Food and Agriculture Organization of the United Nations

Standard operating procedure for soil organic carbon

Walkley-Black method

Titration and colorimetric method

GLOBAL SOIL PARTNERSHIP

SOIL ORGANIC CARBON WALKLEY-BLACK METHOD

Titration and Colorimetric Method

VERSION HISTORY

N°	Date	Description of the modification	Type of modification
01	30 July 2019	Finalization of the draft version	Compilation of all inputs received by RESOLANs
02	28 October 2019	Final review of the SOP at the 3rd GLOSOLAN meeting	Revision of steps in the SOP, final discussion and agreement
03			
04			

Modify by	Revision	Approval date	Validated date
GLOSOLAN SOP Tech. W.G. Leader: G. Nilo, Philippines	3rd GLOSOLAN meeting	3rd GLOSOLAN meeting	27 October 2019

Contents

1. Brief introduction to soil organic carbon (SOC) 3
2. Scope and field of application 4
3. Principle 5
4. Apparatus 6
   4.1. For Titration Method 6
   4.2. For Colorimetric Method 7
5. Materials 8
   5.1. For Titration Method 8
   5.2. For Colorimetric Method 9
6. Health and safety 10
   6.1. Personnel safety 10
   6.2. Chemical hazard 10
7. Sample preparation 10
8. Procedure 11
   8.1. Titration Method 11
   8.2. Colorimetric Method 13
9. Calculation 15
   9.1. Titration Method 15
   9.2. Colorimetric Method 16
10. Quality assurance/Quality control 17
   10.1. Accuracy Test 17
   10.2. Precision Test 17
   10.3. Control Chart 18
11. Reference documents 19
12. Appendix I. Acknowledgements 21
13. Appendix II. List of Authors 21
14. Appendix III. Contributing laboratories 22

1. Brief introduction to soil organic carbon (SOC)

Soil carbon is probably the most important component in soils as it affects almost all soil properties. Carbon, as soil organic matter, alters the physical, chemical, and biological properties of soils. Soil organic matter refers to all decomposed, partly decomposed and undecomposed organic materials of plant and animal origin. It is generally synonymous with humus although the latter is more commonly used when referring to the well decomposed organic matter called humic substances. Soil organic matter is a primary indicator of soil quality. Improvements in soil organic matter create a more favourable soil environment, leading to increases in plant growth. Higher soil organic matter levels cause the soil to retain more water that results in better crop yields, reduces soil erosion, increases plant nutrient retention and increases biological diversity. Moreover, improved aggregation of soil particles results in better soil structure, allowing for movement of air and water through the soil, as well as better root growth. More stable soil structure results in less soil erosion, which retains nutrients on the land and protects water quality.

Soil organic carbon contributes to the cation exchange capacity of a soil. These cation exchange sites are important for retention of nutrients such as calcium, magnesium and potassium. Soil organic carbon often also provides binding sites for many anthropogenic organo chemicals, thus minimizing leaching of hazardous chemicals through the soil profile or making them less bioavailable, which reduces toxicity.

Increased soil organic carbon enhances the biomass and diversity of the soil biota. Since the soil microbial community drives many of the nutrient transformations in soil, plant nutrient availability is often enhanced with the increase in microbial biomass and microbial activity of the soil.

In soil classification, the content of organic carbon of mineral horizons can be estimated from the Munsell colour of a dry and/or moist soil, taking the textural class into account. This estimation is based on the assumption that the soil colour (Munsell value) is due to a mixture of dark coloured organic substances and light coloured minerals. This estimate does not work well in strongly coloured subsoils. It tends to overestimate organic carbon content in soils of dry regions, and to underestimate the organic carbon content in some tropical soils. Therefore, the inferred organic carbon status of a soil should always be locally checked as it is only a rough estimate.

Note for soil classification purposes: (Food and Agriculture Organization of the United Nations, 2006)

*¸Organic carbon content of ≥ 0.6 percent → mollic and umbric horizon.
*¸Organic carbon content of ≥ 1.5 percent → voronic horizon.

(Note: the ratio of organic carbon to organic matter is about 1:1.7– 2.0)

In all these situations, it is important to have high quality organic carbon data (i.e. with low uncertainties). Luckily, the methods to measure organic carbon are rather easy to run but a special effort should be made by soil analysis laboratories to provide the best possible quality data. This will allow monitoring of changes in SOC at both local and regional scales and also give a better idea of the future scenarios, not only for SOC content but also for atmospheric CO_2 evolution.

2. Scope and field of application

This protocol applies to the determination of the Oxidizable Organic Carbon content in soil. Organic carbon content is calculated from the amount of chromic ion (Cr^{3+}) formed, using a titration or colorimetric method, the presence of chloride (>0.5% Cl^-) will produce a positive interference in saline soils. The bias resulting from the presence of chloride can be corrected if required (Rayment and Lyons, 2011). This method is described in Nelson and Sommers (1996) and the test method described here does not routinely apply correction for chloride.

3. Principle

The determination of soil organic carbon is based on the Walkley & Black chromic acid wet oxidation method. Oxidizable organic carbon in the soil is oxidised by 0.167 M potassium dichromate ($K_2Cr_2O_7$) solution in concentrated sulfuric acid. The heat of reaction raises the temperature which is sufficient to induce substantial oxidation.

Chemical reaction is as follows:

$$2\ Cr_2O_7^{2-} + 3\ C^0 + 16\ H^+ \leftrightarrow 4\ Cr^{3+} + 3\ CO_2 + 8\ H_2O$$

The Cr2O72- reduced during the reaction with soil is proportional to the oxidisable organic C present in the sample. The organic carbon can then be estimated by measuring the remaining unreduced dichromate by back-titrating with ferrous sulphate or ammonium ferrous sulphate using diphenylamine or o-phenanthroline-ferrous complex as an indicator.

$$6\ Fe^{2+} + Cr_2O_7^{2-} + 14\ H^+ \leftrightarrow 2\ Cr^{3+} + 6\ Fe^{3+} + 7\ H_2O$$

Alternately the organic carbon can be calculated from the amount of chromic ion (Cr^{3+}) formed, using a colorimetric procedure measuring absorbance at 588 nm (after Sims and Haby 1971). An advantage of this procedure over the titrimetric method is that accurate standardisation of the $Cr_2O_7^{2-}$ solution is not required.

Points to be noted:

1. Recoveries of the total Soil Organic Carbon by this method can typically be between 75 – 90 % in surface soils and will vary with soil type and depth. Walkley & Black found that on the average about 77% of the organic C was recovered by the heat of dilution procedure, and they proposed that a correction factor of 1.3 be used to account for unrecovered organic C;

2. This method is subject to interferences by certain soil constituents that lead to false results with some soils. Chloride, ferrous iron and higher oxides of Mn have been shown to

undergo oxidation-reduction reactions in chromic acid mixtures leading to incorrect values for organic C. The presence of significant amounts of Fe^{2+} or Cl^{-} in soil will lead to a positive error, whereas reactive MnO_2 in soil samples will result in a negative error and low values for organic C. The addition of H_3PO_4 after the sample has cooled helps eliminate interferences from the ferric (Fe^{3+}) ion that may be present in the sample. Chloride interference can be eliminated by washing the soil free of Cl^{-1} before analysis or precipitating the Cl^{-1} as AgCl by addition of Ag_2SO_4 to the digestion acid;

3. For soils that are very high in organic carbon content, the Walkley & Black method may result in low test results, due to the incomplete oxidation of the organic carbon in the sample. Smaller sample weights should be used for samples with very high carbon content;

4. This method is for the determination of organic carbon in soils. It is not applicable to soils containing significant amounts of carbonized materials.

4. Apparatus

4.1. For Titration Method

4.1.1. Analytical balance, with an appreciation of 0.0001 g for the preparation of reagents

4.1.2. Precision balance, with an appreciation dependent on the weight of the sample (Table 1).

4.1.3. Burette 50 mL, with an appreciation of ± 0.02 mL for the titrant solution

4.1.4. Volumetric burette/ dispenser of 10.00 mL ± 0.01 mL, of known uncertainty, to be used with the potassium dichromate solution

4.1.5. Volumetric dispenser, adjusted to 20.0 mL, to be used with concentrated sulfuric acid

4.1.6. Erlenmeyer flasks, 500 mL

4.1.7. Magnetic stirrer and bar

4.1.8. Oven able to reach a temperature of 105ºC
4.1.9. Volumetric flasks; 1000 mL
4.1.10. Glass rod
4.1.11. Beaker; 100 mL, 250 mL
4.1.12. Fumehood – extraction/ventilation
4.1.13. Burette and stand

4.2. For Colorimetric Method

4.2.1. Analytical balance, with an appreciation of 0.0001 g for the preparation of reagents
4.2.2. Spectrophotometer suitable for measuring absorbance at 600 nm wavelength
4.2.3. Centrifuge tubes (can withstand ≥ 130°C of heat) or glass conical tubes, about 50-75 mL capacity
4.2.4. Dispensing or volumetric pipettes, 1mL, 5 mL
4.2.5. Graduated pipettes; 1mL, 2 mL
4.2.6. Calibrated dispenser; 2 mL, 5 mL, 10 mL
4.2.7. Glass rod
4.2.8. Volumetric flasks; 100 mL, 500 mL
4.2.9. Beaker; 100 mL, 250 mL

5. Materials

5.1. For Titration Method

5.1.1. **Deionized water/distilled water, it should have an EC < 1.5*10^{-3} dS m^{-1}**

5.1.2. **Potassium Dichromate Standard, 0.167 M (1.0 N)**

Dissolve 49.04 g of traceable or equivalent analytical grade $K_2Cr_2O_7$ (previously dried at 105°C for 2 hours and cooled in a desiccator to room temperature) in deionized/distilled water, and dilute the solution to a volume of 1000 mL.

5.1.3. **Sulfuric Acid, Concentrated (not less than 96%) - For Titration and Colorimetric Method**

If Cl^- is present in soil, add Ag_2SO_4 to the acid at the rate of 15 g per liter.

5.1.4. **Phosphoric Acid, 85%** (If Diphenylamine indicator is used)

The phosphoric acid is added to form a complex with the interfering iron (III), providing a sharper color change of the indicator.

5.1.5. **Indicator** (either 5.1.5.1 or 5.1.5.2 can be chosen)

5.1.5.1. **o-Phenanthroline - Ferrous Complex, 0.025 M**

Dissolve 1.485 g of o-phenanthroline monohydrate (analytical grade) and 0.695 g of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) (analytical grade) in deionized/distilled water. Dilute the solution to a volume of 100 mL.

The o-phenanthroline-ferrous complex is also available under the name of Ferroin from the G. Frederick Smith Chemical Co. (Columbus, Ohio).

5.1.5.2 **Barium diphenylamine sulfonate Indicator, 0.16% aqueous solution**

5.1.6. **Titrant (either 5.1.6.1 or 5.1.6.2 can be chosen)**

5.1.6.1 **Ferrous Sulphate ($FeSO_4$) solution, 0.5 M**

Dissolve 140 g of analytical grade $FeSO_4 \cdot 7H_2O$ in deionized/distilled water, add 15 mL of concentrated sulfuric acid, cool the solution, and dilute it to a volume of 1000 mL with deionized/distilled water. Standardize this reagent daily by titrating it against 10 mL of 0.167 M (1 N) potassium dichromate.

5.1.6.2 **Ferrous Ammonium Sulphate, 0.5 M**

Dissolve 196 g of analytical grade $(NH_4)_2$ $Fe(SO_4)_2.6H_2O$ in 700 mL of distilled water, add 20 mL of concentrated sulfuric acid, cool the solution, and dilute it to a volume of 1000 mL with distilled water. Standardize this reagent daily by titrating it against 10 mL of 0.167 M potassium dichromate.

Note: The Fe^{2+} in both solutions oxidizes slowly on exposure to air so it must be standardized against the dichromate daily. Prepare a new solution every 30 days.

5.2. For Colorimetric Method

5.2.1. **Deionized water/distilled water, it should have an EC < $1.5*10^{-3}$ dS m^{-1}**

5.2.2. **Potassium Dichromate, 10% (0.34 M)**

Dissolve 50.0 g of traceable or equivalent analytical grade $K_2Cr_2O_7$ in 500 mL deionized/distilled water.

5.5.3 **Sucrose Standard, 4 mg C/mL**

Weigh 0.95 g sucrose (dried at 105°C for two hours) and dissolve in 100 mL deionized/distilled wáter.

6. Health and safety

This procedure involves the use of hazardous chemicals. Refer to laboratory safety guidelines or Material Safety Data Sheet (MSDS) before proceeding.

6.1. Personnel safety

Safety glasses, gloves and lab coats must be worn when handling any chemicals.

6.2. Chemical hazard

1. Potassium dichromate is an inorganic compound that emits toxic chromium fumes upon heating. Potassium dichromate is highly corrosive and is a strong oxidizing agent. This substance is a known human carcinogen and is associated with an increased risk of developing lung cancer.

2. Sulfuric acid: Keep away from naked flames/heat. Measure the concentration in the air regularly. Carry out operations in a fumehood with exhaust/ventilation. Do not discharge the waste into the drain. **Never dilute by pouring water into the acid. Always add the acid to the water**.

3. Hygiene: Wash hands and clean other exposed areas with mild soap and water after using all chemical reagents

4. All titrations and handling of chemicals to be undertaken in a fume hood.

7. Sample preparation

Air dry soil sample and sieve to ≤ 2.0 mm size.

8. Procedure

8.1. Titration Method

8.1.1. Weigh 1.0 g of air dried soil (adjust if necessary, see guideline recommended from Table 1) into a 500 mL erlenmeyer flask.

8.1.2. Add 10 mL of 0.167 M $K_2Cr_2O_7$ and swirl the flask gently to disperse the soil in the solution.

8.1.3. Then with care, rapidly add 20 mL concentrated H_2SO_4, directing the stream into the suspension.

8.1.4. Immediately swirl the flask gently until soil and reagents are mixed, then more vigorously for a total of 1 min.

8.1.5. To minimize heat loss, allow the flask to stand on an insulated sheet for 30 min in a fume hood.

8.1.6. Add 200 mL of water to the flask

Remark: Filter the suspension using an acid resistant filter paper (e.g. Whatman No. 540), if experience shows that the end point of the titration cannot otherwise be clearly discerned.

8.1.7. Add 10 mL of 85% H_3PO_4.(if barium diphenylamine sulfonate indicator is used)

8.1.8. Add three to four drops of o-phenanthroline indicator or barium diphenylamine sulfonate indicator and titrate the solution with 0.5 M $FeSO_4$ solution or 0.5 M $(NH_4)_2 Fe(SO_4)_2.6H_2O$

8.1.9. As the end point is approached,

8.1.9.1. **"Ferroin" Titration**, when using the o-phenanthroline indicator, the solution takes on a greenish cast and then changes to a dark green. At this point, add the ferrous sulfate heptahydrate drop by drop until the color changes sharply from blue to red (maroon color in reflected light against a white background).

8.1.9.2. "**Diphenylamine" Titration**, when using the diphenylamine indicator, near the end-point the color changes to deep violet-blue; slow down the titration by adding the ammonium ferrous sulphate dropwise. At the end-point the color changes sharply to brilliant green.

Determine 1-3 blanks in the same manner, but without soil, to standardize the $K_2Cr_2O_7$.

8.1.10 Compute for the %OC with the computation given at *section 9.1* and report as oven-dry basis with two (2) decimal places.

Table 1. Recommended weight of sample for analysis

Weight, g	OC, %	Color
0.1	>2	black, dark gray, dark brown
0.25	≤2	brown - dark brown, gray - dark gray
0.5	<0.6	Brown

Note: Above is just a guide for determining the appropriate weight to be used for each sample based on soil color. % OC may vary per soil color type. Generally, dark colored soils which are described as dark brown to black show a higher content of carbon and nitrogen than soils that are lighter in color.

Manual Potentiometric Titration

1. Set an expanded scale pH/mV meter with a platinum electrode and calomel reference electrode to read E (mV). Insert the electrodes and temperature compensator in the solution

and stir with a magnetic stirrer. Tall form beakers can be used as an alternative to Erlenmeyer flasks giving more room for the electrodes, temperature compensator and burette.

2. Using one of the unknowns, plot a titration curve by recording values of measured E (mV) and mL titrant (0.5 M $FeSO_4$ or 0.5 M $(NH_4)_2$ $Fe(SO_4)_2.6H_2O$ added from a burette. The end point is then found on the point of inflexion on the curve (approximately 750 mV). Subsequent titrations are discontinued when this point is reached, and the corresponding titrant consumption is then measured. If over 8 mL of the 10 mL of the dichromate has been reduced, the determination must be repeated with a smaller amount of soil sample.

Automatic Potentiometric Titration

Use an auto titrator with a platinum electrode to the mV terminal and calomel reference electrode to the glass electrode terminal. Use a 25 mL autoburette for the 0.5 M $FeSO_4$ or 0.5 M $(NH_4)_2$ $Fe(SO_4)_2.6H_2O$ titrant.

The titration is carried out by first plotting a titration curve as described above and then automatically titrating to the end-point (approximately 750 mV) thus determined. Titrator settings should follow the Titrator Equipment Handbook.

If over 8 mL of the 10 mL of the dichromate has been reduced, the determination must be repeated with a smaller amount of soil sample.

8.2. Colorimetric Method

8.2.1. Preparation of Standards curve

8.2.1.1. Prepare a set of sucrose standards (0-8 mg C) as specified in the table below in centrifuge tubes. Volumes of sucrose standard and deionized/distilled water corresponding to the mass of organic carbon

8.2.1.2. To each tube, add 2.0 mL 10% $K_2Cr_2O_7$ (0.34 M) solution and mix.

8.2.1.3. Add 5.0 mL H_2SO_4, cool and stand for 30.0 minutes.

8.2.1.4. Add 18.0 mL deionized/distilled water to the tube.

Table 2. Standard Preparation

Mass of OC. (mg)	Sucrose Standard (4 mg C/mL) (mL)	H_2O (mL)
0	0.00	2.00
1	0.25	1.75
2	0.50	1.50
3	0.75	1.25
4	1.00	1.00
5	1.25	0.75
6	1.50	0.50
7	1.75	0.25
8	2.00	0.00

8.2.2. Preparation of Samples

8.2.2.1. Weigh 0.5 g soil sample (refer to Table 1 if sample mass is to be modified)

8.2.2.2. Add 2.0 mL 10% (0.34 M) $K_2Cr_2O_7$ solution and mix

8.2.2.3. Add 5.0 mL H_2SO_4, cool and stand for 30.0 minutes.

8.2.2.4. Add 20.0 mL water to the tube. Mix and stand overnight.

8.2.3. Measurement

Read the absorbance of the calibration standards and samples in a spectrophotometer set at 600 nm wave length.

When the correlation coefficient of the calibration curve is equal to, or greater than, 0.9990, proceed with the analysis of samples. Otherwise, verify that the standards and reagents were correctly prepared, the instrument is functioning properly, and that the instrument set-up is correct. Corrective actions must be taken and details of corrective action recorded.

8.2.4 Reporting

Compute for the %OC with the computation given at *section 9.2* and report as oven-dry basis with two (2) decimal places.

9. Calculation

9.1. Titration Method

From the equation:

$$2\ Cr_2O_7^{2-} + 3\ C^0 + 16\ H^+ \leftrightarrow 4\ Cr^{3+} + 3\ CO_2 + 8\ H_2O$$

1 mL of 1 N dichromate solution is equivalent to 3 mg of carbon

After the reaction, the excess Cr_2O_7 is titrated with 0.5 M $FeSO_4$ or 0.5 M $(NH_4)_2Fe(SO_4)_2.6H_2O$

$$6\ Fe^{2+} + Cr_2O_7^{2-} + 14\ H^+ \leftrightarrow 2\ Cr^{3+} + 6\ Fe^{3+} + 7\ H_2O$$

$$Organic\ C,\% = \frac{(V_{blank} - V_{sample}) \times M_{Fe^{2+}} \times 0.003 \times 100 \times f * mcf}{W}$$

where:

V_{blank} = volume of titrant in blank, mL

V_{sample} = volume of titrant in sample, mL

$M_{Fe^{2+}}$ = concentration of standardized $FeSO_4$ or $(NH_4)_2Fe(SO_4)_2.6H_2O$ solution, molarity

0.003 = carbon oxidised (shown below)

$$= \frac{12\ g\ C}{mole} \times \frac{1\ mole\ K_2Cr_2O_7}{6\ moles\ FeSO_4} \times \frac{3\ moles\ C}{2\ moles\ K_2Cr_2O_7} \times \frac{1\ L}{1000\ mL}$$

f = correction factor, 1.3

W = weight of soil, g

mcf = Moisture correction factor *(refer to SOP for Moisture Content to compute for the mcf value)*

Note: An oxidation correction factor of 1.3 is required because, on average, only about 77% of organic carbon is recovered by this method. However, it should be considered that the value of this factor is very variable, since it is conditioned by the type of soil and by the nature of the organic matter.

9.2. Colorimetric Method

$$\%\ OC = \frac{mgC_{sample} - mgC_{blank}}{w,mg} \times f \times mcf \times 100$$

where:

% OC = Organic Carbon content of the soil, %

mg C_{sampl} = Analyte/concentration of C in sample

mg C_{blank} = Analyte/concentration of C in blank

W = Mass of air dry sample, mg

f = Correction factor, 1.3

mcf = Moisture correction factor *(refer to SOP for Moisture Content to compute for the mcf value)*

10. Quality assurance/Quality control

10.1. Accuracy Test

10.1.1. Participate in an Inter-laboratory Proficiency Test at least once a year. The PT z-score should be less than 2. If not, identify root cause, develop corrective and preventive actions, and address the problem.

10.1.2. Perform replicate analyses of the Certified Reference Material (CRM). Compare result of own laboratory with results of other laboratories as provided in the performance analysis report, or CRM certificate. The own laboratory result is considered accurate when it falls within the reported 95% confidence interval of the target value.

10.2. Precision Test

Perform replicate analysis of 10% of samples in a test batch. Calculate the Percent Relative Standard Deviation (%RSD) to determine the precision of replicate analyses is within specification. Compare result with the target precision for the analyte concentration (Table 3).

$$\% RSD = \frac{s}{\bar{x}} \times 100$$

Where: s = standard deviation of the replicate result
$\bar{x}$ = mean

Table 3. Expected precision (repeatability) as a function of analyte concentration

Analyte, %	Analyte ratio	Unit	RSD, %
100	1	100%	1.3
10	10^{-1}	10%	1.9
1	10^{-2}	1%	2.7
0.01	10^{-3}	0.1%	3.7
0.001	10^{-4}	100 ppm (mg/kg)	5.3
0.0001	10^{-5}	10 ppm (mg/kg)	7.3
0.00001	10^{-6}	1 ppm (mg/kg)	11
0.000001	10^{-7}	100 ppb (µg/kg)	15
0.0000001	10^{-8}	10 ppb (µg/kg)	21
0.00000001	10^{-9}	1 ppb (µg/kg)	30

Source: AOAC Peer Verified Methods Program. Manual on Policies and Procedures (1998). AOAC International Gathersburg. MD.

10.3. Control Chart

Analyze at least a duplicate of the Check Sample or Internal Reference Material for every batch of analysis. Plot the result in the control chart. Monitor for out of specified limits. If out of specified limit is observed, identify root cause, develop corrective and preventive actions.

11. Reference documents

AOAC. 1998. *AOAC Peer Verified Methods Program. Manual on Policies and Procedures*. AOAC International Gathersburg. MD.

B. Magnusson & U. Ornemark. 2014. *Eurachem Guide: The fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics.*

Bowman, R.A. 1998. *A Re-evaluation of the Chromic Acid Colorimetric Procedure for Soil Organic Carbon. Commun*. *Soil Sci. Plant Anal*., 29(3&4): 501-508.

Brown, P.E. & O'Neal, A.M. 1923. *The Color of Soils in Relation to Organic Matter Content*. Research Bulletin No. 75. Retrieved from Agricultural Research Bulletin-v005-b075.pdf.

FAO. 2006. *Guidelines for soils description. Fourth edition*. Food and Agriculture Organization of the United Nations, Rome, Italy.

Garfield, F.M. 1991. *Quality Assurance Principles for Analytical Laboratories*. AOAC INTERNATIONAL

Nelson, D.W. & Sommers, L.E. 1996. *Total Carbon, Organic Carbon and Organic Matter.* In D.L. Sparks (Ed.), Soil Science Society of America, Book Series 5. Methods of Soil Analysis Part 3, Chemical Methods. Madison, Wisconsin: Soil Science Society of America, Inc.

Rayment, G.E. & Lyons, **D.J.** 2011. *Soil Chemical Methods - Australasia*. CSIRO publishing, Australia

Sims, J. & Haby, V. 1971. Simplified Colorimetric Determination of Soil Organic Carbon Matter. *Soil Science*, 112(2): 137-141

Walkley, A. & Black I.A., 1934. An examination of the Degtjareff Method for Determining Soil Organic Matter, and a proposed Modification of the Chromic Acid Titration Method. *Soil Science,* 37(1): 29-38

Walkley, A. 1947. A Critical Examination of a Rapid Method for Determining Organic Carbon in Soils – Effect of Variations in Digestion Conditions and of Inorganic Soil Constituents. *Soil Science*, 63(4): 251-264

12. Appendix I. Acknowledgements

GLOSOLAN thanks the Asian Soil Laboratory Network (SEALNET), the Latin American Soil Laboratory Network (LATSOLAN) and Ms. Nopmanee Suvannang, GLOSOLAN Chair for preparing the first draft of this SOP. GLOSOLAN also thanks the Regional Soil Laboratory Networks (SEALNET, LATSOLAN, AFRILAB and EUROSOLAN) that reviewed this SOP at their annual meetings and the participants in the 3rd GLOSOLAN meeting (28-30 October 2019).

13. Appendix II. List of Authors

Main authors (in alphabetical order):

- Mr. Bergil G. Bernaldo, Bureau of Soils And Water Management Laboratory Services Division, **Philippines**
- Ms. Floria Bertsch, CIA-UCR, **Costa Rica**
- Ms. Gina P. Nilo, Bureau of Soils And Water Management Laboratory Services Division, **Philippines**
- Ms. Nopmanee Suvannang, GLOSOLAN Chair, **Thailand**
- Mr. Rob De Hayr, Department of Environment and Science, Science Division, Chemistry Centre, **Australia**

14. Appendix III. Contributing laboratories

GLOSOLAN thanks the following laboratories for completing the GLOSOLAN form on the method and providing information on their Standard Operating Procedure for the Walkley & Black Method (titration and colorimetric methods), which were used as baseline for doing the global harmonization:

From the Asian region:

- ICRISAT, **India**
- ICAR-Indian Institute of Soil Science, **India**
- Department of agriculture land management (DALaM), **Laos**
- Department of Agricultural Research (DAR), **Myanmar**
- Fertilizer Company Limited, **Pakistan**
- Department of Soil and Environmental Sciences, The University of Agriculture, Peshawar, **Pakistan**
- Bureau of Soils and Water Management, **Philippines**
- DA Regional Field Office 3-ILD-Regional Soils Laboratory, **Philippines**
- Horticultural Crops Research and Development Institute , Department of Agriculture, **Sri Lanka**
- Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, **Thailand**
- Soil Analysis Technical Service Group, Office of Science for Land Develoment, Land Development Department, **Thailand**

From the Pacific region:

- Fiji Agricultural Chemistry laboratory, **Fiji**
- The University of the South Pacific. Alafua Campus, **Samoa**

From the Near East and North African region:

- Soil and Water Research Institute, **Iran**
- Ministry of science and technology, Directorate of agricultural research, soil and water resources center, soil chemistry and desertification department, **Iraq**
- Agricultural Research & extension Authority, Renewable Natural Resources Research Center, **Yemen**

From the African region:

- Soil and Plant Analytical laboratory, **Botswana**
- Institute of Agricultural Research for Development, **Cameroon**
- National Laboratory for Diagnosis and Quality Control of Agricultural Products And Inputs, **Cameroon**
- LASEP/ITRAD, **Chad**
- National Soil Testing Center (NSTC), **Ethiopia**
- CSIR-Soil Research Institute, **Ghana**
- KALRO Soil Laboratories, Kabete, **Kenya**
- University of Eldoret, **Kenya**
- Department of Agricultural Research, **Lesotho**
- Laboratoire des Radioisotopes, **Madagascar**
- Department of Agricultura Research Services, Chitedze Agricultural Research Station, **Malawi**
- Farming & Engineering Services (FES), **Malawi**
- Lilongwe University of Agriculture and Natural Resources, **Malawi**
- Department of Agricultura Research Services, Chitedze Agricultural Research Station, **Malawi**
- IIAM/CZnd/ Nampula, **Mozambique**
- Institut National de la Recherche Agronomique du Niger (INRAN), **Niger**
- Nigeria Institute of Soil Science (NISS), National soil and fertilizer reference laboratory kaduna, **Nigeria**

- Internation Institute of Tropical agriculture, **Nigeria**
- RAB, **Rwanda**
- IRD, **Senegal**
- Tanzania Agricultural Research Institute (TARI) - Mlingano, **Tanzania**
- Institut Togolais de Recherche Agronomique, **Togo**
- University of Zambia, **Zambia**
- Zambia Agriculture Research Institute, **Zambia**
- Soil Science & Agric. Engin, University of Zimbabwe, **Zimbabwe**
- Zimbabwe Sugar Association Experiment Station, **Zimbabwe**
- Chemistry and Soil Research Institute, **Zimbabwe**

From the European region:

- Institute of Soil Science, Agrotechnologies and Plant Protection Department of Agrochemistry, Agroecology and Soil Tillage, **Bulgaria**
- University of Zagreb, Faculty of Agriculture, Department of General Agronomy, **Croatia**
- SYNLAB Analytics & Services Germany GmbH, **Germany**
- Food Chain Safety Centre Non-profit Ltd., Soil Conservatory Laboratory, Velence, **Hungary**
- Kosovo Institute of Agriculture, **Kosovo**
- Laboratory of forest environment at Latvian State Forest Research Institute "Silava", **Latvia**
- Chemisch Biologisch Laboratorium Bodem, **Netherlands**
- Instituto Politécnico de Castelo Branco/Escola Superior Agrária, **Portugal**
- Instituto Nacional de Investigação Agrária e veterinária (INIAV), **Portugal**
- INCDPAPM - ICPA Bucharest - Laboratory for Physical and Chemical Analysis, **Romania**
- Soil and Fertilizer Laboratory of Department of Soil Science and Plant Nutrition (SOFLER-TR), University of Ankara, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, **Turkey**
- Soil Fertilizer and Water Resources Central Research Institute, **Turkey**
- Environmental Research Laboratory, **United Kingdom**

From the Eurasian region:

- Laboratory of soil-agrochemical analysis, **Kazakhstan**
- Soil Science Faculty, Lomonosov Moscow State University, **Russian Federation**
- Institute of Biology of Komi Science Centre of the Ural Branch of the Russian Academy of Sciences (IB Komi SC UB RAS), **Russian Federation**

From Latin America:

- Instituto Nacional de Investigaciones Agrícolas. Centro Nacional de Investigaciones. Agropecuarias: INIA-CENIAP, **Bolivia**
- INTA (Instituto Nacional de Tecnología Agropecuaria)- Instituto de Suelos – Laboratorio, **Brazil**
- Laboratorio Químico de Suelos y Plantas, Universidad de Concepción, **Chile**
- Agricultural Land Management Division/ Soil Health Plant Tissue Water Laboratory, **Jamaica**
- ECOSUR, **Mexico**
- Laboratorio de Fertilidad de suelos y Agua del Instituto de Investigación Agropecuaria de Panamá (IDIAP), **Panama**
- Laboratorio de Suelos y Aguas, Dirección General de Recursos Naturales - Ministerio de Ganadería Agricultura y Pesca. (DGRN-MGAP), **Uruguay**
- Instituto Nacional de Investigaciones Agrícolas. Centro Nacional de Investigaciones Agropecuarias: INIA-CENIAP, **Venezuela**

GLOBAL SOIL PARTNERSHIP

CA7471EN/1/01.20