Manual For
Soil Measurements For The Bangladesh Forest Inventory

MANUAL FOR SOIL MEASUREMENTS FOR THE BANGLADESH FOREST INVENTORY

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1 INTRODUCTION

The Bangladesh Forest Inventory is a multipurpose inventory aiming at providing information for resource and carbon inventory, including soil carbon stocks. This manual manual is prepared for Bangladesh Forest Inventory (Iqbal, Kuegler et al. 2016) that will support the Government of Bangladesh in establishing updated forest management plan and climate change mitigation and adaptation strategy. There are numbers of methods for measuring forest resouces and C stocks; however our focus is to adapt international standards per guidelines of IPCC. The international standards commonly divide forests into five carbon pools: 1) aboveground and belowground biomass of live trees, 2) non-tree vegetation, 3) dead wood, 4) forest floor (litter), and 5) soil.

In this manual, we provide standardized methods for field measurement and analysis of soil organic C in forest ecosystems based on previous studies and existing soil organic C protocol. Soils are of belowground C pools that contain huge carbon in the form of carbon-rich organic compounds called soil organic matter. This belowground C pool consists of, not only sediments, living and decomposing roots and litter. While the aboveground biomass, mainly from the tree component is being estimated using allometric models and dendrometric measurements, the soil C stock is being estimated based on soil texture, bulk density, soil OC content and pedotransfer functions.

Because of the importance of reporting to international conventions, in particular to the UNFCCC, the soil layer 0-30cm is considered in priority in this manual. However, in the mangrove ecosystems (Sundarbans and Coastal plantation zones), a particular attention is given to 0-100 cm because of deep organic-rich soils in mangrove forests. This document complements the BFI field inventory manual (BFD 2016).

1.1 PURPOSES OF THIS MANUAL

This manual is developed to train crews and guide the soil data collection process from the field and provide guidance for the soil laboratory analysis. This manual will also be used to support the quality assurance and quality control process for the BFI. The QA/QC procedure is explained in the QA/QC manual (Rahman, Birigazzi et al. 2016).

1.2 ORGANIZATION OF THIS MANUAL

The manual outlines the rationale and design for sampling soil and litter in the field and analyzing the data for soil texture, bulk density and %OC, and litter. The structure of manual is:

1. Soil and litter assessment: Provides information of sampling design at plot level.
2. Soil and litter sampling: Introduces the need of soil and litter measurement for forest inventory and provides the guidance for measuring the samples at subplot level.
3. Laboratory analysis: Outlines the steps to analyse the samples in laboratory.
4. Appendices: Provides supplementary information and guidance for data collection.

ITEM NUMBER; DATA ITEM NAME:
   [table_column name]

In addition, the following information is given for each data item:

When collected	Specific criteria for when variable is recorded
Field width	X digits
Tolerance	Acceptable range of measurement
Values	Legal values/codes variables

Data elements, descriptions of when to collect the data elements, field width, tolerances, and values.

Field width designates the number of columns (or spaces) needed to properly record the data element.

Tolerances may be stated in +/- terms or number of classes for ordered categorical data elements (e.g., +/- 2 classes); in absolute terms for some continuous variables (e.g., +/- 1 cm); or in terms of percent of the value of the data element (e.g., +/- 10 percent of the value). For some data elements, no errors are tolerated (e.g., PLOT NUMBER).

1 SOIL AND LITTER ASSESSMENT UNDER THE BANGLADESH FOREST INVENTORY

1.1 SOIL AND LITTER SAMPLING AT PLOT LEVEL

As soils are heterogeneous, variation is frequently observed. Sample collection from only one spot of the field/area will not be representative. We establish a plot, which is situated in a systematic grid across the forest, at regular intervals of latitude and longitude, and consist of three circular subplots, oriented as a center subplot with two more subplots oriented in two cardinal directions from the center (north and east). This clustered sample units gives a composite sample for three subplots for each plot in the case we have only one land feature, If different land features at the centre of each selected subplot exist, soil samples for soil OC and bulk density are not composed (Figure 1). The main variables of interest for the soil component under the Banlgadesh Forest Inventory, (ie. soil texture, bulk density and organic carbon) are outline in the next section.

Selected subplots for soil data collection

Composite soil sample for the three selected subplot

Two composite soil samples in the case of two different land features

Three soil samples in the case of three different land features
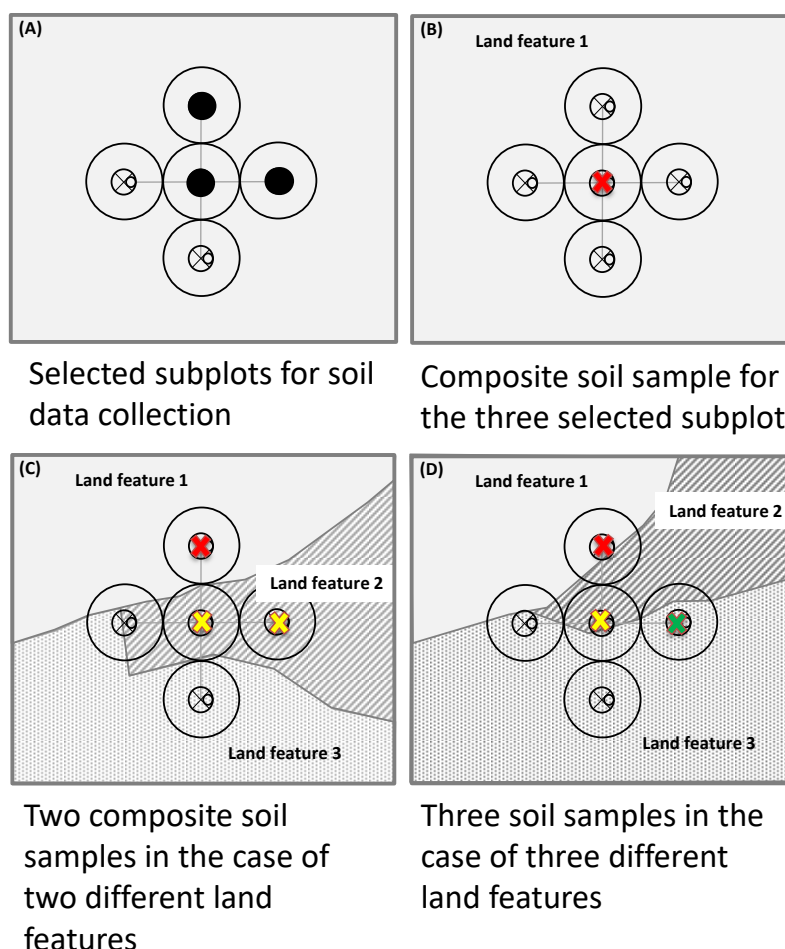
Figure 1: Soil sampling depending on the presence of land features

Sampling of soil and surface materials, are only practical when the soil surface is exposed to the air. For mangrove soil, the sampling window may last about 4 hours due to tides. Thus, this manual is designed at an efficient sampling method based on several experimentations with different techniques or soil sampling equipment in different environmental condition. Autogenerated code for the soil and litter samples to be tagged on the samples until laboratory analysis. A skilled personel, who know how to collect soil sample, and labour are needed for this sampling work in the field.

2 SOIL AND LITTER SAMPLING AT SUBPLOT LEVEL

Soil testing, in a broad sense, is any chemical or physical measurement that is made on a soil. Soil testing includes collection of soil samples, sample preparation, laboratory analyses, calibration and interpretation of the tests. Soil analysis is conducted both in the field and in the laboratory. Colour is determined in the field by visual assessment and with the aid of an appropriate colour chart. Further analysis related to texture and bulk density will be collected in the laboratory. Processes must be followed to ensure soil samples collected for laboratory analysis are prepared and stored appropriately.

A soil sample should be representative of the area from which it is taken to ensure accurate results. The error in sampling in the field is generally greater than error in the laboratory analysis.
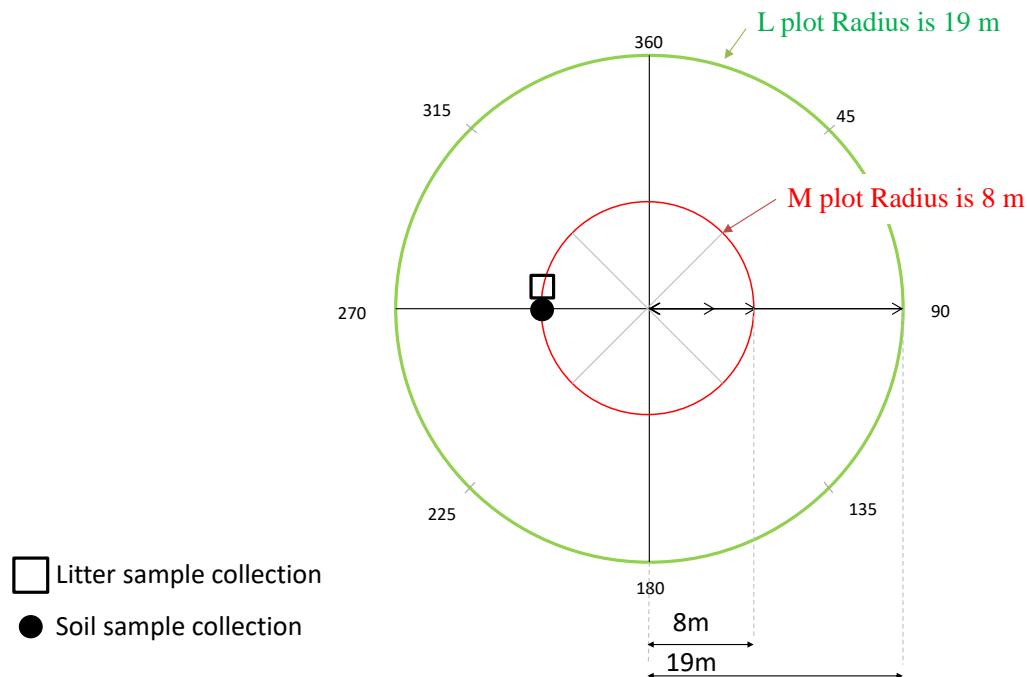
Figure 2: Soil and litter sample data collection

Some considerations when taking soil samples:

- **Label** : Each sample must be labeled and stored to ensure its results are accurately documented and attributed to the correct location

- **Documentation**: It is recommended to document the appearance of the core, especially a core with unique appreance. Photograph the entire core from top to bottom and note its interesting features, for example the presence of roots or other materials. Provide detailed photographs of core section next to the measuring tape included to provide scale.

- **Sample preservation**: Ideally, samples are placed in the drying oven facility the same day of collection. If not possible samples can be air-dried and place in the sun during the day. For soil sample, dry soil should be air-dried at room temperature by spreading on a brown paper for soils having moisture content at around field capacity or on plastic sheet for wet soils.

Figure3. Include the measuring tape when taking photos of soil samples (© Frida Sidik, FAO)

2.1 SOIL PROPERTIES

2.1.1 TEXTURE

The soil is composed by small particles, which vary in size, shape and chemical composition. These particles fall into three groups of soil seperates: sand, silt, and clay. The proportion of the different soil separates in a soil defines its soil texture, which comprise of 12 classes (Figure 4). Although sand, silt and clay are the generally recognized size-groups of soil particles, there is no uniformity in characterizing them with their effective diameter. Different systems are used in the classification of soil particles into different size-groups. Here, we have considered the size-groups of particles advocated by the United States Department of Agriculture (USDA) i.e. the effective diameter of coarse sand is 2 to 0.05 mm, that of silt is 0.05 to 0.002 mm and that of clay is below 0.002 mm. The settling time of the particles are calculated on the basis of above sizes.
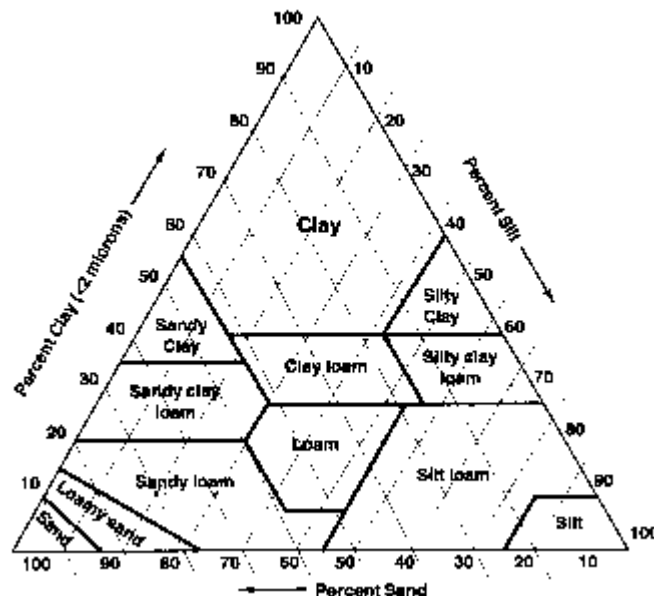
Figure 4. Soil textural triangle and textural classes

Fractionation of soil particles into different size-groups is based on principles of sedimentation. When a mixture of particles of different sizes, after through mixing, is allowed to settle in a fluid, the rate of settling of different particles depends on the effective diameter of the particles i.e. the distance traversed by a falling particle in a given time is dependent on the size of the particles, other variables such as viscosity of the fluid, temperature etc. remaining constant.

Soil texture has role in determining the physical properties of soil, including bulk density, porosity, structure, and water infiltration, and soil resistance to erosion.

2.1.2 SOIL BULK DENSITY

Bulk density is defined as the dry weight of soil per unit volume of soil, expressed in g cm^3. This is a basic soil property that is usually used to represent the soil weight and soil physical, chemical and biological properties in volumetric basis. Bulk density is also important because it reflects the function of soil porosity, structural support, soil aeration and soil compaction. For this reason it is often used as a measure of soil structure.

Bulk density varies with structural condition of the soil, particularly that related to packing, and also on the amount of organic matter, and cultural practices. Ideally, soil has one-half of it is solids, and one-half is pore space. Organic matter is a very small portion of the solids and has a lower bulk density than mineral, which ranges from 1.0 to 1.8 g cm^{-3}. Sandy soil tends to have higher bulk density and lower porosity than clayey soil. High bulk density is an indicator of soil compaction, as well as a low soil porosity that may restrict the root growth, as movement of air and water is limited.

Bulk density has highly correlation with OC content, thus they are used to estimate the carbon pool. As subsurface layers are more compacted and have less organic matter and less root penetration compared to surface layer, bulk density tends to increase with soil depth.

2.1.3 SOIL ORGANIC CARBON CONCENTRATION

All soils contain both organic and mineral components. Organic soil appears dark and has a high concentration of decomposing plant fragments originated from plant litter. The fully decomposed organic matter, so called humus, is formed through processes involving microbial oxidation that leads to C loss. The proportion of soil humus C lost by oxidation varies among the soil management regime and soil types. Humus is also the primary coloring agent in soil that aids the absorption of heat from sunlight on soil surface. The soil color tells the amount and chemical form and serves as indicator of natural drainage condition.

The OC is usually high in the top horizons because this layer receives leaf litter and nutrients extracted by roots from deep layers. This layer is also the most vulnerable to land-use change, therefore many carbon assessments, sampling is usually limited to the surface layer (0-30 cm). In mangroves and peat swamps, deeper soil collection is needed, at least in the top one meter because they often have deep organic-rich soils with little vertical differentiation in OC content or bulk density in the top meter. Soil is commonly the largest C pool of these ecosystems and OC content on deeper layer may be affected by land-use due to drainage and oxidation. Thus, some features need to be recorded in the field during collection of soil sample because they are likely to influence the carbon content, which are:

1. Vegetative species and density, as well as cultivated crops;

2. Soil profiles: the depth of water table, thickness of soil horizons in case of soil profile study, depth of topsoil, colour of topsoil, relief, slope, texture of the soil by feel method;

3. Geomorphological features;

4. Hydrological condition.

2.2 SOIL DATA COLLECTION

2.2.1 SOIL MOISTURE

Soil moisture of surface floor should be described under visual assessment for each sampled subplot.

[soil_moist]

When collected:	All	
Field width:	5 digits	
Tolerance:	No errors	
Values:	Code	Description
	1	Wet
	2	Moist
	3	Dry

2.2.2 SOIL COLOR

Soil colour should be described using the Standard Munsell Soil Colour Chart.

A sample of a page from Munsell Soil Colour Chart is shown in **Error! Reference source not found.**. The appendix shows that the hue is 10YR. If our sample corresponds to the last chip of the sixth row, then the soil has a value = 3, and chroma = 6. Hence, the soil colour notation will be '10YR 3/6'.

[soil_col]

When collected:	All	
Field width:	5 digits	
Tolerance:	No errors	
Values:	Code	Description
		See Appendix 1

2.2.3   SOIL DEPTH OF HUMUS

On each sampling plot, the organic litter covering the soil surface should first be carefully removed to expose the surface dark-coloured humus-rich mineral soil – sometimes referred to as the "duff" or "humus" layer. Then the depth of the dark coloured soil should be measured accurately with a ruler or tape on the vertical side of soil core for soil texture.

[soil_dark_depth]

When collected:	All
Field Width:	3 digits
Tolerance:	+/- 2 cm
Values:	0001 to 100 cm

2.2.4   SOIL TEXTURE

In the field:

- Scrape away the surface litter and weeds from the sampling spot with the help of a spade/belcha. This will ensure samples are free from stubbles, grass, rubbish, plant roots, stems etc.

- Insert the auger by rotating clockwise and then pull it out slowly.

- Slice the core sample into three segments: 0–15 cm and 15–30 cm using the knife.

- Put the sample in the plastic bag or jar and label it as Plot/Subplot/Depth/TEXT. For example P 1/SP 2/0-15/TEXT for Plot 1 Subplot 2

For hard soil using open pit method:

- Open a pit up to 40 cm depth. Mark the depth of sampling (0-15 cm and 15-30 cm) with a field knife by seting a measuring tape (Figure APPENDIX 5).

- Collect the surface sample (0-15 cm) using spade or shovel.

- After collection of the surface sample, remove the top soil and take the sample at 15-30 cm depth.

- Put the sample in labeled plastic bag.

For muddy soils using auger (Mangrove forest only)

- Steadily insert the open-faced auger vertically and twist the auger a few times.

- Gently pull auger out of soil. If the sample is disturbed or consists of coarse roots, clean the auger and try to collect a new core.

- Slice the soil into three segments: 0–15 cm, 15–30 cm, and 30-100 cm and cut away from the auger face.

- Put the sample in the plastic bag or jar and label it as follow:

  Plot / Subplot / Depth / TEXT.

For example   P 1/ SP 2   /   0-15   /TEXT for soil sample for texture for 0-15cm in subplot 2 of plot 1.

Prior to the lab (when in decentralized office):

- Mix the sub-samples according to their respective group depths. Mix thoroughly so all samples are completely homogeneous.

- Split the sample into four parts.

- Discard three parts of the sample and then thoroughly mix the rest, then place in plastic jar and label it as:

" P NN / NN – NN* / TEXT " (*depth)

[soil_text]

When collected:	All
Field width:	Text
Tolerance:	No error
Values	Max 50 characters

2.2.5   BULK DENSITY

Samples are taken from relatively undisturbed cores with minimal soil compaction. Samples should be taken at the points where there are not visible soil cracks (undisturbed). If a core is taken and it is loose, cracked or disturbed, another core should be taken near by the sample location. As the bulk density will be used to calculate the organic carbon content, we use the same samples taken for organic carbon analysis. Always clean the equipment after use to avoid contamination from previous sample collection.
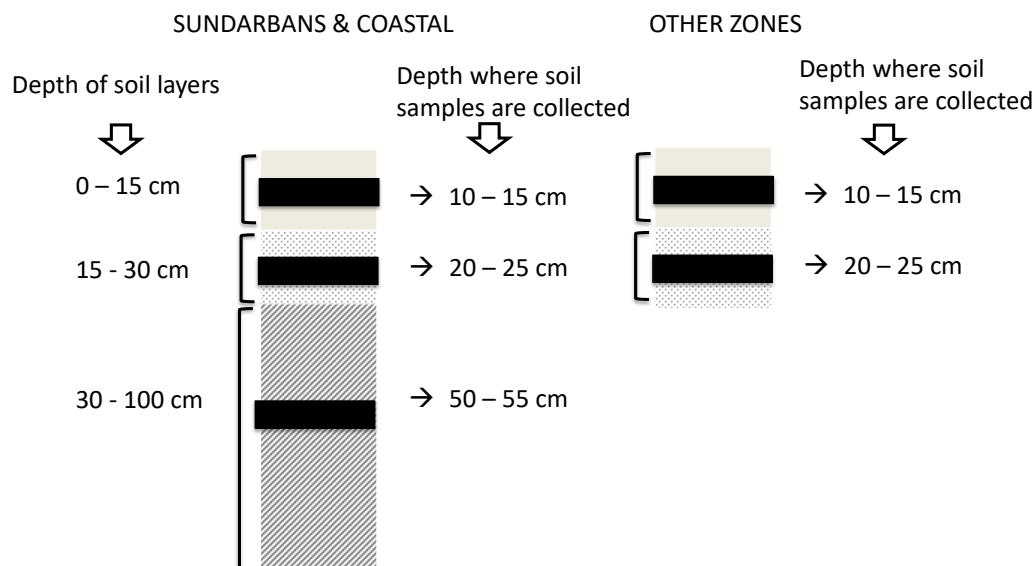

Figure 2: Collection of soil samples for bulk density measurement in different zones

METHOD FOR MUDDY SOILS USING AUGER (MANGROVE FOREST ONLY)

- Steadily insert the open-faced auger vertically and twist the auger a few times.

- Gently pull auger out of soil. If the sample is disturbed or consists of coarse roots, clean the auger and try to collect a new core.

- Cutting the soil away from the auger face. Take the unused parts for soil texture (see the method above) and gently clean and flat the surface of soil core.

- Subsamples the soils at depth 0-15, 15-30, and 30-100 cm by taking a 5 cm sample with consistent depth interval mid point of each subsample depth (Figure 5). For example, when sampling the 0-15 cm depth, the sample would ideally come from the 5-10 cm depth. Use the measuring tape and blade for measuring and marking the depth interval.

- Put it in plastic bag firmly and rolled it with the label.

- Label it with permanent marker as: Plot / Subplot/Depth/BDC. For Bulk density for the soil layer 5-10cm in subplot 2 of plot 1 label as follow:

P 1/Sp 2/ 5-10 /BDC

METHOD FOR DENSE SOILS USING CORE SAMPLER

- Push the ring into the soil vertically using a rubber mallet.

- To avoid the chances of compactness, use extra ring to ensure that sample ring is completely inserted into the soil and filled with undisturbed soil sample.

- Carefully extracted the sample from the soil using a field knife, shovel or spade or belcha.

- Collect the ring that is inserted at 5 – 10 cm. This sample represents the 0 – 15 cm interval written in the field form.

- Level the surface of the soil sample using a sharp knife to ensure that the soil is completely level with edge of the corer. The core should be completely full, however there should be no excess soil. Cut any protruding root debris away.

- Measure the depth of the pit and insert more rings to obtain the sample at 20 – 25 cm (the bottom ring). This sample represents the 15 - 30 cm interval written in the field form.

- Collect the samples by following the previous steps.

- Label the core with permanent marker as Plot/Subplot/Depth/BDC. For example P_1/S_2/5-10/BDC for Plot 1 Subplot 2

[soil_bd]

When collected:	All
Field width:	Text
Tolerance:	No errors
Values:	Max 50 characters

2.2.6 SOIL NOTES

Record notes to explain any information that may need clarification for soil analysis (e.g., presence of roots, etc.). In particular, provide any information related to sample extraction for the bulk density.

[soil_notes]

When collected:	All
Field Width:	2000 characters
Tolerance:	N/A
Values:	Single words or abbreviated sentences

2.3 LITTER SAMPLING

2.3.1 LITTER COLLECTION

In the field:

- Mark out a 1 m x 1 m square at each subplot, near the place where the soil core is collected.

- Collect the dead leaves, flowers, fruits, seeds, and bark fragments (excluding woody particles).

- Put the samples in the plastic bag and label it with permanent marker as Plot/Subplot. For example P 1/S 2 for Plot 1 Subplot 2

[litter_code]

When collected:	All
Field width:	Text
Tolerance:	No errors
Values:	Max 50 characters

2.3.2 LITTER NOTES

Record notes to explain any information that may need clarification for litter analysis. In particular if any event (climatic or human) has influenced the quantity of litter.

[litter_note]

When collected:	All
Field Width:	2000 characters
Tolerance:	N/A
Values:	Single words or abbreviated sentences

3 LABORATORY ANALYSIS

3.1 LABORATORY PREPARATION

3.1.1 PREPARATION OF SOIL SAMPLE IN THE LABORATORY

1. **Wet weight**: Weight the sample to obtain the wet weight. Stones, brick pieces, crop residues etc. should be removed before oven dry, weighed separately, and recorded.

2. **Drying**: The soil samples for carbon analysis should be dry at 60 °C to constant mass (48-72 hours). Prior the oven dry, samples can be air dried to reduce the oven time. Let the samples cool at room temperature in desiccator for about an-hour and record the final oven-dry weight for bulk density. The composite soil samples can be air dried in shade at room temperature until no more water present in the samples.

3. **Grinding**: Particle size should be reduced to the fineness form in order to obtain sample homogeneity that is necessary for total chemical analysis. Soil aggregates are homogenized using wooden roller or porcelain mortar.

4. **Sieving**: The ground soil material was sieved through a 2 mm (10 mesh) sieve. Stones or gravels remaining on the screen should be ignored and discarded.

5. **Storage**: The prepared soil samples should be preserved in labeled screw-cap jars / plastic pot in the laboratory.

3.1.2 PREPARATION OF FOREST LITTER SAMPLE IN THE LABORATORY

1. **Wet weight**: Weight all the samples from subplot to obtain the wet weight of litter.

2. **Composite**: Take the sample randomly from each subplot to make a well mixed 30 g of representative subsample

3. **Drying:** Oven dry the composite sample at 60 °C to constant mass (48-72 hours). Prior the oven dry, samples can be air dried to reduce the oven time. Let the samples cool at room temperature in desiccator for about an-hour and record the final oven-dry weight.

4. **Ratio determination**: Calculate the ratio between wet and dry mass of the subsample for scaling-up the biomass of the bulk sample.

5. **Grinding**: Litter sample should be broken up by grinding with mortar.

6. **Storage**: Keep the sample in labeled screw-cap jars for C organic analysis.

3.2 DETERMINATION OF SOIL TEXTURE

3.2.1 DETERMINATION OF SOIL TEXTURE BY HYDROMETER METHOD

Mechanical analysis of soil refers to the procedures employed to identify soil separates by determining the mechanical composition, the percentage distribution of different sized-particles, of soil. This process records the time of which a specific weight of soil particles fall to the bottom of a tall cylinder filled with water. Mechanical analysis consists essentially of two distinct operations, namely, (i) Dispersion of soil and (ii) Fractionation of the sample i.e. grading the soil particles into different size-groups. A textural triangle or table (Figure 2) can be used to determine soil textural class from the results of a mechanical analysis.

Dispersion of soil has to be made to ensure the separation of primary particles from each other as aggregation by cementing agent results in the formation of particles of larger sizes from those of smaller dimensions. Thus, treatments that affect the destruction of cementing agents (organic matter, $CaCO_3$ and other flocculating agents) are effective measures to break down the secondary particles (aggregates) into primary particles is also termed as "pre treatment". Subsequently, the primary particles are to be kept in dispersed condition during fractionation of the sample. This may be achieved by adding a suitable peptizing or deflocculating agent to the soil suspension. According to the Soil Division of USDA, sodium hexa-metaphosphate is used.

STEPS FOR DETERMINING SOIL TEXTURE:

1. Weight the aliliquot of air-dry, soil sample equivalent to 40g oven-dry weight. Place the soil in a 600 ml beaker and add 20 ml 30% H_2O_2 solution. Cover the beaker with a clock-glass to prevent loss by splitting due to initial violent reaction.

2. After the initial violent reaction has subsided, place the beaker on a boiling water bath. Care must be taken to avoid frothing over. [Further addition of H2O2 may be necessary if much organic matter is present.]

3. When the organic matter has been completely decomposed, cool the beaker and wash down any soil material sticking to the clock glass. Add 100 ml of 5% calgon solution, cover the beaker with watch glass and leave overnight.

4. Transfer all the contents of the beaker to the dispersion cup by repeated washing and taker care that no soil material is lost during transfer; and make the volume up to 400 ml.

5. Place the dispersion cup in position under the motor immersing the stirrer of the motor in the soil suspension, and stir for 5 minutes. Remove the cup from the dispersion unit, wash the stirrer and collect the washing in the cup.

6. Transfer the whole contents of the cup to the sedimentation cylinder by repeated washing with distilled water carefully without losing any soil material.

7. Fill up the sedimentation cylinder up to 1000 ml with distilled water and mix the content thoroughly with the stirer.

8. Place the cylinder in settling position, insert the hydrometer and start the timer. After 40 seconds, record the hydrometer reading and note the temperature of the suspension, without disturbing the suspension. Again record the hydrometer reading and the temperature of the suspension exactly after 2 hours of settling.

CORRECTION OF HYDROMETER READING:

Place the clean hydrometer after reading the densities of soil suspension in a sedimentation cylinder containing 100 mL calgon solution in 1000 ml volume and record the hydrometer reading. This reading has to be deducted from those taken at 40 seconds and two hour intervals on soil suspension. These readings (after calgon correction) are to be corrected further as follows.

If 't' is the temperature of the suspensioin degrees centigrade during hydrometer reading, :

Equation 1. Corrected hydrometer reading : $(Reading\ of\ soil\ suspension - Blank\ reading) + ((t - 19.4) * 0.3)$

Equation 1. % sand : 100 – { (Corrected hydrometer reading at 40 sec x 100)/Oven dry weight of 40

G air-dried soil}

Equation 2 % clay : { (Corrected hydrometer reading at 2 hour x 100)/ Oven dry weight of 40

G air-dried soil}

Equation 3. % silt : $100 - (\%\ sand + \%\ clay)$

3.3 DETERMINATION OF BULK DENSITY

The most important consideration for bulk density determination is a known volume of the sample taken in the field. Therefore appropriate care must be taken when collecting the sample to ensure it is accurate as possible. Volume can be obtained from mathematical formula or directly from the reading. When using the mathematical formula, diameter of the top and bottom horizon of subsample and subsample depth should be known.

For the cylinder with same top and bottom horizon and half open face:

Equation 4. Sample volume (m⁻³) : $V = 0.5 * \pi * r^2 * h$

Equation 5. Bulk density (g m⁻³) : $\frac{dry\ mass\ (g)}{sample\ volume\ (m3)}$

STEPS FOR DETERMINING BULK DENSITY:

1. Transfer the soil to a container, place it in an oven at 105°C. Lower tempeture (ie. 60 °C) is favourable for bulk density samples that will be also used for C organic analysis.

2. After the samples are dried at 60 °C for at least 24 hours or stable weight is reached (no water remains in samples), cool it to room temperature before weighing.

3. Weight it and take the weight as sample dry mass. Note that the inorganic carbon matters (e.g., carbonate shells) should not be removed prior to bulk density analysis.

3.4 DETERMINATION OF ORGANIC CARBON

Three parameters must be obtained for soil organic carbon analysis, ie soil depth, soil bulk density, and soil OC content (%). The calculation of soil carbon mass per sample is:

Equation 6. <u>Soil C (Mg ha⁻¹) :</u> bulk density $(\mathbf{g}.\mathbf{m} - 3) * \textbf{soil depth } (\mathbf{cm}) * \%\mathbf{C}$

The organic carbon content (%OC) can be determined by several methods, including elemental analyzer (CHN analyzer), combustion for loss on ignition (LOI), and wet chemistry techniques (Figure 6). The use of these options depends on the availability of equipment, time and budgets.
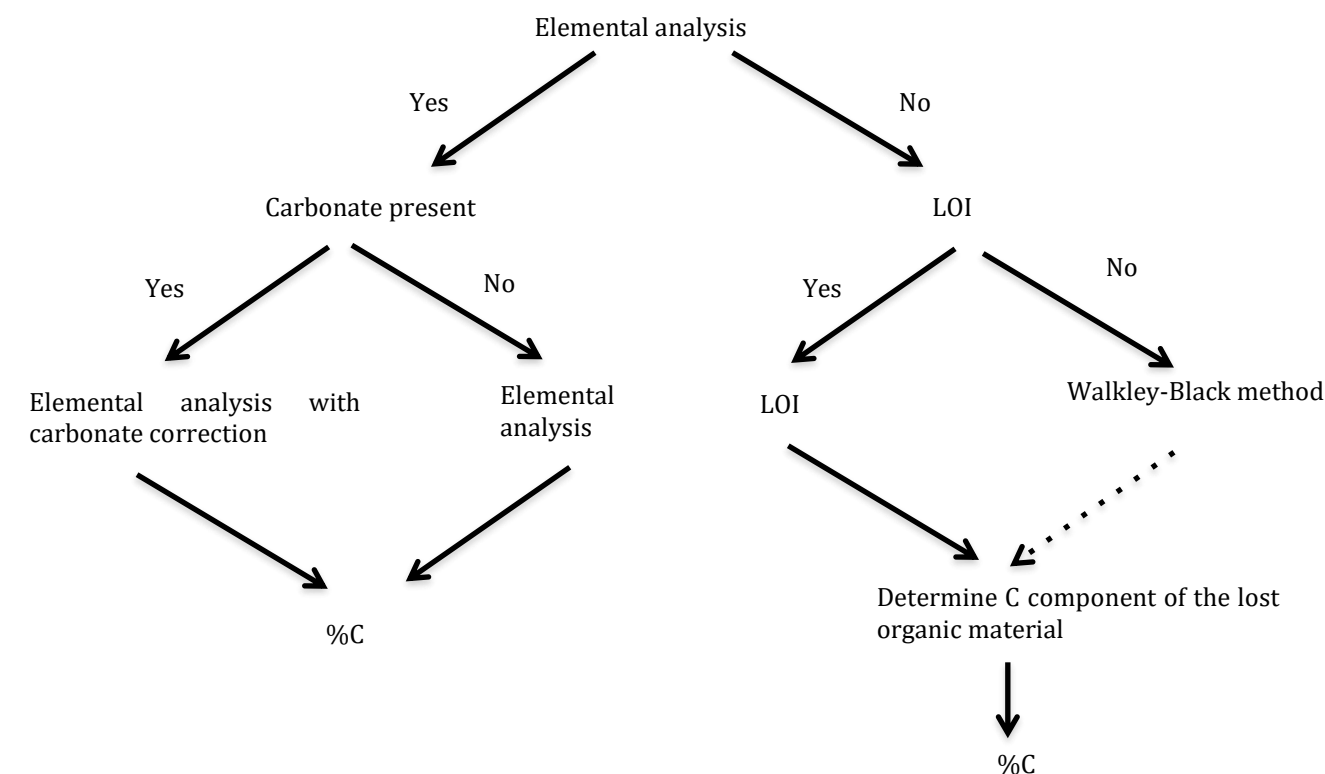
Figure 3. Options to determining the method used for soil organic C component (Howard et al, 2014).

Dry combustion method loss on ignition (LOI)		Wet combustion method H_2O_2 and dichromate digestion (Walkley-black method)	
PROS	CONS	PROS	CONS
Semi-quantitative measure of organic carbon content; low cost and simple technology.	Percent organic carbon determined from empirically derived relationships between carbon and organic matter.	Semi-quantitative measure of organic carbon content; low cost and simple chemistry.	H_2O_2 does not always digest carbon equally; produces hazardous waste.

Table 1 Comparison of laboratory analysis for %OC (adopted from Howard et al, 2014).

3.4.1 DETERMINATION OF ORGANIC CARBON BY LOSS ON IGNITION

The percent loss on ignition technique (often referred to as % LOI) is a measure of the mass of sample lost (e.g., oxidised and lost as gas, or volatilised) when heated to high temperatures. LOI represents the loss of organic matters and not solely the loss of C, thus, a relationship needs to be determined to relate % LOI to % C. An equation must be constructed that relates organic matter content (% lOI) to the organic C content (% OC) of the same sample by sending a limited number of samples for organic C analysis using an elemental analyser or by using a value from the literature for a study location/type. However, large range in the ratio of % C organic to organic matter (% LOI) may result in possible sources of

error in estimating organic C, so it is suggested to sending a few samples to a laboratory for elemental analysis for better accuracy.

Steps for determining %OC :

1. Homogenise the sample and break the large clumps with a spatula. Remove any large items (eg. stones, roots)
2. Take 2 g of sample and put it in ceramic crucible.
3. Heat the sample to combustion at 450 °C for 4–8 hours to ensure that only organic C is oxised.
4. Record the final mass after the combustion.

Equation 7. % loss on Ignition : $\left[\frac{mass\ B(mg) - mass\ A\ (mg)}{mass\ B(mg)}\right] * 100$

Where:

Mass A = dry mass after combustion (mg)

Mass B = dry mass before combustion (mg)

3.4.2 DETERMINATION OF ORGANIC CARBON BY WALKLEY AND BLACK'S WET OXIDATION METHOD

The organic fraction of the soil C includes plants, animals and microorganisms in all stages of decomposition. In dry combustion method, the C in the soil is oxidized to carbon dioxide at a very high temperature in a furnace. In wet oxidation method C is also oxidized to carbon dioxide and the amount loss is determined indirectly by titration (redox titration). The principle is that, organic C compounds are highly reducing substance is employed in the determination of organic C which reduces solution of oxidizing agents when digested with it. The method is known as wet combustion or wet oxidizing method for determining soil organic C.

STEPS FOR DETERMINING OC:

1. Prepare the chemicals:

4. Standard potassium dichromate solution (Preferably Normal (N), may be Molar (M)) (49.03 g $K_2Cr_2O_7$ in 1 l solution).

5. Ferrous solution (Prepare by dissolving 278 g $FeSO_4.7H_2O$ crystals in distilled water followed by an addition of 15 ml concentrated H_2SO_4 and diluting up to 1 l).

6. Concentrated sulphuric acid.

7. Glacial phosphoric acid

8. Sodium fluoride powder.

9. Diphenylamine indicator solution [Dissolve 0.5 g Diphenylamine in a mixture of 100 ml concentrated H_2SO_4 and 20 ml distilled water (add acid to water)].

10. Weight 0.5 g (dark soil) or 2 g (mineral soil) of sample, which has been passed through a 75 mesh sieve, and transfer the soil to a 500 ml clean dry conical flask (quantity of x depends on soil type).

11. Add 10 ml of standard potassium dichromate solution with a pipette and 10 ml of concentrated sulphuric acid with a measuring cylinder and mix thoroughly. (If the colour of the solution changes to green, add an additional 10 ml of $K_2Cr_2O_7$ solution. It is better to restart the procedure using lesser amount of soil). Allow the flask to cool on a sheet of asbestos with occasional shaking for half an hour or till the content becomes cool.

12. Add 150 mL distilled water, 10 ml phosphoric acid and 0.2 g of sodium fluoride powder to the content of the flask.

13. Add 2 ml (60 drops) of diphenylamine indicator solution. The colour of the solution will be deep violet. Additional amount of indicator may be necessary. Cool the flask again.

14. Titrate the excess of chromic acid left in the flask with the help of standard ferrous sulphate solution from burette. At the end point the colour of the solution will change to deep bottle green.

15. Record the amount of ferrous sulphate solution required in the titration.

16. Run a blank experiment in the same way with all the reagents except soil. Record the amount of ferrous sulphate solution (B, see the field form, APPENDIX 3) required blank experiment. From the data of blank experiment the strength of FeSO4 solution can be determined).

Strength: Substance taken in certain volume of solution/Substance required to prepare that volume of standard solution.

Strength of Potassium dichromate :

(49.03 g $K_2Cr_2O_7$ in 1L solution/ 49.03 g $K_2Cr_2O_7$ to prepare 1 L 1N solution = 1N solution).

Equation 9. Strength of Ferrous Sulphate (data from blank determination) :

$$V1 \ x \ S1 = \ V2 \ x \ S2$$

Where :
V_1 = Volume of $K_2Cr_2O_7$
S_1 = Strength of $K_2Cr_2O_7$
V_2 = Volume of $FeSO_4$
S_2 = Strength of $FeSO_4$

Calculation of Carbon:

1l of N $K_2Cr_2O_7$ solution $\equiv$ 1l $FeSO_4$ solution $\equiv$ 3 g OC
1 ml N $K_2Cr_2O_7$ solution $\equiv$ 1ml $FeSO_4$ solution $\equiv$ 0.003 g of C.

Thus, the amount of C in soil, expressed as percent, oxidized by N $K_2Cr_2O_7$ solution:

Equation 10. % OC :

$$\left[\frac{(B-T)*f}{W} * \ 0.003 * 100 \right]$$

Equation 11. % OC in soil :

$$\left[\frac{(B-T)*f}{W} * \ 0.003 * 1.3 * 100 \right]$$

$$\left[\frac{(B-T)*f}{W} * \ 0.39 \right]$$

Where:
B = Amount of N $FeSO_4$ solution required in blank experiment (ml)
T = Amount of N $FeSO_4$ solution required in experiment with soil (ml)
f = Strength of $FeSO_4$ solution
W = Weight of soil taken

It has been estimated that only about 77% C of the organic compounds in soil is oxidized by normal $K_2Cr_2O_7$ solution. So, 0.003 should be multiplied by 1.3 to get the total percentage of C present in the soil.

$$\%OM = \% \ OC \times 1.72$$

A sample of a page from Munsell Soil Colour Book is shown below. The page shows that the HUE is 10YR. If our sample corresponds the last chip of the sixth row, then the soil has a value= 3, and chroma =6. Hence, the soil colour is quoted as '10YR/3/6'.
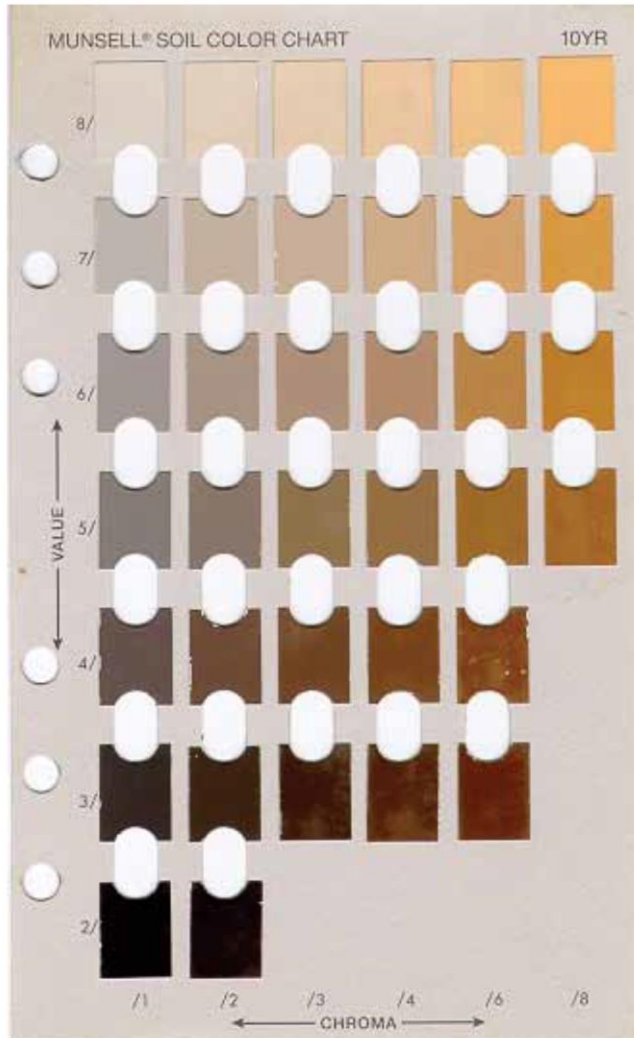
PLOT NUMBER	
LITTER AND SOIL	

SUBPLOT NUMBER	DEPTH OF LAYER INTERVAL (cm)	SOIL MOISTURE	SOIL COLOR	SOIL DEPTH OF HUMUS	SOIL TEXTURE	DEPTH OF SOIL COLLECTION	BULK DENSITY	NOTE SOIL	LITTER	NOTE LITTER
1	0-15					5-10				
1	15-30					20-25				
1	30-100					50-55				
2	0-15					5-10				
2	15-30					20-25				
2	30-100					50-55				
3	0-15					5-10				
3	15-30					20-25				
3	30-100					50-55				

F7B– LITTER AND SOIL   IN OTHER ZONES

PLOT NUMBER (711)	
LITTER AND SOIL (12)	

SUBPLOT NUMBER	DEPTH OF LAYER INTERVAL (cm)	SOIL MOISTURE	SOIL COLOR	SOIL DEPTH OF HUMUS	SOIL TEXTURE	DEPTH OF SOIL COLLECTION	BULK DENSITY	NOTE SOIL	LITTER	NOTE LITTER
1	0-15					5-10				
1	15-30					20-25				
2	0-15					5-10				
2	15-30					20-25				
3	0-15					5-10				
3	15-30					20-25				
4	0-15					5-10				
4	15-30					20-25				
5	0-15					5-10				
5	15-30					20-25				

FORMS FOR LABORATORY ANALYSIS

Table for bulk density and LOI

PLOT	SUBPLOT	LAYER	VOLUME (cm^3)	WET WEIGHT (g)	DRY WEIGHT (g)	BULK DENSITY	WEIGHT LOI	(mg)	%LOI
							BEFORE	AFTER	

Table for texture

PLOT	SUBPLOT	LAYER	READING	% SAND	% CLAY	%SILT

Table for litter

PLOT	SUBPLOT	WET WEIGHT (g)	COMPOSITE (g)		RATIO
			WEIGHT	DRY	
1	1				
	2				
	3				
2	1				
	2				
	3				

Table for data OC by Walkley and Black's wet oxidation method

Experiment with soil

No. of observation	Initial burette reading (IBR) (a)	Final burette reading (FBR) (b)	Difference (b - a)	Mean
1.				
2.				
3.				

Experiment without soil

No. of observation	Initial burette reading (IBR) (a)	Final burette reading (FBR) (b)	Difference (b - a)	Mean
1.				
2.				
3.				

APPENDIX 4. LIST OF EQUIPMENT

Items	Analysis	Function
Soil Auger (140 cm)	Bulk density - Carbon sampling	To collect samples in mangroves
Plastic bag (Zip lock) medium size	Bulk density - Carbon sampling	Sample media
Plastic bag (Zip lock) large size	Bulk density - Carbon sampling	Sample media
Kitchen knife	Bulk density - Carbon sampling	To slice the samples mangroves
Plastic Jar (200g) small	Bulk density - Carbon and Texture sampling	Sample media
Plastic Jar (500g) large	Texture sampling	Sample media soil composite to lab
Soil Core Rings	Bulk density - Carbon sampling	Sample media
Soil Core Sampler	Bulk density - Carbon sampling	To insert the rings
Hammer	Bulk density - Carbon sampling	To insert the rings
Wood	Bulk density - Carbon sampling	To insert the rings
Shovel Garden Shovel	Bulk density - Carbon sampling	To keep a set of samples per plot
Bangla Shovel	Bulk density - Carbon sampling	To dig the soil
Soil Auger (30 cm)	Texture sampling	To collect samples
Soil Color chart (water proof)	Soil colour	To identify soil colour
Transect	Litter	To transect litter
Plastic bags	Litter	Sampe media
Garden secateurs	General	To clear soil surface
Large Plastic sheet	General	To place the instrument in field
Knife	General	To cut samples
Dry cloth	General	To clean instrument
Synthetic cloth - chamois	General	To clean instrument
Lubricant	General	To clean instrument
Small stool	General	To prapare samples
Bucket (20L)	General	To collect water or prapare composite
Masking tape	General	To label the samples
Permanent marker	General	To label the samples

STEPS FOR SOIL TEXTURE COLLECTION

SOIL COLLECTION USING AUGER

Insert the auger by rotating clockwise to a depth of at least 30cm, and then pull it out slowly.	Place the auger in the big plastic sheet and collect the sample	Put the sample in plastic bag or jar

SOIL COLLECTION FOR HARD SOIL

Dig a pit and measure the depth of soil	Mark the depth of sample layer and collect the sample using spade	Put the sample in plastic bag or jar

APPENDIX 6. STEPS FOR SOIL BULK DENSITY COLLECTION

Push the core sampler into the soil	Extract the sample using a field knife or shovel	Level the surface of the soil sample on both sides and ensure that soil is completely level with edge of the core.

4 REFERENCES

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