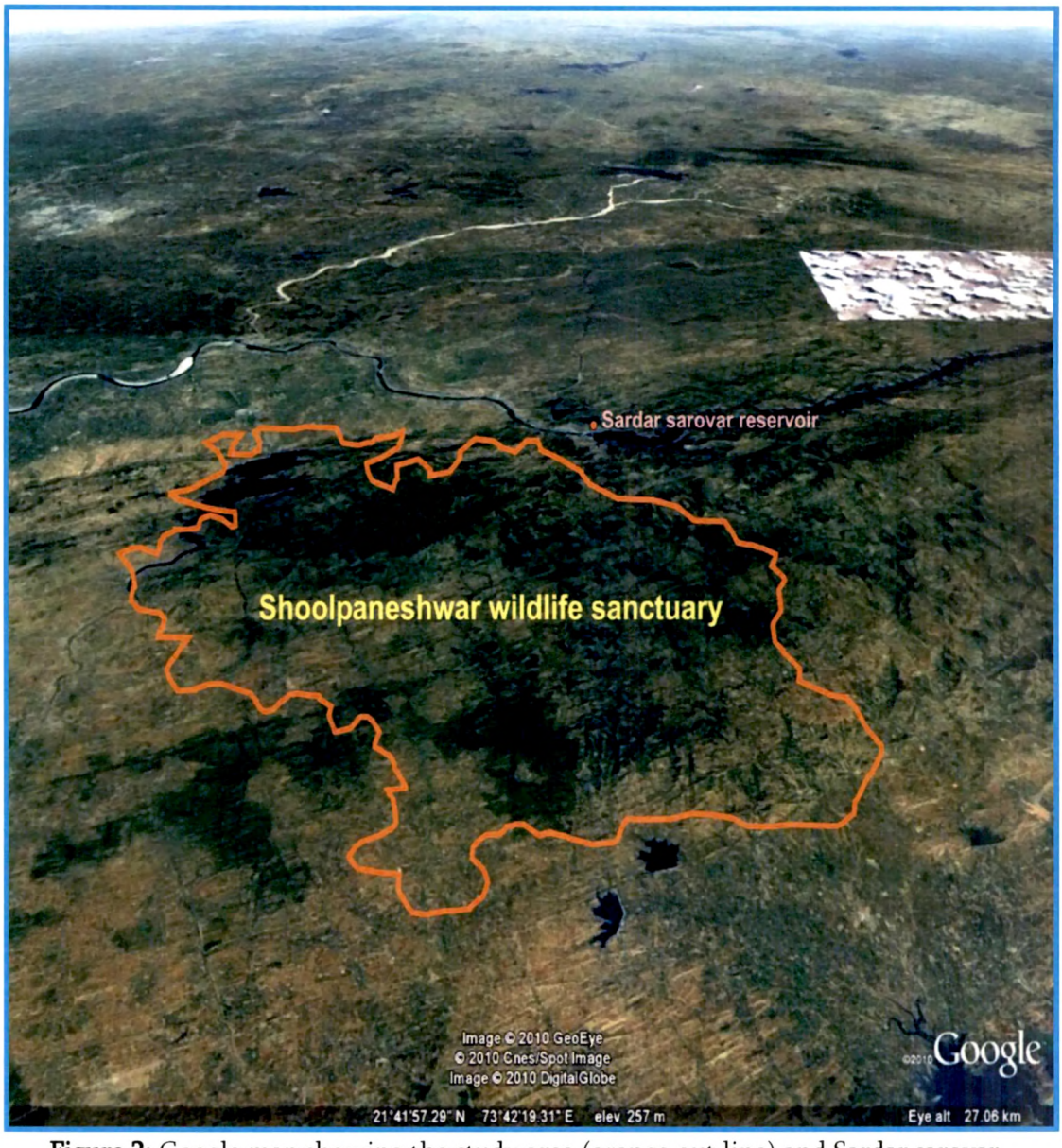


Figure 2: Google map showing the study area (orange out line) and Sardar sarovar reservoir situated in Narmada district.

Figure 3: Photographs showing the teak (a), bamboo (b) mixed cover (c), including herbaceous vegetation, during post monsoon season and trees (d) shed their leaves during summer season at the shoolpaneshwar wildlife sanctuary.

2.1 Geology and soil

The sanctuary falls within the lower narmada valley. Geologically it includes the precambrian basement metamorphics and granites, cretaceous Bagh beds and their fresh water equivalents, Deccan trap and associated intrusive, tertiary sedimentary deposits and Quaternary alluvium (Deota, 1991). The major geological formations met within the study area in sequence of deposition are Bagh limestone, sand stones, the deccan trap lava flows and the alluvial depsoits. The sub-aerial weathering of the basalts has given rise to lateritic rocks in some parts, mostly occuring as a thin cover of lateritic soil (Binal, 2008). The soils in this area vary in colour, texture, depth and stoniness depending upon the base rock and topography. The light grey, yellowish brown and reddish brown colour soils were found in the gentle slopes, hill tops and modulating plains of the study area respectively. Alluvium deposits of clay-loam type are also seen with light brown to grey / black colour (Gujarat state Forest Department, unpublished data). According to Merh, (1995), soils at the study area are classified as Entisols (Figure 4). Entisols have developed over traps, granite, gneiss, quartzite, alluvium. They are light grey, grayish brown and reddish brown in colour, have formed under tropical climate marked by annual precipitation 55 to 950 mm with the mean temperatures of 25^{0}C to 26^{0}C. The Entisols of Gujarat taxonomically represent Ustorthents, Ustipsamments and Ustifluvents (Merh, 1995).

2.2 Climate

The climate of the study area shows three well defined seasons viz., summer (March to June), monsoon (July to October) and winter (November to February). From March onwards the temperature starts rising and reaches a maximum in May (42^{0}C). January is the coldest month of the year with temperature ~8^{0}C. The month of October acts as a transition period between monsoon and winter season. The area is under the south west monsoon regime and receives much of the rainfall between July to October. It receives an annual rainfall in the range of

900 - 1200 mm. Humidity levels are maximum in the monsoon (June-October) and between 15-30% (dry environment) for the rest of the year. Influence of seasonal variations is seen on the vegetal cover.

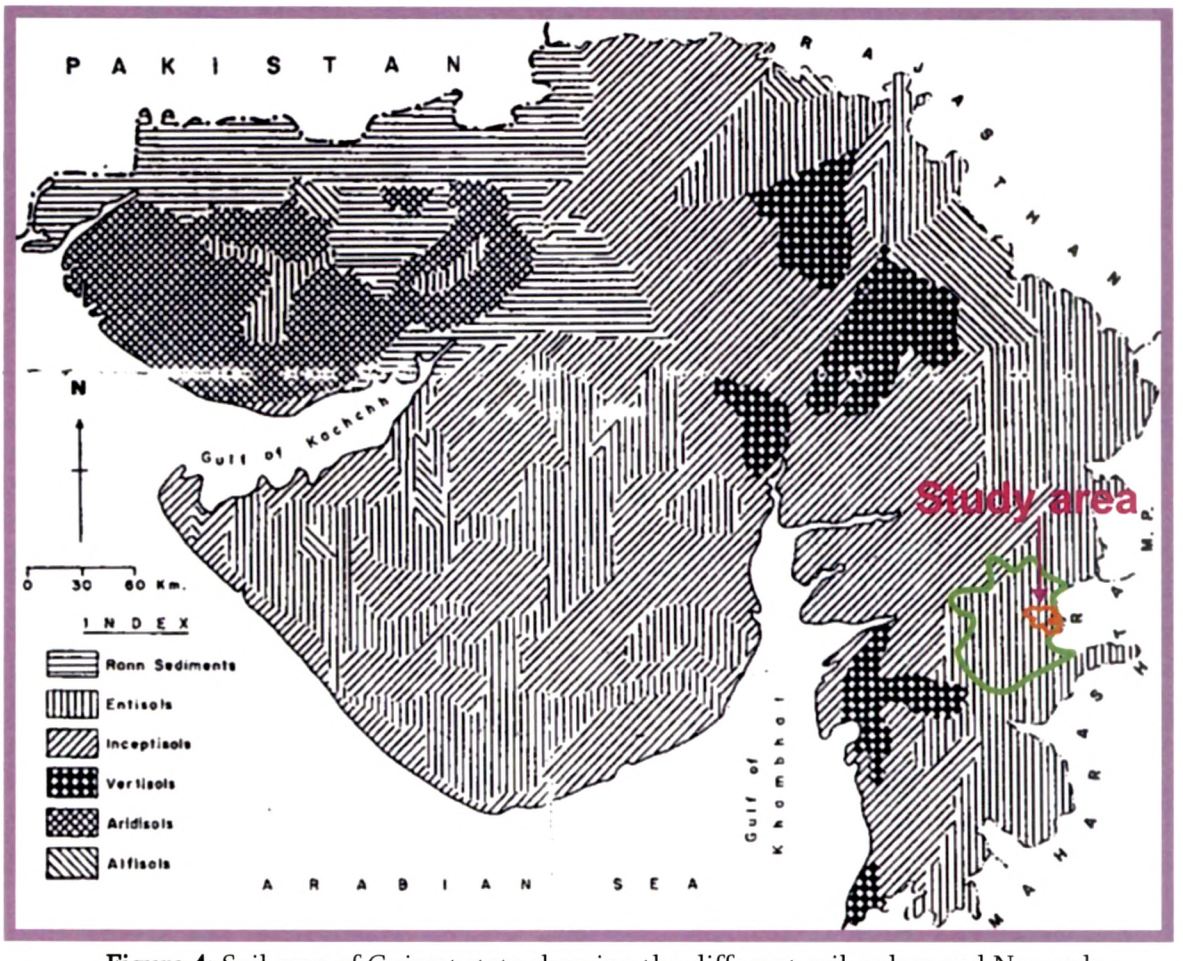


Figure 4: Soil map of Gujarat state showing the different soil orders and Narmada district (green out line) along with the study area (orange outline inside the Narmada district) falls in the order of Entisols (Soil map of Gujarat after Merh, 1995).

2.3 Vegetation

According to the Holdridge life zone system of classification the vegetal cover of the study area falls in tropical dry forest (Figure 5). Vegetal cover is dominated by *Tectona grandis* Linn. (Teak) and *Dendrocalamus strictus* Nees. (Bamboo). Rest of the area is occupied by a variety of trees such as *Mangifera indica* Linn., *Madhuca indica* Linn., *Terminalia bellerica* Roxb., *Pongamia pinnata* (Linn) Pierre., *Wrightia tinctoria* R.Br., *Bauhinia racemosa* Lamk., *Morus alba* Linn., *Anogeissus latifolia* Wall., *Garuga pinnata* Roxb., *Lannea coromandellica* Houff., *Mitragyna Parvifolia* Korth., *Butea monosperma* Lamk., *Aegle marmelos* Corr., *Nyctanthes arbortristis* Linn., Density of vegetation in the sanctuary is ~650 ha^{-1} teak trees, ~350 ha^{-1} bamboo clumps, and ~650 ha^{-1} mixed trees. The average age of the trees is ~60 years (unpublished records). Most of the plants in the study area are deciduous and shed their leaves in summer and reproduce new leaves during the monsoonal months (Figure 3). During monsoon and post monsoon periods (up to October) the ground vegetation become more prominent and the entire area of the sanctuary gives an impression of a semi-evergreen type of forest (Sabnis and Amin, 1992). The dominant ground cover species of the ground cover are *Sida acuta* Burm., *Corchorus fascicularis* Lam., *Abutilon indicum* Sweet., *Vernonia cinerea* Lees, *Alternanthera sessilis* R.Br., *Solanum nigrum* Linn., *Acalypha indica* Linn., *Oldenlandia corymbosa* Linn., *Clitoria ternatea* Linn., *Tephrosia purpurea* Pers., *Haplanthus verticillaris* Nees., *Boerhavia diffusa* Linn., *Antigonon leptopus* Hook. & Arn., *Euphorbia hirta* Linn., *Aeruva javanica* (Burm.f.) Schult., *Tridax procumbens* Linn., *Achyranthes aspera* Linn, *Amarantus spinosus* Linn., *Amarantus viridis* Linn., *Peristrophe bicalyculata* Nees, *Blumea membranacea* DC., *Cassia tora* Linn. Herbaceous covers complete their lifecycle by the end of January / February.

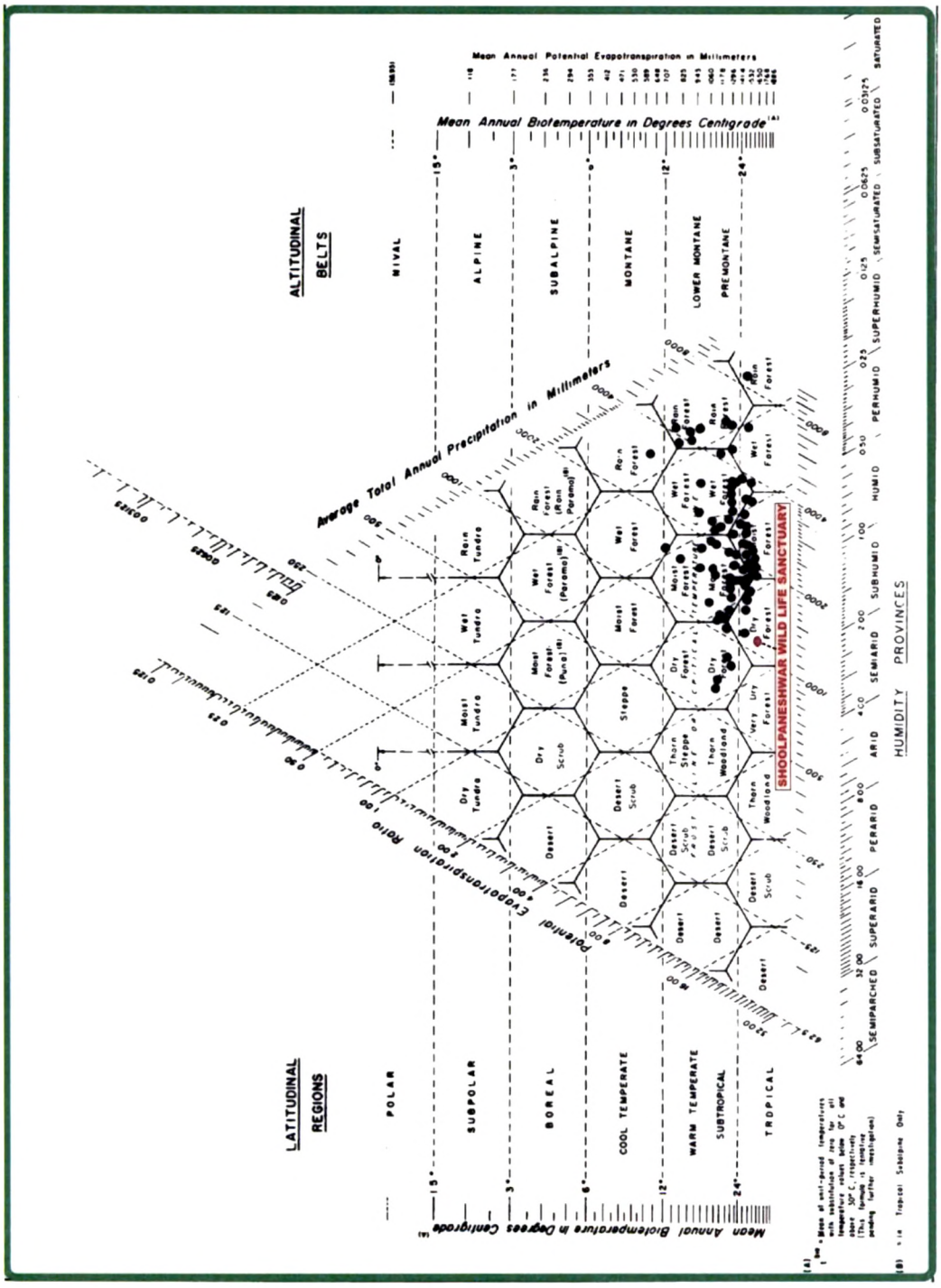
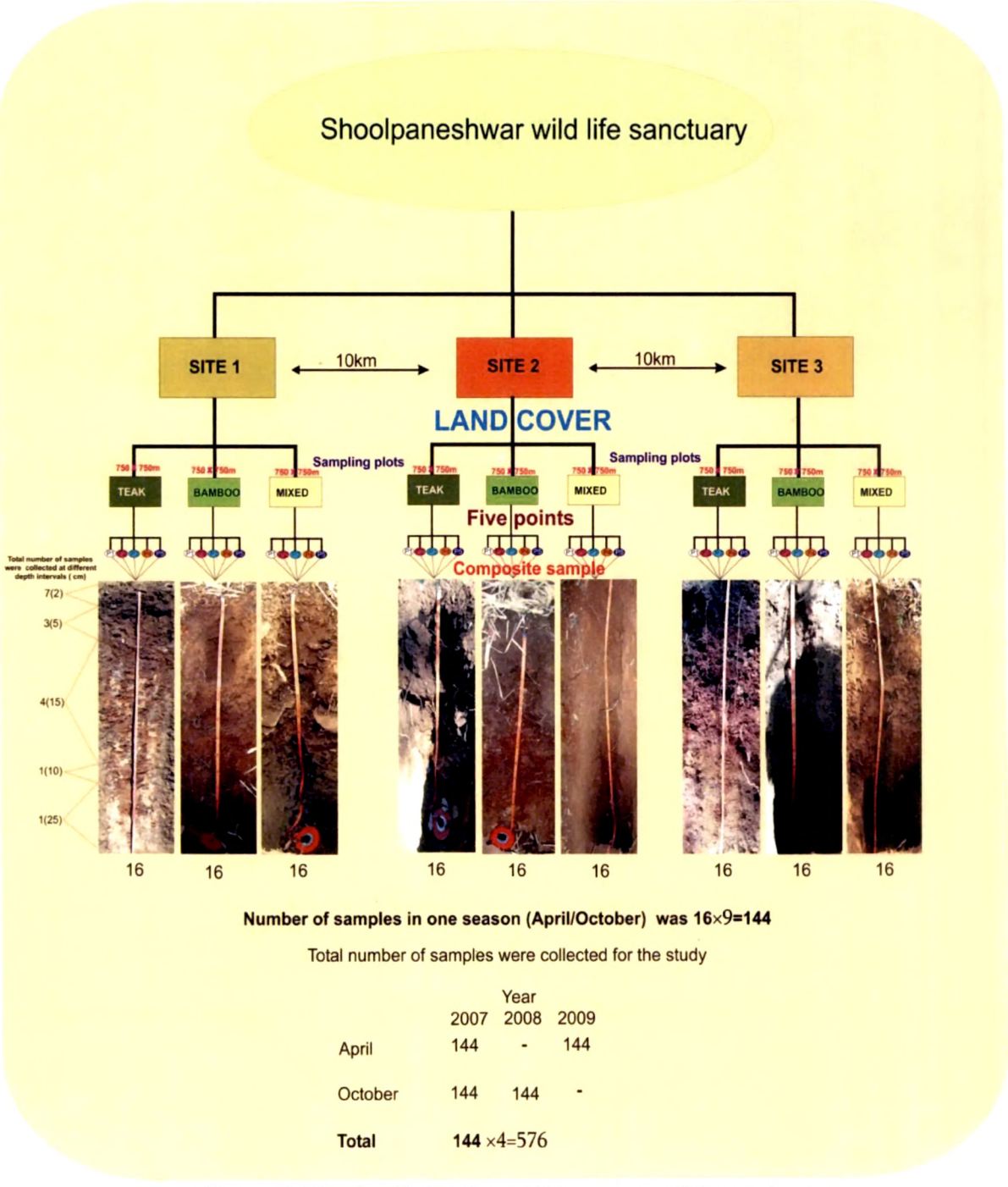


Figure 5: Diagram of the Holdridge life zone system for classifying world plant formations showing the shoolpaneshwar wild life sanctuary in the dry forest zone (after Brown and Lugo, 1982).

2.4 Soil sampling

The detailed soil sampling pattern of the study is illustrated in Figure 6. Soil samples were collected from teak, bamboo and mixed cover site in the sanctuary by trench method (Figure 7). These three types are considered as dominant covers of the sanctuary for the past 75-100 years. The sampling plots were of 750 m × 750 m size and were picked up for each land cover at each of the selected sites. The distance between two adjacent sites was about 10 km. The selected plots did show homogeneity for the kind of vegetal cover they were chosen. At each selected site five points were dug out to collect soil samples up to 125 cm depth with different increments. Based on our preliminary survey, 16 soil samples were collected sequentially up to a depth of 125 cm in the following manner. Seven samples at 2 cm intervals up to a depth of 14 cm, three samples beyond 14 cm and up to 30 cm at 5 cm intervals, four samples beyond 30 cm and up to 90 cm at 15 cm intervals, one sample beyond 90 cm but below 100 cm and one sample beyond 100 cm but below 125 cm with 25 cm interval. Soil samples coming from all the layers of five different points were pooled and labeled as a composite sample. Totally three composite samples for each land cover were collected. Sampling was done during the years 2007-2009. Taking the seasonality into consideration, sampling was done in the months of April (summer) and October (post monsoon) (Figures 8-10). Sampling was done four times (two coming from summer and the other two from post monsoon). A total of 576 soil samples were collected from three types of land cover with different depth intervals for the study (Figure 6). The collected soil samples were brought to the laboratory in sealed bags, air dried and stored in a cool, dry room.



Number of samples in one season (April/October) was 16×9=144

Total number of samples were collected for the study

	Year		
	2007	2008	2009
April	144	-	144
October	144	144	-
Total	**144** ×4=576		

Figure 6: The detailed soil sampling pattern of the study area.

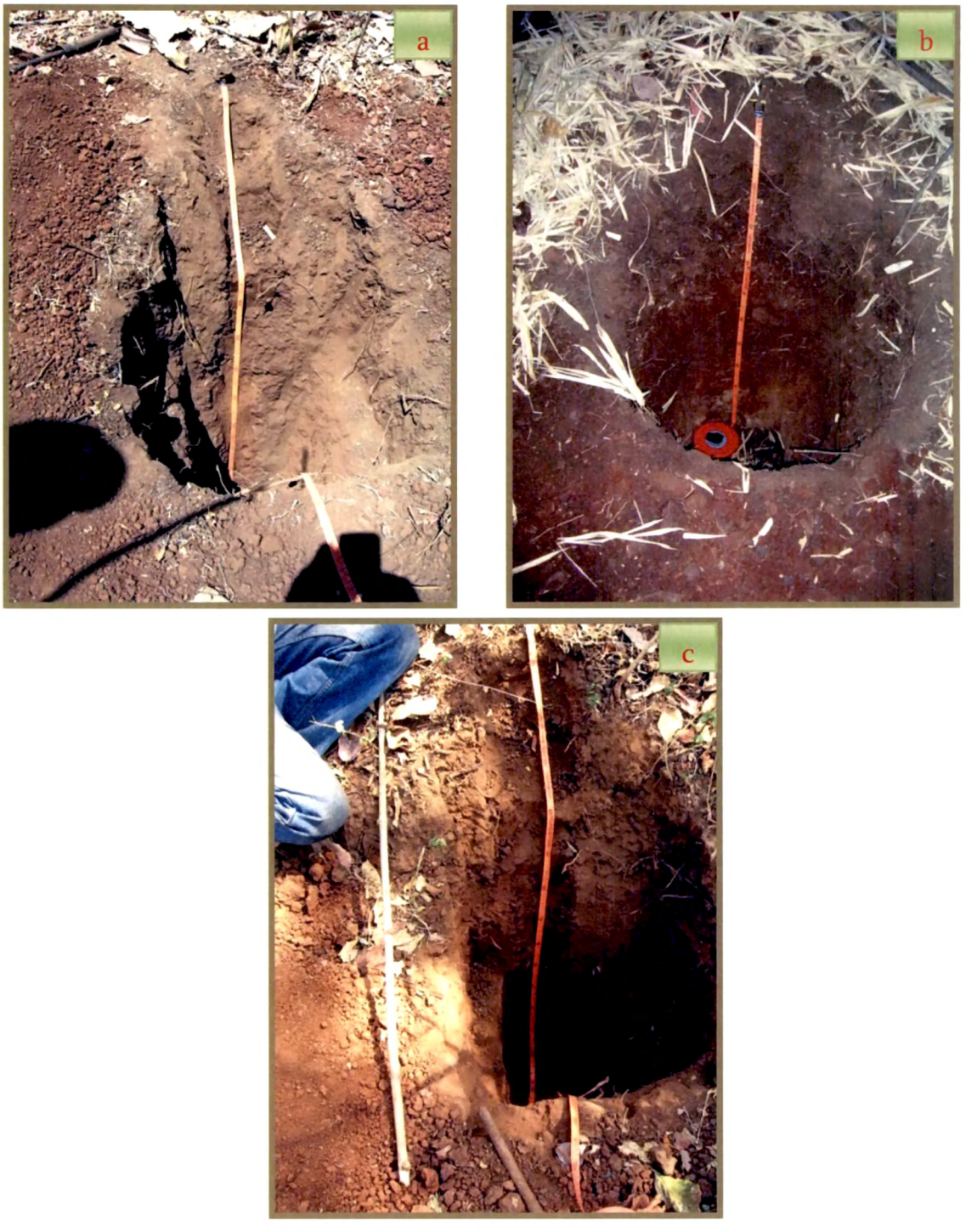


Figure 7: photographs showing the trench made in the teak (a), bamboo (b) and mixed cover (c) sites.

Figure 8: Teak cover during summer (April) and post monsoon season (October) at the study area.

Figure 9: Bamboo cover during summer (April) and post monsoon season (October) at the study area.

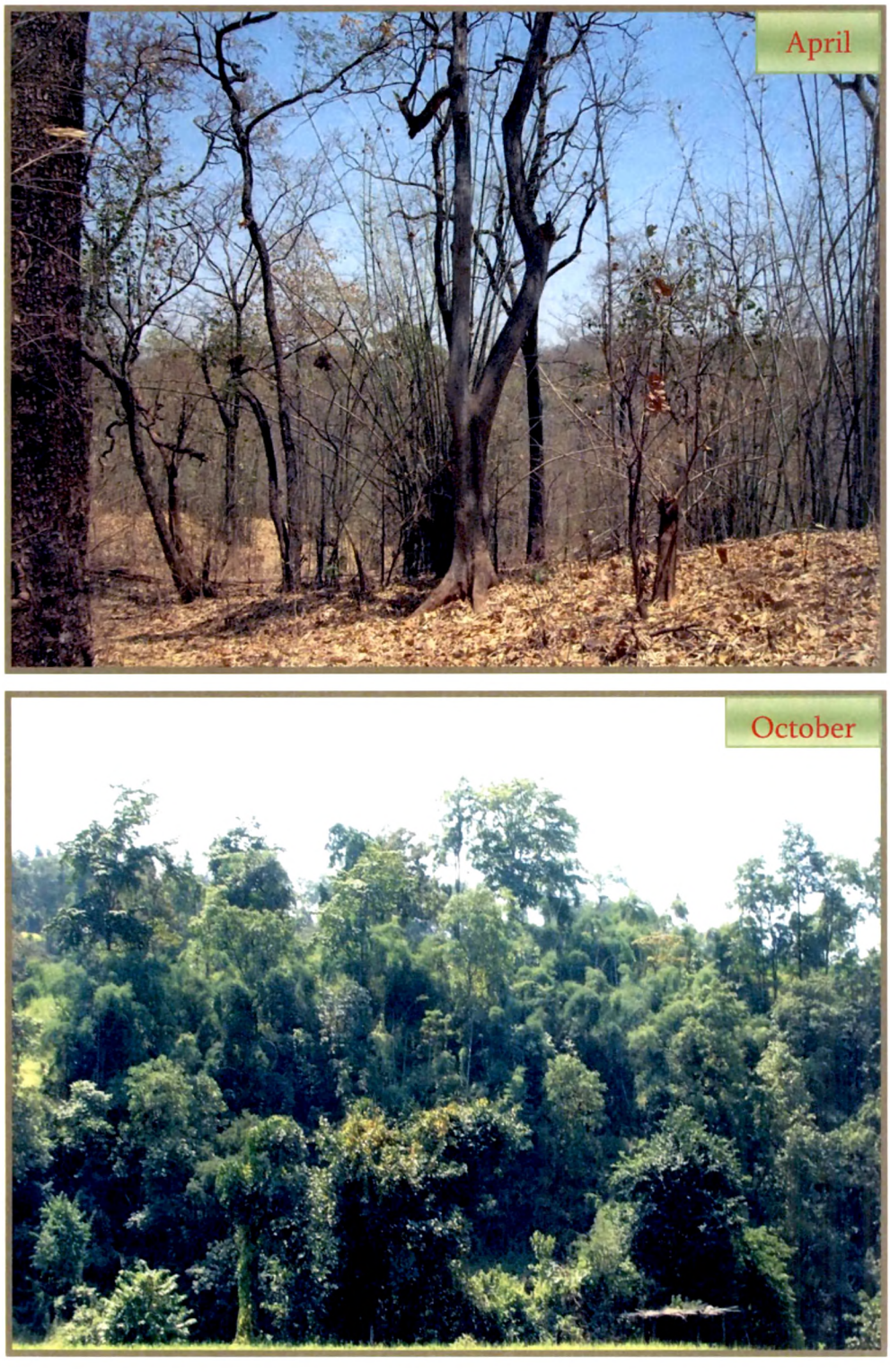


Figure 10: Mixed cover during summer (April) and post monsoon season (October) at the study area.

2.5 Soil analyses

The collected soil samples from three types of vegetal / land cover were analyzed for different the designated physical, chemical and biological parameters to understand the carbon sequestration of three types of land cover. The following soil parameters were analyzed;

Soil physical parameters

1. Soil pH
2. Particle size distribution
3. Soil bulk density

Soil chemical parameters

1. Soil organic carbon (SOC)
2. Hydrofluoric acid (HF) soluble carbon
3. Proton (^{1}H) Nuclear magnetic resonance (NMR) analysis
4. Carbon isotopes analysis (^{13}C and ^{14}C) analysis

Soil biological parameters

1. Litter decomposition
2. Soil respiration
3. Microbial biomass carbon (MBC)

2.6 Soil physical parameters

2.6.1 Soil pH

Soil pH was measured in the collected composite soil samples, at different depths, from teak, bamboo and mixed land cover sites. Soil pH was measured at a soil: water ratio of 1:5 (weight / volume). Air-dried soil (10 g) and 50 ml of distilled water were shaken together for 1 minute, left to settle for 5 minutes, was measured by using a pH meter.

2.6.2 Particle size distribution

Particle size separation of the soil samples was done by pipette method (Kilmer and Alexander, 1949). Particle size analysis was done in the following collected composite soil samples at different depths of teak, bamboo and mixed cover sites. They are 0-2, 4-6, 8-10, 12-14, 20-25, 45-60, 60-75, 75-90, 90-100 and 100-125 cm. Briefly, the air dried samples were first screened to remove all the plant materials and roots. Then the soil sample was passed through a 2 mm sieve, weighed and transferred into 250 ml beaker. About 50 ml of distilled water and 5 ml of 30% H_2O_2 were added into the beaker for removing the OM. Samples were stirred continuously. This step was repeated up to the cessation of frothing. Beakers were kept on a hot plate at 70^{0}C for 30-45 minutes and ensured that OM was completely removed from the sample by adding extra H_2O_2 if necessary. The supernatant was decanted carefully. After that, 100 ml of distilled water along with 10 ml of calgon solution (Sodium hexameta phosphate (35.7 g) and sodium carbonate (7.94 g) dissolved in 1000 ml of distilled water) was added to the samples and kept for over night. The samples were filtered through 53 μm sieve and the leftovers were washed with water. The particles on the 53 μm sieve were transferred into petri plates covered with aluminium foil and it was oven dried at 70^{0}C overnight and weighed. The filtrate (particles passed through the 53 μm sieve) was transferred into a 1000 ml measuring cylinder and filled up with distilled water to the 1 litre

mark. The suspension was stirred vigorously for about half a minute, so that the particles were evenly distributed throughout the cylinder. The 2 μm particles (clay) are pipetted at a 10 cm depth after a predetermined settling time (usually six to six and half hours). Pipetted sample was transferred into a pre-weighed aluminium foil covered pertiplate. The petriplate was oven dried at 70°C overnight and weighed. The sand, silt and clay was calculated by the following equations

Calculations

Sand

Sand (%) = weight in grams of fractions on 53 μm sieve / Total dry weight of *sample × 100*

Silt

Silt (%) is obtained by subtracting the sum of the percentage of sand and clay from 100.

Clay

Clay (%) = A-B / 10 × 100

Where, A= weight in grams of pipetted fraction, B= weight correct for dispersing agent (in grams), 10 is the initial weight of sample.

2.6.3 Soil bulk density

Soil bulk density of the collected composite samples was determined by using the soil calculator developed by Metherell et al. (1993). The equations used in this calculator to compute the values are modified equations obtained from the "Soil texture triangle hydraulic properties calculator" available online at http://hydrolab.arsusda.gov/soilwater/Index.html. Figure 11 shows the typical soil calculator used in CENTURY version 4.0 to calculate the soil physical parameters (including soil bulk density). The present study determined soil bulk density of

the collected composite samples at different depths (0-2, 4-6, 8-10, 12-14, 20-25, 45-60, 60-75, 75-90, 90-100 and 100-125 cm) under three land cover types. For this, sand, silt and clay proportions of the selected soil samples were fed in the soil calculator to obtain soil bulk density. The data are expressed as gram per cubic centimeter (g cm^{-3}).

Figure 11: Soil calculator developed by Metherell et al. (1993) for CENTURY version 4.0 to calculate soil physical parameters.

2.7 Soil chemical parameters

2.7.1 SOC analysis

The Walkley-Black's (WB) method (1934) has long been a standard method for measuring OC in forest soil samples. Prior to wet oxidation the air dried soil samples were passed through 2 mm sieve to remove any plant materials and grounded into fine powder with the help of mortar and pestle. A known amount (0.5 g) of soil was taken in a 250 ml conical flask and it was treated with 10 ml of 0.167M potassium dichromate ($K_2Cr_2O_7$) and 20 ml of concentrated sulphuric acid (H_2SO_4). The mixture was swirled gently and allowed to stand for 30 minutes in a dark place. Subsequently 100 ml of distilled water was added into the mixture. Afterwards, 10 ml of orthophosphoric acid (85%, H_3PO_4), and 0.2 g of sodium fluoride (NaF) was added into the mixture. Then few drops of ferroin indicator (1,10 phenanthroline (3.71g) and ferrous sulphate (1.74g, $FeSO_4 \cdot 7H_2O$) dissolved in 250 ml of distilled water) were added into the mixture. Finally the mixture was titrated against 0.5 M ferrous ammonium sulphate (FAS) ($Fe(NH_4)_2(SO_4)6H_2O$) solution. During each titration, the colour of the solution was yellow-orange to light green at first and changed to turbid grey and finally to a burgundy colour at the end point. The percentage of easily oxidizable organic carbon was calculated as follows:

$$\text{Easily oxidizable organic carbon (\%)} = \frac{(B\text{-}S) \times M \text{ of FAS} \times 12 \times 100}{\text{Gram of soil} \times 4000}$$

Where B is ml of ferrous ammonium sulphate solution used to titrate the blank, S is ml of ferrous ammonium sulphate solution used to titrate sample, M is the molar of ferrous ammonium sulphate solution used and 12/4000 is miliequivalent weight of carbon in grams.

A correction factor (1.32) has been applied to correct the incomplete oxidation of the sample (Lettens et al., 2007).

SOC content of each depth (g C m^{-2}) was calculated as (Schlesinger, 1990)

$$\text{SOC content (g C m}^{-2}) = \sum_{i=1}^{k} OC_i \times BD_i \times D_i \times 10{,}000$$

Where OC_i is the organic carbon content (%) in layer i, BD_i is the bulk density (g cm^{-3}) of layer i, D_i is the thickness (m) of the layer i and 10,000 is the factor used to convert the SOC value from 1 cm^2 to 1 m^2 (100 × 100 cm^2 =1 m^2). Thus SOC carbon content finally converted into tonnes per hectare for each depth by again multiplying with 10,000 (100 × 100 m^2 =1 hectare).

2.7.2 Physical fractionation

Physical fractionation was done by the method developed by Six et al. (2002) to fractionate the soil samples. Soil samples collected at different depths (0-2, 2-4, 4-6, 6-8, 8-10, 10-12, 12-14, 20-25, 30-45, 60-75, 75-90, 90-100 and 100-125 cm) under teak, bamboo and mixed cover sites were physically fractionated into four different size fractions (pools) by wet sieving (Figure 12). Briefly, a quantity (100 g) of air dried soil samples were submerged in distilled water for 30 minutes and the floating materials were removed from the water by decanting technique. The wet sieving was done manually by flushing the distilled water and moving the sieves up and down for 2-5 minutes. Gravels, pebbles and organic materials on 2000μm sieve were removed after the 2-5 minutes cycle. Then the soil samples were physically fractionated into four different pools >500-2000 μm, >250-500 μm, >53-250 μm and <53 μm by passed through four different size of sieves (Figure 12). The fractions that remained on the sieves were collected in an aluminium foil covered petri plates and oven dried at 70°C overnight. SOC was estimated for all the fractions.

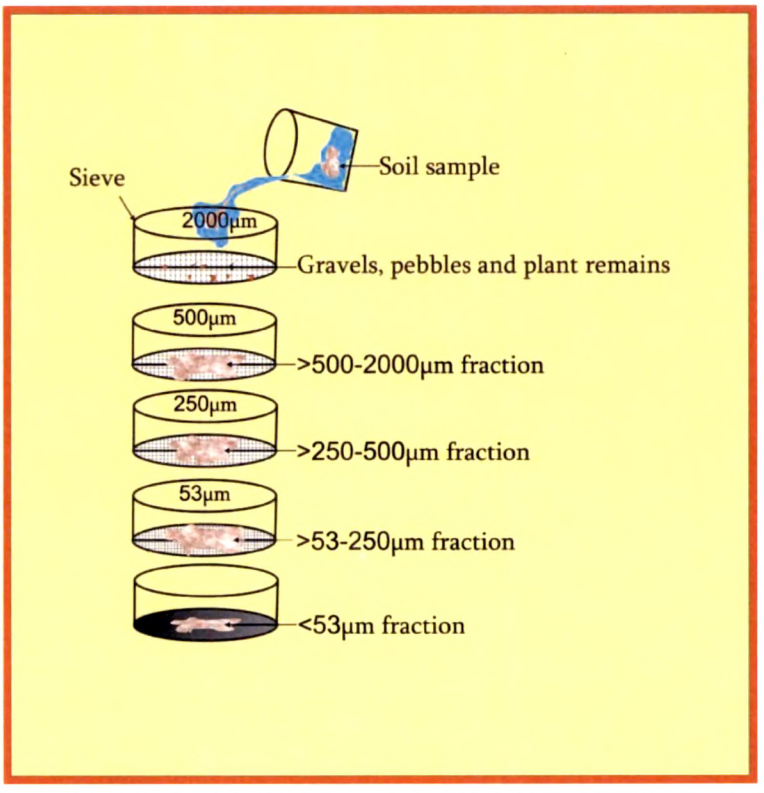


Figure 12: Schematic illustration of physical fractionation method.

Fractionated soil samples (>500-2000 µm, >250-500 µm) were referred together as mobile pool and samples of >53-250 µm and <53 µm were designated as recalcitrant pool (Figure. 7). These designations were made on the basis of published reports (Six et al., 2002; Chevallier et al., 2004; Arrouays et al., 2006; Shrestha et al., 2007).

2.7.3 Proton NMR analysis of SOC

Air dried soil samples at two different depths of 0-2 and 12-14 cm of teak and bamboo were screened for any remnants of root / leaf material by passing through a 2 mm sieve. Physical fractionation pools such as mobile (>250-2000 µm) and recalcitrant (<250 µm) were also used for analysis. Known amounts (40-60 g) of soil were weighed and SOC was extracted by magnetically stirring the soil samples continuously for one hour in a mixture of methanol and chloroform (50:50). The

samples were allowed to settle and subsequently filtered through Whatman filter paper. Methanol: chloroform extract was evaporated to dryness under liquid nitrogen. Proton NMR analysis was carried out at Department of chemistry, S.P. University, V.V. Nagar, Gujarat. INDIA. 3-5 mg of this dried sample was solubilized in deuterated solvents (chloroform-d and Methanol-d_4). Solubilized samples were subjected to NMR analysis by using a Bruker 400 MHz Proton (^1H) NMR Spectrophotometer (Bruker instruments Inc., Karlsruhe, Germany). Spectra were classified into the chemical shift regions: 0.5-2 ppm Alkyl group; 3-5 ppm O-alkyl; 7-8 ppm Aromatic; and 10-12 Carboxyl group (Silverstein and Webster, 2002).

2.7.4 Carbon isotopes analysis

Carbon has two stable isotopes (^{13}C and ^{12}C) and a radioactive isotope (^{14}C) that are useful tracers in the study of the soil carbon cycle.

2.7.4.1 Stable carbon isotopes ($\delta^{13}C$) analysis

Stable carbon isotopes ($^{13}C/^{12}C$) were determined in litter and soil samples (at 0-2 cm, 8-10 cm, 20-25 cm, 45-60 cm, 75-90 cm and 90-100 cm) of teak, bamboo and mixed cover. Litter was grounded into fine powdered before injection into mass spectrometer. The soil samples were first treated with 10% HCL for several hours to remove carbonates, washed with distilled water several times to get neutral pH and dried in oven at 70°C for 24 hours. Stable carbon isotope analysis was carried out at Physical Research Laboratory (PRL), Ahmedabad, Gujarat. INDIA. CuO powder and silver foil were added to the dried and powdered samples and then they were combusted at 900°C to produce CO_2. The CO_2 produced was cryogenically purified in several steps and then injected into mass spectrometer (GEO-20-20). The carbon isotope ratios are calculated in δ-notation on a per mill basis (parts per thousand, ‰), which is defined as the $^{13}C/^{12}C$ ratio of the sample relative to the international Vienna Pee Dee Belemnite (V-PDB) standard. $\delta^{13}C$ of the samples were calculated by the following equation.

$$\delta^{13}C\ (‰) = [(^{13}C/^{12}C)_{sample}/ (^{13}C/^{12}C)_{standard} - 1] \times 1000$$

The external precision of the instrument is 0.1% for $\delta^{13}C$.

2.7.4.2 Radiocarbon age analysis

The soil samples collected at three different depths (0-2, 45-60 and 90-100cm) under teak cover were used to calculate the age of SOC. ^{14}C analysis was carried out at PRL, Ahmedabad, Gujarat. INDIA. The radiocarbon dates of soil organic carbon are obtained by combusting a known quantity (~500g) of bulk samples. All the samples were sieved with a 2 mm mesh, de-carbonated with 10% HCl, neutralized with distilled water, dried and then combusted at 900^{0}C in a quartz tube under pure oxygen flow at a pressure of about 2-3 cm of Hg column. CO_2 produced was purified in several steps and synthesized to benzene, radioactivity of which was counted in a liquid scintillation counter (Quantulus 1220). Fractionation correction ($\delta^{13}C$ w. r. t. PDB) has been applied to all samples taking an aliquot of CO_2 and measuring in a mass spectrometer (GEO 20-20). Ages were calculated using ^{14}C half life of 5730 years and rounded to the nearest 10 following Yadava and Ramesh (1999). An error of 1 sigma is also reported with all radiocarbon ages. Results are expressed in present modern carbon (pMC, 100 pMC =1950AD) and calendar ages are also calculated using online radiocarbon calibration software Calib 6.0 (http://calib.qub.ac.uk/calib/calib.html) (Reimer et al., 2009; Stuiver and Reimer, 1993).pMC : percent modern carbon defined by pMC = $(A/A_o)\times100$, where A and A_o are present and initial activity of the sample Radiocarbon age in years BP is given by the relation

$$t = 8268 \ln\left(\frac{A_0}{A}\right)$$

All the errors are at 1 sigma level. Mean residence time (MRT) of SOC was calculated using calendar ages with standard (NBS Oxalic acid II) activity corrected for decay from 1950 to the year of sample collection.

2.7.5 Estimation of Hydrofluoric acid (HF) soluble carbon

In this analysis SOC from bulk soil samples was extracted from three types of land cover at six different depths by using hydrofluoric acid method (Eusterhues et al., 2003). The soil samples used for $\delta^{13}C$, radiocarbon age analysis and litter bag experiment study of three types of land cover were subjected to HF analysis. The HF treatment was performed by adding 20 ml of 10% HF to 2 g of ground bulk sample. The suspension was shaken for 2 hour at room temperature in a mechanical shaker. After that, it was centrifuged and supernatant removed. This procedure was repeated five times and then the sediment was washed five times with distilled water to remove salts and residual HF. Afterwards, the samples were air dried and weighted. The concentration of OC was determined by wet oxidation method (see SOC analysis). The amount of HF-soluble OC in the sample was obtained by subtracting the amount of HF-resistant soil OC from total organic carbon (not treated with HF). The difference between total organic carbon content and HF-resistant OC is called HF-soluble OC.

2.8 Soil biological parameters

2.8.1 Litter collection

Litter (includes leaves, twigs and flowers) was collected from teak, bamboo and mixed cover sites at quarterly intervals for one year (Figures 13-14). At each time of sampling 1 m^2 quadrats were randomly laid on the forest floor. The litter that fell in those areas were picked up and transferred into plastic bags. The collected litter was oven-dried and their dry weights were measured. At each site five to eight quadrats were laid. Extreme values were discarded while pooling the data. It was ensured that there was no repetition of areas. About 85-90% of the litter consisted of leaves. The rest consisted of dried twigs. The measurement for each vegetal cover is the average of all these measurements.

Figure 13: Litter layer of teak (a) and bamboo (b) cover plots during summer season (June).

Figure 14: Litter layer of mixed cover plot during summer season (June) in the study area.

2.8.2 Litter decomposition experiment

Litter decomposition was monitored by litter bag experiment. Litter bags having perforations of different sizes (recommended sizes) on two sides were used. Litter bags consisted of a 0.5 mm mesh at the bottom and 1 mm mesh at the top. Three bags were kept for each type of leaf litter at each depth. A total of 135 litter bags were used for this experiment. Each litter bag was filled with 50 g of air dried litter (only leaves) collected from the floors of teak, bamboo and mixed vegetation. These bags were placed at 2-5 cm, 22-25 cm, and 47-50 cm depth with minimum possible disturbance. To understand the microorganism specificity towards litter, samples of bamboo and teak were interchanged between the two vegetal covers and kept at same depths at different points. Litter bags were exhumed carefully at the three intervals of 90, 220 and 320 days and the samples were carefully transported to the laboratory. Litter left in the bags was carefully picked up taking due care to see that it was not mixed up with soil and weighed after air drying.

Soil samples also were taken every time from the vicinity of litter bags to observe variations in SOC content. SOC was estimated in all these samples. Litter mass loss rate (%) were calculated by following formula.

Litter mass loss (%) = $LM_i - LM_r / M_i \times 100$

Where, LM_i is initial litter mass (weight in grams) and LM_r is remaining litter mass (weight in grams).

2.8.3 Soil Respiration

The present study measured soil respiration of three types of land cover in the field using an alkali absorption method (Anderson, 1982) in three different seasons (summer, monsoon and winter). All the plant parts were cleared before the measurement. Five cylindrical plastic chambers (of 18 cm diameter and 20 cm height) were randomly placed in teak, bamboo and mixed vegetal cover plots. Each plastic container was inserted to a depth of 3 cm into the soil surface. The set up was shielded from direct sunlight. CO_2 efflux was collected in small plastic beakers with 20 ml 1M NaOH over a 24 hour period. The amount of CO_2 absorbed was estimated by titrating with 1M HCl using phenolphthalein as an indicator. It was done for the five samples taken at each site in each season.

Calculation

The CO_2 evolution rate was calculated by the following formula.

C or CO_2 (mg) = (B-V) NE

Where, B is the HCL (ml) needed to titrate the NaOH solution from the control, V is the HCl (ml) needed to titrate the NaOH solution in the plastic beakers exposed to the covered soil surface, N= 1.0 (HCl normality) and E is the equivalent weight (22 for CO_2 and 6 for C). The data are expressed as milligrams of CO_2 per square meter per day (mg CO_2 m^{-2} day^{-1}).

2.8.4 Estimation of microbial biomass carbon

Microbial biomass carbon (MBC) content in the soils of three types of land cover was measured. It was also estimated in soil samples coming from litter decomposition experiment. MBC was estimated by chloroform fumigation extraction method (Witt et al., 2000). Briefly, 20 g of dried soil samples were taken in 250 ml Schott bottles. Nearly 10ml of distilled water was added for moistening and triggering microbial activity. Control samples were extracted immediately with 0.5 M K_2SO_4. Samples were placed on a shaker for an hour. The solution was filtered and carbon content of the filtrate was estimated by wet oxidation method. In another bottle 3 ml of ethanol free chloroform was added and sealed. After incubating for 24 hours in darkness, the bottles were opened to evaporate chloroform. 0.5 M K_2SO_4 was added as mentioned earlier and carbon content was estimated. MBC was calculated as the difference in organic carbon content between fumigated (C_f) and unfumigated soils (C_{uf}).

$$MBC\ (g\ kg^{-1}) = C_f - C_{uf}$$

2.9 Statistical analysis

Descriptive statistics (means and standard error of the mean) were calculated for soil physical, chemical and biological parameters of soil samples collected from three types of vegetal cover. Throughout the thesis, error bars and error of mean values (±X) indicate the standard error of the mean. Analysis of variance (two way-ANOVA) was performed to test for significance ($P<0.01$ and $P<0.05$ level) differences in SOC (at different depths and three types of land cover), physically fractionated SOC pools (between pools, at different depths and three types of land cover), litter fall (between months and three types of land cover), soil respiration (between seasons and three types of land cover), HF soluble carbon (at different depths and three types of land cover) and SMBC (at different depths and three types of land cover). All the statistical analyses were done using SPSS 15.0 software for windows (version 2007). The relationship between log transformed

SOC at different depths and $\delta^{13}C$ of SOC was examined by single linear regression analysis for three types of land cover. The linear regression analysis was used to relate $\delta^{13}C$ of SOC at different depths to abiotic variables such as soil pH, sand, silt and clay. In addition, the linear regression analysis was used the beta (β) values to abiotic variables to understand the effects of these variables on changes in $\delta^{13}C$ values in soils. The relationship between HF-soluble carbon and MRT was examined by linear regression analysis for teak land cover. Also the relationship between $\delta^{13}C$ of SOC and HF-soluble was examined by linear regression analysis for three types of land cover.