CHAPTER- 3

MATERIAL AND METHODS

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➢ Measurement of AGB, BGB and litter fall
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➢ Determination of physicochemical parameters of soil
➢ Estimation of community exchange of CO₂ fluxes in the compartments
➢ Rate of litter fall
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➢ Determination of thermal properties of wood by TGA-DSC
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3.1 Sampling of air

Air samples were collected from 10m and 20m at station 1, 2 and station 4 and from 10 and 20 m with the help of a portable air sampler (AS2- Technovarian) at a rate of 21 min\(^{-1}\) (for CO\(_2\)) and drawn into a pre-evacuated glass-sampling bulb of 150 ml capacity fitted with rubber septum. Soon after collection glass sampling bulbs were sealed properly with parafilm and kept in a cool and dry place and transported to the laboratory for the determination of CO\(_2\) by GC (Varian 3800). For diurnal variation, air samples were also collected at every 3 hours interval covering 24 hrs.

Micrometeorological parameters like air temperature, atmospheric moisture content, atmospheric pressure and wind velocity at these stations were recorded simultaneously by using probes and an anemometer connected with computerized weather station (Model No. Davis 7440). Climate sensors were scanned in every 5min and 1hr average were recorded. Micrometeorological data were procured from local meteorological office (Alipore, Kolkata) and incoming radiation, height of mixed layer depth were obtained from NOAA, ARL data–base (http://www.arl.noaa.gov/ready.html).

3.2 Measurement of atmospheric CO\(_2\)

Gas chromatographic method:

1ml of air sample or standard gas was injected into the injection port of the gas chromatography (Varian CP 3800) with the help of gastight syringe. Carbon dioxide was converted to methane after passing through Methanizer (Nickel catalyst system, Model No. MTN-1) maintained at 350°C. Temperature of chrompack capillary column (12.5 m, 0.53 mm) and FID were maintained at 50 and 150°C, respectively. Followed by its
determination with FID standard carbon dioxide (320 ppm), procured from EDT Instruments Ltd (109 Swan Street, Leicestershire, UK) was used for calibration. Finally mixing ratio of CO₂ in the air sample was determined by comparison of peak areas for samples and standard. The relative uncertainty on CO₂ measurement was found to be ± 0.063.

3.3 Measurement of AGB, BGB and litterfall

Out of the several approaches (Whittaker and Marks, 1975) both allometric technique for above ground biomass increase and micrometeorological approaches for whole community CO₂ gas exchange were applied for the measurement of primary productivity in the Sundarban mangrove forest.

For allometric studies more than 100 trees covering all species, total weight, wood specific gravity (oven dry weight over green volume), height (H) and tree circumference at breast height (cbh) at 1.3m height were measured. Trees of each species were felled (three trees per cbh class) and after collection of above ground biomass (AGB) tree roots were dug up as complete as possible for the measurement of below ground biomass (BGB). Based on field measurements, different cbh classes of trees were recognized: ≤10, 10-20, 20-30, 30-40, 40-50, 50-60, >60 cm. Trees of each of the species were considered (three trees per cbh class) in the forest. Quadrates (10mx10m) were selected randomly in west Sundarbans: Lothian Island North (Stn.1, four quadrates), Lothian Island South, Ecocamp (Stn.2, four quadrates), Prentice Island (Stn.3, two quadrates), and in east Sundarbans: Bonnie camp (Stn. 4, two quadrates) and Halliday Island (Stn.5, four quadrates) for cbh measurements of mangrove trees (Fig. 2.2). Above ground biomass (AGB) was obtained from allometric equations and data were used to extrapolate over the
entire mangrove forest. The below ground biomass (BGB) of live root material was collected from the soil core (1x1x0.45m) from each quadrate. The powdered soil samples were sieved in 2 mm mesh size (mesh no 20) and washed with a fine jet of water.

Five quadrates of 10x10m size in the footprint of the observatory tower selected randomly along a transect covering a plot (54 ± 21 trees in 0.01 ha quadrate) of 1 ha. Different mangrove species were marked and measured for cbh increment at one year interval (September, 2009 - September, 2010) at Stns 1, 2 and 4. Annual increment of above ground biomass was estimated from increased diameter (dbh) using allometric equations.

Litter fall was collected from a trap (3x3m) made of nylon screen and suspended below the canopy from branches of the trees at height sufficiently above the ground to avoid tidal inundation. Litters were collected monthly using 10 such type of traps placed randomly inside the deep forest. All samples of AGB, BGB and litter were oven dried at 60° C for the determination of their dry weight.

Carbon was estimated in different components of dried plant material (stem, root, leaf, litter) using CHN Analyzer (2400 series-11, Parkin-Elmer).

Images of different sampling types during field-work are shown in Fig 3.1.

3.4 Measurement of soil CO$_2$ and CH$_4$ emission

The enclosed static chamber technique (Bartlett et al. 1987; Van der Nat and Middelburg, 2000) was used to measure soil CO$_2$ and methane (CH$_4$) emission. Estimation of soil CO$_2$ emission by chamber technique does not differentiate between autotrophic and heterotrophic soil respiration. Although mangrove ecosystems are
generally considered net autotrophic and have high productivity and low ratio of sediment respiration to net primary production (Yong et al. 2011).

3.5 Determination of physicochemical parameters of soil

Core samples were collected by using a corer made of stainless steel (5.5 cm i.d.) during three seasons at different depths up to 30 cm from surface to estimate soil physicochemical parameters. Sediment was placed in a screw capped centrifuge tube with septum and pore water was separated for salinity measurement avoiding air contact by means of centrifugation (30 min, 5000 rpm). Then soil samples were extracted in potassium chloride (2 mol L\(^{-1}\)) for the determination of total inorganic nitrogen (TIN) and total inorganic phosphorus (TIP) by spectrophotometric method (Riley et al. 1995, Grasshoff, 1983; APHA, 1995; Chang and Jackson, 1957). A relative error of accuracy was ±2% for phosphate; ±3% for nitrate

3.5.1 Determination of salinity (pore water)

Salinity of the sample water was determined by Mohr- Knudsen titration method. 15 ml of sample was taken in a clean glass conical flask with the help of a cleaned and dry pipette. About 25 ml of chloride free distilled water was mixed with the sample and 6 drops of 8% potassium chromate solution was added as indicator. Whole mixture was stirred with adjusted speed of magnetic stirrer and silver nitrate solution (36.75 g L\(^{-1}\)) was added drop by drop from the piston burette, until first sign of a constant colour change. When a slight flesh red colour appeared which persisted for 30 seconds, it was the end point of the titration. All the chlorides present in the sample were reacted with Ag\(^+\) ions and got precipitate. Excess chromate ion reacted with silver ion and formed the red precipitate. Every sample was titrated thrice to get the mean titer value. Standard
seawater of chlorinity \( 19.374 \times 10^{-3} \) was also titrated following the same procedure. Chlorinity of the sample water was then calculated as follows:

\[
\text{Cl}(x \times 10^3) = \text{volume of AgNO}_3 \text{ required to titrate the sample (in dm)} \times f,
\]

Where, \( f = 19.375 / \text{vol. of AgNO}_3 \text{ required to titrate standard sea water (in dml)}, \)

\( \text{dml} = \text{double ml} \), and 15 ml of each of sample and standard sea water were taken for titration.

From the knowledge of chlorinity, salinity was calculated using the Knudsen relation:

\[
\text{S} (x \times 10^3) = 1.80655 \times \text{Cl} (x \times 10^3)
\]

**3.5.2 Dissolved Inorganic Nitrate -Nitrogen**

All nitrates present in the sample water were converted to nitrite by reduction. A glass column packed with copper coated cadmium cheeps was used for reduction. Method based on the formation of azo dye. The method for determination of total nitrate and nitrite consists of treating 100 ml of water sample was mixed with ammonium chloride solution (2 ml of 25%) and passed through the amalgamated cadmium reduction column with a speed of 2 drops s\(^{-1}\). The eluent (50 ml) collected from the column was then treated with 1 ml solution of sulphanilamide; the resultant diazonium ion was coupled with 1ml of \( N – (1\text{-naphthyl-ethylene diamine dihydrochloride to give an intensely pink dye. The absorbance of the resulting pink solution was measured photometrically at 543 nm against a reagent blank. Efficiency of the reduction column (>90%) was tested periodically with standards and was subjected to identical treatment in each batch. The concentration of total nitrate and nitrite was computed from calibration curve. \)
3.5.3 Inorganic Phosphate- phosphorus

Concentration of phosphate- phosphorus in soil sample was determined using acidified molybdate solution and ascorbic acid. 35 ml of sample water was treated with 1 ml mixed reagent (mixture of 0.073 M ammonium molybdate and 9.1 N H₂SO₄ and a small portion of potassium antimonyl tartrate) followed by the addition of 1ml 0.4 M ascorbic acid solution. Dissolved inorganic phosphate present in seawater was converted to the formation of phosphomolybdate complex with acidified molybdate reagent, which on reduction with ascorbic acid formed a highly colored molybdenum blue compound. The absorbance of the resultant molybdenum blue was measured spectrophotometrically at 882 nm against reagent blank. Samples and standards were subjected to identical treatment in each batch. Turbidity blank was used whenever it was necessary. The concentration PO₄³⁻- P was then computed from calibration curve.

3.5.4 Determination of Ammonia in soil [Phenol – hypochlorite method (Grasshoff, 1983)]

50 ml of suitably diluted soil extracted solution was taken and 2ml phenol solution (10gm phenol in 100ml ethyl alcohol) was added followed by addition of 2ml nitroprusside solution (0.5 gm sodium nitroprusside per 100 ml deionized water) and 5 ml oxidizing reagent (mixture of 10 gm sodium citrate and 1gm NaOH in 100ml deionized water and 25 ml sodium hypochlorite solution, 3.5%). Sample was mixed well by swirling between the additions. The whole mixture was placed in thermostat (maintained at 40 – 45°C) for 30 minutes and was kept in room temperature for more than one hour to develop blue color. A series of working standard solution (1 – 5 μM) was prepared and were treated as the same way of sample for calibration. The method essentially consists
of formation of monochloroamine by the reaction of ammonium with hypochlorite, which on reaction with phenol in alkaline medium (pH ≥ 10) in presence of trisodium citrate and nitroprusside (catalyst) gives an intensely colored indophenol blue. Precipitation of Ca and Mg is prevented by the use of citrate as complexing reagent. Ammonia – free deionized water was used throughout the procedure. The absorbance of the resulting blue color was measured at 630 nm against a reagent blank and the concentration of NH₄⁺ - N was computed from the calibration curve.

3.5.5 Determination of Organic Carbon in soil (Walkey and Black, 1934, Shrawat, 1982)

One gram 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 solution (1.25 gm silver sulphate dissolve in 100ml concentration sulphuric acid) 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 salt solution (393.13 gm ferrous ammonium sulphate dissolve in 50 ml concentrated sulphuric acid 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 organic carbon.

% of organic carbon = \( \frac{(V_1 - V_2)}{W} \times 0.003 \times 100 \)

Where \( V_1 \) = Volume of Mohr salt required to titrate

\( V_2 \) = Volume of Mohr salt required to titrate 10ml potassium di-chromate as blank

\( W \) = Weight of the sediment sample taken

% of organic matter = % of organic carbon × 1.724
Glucose was used as a standard for labile organic matter in the sediment and 97.6% accuracy and 3.14% coefficient of variation were achieved for the determination.

3.5.6 Organic matter (HA & FA) analysis in sediment

Humic acid (HA) and fulvic acid (FA) were extracted from 0.5 g of dried sediment by the Methyl Isobutyl Ketone method (MIBK) (Rice, A.J. and Mac carthy, P. 1989). 20g of the sediment sample was added to 0.5 (M) NaOH solutions and stirred for 24h. in the nitrogen atmosphere. The mixture was filtered through GF/C glass filters. The filtrate was transferred to a separatory funnel along with 75 ml MIBK and acidified to pH 2-3 with concentrated HCl. The filtrate was shaken vigorously. Humic acid enters into the MIBK phase as a suspension, leaving the fulvic acid in the aqueous phase. The aqueous phase was collected and dried in a rotary evaporator under vacuum. The residue left was extracted with absolute alcohol. The extract was dried in a rotary evaporator to get solid fulvic acid. A fresh aliquot of 0.5 (M) NaOH was added to the MIBK phase in the separatory funnel and shaken vigorously. The humic acid was extracted from the MIBK phase into the aqueous alkaline phase. The alkaline phase was again acidified to get the precipitation of humic acid.

The concentration of humic (HA) and fulvic (FA) acid in the centrifugate were estimated by Fluorescence method using LS-50 Luminescence spectrophotometer. FA (Excitation 313 and Emission 425 nm) and HA (Excitation 392 and Emission 484nm) procured from Aldrich. Co. was used as standard (Ghatak et al. 2002; Leifer, 1988, Silva et al. 1994)).
3.5.7 Soil texture analysis

Sediment samples were collected with the help of stainless steel soil corer and as soon as possible after collection of sediment samples transported to the laboratory and were spread for air drying. After drying of the soil to constant weight, stones and other similar objects were removed and the soil was ground to break up aggregates and crumbs. The soil was then used by cone-quadrate method to analyze different parameters.

Sediment samples were collected from the different sampling sites of Sundarbans. Air-dried samples were used for the grain size analysis following pipette method (Piper, 1950). 25 gm of air-dried soil was treated with 6% hydrogen peroxide (H₂O₂) and 2 (N) HCl successively and was transferred to a cylinder (2 lts.) with 250 ml distilled water and a 0.5% sodium hexametaphosphate solution after having been mixed in a beaker for 10-15 minutes with a mechanical stirrer. H₂O₂ is used to remove the organic matter from the sample while sodium hexametaphosphate used to retard biogenic growth by coagulating the soil. The mixture was diluted upto 1250 ml graduation mark. With the help of a 20 ml pipette first and second samples were taken at proper time interval; they were dried in a silica crucible and weighed to get percentage of silt, clay and sand (Piper, 1950).

3.5.8 Ph and Redox potential measurement

pH and Eh (redox potential) were measured by gently lowering the electrodes into the sediment (Vischer et al. 1991).
3.6 Estimation of community exchange of CO₂ fluxes

The rate of CO₂ exchange between the atmospheric and biosphere (\(F_{AF}\) and \(F_{FA}\)) were calculated from the concentration difference (\(\chi\)) between 10 and 20 m: \(\Delta \chi = \chi_{10} - \chi_{20}\), aerodynamic \(r_a\) and surface layer \(r_s\) resistance. With exchange velocity, \(V_c\), defined as \(1/(r_a + r_s)\), net flux, \(F\), was calculated using the relation (Barrett, 1998):

\[
F = V_c \Delta \chi.
\]

Negative \(F\) indicates net transfer from the atmosphere to the biosphere and positive \(F\), for emission. The aerodynamic resistance, \(r_a\), describes the resistance to transport between the reference height \((Z)\) at which the concentration is measured and the quasilaminar layer next to the receiving surface and can be evaluated as (Wesely and Hicks, 1977):

\[
r_a = \frac{\ln (Z/Z_0) - \psi_c}{k u^*}
\]

Here, \(Z_0\) is roughness height and was determined from the intercept (\(\ln Z0\)) of the straight line obtained by plotting \(\ln Z\) versus \(u\). \(k\) is the dimensionless Von Karman constant (0.4), and \(\psi_c\) is a correction function for atmospheric stability, which serves to increase \(r_a\) for stable condition and to decrease it for unstable condition. The equations for the correction functions are (Wesely and Hicks, 1977):

\[
\psi_c = -5Z/L \text{ for } 0<Z/L<1 \text{ (stable condition) and }
\psi_c = \exp[0.0598+ 0.39 \ln(-Z/L)- 0.09 \ln(Z/L)]^2 \\
\text{for } 0>Z/L>-1 \text{ (unstable condition).}
\]
The correction functions are expressed in terms of a stability parameter \(Z/L\), in which \(Z\) is the height and \(L\) is the Obukhov Scale length. The friction velocity, \(u^*\), was estimated from the wind velocity at 10 and 20m in the following manner:

\[
u^* = k(u_{10} - u_{20})/ \ln(Z_{20} - Z_{10}),\]

where \(k\) is the Von Karman constant. \(Z_0\) was determined from the intercept (lnZ0) of the straight line obtained by plotting lnZ versus \(u\). 80% of the average height (10 m) of the mangrove plants was considered to calculate displacement length, \(d\) (Panofsky and Dutton, 1984). Gradients of wind velocity and temperature observed over the canopy at 10 and 20m for computing aerodynamic resistance were found well within the limit of sensitivity (± 0.01 °C and ± 0.01 ms\(^{-1}\)) of the used sensors. Gradients of wind velocity and temperature observed over the canopy at 10 and 20m for computing aerodynamic resistance were found well within the limit of sensitivity (± 0.01 °C and ± 0.01 ms\(^{-1}\)) of the used sensors.

Considering the stability classes of Pasquill: A–F (Pruppacher and Klett, 1997) the scale length, \(L\), was evaluated using the following relation:

\[
1/L = a + b \log Z_0,
\]

where ‘a’ ranges between 0.035 and -0.096 and ‘b’ ranges between 0.029 and -0.036 (Golder, 1972).

Pasquill stability classes in terms of wind speed, insolation and state of sky were as follows, D–F (stable) for post-monsoon, B–D (unstable) for pre-monsoon and E, F for monsoon in the nighttime and A–C (unstable) for post-monsoon, E, F (unstable) for pre-monsoon and B (unstable or occasionally stable) were observed in the daytime. For
surface layer resistance \( (r_s) \), following relations with surface transfer function, \( B'^1 \) (Wesely and Hicks, 1977) and \( u^* \) were used:

\[
kB'^1 = 2(K/Dc)^{2/3} \quad \text{and} \quad rs = B'^1/u^* \quad \text{(for forest cover)},
\]

where \( k \) is the Von Karman constant; \( K \) is the thermal diffusivity of air and \( D_c \) is the molecular diffusivity:

\[
D_c = 0.115 (T_2/273)^{1.5}, \quad \text{where} \ T_2 \text{is the absolute temperature at 20m height (Ganguly et al., 2008).}
\]

Only data from runs in near neutral stability were considered to minimize error (Mukhopadhyay et al., 2002). Advective error varied between 0.001 and 7.15% and storage error during stable condition varied between 4.2 and 21.6%. The flux values reported here are to be considered as estimates rather than absolute values. From the difference between night time CO\(_2\) efflux from the forest and soil emission, plant respiration was calculated.

### 3.7 Rate of litter fall

From monthly measurement of litter fall, C flux from forest to soil is calculated and expressed as \( \mu g \ C \ m^{-2} \ s^{-1} \).

### 3.8 Estimation of total C stock in different compartments

**Atmospheric C stock** (\( M_A \)): Total carbon stock in the boundary layer over 4264 km\(^2\) reserved forest (\( M_A \)) was computed from the mean CO\(_2\) concentration.

**Forest C stock** (\( M_P \)): The mean carbon stock in terms of AGB and BGB obtained from different quadrate values was expressed in mega gram carbon per hectare (Mg C ha\(^{-1}\)).
considering total terrestrial area of Sundarbans to be 4264 km². Total forest biomass is converted to equivalent amount of carbon stock ($M_F$).

**Soil C stock ($M_s$):** There is a well established set of methods for measuring soil carbon pools (Post et al. 1999). In the mangrove sediment, the long term rate of accumulation of soil organic carbon/humic material ($M_s$) is calculated from bulk density ($\rho = 2.5$ g cm$^{-3}$) and the percentage of organic carbon/humic material at each depth in the sediment profile of depth 0 – 30 cm.

$$\text{Organic C or Humic material (g cm}^{-2}) = \sum_{D=0}^{30} 100 \cdot \rho(D) \cdot P(D)$$

where $\rho(D)$ and $P(D)$ are the bulk density in gm cm$^{-3}$ and the percentage of humic organic material at the depth D (cm).

The long term average rate of accumulation is obtained by dividing the humic carbon content (50%) by radio carbon data of humic materials which is frequently 1000 yrs (Schlesinger, 1990).

**3.9 Measurement of mangrove wood density and chemical composition**

Different wood species in triplicate were used for both physical and chemical study. Powdered wood samples (5 g) were sieved (200µm) and were used for analysis. Density ($\rho$) of blocks (2x2x4 cm) cut from wood discs was determined dividing sample oven dry weight by the dry sample volume (Haygreen J. G. and Bowyer J. L., 1996). In this study density was given in kg m$^{-3}$.

The relative distribution of cellulose and lignin in wood dust was examined by spectrophotometric method (Yemm EW, Willis AJ.1954; Sluiter 2010).
3.9.1 Determination of Carbohydrates (cellulose standard)

50 mg of the dry samples were weighed and homogenized with 5ml of 2.5 N HCl and placed over a boiling water bath for one and a half hours. After subsequent treatment of homogenized mixture with solid sodium carbonate, anthrone reagent was added and absorbance of the green colored solution was measured at 630 nm. Carbohydrate percentage was determined using extra pure glucose as standard.

3.9.2 Determination of total Lignin

In this method the samples were extracted in 72 % of sulphuric acid at 60 °C in a water bath. Upon completion of hydrolysis, mixture was diluted (4% sulphuric acid) and heated in autoclave for ½ hr at 120 °C. The mixture after filtration under vacuum was used for the measurement of absorbance at 320 nm for acid soluble lignin (ASL) and residue was used for the measurement of acid insoluble lignin (AIL).

3.9.3 Determination of ash content:

For determination of ash content, 1g of the sample was taken and it placed in a silica crucible which was weighed very accurately. Next, the weighed crucible was subjected to heating at 550°C in a furnace for three hours. The sample was given ample time to cool down and it was weighed again. From the difference in the final and the initial weights of the crucible, the ash content was evaluated and expressed in percent.

3.9.4 Estimation of moisture:

2 g of the wet samples were taken in flat dishes and kept for 48 hours in hot air oven at 100-110 ° C and weighed and the loss in weight was regarded as a measure of moisture content (Indrayan et al. 2005).
3.9.5 Determination of total carbon, phosphorous and nitrogen

Carbon and nitrogen were examined using CHN Analyzer (2400 Series-11, Perkin Elmer). Total phosphorous was analyzed by spectrophotometric method after the oxidation of the sample using perdisulphate oxidation method. (Mc Lellan R, Acree TE 1992).

3.9.6 Estimation of Protein

Total protein was calculated multiplying the evaluated nitrogen (N %) by 6.25 (Nagy et al. 1990).

3.10 Determination of cellulose and lignin by FTIR

FT-IR spectra were recorded with a resolution of 4 cm⁻¹ on solid samples (2mg) in KBr pellets using L120-000A infra red spectrophotometer (Parkin-Elmer). DTGS detector was used. The concentration of standard cellulose (E.Mark) in the pellets were varied between 1 and 5 mg/100mg KBr. Spectral ranges of 4000-2500 and 1700-800 cm⁻¹ were used for multiple regression analysis with cellulose concentration (Nuopponen et al 2006) and contribution for cellulose was established. Cellulose concentration data obtained from FTIR were compared with analytical data.

3.11 Determination of thermal properties of wood by TGA-DSC

Thermogravimetric Analysis (TGA) was carried out under constant nitrogen flow (90 ml/min.) at a heating rate of 20 °C/min. using a DSC-TGA thermo balance (SDT Q600 V8.2 Build 100) The heating scans were prepared on 4 mg of the sample in the
temperature range of 25-800 °C. Calorific values and FVI (fuel value index) were calculated from the following relations, respectively (Demirbas A. 1997; Mainoo A A., Ulzen-Appiah F. 1996)

\[
CV\ (MJ/kg) = 0.312(FC) + 0.1534(VM),
\]

\[
FVI = \text{calorific value (cal/g)} \times \text{sp. gravity (g/cc.)} \times \%\ \text{lignin}
\]

Moisture content (%)

Where, FC = Fixed carbon and VM = Volatile matter in percentages.

3.12 Application of statistical software (MINITAB, MATLAB)

Microsoft word and Excel were used for data processing. Adobe Photoshop, Microsoft Office paint was used to edit and prepare different figures. The different sample data were used for different statistical analysis (e.g. stepwise regression analysis, Factor analysis, 't' test, etc.) which were done by MINITAB (version 13.1) and model equations were simplified by using MATLAB, Mathworks, (version 7.1) statistical package during the study.
Fig. 3.1: Images during research work