Laboratory Manual for Aboveground Understory and Soil Organic Carbon Analysis

Department of Forest & Park Services
Ministry of Agriculture & Forests

Table of Contents

Figures

Tables

FOREWORD

I am very happy that the collaboration between our department and the Department of Agriculture has resulted in the development of this laboratory manual for organic carbon analysis of plants and soil.

This laboratory manual fulfills three important purposes:
1. It serves as an important reference for organic carbon analysis of both plant and soil in the country
2. It provides institutional support for pursuing periodic national forest inventory
3. It expands the scientific and technical capacity of both forestry and agriculture sector

This is one of the important building blocks for institutionalizing **measuring, reporting and verification** (MRV) of forest carbon and consequently fulfilling our commitment to remain carbon neutral.

For this, I would like to thank the Department of Agriculture, and particularly Dr. Karma Dema, Program Director of National Soil Service Centre and Mr. Jamyang, Specialist at Soil and Plant Analytical Laboratory for being receptive to the collaboration. I remain highly appreciative and grateful for the support and hard work rendered by Mr. Jamyang and his team at SPAL in analyzing almost 2000 samples of plant and soil collected from our NFI plots and for bringing out this laboratory manual. The forest carbon assessment wouldn't be complete without the inclusion of above ground understory and soil carbon and therefore, their contribution to the effort is very significant.

I would like to congratulate Mr. Lobzang Dorji, Chief Forestry Officer at Forest Resources Management Division (FRMD) and his NFI team for successfully leading the collaboration with NSSC and for jointly producing this manual. I understand this manual highlights the expansion of forestry sector beyond timber and timber volume;and enhances our capacity to account for the role of forests in sequestering carbon.

I would also like to share my appreciation to BMUB, GIZ and ICIMOD for supporting our efforts on forest carbon assessment through the REDD+ Himalayas project and for supporting the development of such knowledge products.

This knowledge product will be useful to many other agencies, students, scholars and researchers interested in above ground understory plant and soil carbon analysis procedures.

Phento Tshering
Director

ACRONYMS AND ABBREVIATIONS

ABG	Aboveground
BMUB	Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety
BTFEC	Bhutan Trust Fund for Environmental Conservation
CI	Confidence Interval
cm	Centimeter
^{0}C	Degree centigrade/celcius
DoFPS	Department of Forests and Park Services
EU	European Union
FAO	Food and Agriculture Organization
FCPF	Forest Carbon Partnership Facility
FRMD	Forest Resources Management Division
g	Gram
GCCA	Global Climate Change Alliance
GIS	Geographic Information System
GIZ	Deutsche Gesellschaft für Internationale Zusammenarbeit - German Corporation for International Cooperation
GS	Growing Stock
Ha	Hectare (unit of area)
ICIMOD	International Centre for Integrated Mountain Development
JID	Job Identification Number
KG or kg	Kilogram
KM or km	Kilometer
m^3	Cubic meter (unit of volume measurement)
Mc	Moisture correction factor
ml	Milli liter
Mg/ ha	Megagrams per hectare
Mg C/ha	Megagrams of Carbon per hectare or Tonnes of carbon per hectare
MRV	Measurement, Reporting and Verification
MoAF	Ministry of Agriculture and Forests
MoE	Margin of Error
NFI	National Forest inventory
NFMS	National Forest Monitoring System
NSSC	National Soil Service Centre
REDD	Reducing Emission from Deforestation and Degradation of forests
RGoB	Royal Government of Bhutan
RNR SSP	Renewable Natural Resources Sector Support Programme
R-PP	REDD Readiness Project
SPAL	Soil and Plant Analytical Laboratory
SNV	Netherlands Development Agency
UN REDD -TS	UN REDD Targeted Support
UN	United Nations
WB	World Bank

Chapter I:
Introduction

Developed with the intention to provide transparency to the procedures adopted for National Forest Inventory (NFI) of Bhutan (2012-2015) and to serve as institutional reference for future NFI, this is a complementary document to the field manual "A Field Guide for Aboveground Understory (ABG) and Soil Carbon Assessment" that was developed by Forest Resources Management Division in 2014.

This manual provides step by step procedure for handling and processing of plant and soil samples in the laboratory. Together with the field manual, it serves as the complete guidance on carrying out the organic carbon assessment of aboveground understory and soil samples.

This is an important result of the on-going collaboration between Forest Resources Management Division, Department of Forests and Park Services and Soil and Plant Analytical Laboratory (SPAL), National Soil Service Centre, Department of Agriculture of Ministry of Agriculture and Forests (MoAF) on ABG and soil carbon analysis. This manual thus presents the standard analysis procedures being adopted and implemented at SPAL, applied to the overall ABG and soil carbon assessment methodology developed by FRMD and soil scientists of USDA-Forest Service and theUniversity of Washington of USA.

The document will be useful to any future NFI work, academician, researchers and students in the country, interested in studying or conducting a research on organic carbon.

Chapter II:
Procedures for aboveground understory plant carbon assesment

PRINCIPLE:

With climate change and gobal warming issues, it is felt increasingly important to estimate the carbon stock of forest soil and vegetation. This chatpter therefore, deals in assessing the carbon content of aboveground understory plant samples. For this, fresh plant samples need to be processed in the laboratory and dry matter estimated. Plant dry matter refers to the weight of dry sample after removing all moisture at 70°C and is expressed as percentage of fresh weight. An accurate assessment of dry matter is important to measure biomass per specified plot or area, which is essential for estimation of carbon content in aboveground understory sample using a factor of 1.75. In general, approximately half of plant biomass is considered to be made of carbon molecules.

The standard procedure for assessing the dry weight and finally estimating the carbon tonnes per hectare are presented below.

Fig.1.Samples in a sack

Fig.2a. Samples sorting

1. Samples sorting by client

1.1 Upon reaching plant samples in the laboratory, a client should first do the sorting of the samples which are brought in the sacks Figure 1, and arrange them in sequence (Fig.2a) according to their identification code or labels as listed (Fig.2b) in the Sample Submission Form as in Figure 3. If not already filled in the Sample Submission Form the client has to fill-in the FORM as he or she wants the results to appear.

If needed SPAL technician can provide helping hand and guide how samples are to be arranged.

1.2 After the samples are arranged the client can then hand over them to the Laboratory Sample Receptionist who will check if they are carefully packed in a linen or a plastic bag and clearly labeled for identification. Any sample not properly labeled, or labeled but not clear and discrepancies arise, such samples will be rejected unless the person handing in could clarify the confusion.

Fig.2b. Samples arranged

The Sample Receptionist will also cross check whether the number of samples and their identification Code listed in the Sample Submission Form tally with the physical count and actual code on the samples

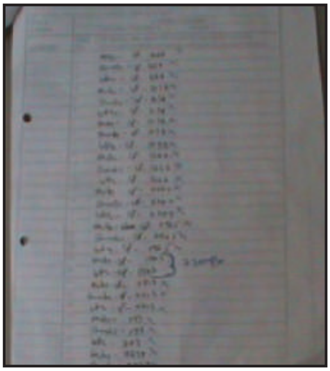


Fig.3. Filled sample submission Form

2. Samples reception and registration by the Laboratory staff

The Sample Receptionist should cross check whether the number of samples submitted matches with their identification Code listed in the Sample Submission Form (Fig 3) with the physical count and actual code provided in the samples list. Any mismatch should be either instantly corrected or discarded. The Sample receptionist should also check if the contact address provided in the Form is correct

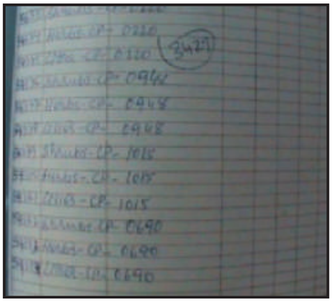


Fig.4. Plant Sample Register

The sample receiving staff should then transcript the information from the Sample Submission Form Fig 3 into the Sample Registration Form Fig 4. This in turn should be entered into the SPAL's Database computer Fig 5 to assign Lab

Number and Job Identification Number (JID). This JID may be used by the client for future reference for any inquiry. Once lab number is assigned to respective samples the samples are then processed as in section 3 below.

The completed Sample Submission Form is signed and photocopied. One copy is filed and the other is returned to the originator. This is to facilitate the better communication.

3. Sample processing: Oven drying and dry matter estimation

Samples received for Carbon analysis are often heterogeneous due to variation in stem, leaf content and the chopping method used. The laboratory attempts to minimize this variation by thorough chopping of larger sample pieces down to a uniform size where possible. In order to estimate the plant Carbon follow the below steps.

3.1 Using digital electronic balance (Fig.6), weigh the plastic tray or the linen bag. (Fig.7)
3.2 Transfer the sample into the plastic tray or in the linen bag.
3.3 Weigh the fresh sample + the container Fig.8.
3.4 Record the weight of the wet sample.
3.5 Weigh a sub-sample of the wet sample+ container
3.6 Put the sample in the ventilited electric oven Fig.9, for drying
3.7 Set the temperature to 70 °C and heat over night
3.8 Next morning switch off the oven and let it cool.
3.9 Weigh the sample + container to obtain the weight of the dry sample Fig 10.
3.10 Record all the values as in Fig 11.

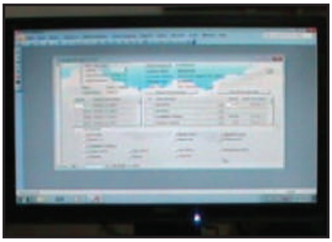


Fig.5. SPAL's Database computer


Fig.6. Electronic balance


Fig.7. Weight of an empty tray


Fig.8. Tray plus fresh sample


Fig.9. Tray plus fresh sample in oven

4. Estimating carbon content in ABG understory

Enter the values obtained in this process into the calculation worksheet (in Excel) to estimate Aboveground Understory carbon content expressed in Mg C/ha.

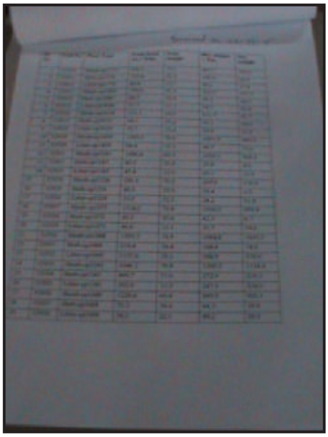


Fig.11b Complete weight record


Fig.10.Weight of tray plus dry sample

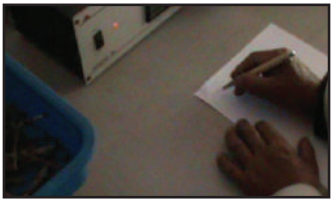


Fig.11a. Record weight of dry sample

Table 1. Estimating carbon content of plant materials(shrubs, herbs and litter) using the template in Microsft excel

Field work				**Lab work**			**Calculated shrub biomass**		**I. Cal-culated shrub C**
A. All shrubs + tared total wet wt (kg / 25m^2)	B. Tared wt (eg, ropes, tarp) (kg)	C. All shrub wet wt (kg / 25m^2)	D. Subsample of shrubs wet wt (kg)	E.~ 70C ovendried subsample wt dry wt (kg)	F. dry wt / wet wt fraction (dry kg / wet kg)	G. Field Moisture Content H2O (%)	H. Estimated dry wt i. (kg / 25m^2)	ii. (Mg / ha)	i. (Mg C/ha)
(Enter actual measurement here)	(Enter actual measurement here)	formula: A-B	(Enter actual measurement)	(Enter actual measurement)	formula: E/D	formula: ((D-E)/D)*100	formula: F*C	formula: H(i)/25* 10000/1000	formula: H(ii)/2

Table 2. Estimating carbon content of herbs sampled from 1meter by 1meter plot

Field work				**Lab work**			**Calculated shrub biomass**		**I. Calcu-lated herb C**
A. All herbs + tared total wet wt (kg / 1m^2)	B. Tared wt (eg, ropes, tarp) (kg)	C. All herb wet wt (kg / 1m^2)	D. Subsample of herbs wet wt (kg)	E.~ 70C ovendried subsample wt dry wt (kg)	F. dry wt / wet wt fraction (dry kg / wet kg)	G. Field Moisture Content H2O (%)	H. Estimated dry wt i. (kg/1m^2)	ii. (Mg/ha)	i. (Mg C/ha)
(Enter actual measurement here)	(Enter actual measurement here)	formula: A-B	(Enter actual measurement)	(Enter actual measurement)	formula: E/D	formula: ((D-E)/D)*100	formula: F*C	formula: H(i)/1* 10000/1000	formula: H(ii)/2

Table 3. Estimating carbon content of organic matter/detritus of forest floor(FF) from 20cm by 20cm plot

Field work				**Lab work**			**Calculated shrub biomass**		**I. Calculated herb C**
A. All FF + tared total wet wt (g/400cm^2)	B. Tared wt (eg, ropes, tarp) (g)	C. All shrub wet wt (g/400cm^2)	D. Subsample of shrubs wet wt (g)	E.~ 70C ovendried subsample wt dry wt (g)	F. dry wt / wet wt fraction (dry g / wet g)	G. Field Moisture Content H2O (%)	H. Estimated dry wt		
							i. (g/400cm^2)	ii. (Mg/ha)	i. (Mg C/ ha)
(Enter actual measurement here)	(Enter actual measurement here)	formula: A-B	(Enter actual measurement)	(Enter actual measurement)	formula: E/D	formula: ((D-E)/D)*100	formula: F*C	formula: H(i)/400*10000*10000/1000000	formula: H(ii)/2

Chapter III:

Procedures for soil organic carbon analysis

1. Soil sample sorting by the client

1.1 Upon reaching soil samples in the Laboratory Fig 12, a client should first do the sorting of the samples Fig 13 and arrange them in sequence with their identification code or labels as listed in the Sample Submission Form Fig 14. If not already filled in the Sample Submission Form the client has to fill-in the FORM as he or she wants the results to appear. If needed SPAL technician can provide help and guide how samples to be arranged.

1.2 Once the samples are arranged the client can then hand over them to the Laboratory Sample Receptionist.

Fig.12. Bulk soil samples

Fig.13. Sorting samples

2. Samples reception and registration by Laboratory staff

2.1 The Laboratory Sample Receptionist should check whether they are properly packed in a plastic bag and clearly labeled with identification code. Any sample not properly labeled, or labeled but not clear will be rejected unless the person handing in could clarify the confusion.

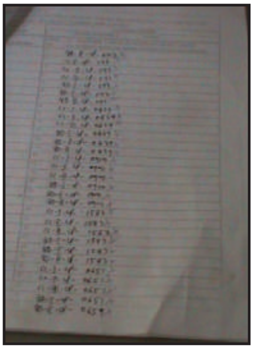


Fig.14 Sample Submission Form

Fig.15. Soil Registration Log book

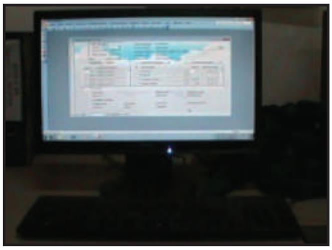


Fig.16. SPAL's Database computer

Fig.17. Samples for drying

Fig.18. Drying soil samples

2.2 The Sample Receptionist will also cross check whether the number of samples and their identification Code listed in the Sample Submission Form tally with the physical count and actual code on the samples. Any mismatch should be instantly corrected.

2.3 The Sample receptionist should also check if the contact address provided in the Form is correct.

2.4 The sample receiving staff should then transcript the information from the Sample Submission Form Fig 14 into the Sample Registration Form Fig 15. This in turn should be entered into the SPAL's Database computer Fig 16 to assign Lab Number and Job Identification Number (JID). This JID may be used by the client for future reference for any inquiry. Once laboratory number is assigned to respective samples the samples are then processed as in section 3 below.

2.5 The completed Soil Submission Form is signed and photocopied. One copy is filed and the other is returned to the originator. This is to facilitate the better communication

3. Soil samples processing

3.1 Transfer the samples Fig 17 into the drying room and dry the samples as in Fig 18.

3.2 Once air drying is completed Segregate the rocks greater than 2 mm from the soil sample Fig 19.

3.3 Weigh the stones + tarewt (eg, a tray or a plastic bag)Record the weight of the stones as in Fig 19.

3.4 Take the separate weight of the container.

3.5 Sieve the soil Fig 20a, and grind the soil in mortor and pestle Fig 20b if needed. Weigh the soil that has passed through 2mm sieve Fig 21.

3.6 Take the weight of the container, if different from that of serial number 3.4.

3.7 Record the weight of the soil sample Fig 22.

3.8 Transfer the 2 mm soil sample into a plastic bottle Fig 23, along with its ID label as in Fig 24. The sample is now ready for soil carbon analysis.

3.9 Eventual printed results will apper as in Fig 25.

Note the total weight of stone, rocks, and total weight of the soil close to 0.01 g accuracy.


Fig.19. Stones Seggregated from soil and weighed


Fig.20a. Sieving soil sample


Fig.20b. Grind sample with mortar and pestle

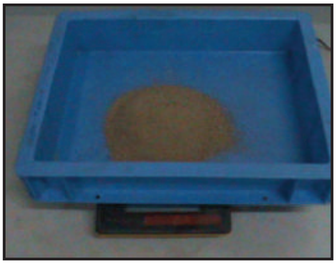


Fig.21. Taking soil weight

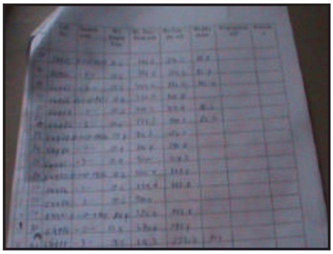


Fig.22. Stone & soil weight recorded


Fig.23. Soil sample bottled


Fig.24. Soil sample bottled with code

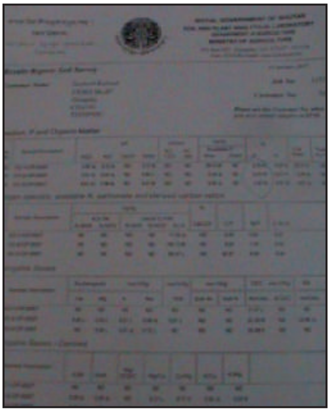


Fig.25. Printed soil Carbon results

4. Soil moisture content determination and correction factor

4.1 Principle

The moisture content is determined by drying the sample overnight at 105 °C. The moisture correction factor is calculated from the moisture content.

4.2. Appartus

Analytical balance with accuracy 0.001 g
Drying Oven
Desiccators filled with Silica gel
Labeled Aluminium tins with lids

4.3. Procedure

4.3.1. Dry an Aluminium tin with its corresponding lid at 105 °C for 1 hour
4.3.2. Cool in a desiccators for 45 minutes
4.3.3. weigh the tin + lid with 0.001 g accuracy (Weight W g)
4.3.4. Transfer approximately 5.0 g of wet soil sample into the tin Fig 26.
4.3.5. Weigh the tin + lid + wet sample with 0.001 g accuracy (Weight A g)
4.3.6. Remove the lid from the tin.
4.3.7. Dry the tin with the sample overnight at 105 °C Fig 27.
4.3.8. Next morning remove the tin from the oven and close it with the corresponding lid Fig 28.
4.3.9. Cool down in a desiccators for about 45 minutes or until a room temperature is attained (see 5.1) Fig 29.
4.3.10. Weigh the tin + Lid + drysample with 0.001 g accuracy (Weight B g) Fig 30
4.3.11. Record the readings in the Moisture Logbook Fig 31.
4.3.12. Enter the recorded values obtained into the SPAL Database Fig 32 for calculating soil carbon results on dry basis.

4.4. Calculations

Percentage moisture air-dry basis

$$\frac{(A-B)\times 100}{(A-W)}$$

Where:
A = Weight of the moisture tin + lid + wet sample
B = Weight of the moisture tin + lid + dried sample
W = Weight of the moisture tin + lid
Record the results in 3 decimal places.

Moisture correction factor (Mc)

$$\frac{100}{(100-Mc)}$$

Where:
M = moisture percentage as calculated above. Record the results in 3 decimals

4.5. Remarks

As oven dried soil attracts atmospheric moisture very rapidly, the tins once removed from the oven should be placed immediately in the desiccators, and should not be left in the desiccators for more than 45 minutes.

The moisture correction factor is used to calculate results on oven-dry basis when soil samples are not dried at 105 °C before analysis. The results of the analyses are multiplied with the correction factor to obtain results on oven-dry basis.

4.6. Reference: USDA, SCS, Analysis No.3A1


Fig.26 Weighing for moisture


Fig.27. Oven drying for moisture


Fig.28. Oven dried sample


Fig.29. Cooling in desiccators

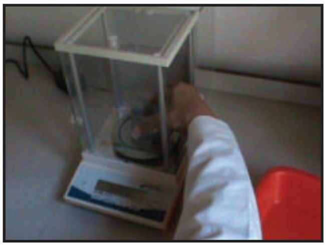


Fig.30. Weighing dry sample


Fig.31. Dry weight recorded

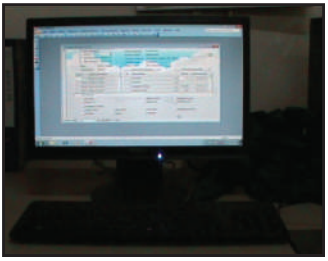


Fig.32. Moisture calculation in Database.

5. Analysis for soil Organic Carbon using Walkley and Black Method

Soil organic carbon can either be analyzed by using WALKLEY AND BLACK Method or with CHN Analyzer. First the procedure for Walkley and Black method is described in detail and then a brief description for CHN628 Analyzer method is presented for soil carbon analysis in section 6.

5.1. Principle

Soil organic matter is oxidized at a temperature of approximately 120 ^{0}C with a mixture of potassium dichromate and concentrated sulphuric acid (wet combustion). The excess potassium dichromate is titrated against ammonium ferrous sulphate with diphenylamine as an indicator. Before titration, phosphoric acid is added to form a complex with the interfering iron(III), providing a sharper colour change of the indicator.

5.2. Apparatus

Burette, graduated in 0.01 ml
Erlenmeyer flask of 500 ml
Mortar and pestle
Sieve of 2.00 mm
Dispenser
Measuring cylinder
Volumetric flask of 1000.0 ml
Wooden pad or a white tile

5.3. Reagents

5.3.1. Potassium dichromate 0.1667 M (1.000 N)

1. Dry about 55 g of $K_2Cr_2O_7$ (AR grade) at 150-200 ^{0}C for 2 hours.
2. Cool in a desiccators.
3. Weigh 49.033 g of dried $K_2Cr_2O_7$
4. Transfer into a 1000.0 ml volumetric flask
5. Dissolve in distilled water.
6. Make up to the mark and mix.

5.3.2. Concentrated Sulphuric acid H_2SO_4 with a concentration not less than 95%

5.3.3 Concentrated O-Phosphoric acid H_3PO_4 with a concentration of 85%

5.3.4 Ammonium Ferrous Sulphate 0.5 M

1 Weigh 196 g of $(NH_4)_2SO_4.FeSO_4.6H_2O$
2 Transfer it into a 1000.0 ml volumetric flask.
3 Add about 700 ml of distilled water and dissolve
4 carefully add 20 ml of concentrated sulphuric acid and swirl
5 Let it cool
6 Make up to the mark with water and mix

Store the solution in a brown bottle (See 5.6.1)

For each series of

determinations standardise against $K_2Cr_2O_7$ (See procedure below)

5.3.5 Diphenylamine indicator 1%

1. Pour 20 ml of distilled water into a 250 ml Erlenmeyer flask
2. Carefully add 50 ml of concentrated sulphuric acid to the Erlenmeyer flask
3. Swirl and let it cool
4. Carefully add another 50 ml of concentrated sulphuric acid to the Erlenmeyer flask
5. Swirl and let it cool
6. Add 1.0 g of Diphenylamine
7. Dissolve and mix Store the solution in a brown bottle (See 5.6.2)

5.4 Procedure

A series consists of 14 samples, including one blank and one standard sample

5.4.1 Grind about 2 g of sample in a mortar so that everything passes through a 0.5 mm sieve.

5.4.2 Weigh 1.000 g of the sieved sample into a 500 ml Erlenmeyer flask (accuracy 0.001 g)Fig 33.

5.4.3 Add 10.0 ml of 0.1667 M potassium dichromate solution with a burette or pipette and swirl the Erlenmeyer flask gently to disperse the soil in the solution.

5.4.4 Start with the blank. Add carefully 20 ml of concentrated sulphuric acid with a dispenser or measuring cylinder Fig 34.

5.4.5 Swirl for 1 minute

5.4.6 Leave the Erlenmeyer flask on a wooden pad for 30 minutes

5.4.7 Add 200 ml of distilled water with a measuring cylinder.

5.4.8 Add 10 ml of phosphoric acid with a dispenser or a measuring cylinder.

5.4.9 Add 1.0 ml of diphenylamine indicator

5.4.10 Titrate the sample with ammonium ferrous sulphate. Near the end point the colour changes to deep violet-blue. Slow down the titration by adding the ammonium ferrous sulphate drop wise Fig 35. At the end point the colour changes sharply to brilliant green (See 5.6.3), and record the reading Fig.36 and use for calculating the carbon analysis in SPAL Database Fig 37.

5.4.11 Only for the Blanks:

After the titration of the blank, record the burette reading.

Add again 10.0 ml of 0.1667 M $K_2Cr_2O_7$ to the titrated blank.

Titrate again with ammonium ferrous sulphate.

This second titration is for standardising the ammonium ferrous sulphate solution.

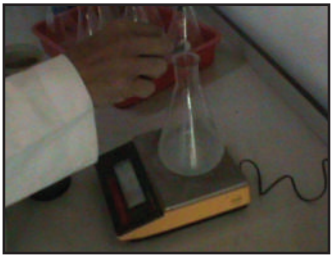


Fig.33. Weighing for carbon analysis

Fig.34. Adding sulphuric acid to sample

Fig.35. Titration

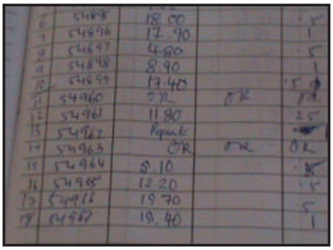


Fig.36. Record the reading

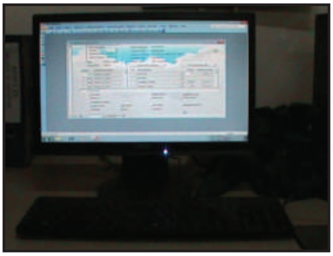


Fig.37. Result calculation in Database

5.5 Calculations

Normality of the ammonium ferrous solution:

$$N = \frac{\mathrm{F\ x\ K}}{\mathrm{T}}$$

Where:
F = ml of potassium dichromate added to the titrated blank
K = Normality of the potassium dichromate
T = ml of ammonium ferrous sulphate used for the second titration of the blank
N = Normality of the ammonium ferrous sulphate.
Record results in 4 decimal places.

Organic carbon content:

$$\%\, O.C = \frac{(\mathrm{B}-\mathrm{A})\ \mathrm{x\ N\ x\ 0.396\ x\ Mc}}{\mathrm{W}}$$

Where:
B = ml ammonium ferrous sulphate used for the first blank titration.
A= ml ammonium ferrous sulphate used for the sample.
N = Normality of the ammonium ferrous sulphate.
W = Weight of the sample in g
Mc= Moisture correction factor
0.396 is a constant factor (See 5.6.5)
Record results in 1 decimal place.

5.5.1. Enter the soil carbon values obtained in this process to estimate soil carbon content expressed in Mg C/ha using the Excel calculation worksheet shown in Table 4.

Table 4. Estimation of C/ha for mineral soil from 10cm by 5cm from depth of 10 cm. (The C/ha will be estimated for three soil depth: 0-10cm, 10-20cm and 20-30cm)

Field work								Lab work						
A	B	C	D	E	F	G	H	I	J	K	L	M	N.(i)	N(ii)
>2mm rock etc + tared total wet wt	>2mm rock sample tared wt (eg, bucket, etc)	>2mm rock wt	<2mm soil + tared total wet wt	<2mm sample tared wt (eg, bucket, etc)	<2mm soil wet wt	<2mm soil subsample for Carbon analysis	<2mm soil subsample for moisture calc.	<2mm soil subsample ~ 105C oven dried wt	<2mm soil dry wt / wet wt fraction	<2mm soil Field Moisture Content	<2mm soil dry wt	<2mm soil Lab Carbon analysis	Calculated Soil (<2mm) Organic C	
(kg/10x5x 10cm)	(kg)	(kg/10x5x 10cm)	(kg/10x5x 10cm)	(kg)	(kg/10x5x 10cm)	wet wt(g)	wet wt(g)	dry wt(g)	(dry g/ wet g)	H2O (%)	(kg/10x5x 10cm)	Soil Organic C (%)	i. (kg C / 50cm2 x 0-10cm depth)	ii. (Mg C/ ha for 0-10cm depth)
Mini	*(Enter actual measurement here)*	*(Enter actual measurement here)*	*(Enter actual measurement here)*	*(Enter actual measurement here)*	*formula: D-E*	*(Enter actual measurement of sub sample taken for carbon)*	*(Enter actual measurement of sub sample taken for moisture)*	*(Enter actual measurement of dry weight here)*	*formula: I/H*	*formula: (H-I)/H*100*	*formula: F*J*	*(Enter the actual SOC measured)*	*formula: M/100*L*	*formula: N/50)* 10000* 10000/ 1000*

5.6 Remarks

5.6.1 Instead of ammonium ferrous sulphate, a 0.5 M ferrous sulphate solution may be used. Ferrous sulphate however, is less stable than ammonium sulphate.

1. weigh 139 g of $FeSO_4.7H_2$) into a 1.0 Erlenmeyer flask
2. Add about 700 ml of distilled water and dissolve.
3. Carefully add 15 ml of concentrated sulphuric acid and mix
4. Transfer the solution into a 1000.0 ml volumetric flask.
5. make up to the mark with distilled water and mix.

Store the solution in a brown bottle.

5.6.2 An alternative indicator is barium diphenylamine sulphonate (0.16 g in 100 ml of distilled water). This indicator is easier to prepare.

5.6.3 If a sample uses less than 4 ml of 0.5 M ammonium ferrous sulphate, corresponding to a Carbon content of 2.5% or higher, repeat the determination using only 0.500 g of soil sample.

5.6.4 If a sample with 0.500 g still uses less than 4 ml of 0.5 M ammonium ferrous sulphate, corresponding to Organic Carbon content of 5.0% or higher, repeat the determination using only 0.500 g of soil sample and double the amounts of potassium dichromate, sulphuric acid, phosphoric acid and distilled water.

5.6.5 The factor 0.396 is derived from:

a Incomplete combustion (1.32)
b Equivalent weight of carbon (3)
c Conversion from milli-equivalents to equivalents (1000)

d Conversion to percentage (100)

$$\text{i.e., } 0.396 = \frac{1.32 \text{ x } 3 \text{ x } 100}{1000}$$

5.6.6 References

Black et.al. Part 2 page 1372-1375
USDA, SCS Analysis No. 6A1

6. Analysis of Soil Organic Carbon using CHN628 Analyzer

The CHN628 Series Elemental Determinator is used to determine nitrogen, carbon, and hydrogen in organic matrices. The instrument utilizes a combustion technique using IR detectors for C (CO_2), H (H_2O) and S (SO_2) and a TCD for N.The instrument features custom Microsoft Windows-based software operated through an external PC to control the system operation and data management. The standard operating procedure for the equipment is as follows:

1. Turn on the CHN628 Analyzer Fig.38
2. Turn on the computer and open 628 Series Software Fig 39
3. Open all gases (oxygen, helium, and compressed air) at gas cylinders Fig.40
4. Wait for the CHN628 furnace and reduction tube temperature to reach to its set temperature 950 °C, and 850 °C.
5. Leave the instrument to be stabilised about 1-2 hours after the CHN628 furnace and reduction tube reach to its temperature set point.
6. Run the system check at Diagnostic page and ensure all leak check is performed.

Fig.38. CHN628 Analyzer

Fig.39. PC to control the system

Fig.40 Gases cylinders

7. Run the Blanks for 3-5 times ensuring the blanks are low and repeatable
8. If the Blanks are high perform a Blank calibration by selecting last three repeatable blank results.
9. Run one time LECO standard to verify the results of carbon, nitrogen and hydrogen.
10. If the results are in the range then proceed with Sample Analysis.
11. For sample analysis press F3 to login sample
12. Enter the sample Name and the mass of the sample will be printed from a balance Fig.41
13. Load the sample in the carousel Fig.42 and Fig 42a
14. Press F5 to analyser the sample.
15. Print the results expressed as %C Fig.43
16. Enter the soil carbon values obtained in this process to estimate soil carbon content expressed in Mg C/ha using the Excel calculation worksheet shown in Table 5.

Fig.41. Balance with sample cup

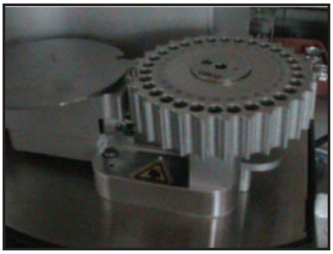


Fig.42. The carousel

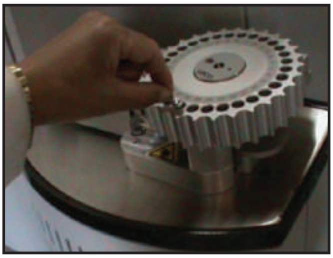
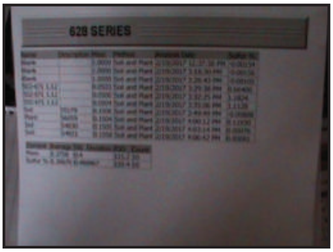


Fig.42a. Loading carousel



628 SERIES

Fig.43. Printed result

Table 5. Estimation of C/ha for mineral soil from 10cm by 5cm from depth of 10 cm. (The C/ha will be estimated for three soil depth: 0-10cm, 10-20cm and 20-30cm)

Field work								Lab work						
A	B	C	D	E	F	G	H	I	J	K	L	M	N.(i)	N(ii)
>2mm rock etc + tared total wet wt	>2mm rock sample tared wt (eg, bucket, etc)	>2mm rock wt	<2mm soil + tared total wet wt	<2mm sample tared wt (eg, bucket, etc)	<2mm soil wet wt	<2mm soil subsample for Carbon analysis	<2mm soil subsample for moisture calc.	<2mm soil subsample ~ 105C oven dried wt	<2mm soil dry wt / wet wt fraction	<2mm soil Field Moisture Content	<2mm soil dry wt	<2mm soil Lab Carbon analysis	Calculated Soil (<2mm) Organic C	
(kg/10x5x 10cm)	(kg)	(kg/10x5x 10cm)	(kg/10x5x 10cm)	(kg)	(kg/10x5x 10cm)	wet wt(g)	wet wt(g)	dry wt(g)	(dry g/ wet g)	H2O (%)	(kg/10x5x 10cm)	Soil Organic C (%)	i. (kg C / 50cm2 x 0-10cm depth)	ii. (Mg C/ ha for 0-10cm depth)
Mini	*(Enter actual measurement here)*	*(Enter actual measurement here)*	*(Enter actual measurement here)*	*(Enter actual measurement here)*	*formula: D-E*	*(Enter actual measurement of sub sample taken for carbon)*	*(Enter actual measurement of sub sample taken for moisture)*	*(Enter actual measurement of dry weight here)*	*formula: I/H*	*formula: (H-I)/H*100*	*formula: F*J*	*(Enter the actual SOC measured)*	*formula: M/100*L*	*formula: N/50)* 10000* 10000/ 1000*

6.17 Reference

CHN628 Carbon/Hydrogen/Nitrogen Determinator Instruction Manual; Version 1.3x, Part Number 200-731.