Training Manual
Sample Processing and Laboratory Analysis for the Development of Allometric Equation

Training Manual: Sample Processing and Laboratory Analysis for the Development of Allometric Equation

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Contact:
Forest Department
Ministry of Environment and Forests
Government of the People's Republic of Bangladesh
Bana Bhaban, Plot No- E-8, B-2
Agargaon, Sher-e-Bangla Nagar
Dhaka-1207
info@bforest.gov.bd

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Disclaimer

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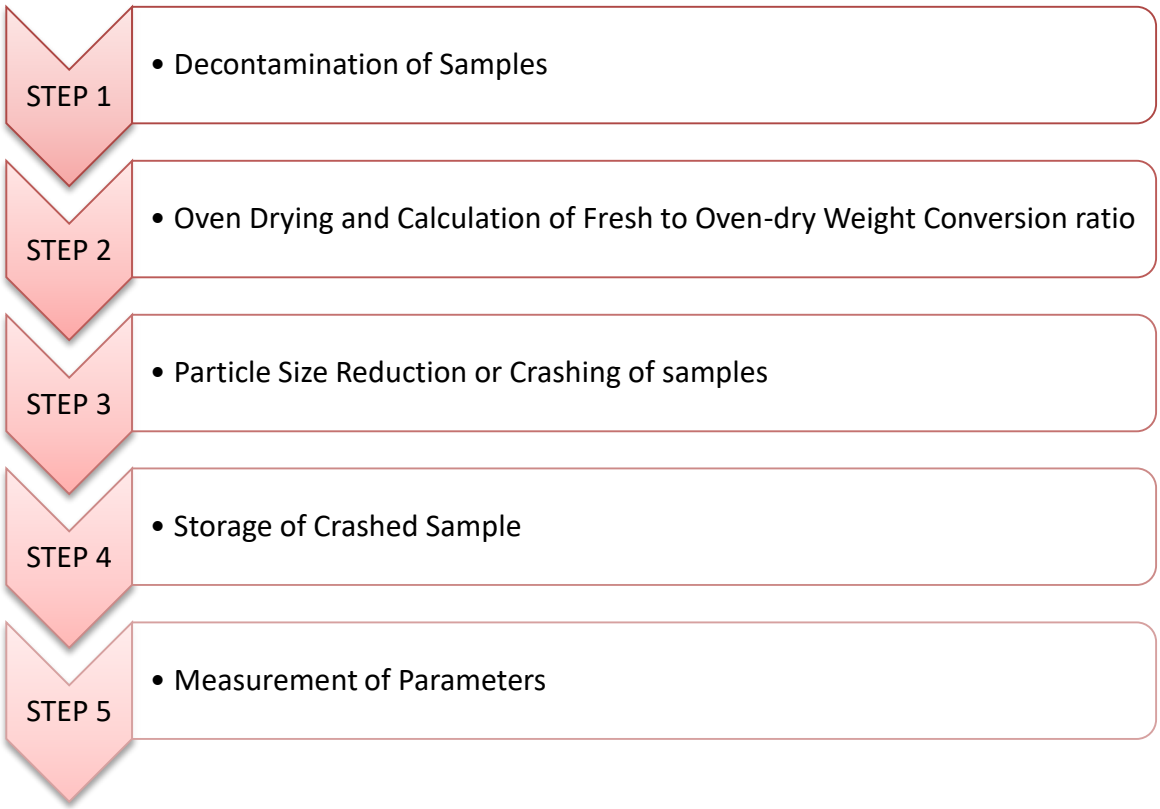
1. INTRODUCTION

Development of allometric equation includes five basic tasks e.g. planning of field work, field work, laboratory analysis, data compilation and selection of best fit equation. Laboratory analysis is a critical task to obtain accurate data and reliable interpretation of results. Therefore, standard methods should be followed during the sample drying, processing (crushing, sieving and storing), analysis of carbon, nitrogen, phosphorus and potassium concentration in plant parts (leaves, branches, bark and stem). Each of the procedure provides opportunities to enhance the accuracy and reliability of the analytical results. Amount of sample collected from the field in terms of weight and proper coding of samples (Appendix 1) are very important issue in sample processing and analysis. Usually 250 g of fresh sample/sub-sample gives good result for oven-drying process, which may homogenize the oven-drying time.

This manual aims to provide guidance to technicians, professionals, and students in sample processing and laboratory analysis for the development of allometric equation. It is believed that this manual will be helpful at present and in the future to ensure accuracy and reliability in analysis of plant samples for the development allometric equation. Sample processing and laboratory analysis for the development of allometric equation consist of following five steps.



2. STEP 1: DECONTAMINATION

2.1. PRINCIPLE

Plant materials must be clean and free from extraneous substances, including soil and dust particles that may influence analytical results. The elements most often affected by soil and dust particles are iron (Fe), aluminum (Al), silicon (Si), and manganese (Mn), especially with seedling and grass crops. Decontamination procedure involves brushing, washing and rinsing of fresh, fully turgid plant samples

2.2 REAGENT AND APPARATUS

- Deionized water
- 0.1 to 0.3% detergent solution (non-phosphate)
- Medium-stiff nylon bristle brush or sponge
- Plastic containers suitable for washing and rinsing tissue samples

2.3 PROCEDURE

- Examine fresh plant tissue samples to determine physical condition and extent of contamination. Unless leaf tissue is visibly coated with foreign substances, decontamination is usually not required except when Fe, Al, Si, or Mn are to be determined
- When Al, Si, Mn, and Fe are not of primary interest, plant leaves should be brushed briskly to remove visible soil and dust particles.
- When plant samples show visible residues from spray applications and when Al, Si, Fe and Mn are elements of interest, leaves should be washed in 0.1 to 0.3% detergent solution followed by rinsing in deionized water.
- The wash and rinse periods should be as short as possible to avoid leaching of nitrate (NO_3), boron (B), potassium (K), and chloride (Cl) from the plant tissue
- After decontamination, samples should be dried immediately to stabilize the tissue and stop enzymatic reactions.

2.4 COMMENTS

- When proper sampling techniques have been utilized, decontamination should be minimized.
- Decontamination is generally not necessary where tissue has been exposed to frequent rainfall and/or not exposed to nutrient or fungicidal sprays.
- Excessive washing is likely worse than no decontamination since soluble elements including B, K, and NOi-N are likely to leach from the tissue.
- Samples should be dipped quickly in the wash and rinse solutions. Recommended time is 15 seconds.

3. STEP 2: OVEN DRYING AND CALCULATION OF FRESH TO OVEN-DRY WEIGHT CONVERSION RATIO

3.1. PRINCIPLE

Moisture is removed from plant tissue to stop enzymatic reactions and to stabilize the sample for accurate weighing.

3.2 APPARATUS

- Forced-air oven

3.3 PROCEDURE

- Warp the individual sample with paper or aluminium foil along with sample ID or code
- Place samples inside the oven and dry at 80 °C until constant weight by checking weight periodically.
- Measure the sample weight using laboratory balance and record in the following prescribed data form 1
- Calculate fresh weight to oven-dry weight conversion ratio and record in the Data form 2
- **Note:** The length of drying time varies with sample nature and its moisture content

3.4 COMMENTS

- Drying at temperature below 80 °C may not able to remove all moisture and may result in poor homogenization and incorrect analytical results.
- Drying temperatures above 80 °C may result in thermal decomposition and reduction in dry weight.
- If samples absorb significant amounts of moisture during grinding, additional drying may be required prior to weighing for analysis.
- Oven dry weight until constant weight requires periodic weighing

Data form 1

Sample ID	Sub-sample fresh weight (kg)	Sub-sample weight at laboratory (kg)	Sub-sample oven-dry weight (kg)	Conversion ratio = $\frac{\text{Oven dry weight (kg)}}{\text{Fresh weight (kg)}}$
Average				

Data form 2

Plant part	Conversion ratio
Leaves	
Leaf containing smaller branches	
Smaller branches	
Bigger branches without bark	
Stem/ bole without bark	
Bark of bigger branches	
Bark of stem/ bole	

4 STEP 3: PARTICLE SIZE REDUCTION OR CRASHING OF SAMPLES

4.1 PRINCIPLE

Plant tissue samples are reduced to 0.5 to 1.0 mm particle size to ensure homogeneity and to facilitate organic matter destruction and other chemical analysis.

4.2 APPARATUS

- Standard Wiley mill equipped with 2 mm, 1 mm, 0.5 mm and 0.25 mm sieve screens and stainless steel contact points, or equivalent high-speed grinder
- Medium bristle brush

4.3 PROCEDURE

- Crash the sample separately and using a brush to clean the grinding apparatus
- The crashed samples should be pass through a 2 mm or 1 mm sieve
- 2 mm or 1 mm sieve is adequate for sample aliquot to be assayed is > 0.5 g
- 0.5 mm sieve is appropriate for sample aliquot to be assayed is < 0.5 g
- About 5 to 10 g of sieved sample are kept for analysis and storage

4.4 COMMENTS

- Uniform grinding and mixing are critical in obtaining accurate analytical results
- Special care should be taken for grinding very small samples and samples with fibrous texture. In these case high speed grinders are preferable
- Most mechanical grinders contribute some contamination of the sample with one or more elements. The extent of contamination depends on the condition of the grinders and exposure time. Stainless steel grinder and cutting apparatus are recommendation to reduce the contamination
- Cutting knives or blades should be maintained in sharp condition and in proper adjustment.

Figure 1: Standard Wiley mill and sieve

5 STEP 4: STORAGE OF CRASHED SAMPLE

5.1 PRINCIPLE

After crashing and sieving, samples should be stored properly to minimize deterioration and to maintain sample integrity for further analytical work.

5.2 APPARATUS

- Air-tight plastic storage containers
- Storage cabinet located in cool, dark, moisture-free environment
- Refrigerator

5.3 PROCEDURE

- After grinding, sieving and homogenization, a representative sample is taken from the ground plant material for analysis and storage. The sample should be placed in a container and securely sealed.
- Containers should be stored under cool and dry condition
- For long-term storage, samples should be sealed air tightened and placed into refrigerator at 4 °C until analyses

5.4 COMMENTS

- If samples are placed in a cool (4°C), dark, dry environment, storage life is indefinite
- Coin envelopes can also be used for sample storage; however, somewhat greater care must be exercised in sample handling to prevent absorption of moisture. Collecting the ground sample in the envelope and immediately placing into a desiccation cabinet or jar will minimize moisture absorption.

Figure 2: Container for sample storing

6. STEP 5: MEASUREMENT OF PARAMETER

6.1 MEASUREMENT OF WOOD DENSITY

6.1.1 PRINCIPLE

Wood density of samples can be estimated from the following formula

$$\text{Wood density (g/cm}^3\text{)} = \frac{\text{Oven-dried Sample weight (g)}}{\text{Volume of fresh sample } (cm^3)}$$

6.1.2 APPARATUS

- Forced-air oven
- Digital balance
- Measuring cylinder
- Slide callipers

6.1.3 PROCEDURE

- Take sub-samples (2.5 cm x 2.5 cm x 7.5 cm) of wood from each stem disk (periphery to the center of disk).
- Measure the sample volume using water displacement method after re-saturation of water or estimate from the dimension of the samples and record in data form.
- Oven-dry the sub-samples at 105 °C until constant weight
- Take oven-dry weight of the sub-samples using high precision laboratory digital balance and record in data form 3.
- Calculate the wood density by using the above equation for each sample and average the estimates to get final wood density.

6.1.4 COMMENTS

- Sample should take from different parts of the tree stem and even different portion of the stem disk
- When measuring the wood volume by water displacement method re-saturation of wood sample should be done by immersing into the water for 24 hours.

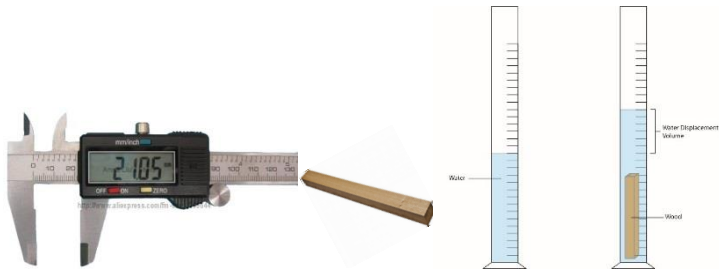


Figure 3: Measurement of wood volume using water displacement method

Data form 3

Sample ID and code	Sub-sample ID and code	Oven-dry weight of sub-sample (g)	Volume of sub-sample (cm^3)	Wood density (g/ cm^3) = $\frac{\text{Oven dry biomass (g)}}{\text{Volume (cm3)}}$

6.2 ORGANIC CARBON DETERMINATION FOR PLANT PARTS

6.2.1 PRINCIPLE

Organic carbon of plant sample can be determined from Loss of Ignition method. The Loss of Ignition (LOI) can be determined with the following formula

$$\text{LOI}\ (\%) = \frac{\text{Loss of weight after ignition (g)}}{\text{Ovendry initial weight (g)}} \text{ x } 100$$

Estimate the organic carbon (%) from the 50% of LOI of the sample (Allen, 1989)

6.2.2 APPARATUS

- Porcelain cup
- High precision digital balance
- Forced-air oven
- Maffle furnace
- Desiccator

6.2.3 PROCEDURE

- Take dry and clean porcelain cup
- Measure the weight of the porcelain cup
- Take approximately 1 g of plant sample in that porcelain cup
- Oven dry the sample with the porcelain cup at 105 °C for 1 hour to make the sample free from moisture
- Take weight of the sample along with the porcelain cup and keep record
- Place the porcelain cup into the Maffle Furnace and raise the temperature slowly up to 450 °C
- Let the sample burn in the furnace for 4 to 6 hour at 450 °C
- After burning, let the furnace cool down and take away the sample and place into a desiccator for further cooling
- Again measure the weight of remaining sample with porcelain cup by the balance and keep all records in data form 4
- Calculate the LOI% and Organic carbon content from the equation.

6.2.4 COMMENTS

- Time for oven-drying of the sample at 105 °C may varies with sample types
- While running the Maffle furnace in laboratory Air Condition should be shut down because the furnace generates considerable heat and the furnace should keep in observation for accidental firing

Figure 4: Porcelain cup, high precision analytical balance and muffle furnace

Data form 4

Sample ID/code	Weight of cup (g)	Sample weight (g)	Cup + sample weight (g)	Cup + Sample weight at 105 °C (g)	Sample weight at 450 °C (g)	Cup + Sample weight at 450 °C (g)	Sample weight at 450 °C (g)	Loss on ignition (%)	Organic carbon (%) = loss of ignition x$\frac{50}{100}$

6.3 KJELDAHL DIGESTION FOR TOTAL NITROGEN

6.3.1 APPARATUS

- Block digester and digestion tube
- JP pump and fume hood
- Micro pipette
- Digital balance
- Filter paper (Whatman no-1)
- Volumetric flux
- Funnel

6.3.2 CHEMICALS

- Potassium sulphate (K_2SO_4)
- Copper sulphate ($CuSO_4$)
- Selenium powder (Se)
- Sulphuric acid (H_2SO_4)

6.3.3 PROCEDURE

- Take 0.1 g of plant sample in the digestion tube
- Add 1.1 g catalyst mixture (Potassium sulphate (K_2SO_4), Copper sulphate ($CuSO_4$) and Selenium powder (Se) in the proportion of 100 : 10 : 1
- Add 3 ml of Sulphuric acid (H_2SO_4) and heat continuously in a block digester to oxidize the organic matter at 200 °C for 15 minutes
- Raise temperature at 400 °C and heat continuously for 30 minutes
- Cool the digest and add distilled/deionized water
- Filter the digest through filter paper (Whiteman No-1) and diluted to 100 ml
- Store the digest in air tight plastic container at 4 °C for long-term storage in refrigerator

6.3.4 COMMENTS

- Catalyst Mixture should have prepared by maintaining the ratio

  $K_2SO_4 : CuSO_4 : Se = 100:10: 1$

  - Take the following chemical with the given amount (for 20 samples)

K_2SO_4	21.62 g
$CuSO_4$	2.16 g
Se	0.22 g

- For the digestion of 20 samples take 65 ml of Sulphuric acid (H_2SO_4) into a beaker and then give 3 ml acid to each digestion tube using pipette

6.3.5 ANALYSIS OF TOTAL NITROGEN

6.3.5.1 APPARATUS

- Volumetric flux
- Beaker
- Micro pipette
- Glass pipette
- Magnetic stir
- Vortex mixture
- Distilled/ Deionized water
- UV-Spectrophotometer
- Test tube

6.3.5.1 CHEMICALS

- $Na_2HPO_4.12H_2O$
- Na-K tartrate
- NaOH (Pellets)
- Na Salicylate
- Na Nitroprusside
- Na hypochlorite (Clorax)
- K_2SO_4
- $CuSO_4$
- NH_4Cl

Figure 5: Block digester and filtration activity

6.3.5.2 PROCEDURE

Task 1: Solution preparation

1.1. Solution 1: Working Buffer Solution (for 180 samples, 5.5 ml for each sample)

$Na_2HPO_4.12H_2O$	35.8 g	Dilute to 1 litter with Distilled Water (DW)	Store in a cold place
Na-K tartrate	50 g		
NaOH (Pellets)	54 g		

1.2. Solution 2: Na salicylate-Na Nitroprusside solution (for 250 samples, 4 ml for each sample)

Na Salicylate	150 g	Dilute to 1 litter with DW	Store in a light resistant bottle
Na Nitroprusside	0.30 g		

1.3. Solution 3: Na Hypochlorite Solution (for 250 samples, 2 ml for each sample)

5.25% Na hypochlorite (clorax)	30 ml	Dilute to 500 ml with DW	Prepare fresh daily

Task 2: Nitrogen Standard solution preparation

2.1. Diluent preparation

K_2SO_4	9.91 g	Dilute to 1 litter with 1.1M H_2SO_4 (60 ml 98% H_2SO_4 in 1L DW)	Store it to prepare standard solution
$CuSO_4$	0.991 g		

2.2. Stock solution preparation (1000 ppm)

Dry NH_4Cl (Dry NH_4Cl at 105°C)	1.9095 g	Dilute to 500 ml with diluent	Nitrogen (N) stock 1000 ppm or mg N/L

Dilute the stock 10 times to prepare 100 ppm standard Nitrogen solution

1000 ppm stock	10 ml	Dilute to 100 ml with diluent	Nitrogen (N) stock 100 ppm or mg N/L

2.3. Graduated standard solution preparation for standard curve

Standard N (ppm)	Amount of 100 ppm N Stock required (ml)	Final Volume (ml)
0 (Blank)	Diluent	-
5	2.5	50
10	5.0	50
15	7.5	50
20	10.0	50

*Working range 0-50 ppm

Task 3: Colorimetric determination of N

- Dilute the digest as required (Generally plant leaf sample is diluted 50 times and 5 times for other parts if 0.1 g plant sample is taken for Kjeldahl digestion)
- Take 1 ml aliquot/diluted aliquot of digest in a test-tube
- Add 5.5 ml of solution-1 and stir with a vortex mixer
- Add 4 ml of solution-2 and mix again
- Add 2 ml of solution-3 and mix thoroughly
- Let stand for 45 minutes at 25 °C (or 15 minutes at 37 °C)
- Do same thing as describe from 2-6 with the graduated standard solution including blank
- After immediate stirring with vortex, read absorbance in a spectrophotometer using a wavelength of 650 nm. Take reading within 120 minutes of sample preparation.
- Prepare standard curve from the absorbance with the standard in the spectrophotometer and use data form 5 for standard
- Record the concentration reading using spectrophotometer in data form 6
- Calculate the Total Kjeldahl Nitrogen (TKN) with the following equation

TKN (mg/g) = (C × df × fv) ÷ (W × 1000)

Where,

C = Concentration obtained from spectrophotometer in ppm or mg N/L
df = Dilution factor (times)
fv = Final volume of the digest (ml)
W = Weight of soil/plant taken in digest (g)

6.3.5.3 COMMENTS

- Dilution of sample should be done with diluent not with the distilled/deionized water
- UV-spectrophotometer should be started 15 minutes before taking reading

Data form 5
Standard

Concentration (ppm)	Absorbance	Equation with R^2 value
0		
5		
10		
15		
20		

Data form 6
Samples

Sample Code	Dilution Factor	Absorbance	Concentration (ppm)

6.4 TRI-ACID DIGESTION FOR TOTAL PHOSPHORUS AND POTASSIUM DETERMINATION

6.4.1 APPARATUS

- Block digester and digestion tube
- JP pump and fume hood
- Micro pipette
- Digital balance
- Filter paper (Whatman no-42)
- Volumetric flux
- Funnel

6.4.2 CHEMICALS

- Concentrated Nitric acid (HNO_3)
- Perchloric acid 60% ($HClO_3$)
- Sulphuric acid (H_2SO_4)

6.4.3 PROCEDURE

- Take 0.1 g of plant sample in the digestion tube
- Add 3 ml concentrated Nitric acid and heat continuously in block digester to oxidize the organic matter at 100 °C for 50 to 60 minutes
- Add 6.4 ml of mixed acid (Nitric acid, Perchloric acid 60% and Sulphuric acid mixed at the proportion of 10:2:1) to the predigested samples and raise the temperature up to 200 °C and digest for 20 minutes
- Cool the digest and add distilled/ deionized water
- Filter the digest through filter paper (Whatman No 42) and diluted to 100 ml
- Store in air tight plastic container and store at 4 °C for long term storage into the refrigerator.

6.4.4 COMMENTS

- For the digestion of 20 samples take 65 ml of Nitric acid into a beaker and then give 3 ml acid to each digestion tube using pipette
- Take the following acids with the given amount (for 20 samples) in a beaker for the preparation of mixed acid

Nitric acid	100 ml
Perchloric acid	20 ml
Sulphuric acid	10 ml

- Then mix the acids carefully and give 6.4 ml of mix acid to each digestion tube using pipette.
- Fume hood and JP pump must be used during Perchloric acid digestion

6.4.5 ANALYSIS OF TOTAL PHOSPHORUS

6.4.5.1 APPARATUS

- Volumetric flux
- Beaker
- Micro pipette
- Glass pipette
- Magnetic stir
- Vortex mixture
- Distilled/ Deionized water
- UV-Spectrophotometer
- Test tube
- Measuring cylinder

6.4.5.2 CHEMICALS

- Ammonium Molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$)
- L-Ascorbic Acid
- Antimony potassium tartrate
- Sulfuric acid (H_2SO_4)
- Potassium bi phosphate (KH_2PO_4)

6.4.5.3 PROCEDURE

Task 1: Solution preparation

1.1. **Solution 1:** Ammonium molybdate solution

Ammonium Molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$)	4 g	Dilute to 100 ml with DW at 50°C on a hot plate	Cool it and store

1.2. **Solution 2:** Ascorbic Acid Solution

L-Ascorbic Acid	2.64 g	Dilute to 50 ml with DW	Store in refrigerator at ~2°C

1.3. **Solution 3:** Antimony potassium tartrate Solution

Antimony potassium tartrate	0.1454 g	Dilute to 50 ml with DW	Store

1.4. **Solution 4**: Sulfuric acid (2.5 M)

Sulfuric acid (H_2SO_4)	35 ml	Dilute to 250 ml with DW	Add acid drop wise to the water

1.5. **Mixed Solution:** Murphy Riley Color developing solution

Solution 4 Solution 1 Solution 2 Solution 3	250 ml 75 ml 50 ml 25 ml	Dilute to 500 ml with DW	Take the reagent accordingly and Mix with a magnetic stirrer. Store in a dark place

Task 2: Phosphorus Standard solution preparation

2.1. Stock solution preparation (50 ppm)

Dry KH_2PO_4 (Dry KH_2PO_4 at 105°C)	0.1098 g	Dilute to 500 ml with DW. Before doing final volume add 3 ml Concentrated H_2SO_4.	Phosphorus (P) stock 50 ppm or mg P/L

2.2. Stock Solution (5 ppm)

50 ppm stock	10 ml	Dilute to 100 ml with DW.	Phosphorus (P) stock 5 ppm or mg P/L

2.3. Graduated standard solution preparation for standard curve

Standard P (ppm)	Amount of 5 ppm P Stock required (ml)	Final Volume (ml)
0 (Blank)	DW	-
0.2	2	50
0.4	4	50
0.6	6	50
0.8	8	50
1.0	10	50

*Working range 0.1-1.0 ppm

Task 3: Colorimetric determination of P

- Dilute the digest as required (Generally no dilution is required if 0.1 g plant sample and 0.5 g soil sample is taken for HNO_3+$HClO_4$ acid digestion)
- Take 10 ml aliquot/diluted aliquot of digest in a 50 ml volumetric flux
- Add 8 ml Mixed Solution (see 1.5)
- Make to a volume (50 ml) with DW, shake and allow 15 min for color development.
- Prepare the standard as same the procedure described in Task 2
- Measure the absorbance of the standards and samples with UV-Spectrophotometer at 880 nm wavelength and record in data form 7.

- Prepare standard curve from the absorbance with the standard in the spectrophotometer with a R^2 approximately 0.99.
- Record the concentration of samples using spectrophotometer and record in data form 8.
- Calculate the Total Phosphorus (TP) with the following equation

$$\mathbf{P\ (mg/g) = (C \times df \times fv) \div (W \times 1000)}$$

Where,

C = Concentration obtained from spectrophotometer in ppm or mg P/L
df = Dilution factor (times)
fv = Final volume of the digest (ml)
W = Weight of soil/plant taken in digest (g)

6.4.5.4 COMMENTS

- Care should be taken when working with acid. It can burn your skin.
- Mixed solution should be made by adding the solution in a sequence of 4, 1, 2, 3.

Data form 7
Standard

Concentration (ppm)	Absorbance	Equation with R^2 value
0		
5		
10		
15		
20		

Data form 8
Sample

Sample Code	Dilution Factor	Absorbance	Concentration (ppm)

6.4.6 FLAME EMISSION DETERMINATION OF POTASSIUM

6.4.6.1 APPARATUS

- Test tube
- Volumetric flux
- Distilled/ Deionized water

6.4.6.2 CHEMICALS

- Flame Photometry Standard 1000 ppm Potassium

6.4.6.3 PROCEDURE

Task 1: Preparation of Solution for Standard Curve

For preparation of standard curve, prepare solution of 0, 5, 10ppm from the stock solution of Flame Photometry Standard 1000 ppm Potassium as follows

Amount of stock solution	Dilution amount	Final Concentration
1 ml	100 ml	10 ppm
0.5 ml	100 ml	5 ppm
Nil	100 ml	0 ppm

- Take 1 ml of 1000 ppm stock K-standard in a 100 ml volumetric flux and fill the flux with distilled water to make a 10 ppm K-standard
- Again take 0.5 ml K standard (1000 ppm) in 100 ml volumetric flux to make 5 ppm K-standard
- Use distilled water as blank

1. **Task 2: Analysis of K in Flame Photometer**
    - Start the flame photometer and run the machine for about 20 minutes before starting measurement
    - Start ignition and control gas output until the color of the flame becomes bluish-green
    - Dilute the digest as required (Adding 1 ml sample and 9 ml distilled water makes the sample 10 times diluted)
    - Take the sample in flame photometer for the measurement of Potassium (K)
    - Take the absorbance of standards and then take the absorbance of each sample.
    - Keep records of all absorbance
    - Calculate a straight-line equation for the concentration as a function of absorbance of K-standards by using excel
    - Then, calculate the K-concentration of each sample by using the standard equation

Finally, calculate the Potassium (K) content of tree components with the following equation

K (mg/g) = (C × df × fv) ÷ (W × 1000)

Where,

C = Concentration obtained from Flame Photometric absorbance in ppm

df = Dilution factor (times)

fv = Final volume of the digest (ml)

W = Weight of soil/plant taken in digest (g)

Data form 9

Standard

Concentration (ppm)	Absorbance	Equation with R^2 value
0		
5		
10		

Data form 10

Sample

Sample Code	Dilution Factor	Absorbance	Concentration (ppm)

6.5 COMPILATION OF BIOMASS DATA

Fresh weight of each part of plant will be multiplied by the fresh to oven-dry weight conversion factor to get the oven-dry weight of the respective plant part

Plant part	Conversion ratio
Leaves	
Leaf containing smaller branches	
Flowers	
Fruits	
Smaller branches	
Bigger branches without bark	
Stem/ bole without bark	
Bark of bigger branches	
Bark of stem/ bole	

Sample tree no	**Fresh weight of tree parts (kg)**												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Leaves	Flowers	Fruits	Leaf containing smaller branches	Smaller branches	Bigger branches with bark	Bigger branches without bark	Bark of bigger branch	Bole with bark	Bole without bark	Bark of bole	Total bark	Total AGB = (1+2+3+4+5+7 +10+12)

Sample tree no	**Oven-dried biomass of tree parts (kg)**												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Leaves	Flowers	Fruits	Leaf containing smaller branches	Smaller branches	Bigger branches with bark	Bigger branches without bark	Bark of bigger branch	Bole with bark	Bole without bark	Bark of bole	Total bark	Total AGB = (1+2+3+4+5+7 +10+12)

6.6 COMPILATION OF CARBON DATA

Oven-dry weight (ODB) of each part of plant will be multiplied by the carbon concentration of respective plant part to get carbon amount in that part

Plant part	Carbon concentration (%)
Leaves	
Leaf containing smaller branches	
Flowers	
Fruits	
Smaller branches	
Bigger branches without bark	
Stem/ bole without bark	
Bark of bigger branches	
Bark of stem/ bole	

Sample tree no	Carbon in plant parts (kg)												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	ODB of Leaves X CC	ODB of Flowers X CC	ODB of Fruits X CC	ODB of leaf containing smaller branches X CC	ODB of Smaller branches X CC	ODB of Bigger branches with bark X CC	ODB of Bigger branches without bark X CC	ODB of Bark of bigger branch X CC	ODB of Bole with bark X CC	ODB of Bole without bark X CC	ODB of Bark of bole X CC	CC in Total bark (8+11)	Total carbon in AGB = (1+2+3+4+5 +7+10+12)

*CC= Carbon Concentration

6.7 COMPILATION OF NUTRIENT DATA

Oven-dry weight (ODB) of each part of plant will be multiplied by the nitrogen concentration of respective plant part to get nitrogen amount in that part

Plant part	Nitrogen concentration (mg/g)
Leaves	
Leaf containing smaller branches	
Flowers	
Fruits	
Smaller branches	
Bigger branches without bark	
Stem/ bole without bark	
Bark of bigger branches	
Bark of stem/ bole	

Sample tree no	Nitrogen in plant part (kg)												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	ODB of Leaves X NC	ODB of Flowers X NC	ODB of Fruits X NC	ODB of leaf containing smaller branches X NC	ODB of Smaller branches X NC	ODB of Bigger branches with bark X NC	ODB of Bigger branches without bark X NC	ODB of Bark of bigger branch X NC	ODB of Bole with bark X NC	ODB of Bole without bark X NC	ODB of Bark of bole X NC	NC in Total bark (8+11)	Total Nitrogen in AGB = (1+2+3+4+5 +7+10+12)

*NC= Nitrogen concentration

6.8 COMPILATION OF PHOSPHORUS DATA

Oven-dry weight (ODB) of each part of plant will be multiplied by the phosphorus concentration of respective plant part to get phosphorus amount in that part

Plant part	Phosphorus concentration (mg/g)
Leaves	
Leaf containing smaller branches	
Flowers	
Fruits	
Smaller branches	
Bigger branches without bark	
Stem/ bole without bark	
Bark of bigger branches	
Bark of stem/ bole	

Sample tree no	Phosphorus in plant part (kg) / (g)												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	ODB of Leaves X PC	ODB of Flowers X PC	ODB of Fruits X PC	ODB of leaf containing smaller branches X PC	ODB of Smaller branches X PC	ODB of Bigger branches with bark X PC	ODB of Bigger branches without bark X PC	ODB of Bark of bigger branch X PC	ODB of Bole with bark X PC	ODB of Bole without bark X PC	ODB of Bark of bole X PC	PC in Total bark (8+11)	Total Phosphorus in AGB = (1+2+3+4+5 +7+10+12)

*PC= Phosphorus concentration

6.9 COMPILATION OF POTASSIUM DATA

Oven-dry weight (ODB) of each part of plant will be multiplied by the potassium concentration of respective plant part to get potassium amount in that part

Plant part	Potassium concentration (mg/g)
Leaves	
Leaf containing smaller branches	
Flowers	
Fruits	
Smaller branches	
Bigger branches without bark	
Stem/ bole without bark	
Bark of bigger branches	
Bark of stem/ bole	

Sample tree no	Potassium in plant part (kg)												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	ODB of Leaves X KC	ODB of Flowers X KC	ODB of Fruits X KC	ODB of leaf containing smaller branches X KC	ODB of Smaller branches X KC	ODB of Bigger branches with bark X KC	ODB of Bigger branches without bark X KC	ODB of Bark of bigger branch X KC	ODB of Bole with bark X KC	ODB of Bole without bark X KC	ODB of Bark of bole X KC	KC in Total bark (8+11)	Total Potassium in AGB = (1+2+3+4+5 +7+10+12)

*KC= Potassium concentration

APPENDIX 1: CODING OF SAMPLES AND SUB-SAMPLES FOR PLANT PARTS

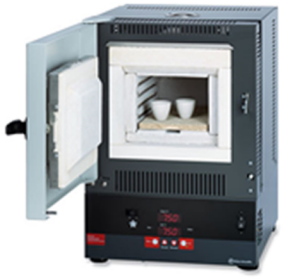
Level 1 (Plant life form)	Code	Example for code of trees	Level 2 (Plant parts)	Code for plant parts	Example of Code for fresh weight	Level 3 (Sub-sample)	Code for sub-samples	Example of code for sub-samples of plat parts
Tree	Tr	Tr1, Tr2 ..Trn	Leaf	L	Tr1L1, Tr1L2…....TrnLn	Leaf	SubL	Tr1SubL1 ….TrnSubLn
			Flower	Fl	Tr1Fl1, Tr1Fl2.….TrnFln	Flower	SubFl	Tr1SubFl1.. TrnSubFln
			Fruit	F	Tr1F1, Tr1F2…....TrnFn	Fruits	SubF	Tr1SubF1.. TrnSubFn
			Leaf containing smaller branches	Lsb	Tr1 Lsb1, Tr1Lsb2…....Trn Lsbn	Leaf containing smaller branches	SubLsb	Tr1 SubLsb1…....TrnSubLsbn
			Smaller branch	Bt	Tr1Bt1, Tr1Bt2...TrnBtn	Smaller branches	SubBt	Tr1SubBt1.. TrnSubBtn
			Bigger branch	Bg	Tr1Bg1, Tr1Bg2 ..TrnBgn	Bigger branches	SubBg	Tr1SubBg1.. TrnSubBgn
			Dead Branch	Bd	Tr1Bd1, Tr1Bd2..TrnBdn	Dead Branch	SubBd	Tr1SubBd1.. TrnSubBdn
			Bole or stem	T	Tr1T1, Tr1T2…….TrnTn	Bole or stem	SubT	Tr1SubT1 ... TrnSubTn
			Bark of bole	Ba	Tr1Ba1, Tr1Ba2..TrnBan	Bark of Bole or stem	SubBa	Tr1SubBa1.. TrnSubBan
			Bark of bigger branch	BaBg	Tr1BaBg1……TrnBaBg	Bark of bigger branch	SubBaBg	Tr1SubBaBg1. TrnSubBaBgn
			Wood Density	WD	Tr1T1WD1…….TrnTnWDn	Wood Density	SubWD	Tr1T1SubWD1…TrnTnSubWDn
Shrub	Sh	Sh1, Sh2..Shn	Leaf	L	Sh1L1, Sh1L2……. ShnLn	Leaf	SubL	Sh1SubL1 ….ShnSubLn
			Flower	Fl	Sh1Fl1, Sh1Fl2.….ShnFln	Flower	SubFl	Sh1SubFl1.. ShnSubFln
			Fruit	F	Sh1F1, Sh1F2…....ShnFn	Fruits	SubF	Sh1SubF1.. ShnSubFn
			Leaf containing smaller branches	Lsb	Sh1 Lsb1, Sh1Lsb2……. Shn Lsbn	Leaf containing smaller branches	SubLsb	Sh1SubLsb1…....ShnSubLsbn
			Smaller branch	Bt	Sh1Bt1, Sh1Bt2...ShnBtn	Smaller branches	SubBt	Sh1SubBt1.. ShnSubBtn
			Bole or stem	T	Sh1T1, Sh1T2……ShnTn	Bole	SubT	Sh1SubT1 ... ShnSubTn
			Bark of bole	Ba	Sh1Ba1, Sh1Ba2..ShnBan	Bark of Bole or stem	SubBa	Sh1SubBa1.. ShnSubBan
Palm	Pa	Pa1, Pa2..Pan	Petiole	P	Pa1P1, Pa1P2……. Panpn	Petiole	SubP	Pa1SubL1 ….PanSubLn
			Rachis	R	Pa1R1, Pa1R1…....PanRn	Rachis	SuR	Pa1SubL1 ….PanSubLn
			Leaflets	Ll	Pa1L1, Pa1L2……. PanLn	Leaflets	SubL	Pa1SubL1 ….PanSubLn
			Flower	Fl	Pa1Fl1, Pa1Fl2.….PanFln	Flower	SubFl	Pa1SubFl1.. PanSubFln
			Fruits	F	Pa1F1, Pa1F2…....PanFn	Fruits	SubF	Pa1SubF1.. PanSubFn
			Bole or stem	T	Pa1T1, Pa1T2…….PanTn	Bole or stem	SubT	Pa1SubT1 ... PanSubTn

ANNEX 1: (CONT..)

Level 1 (Plant life form)	Code	Example for code of trees	Level 2 (Plant parts)	Code for plant parts	Example of Code for fresh weight	Level 3 (Sub-sample)	Code for sub-samples	Example of code for sub-samples of plat parts
Nypa palm	Ny	Ny1, Ny2, .. Nyn	Petiole	P	Ny1P1, Ny1P2……. Nynpn	Petiole	SubP	Ny1SubL1 ….NynSubLn
			Rachis	R	Ny1R1, Ny1R1…….NynRn	Rachis	SuR	Ny1SubL1 ….NynSubLn
			Leaflets	Ll	Ny1L1, Ny1L2……. NynLn	Leaflets	SubL	Ny1SubL1 ….NynSubLn
Liana	Li	Li1, Li2, ..Lin	Leaf	L	Li1L1, Li1L2…….LinLn	Leaf	SubL	Li1SubL1 ….LinSubLn
			Flower	Fl	Li1Fl1, Li1Fl2.….LinFln	Flower	SubFl	Li1SubFl1.. LinSubFln
			Fruits	F	Li1F1, Li1F2…….LinFn	Fruits	SubF	Li1SubF1.. LinSubFn
			Bole or stem	T	Li1T1, Li1T2…….LinTn	Bole or stem	SubT	Li1SubT1 ... LinSubTn
			Above-ground Root	AR	Li1AR1, Li1AR2…….LinARn	Bole	SubAR	Li1SubAR1 ... LinSubTn
Bamboo	Bo	Bo1, Bo2 ..Bon	Leaf	L	Bo1L1, Bo1L2…….BonLn	Leaf	SubL	Bo1SubL1 ….BonSubLn
			Leaf containing smaller branches	Lsb	Bo1Lsb1, Bo1Lsb2……. BonLsbn	Leaf containing smaller branches	SubLsb	Bo1SubLsb1…….BonSubLsbn
			Smaller branch	Bt	Bo1Bt1, Bo1Bt2...BonBtn	Smaller branches	SubBt	Bo1SubBt1.. BonSubBtn
			Bole or stem	T	Bo1T1, Bo1T2…….BonTn	Bole	SubT	Bo1SubT1 ... BonSubTn

Generation and description of ID number

- Plant life forms like Tree, Shrub, Palma, Nypa palm, Liana, and Bamboo coded as Tr, Sh, Pa, Ny, Li and Bo respectively
- 1st, 2nd, 3rd and nth tree coded as Tr1, Tr2, Tr3 ……. Trn; 1st, 2nd, 3rd and nth Shrub coded as Sh1, Sh2, Sh3 ……. Shn; 1st, 2nd, 3rd and nth Palm coded as Pa1, Pa2, Pa3 ……. Pan respectively
- Plant parts like Leaf, Flower, Fruit, leaf containing smaller branches, Smaller branch, Bigger branch, Dead Branch, Bole or stem, Bark of bole, Bark of bigger branch, Wood Density coded as L, Fl, F, Lsb, Bt, Bg, Bd, T, Ba, BaBg, WD respectively
- The code of 1st log of 1st Tree and 4th log of 5th tree will be codes as Tr1T1 and Tr5T4 respectively
- Use “Sub” as code for sub-sample of plant parts
- The code of 1st, 2nd and 3rd sub-samples of leaf from 1st tree and 10th shrub will be Tr1Subl1, Tr1SubL2, Tr1SubL3 and Sh10SubL1, Sh10SubL2, Sh10SubL3 respectively



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