



Food and Agriculture
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Raising confidence in quality measurements from soil laboratories in Sub-Saharan Africa

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**SOIL ORGANIC CARBON PRACTICAL
WALKLEY-BLACK METHOD
(Titration)**



**Practical Presentation
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1.0 Introduction

Walkley and Black Organic Carbon SOP is a classic analytical procedure designed to measure Soil Organic Matter (SOM) in soils/sediments through quantifying Carbon in the given sample.

Also known as HUMUS => are remains of plant and animal residues as well as microbial decomposition or synthesis at various levels (except coal/charcoal) are oxidized.

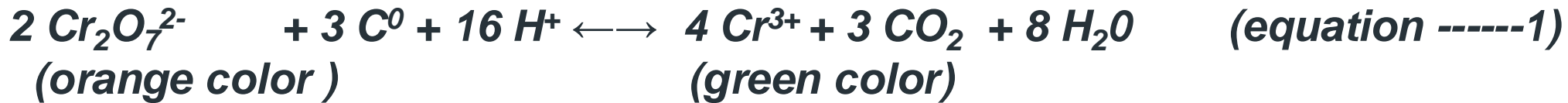
Importance in Quantifying Organic matter present in the soil is linked to:-

- ***Retention of basic cations hence reduction in acidity = nutrient availability => fertility***
- ***SOM means => Soil aggregates, improved structure, aeration, moisture retention etc***
- ***Facilitates microbial processes (mineralization, ammonification, nitrification etc.)***
- ***Reduction in inorganic fertilizers = reduction in pollution = reduced global warming***

2.0 Principle and Scope overview of application

Walkley and Black SOP is based on chromic acid wet oxidation-reduction reaction.

Oxidation – C is oxidized to CO₂ by dichromate ions from 1 N K₂Cr₂O₇ (0.1667 M)



Organic carbon is oxidized by known (volume and Molar concentration of reactant dichromate) put in excess. Excess chromic acid not reduced is determined by back titration - ferrous sulphate solution, using indicator or potentiometric means.

Dichromate ions (1 volume) reacts with soil C under H₂SO₄ (2 volumes) producing an exothermic reaction ideal for oxidation process 120°C.

N.B. As the Cr³⁺ ions in aqueous solution imparts green color (in solid state violet) This is the basis of Walkley and Black Colorimetric Method where soil C after oxidation - reduction reaction can be estimated based on (Beer – Lambert's law).

3.0 Walkley and Black – Titrimetric Method

BACK TITRATION CONDITIONS demand (i) Volatile analyte react with an excess reagent. A titration is conducted on remaining quantity of known solution (reactant) = measuring amount consumed by the analyte => Inverse proportion

Equation 1 shows that with dichromate in excess => there is still potential for oxidation.. Fe²⁺ in Ferrous Ammonium Sulphate /Ferrous Sulphate (will be used as reactant) to quantify unused chromate ions inversely proportional to C

We can thus know the amount of dichromate that was not used initially as determined by back titration.



End point can be determined Potentiometrically of by way of suitable indicator

4.0 Conceptualisation: Walkley and Black Determination of Soil Carbon

It has been debated whether or not the Exothermic reaction of chromic acid (120°C) Conceptualised by Walkley and Black can cover high OC soils. Argument being that soils of higher carbon content > 2% need extra external heat heating.

This is in tandem with Walkley and Black, 1934 , who found that on average 77 % is recovered by heat of dilution only.

With muck soils in colder regions contributing to OM > 25% - Tropical Regions 1-4% SOM Anderson and Ingram, 1993, proposed external heating at 150°C for 30 minutes on hot plate arguing that heat of dilution alone covers only cover 74% of oxidation on high OC soils.

To this end recovery factors often quoted as correction factors have been used e.g. 1.3 being recovery for the 74-77% assumed oxidation recovery factor termed subsequently Total Organic Carbon (TOC) to differentiate from un-factored oxidizable organic carbon.

N.B. also - Assuming that OM contains 58% organic carbon, another conversion factor $100/58 = OM$ i.e. $TOC \times 1.72 = OM$ is widely used in reporting Organic Matter content.

5.0 Choice of methods - Considerations

As conceded, large variations in SOM in soils might call for consideration by comparison of other proposed SOP's

In choosing the best method to follow, the choice should centre on a method that gives nearly accurate results as much as Quality Control and Quality assurance can vouch...backed by PT and References.



5.0 Choice of methods – Considerations (continued)

5.1 Interferences

Should carefully be considered, as much as possible, circumvent the interferences. Chloride has been picked as an example which has interferences in saline soils (see Reagents 7.3). Iron, Manganese also are among others. For iron, as Fe^{2+} is thought to be the problem, it should be negligible given that while available in high clay soils with high water retention, after air drying should be ok. Carbonates which affect LOI is negligible up to 50 % in Walkely and Black

5.2 Precision

Factors that affect how precise we are, link to sample size.

Otherwise with records kept over time, ideal sample weights are derived from history, GPS location...color, site.. and such information can be exchanged in country/region's laboratory to save time such that compromise.

NB. Noting that sample size has been to date been shown not to impact results HENCE NEED TO DECREASE REAGENTS by decreasing sample size.

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6.0 Apparatus for titration method

- 6.1 Analytical balance for preparation of reagents 0,0001 g (0.001g)**
- 6.2 Volumetric pipette or dispenser (10ml) +/- 0.01ml (calibrated regularly)**
- 6.3 Precision balance with appreciation of 0.01g i.e. 2 decimals**
- 6.4 Syringe pipette/ Dispenser 20 ml or (if unavailable 50ml measuring cylinder)**
- 6.5 Erlenmeyer flasks (wide mouthed preferably)**
- 6.6 Magnetic stirrer and teflon coated stirring bar**
- 6.7 Oven for moisture determination (105°C) – pre-calibrated**
- 6.8 Volumetric flasks 1L**
- 6.9 Beakers 100ml and 250 ml**
- 6.10 Burette (preferably auto zero) and burette stand**
- 6.11 Dropper or dropper bottle**

7.1 De-ionized water /distilled water (EC <1.5 uS)

7.2 Potassium Dichromate - Dissolve 49.04g of potassium dichromate previously dried for a minimum of 2 hrs in oven at 105°C and cooled in a dessicator with active silica gel in 1 L volumetric flask and thoroughly mix.

7.3 Sulphuric acid not less than 98 %

(if the soil has appreciable quantities of Cl⁻ add 15g Ag₂SO₄)

N.B. Sulphuric acid is hygroscopic hence it is strongly recommended to use from bottle as it rapidly picks moisture from atmosphere

4. Phosphoric Acid 85% (If diphenylamine indicator is to be used as it provides sharp end point when Fe²⁺ is an interfering ion)

7.0 Reagents (continued)

7.5 Indicator use 5.1 or 5.2

7.5.1 O-phenanthroline – Ferrous Complex

Dissolve 1.485 g orthophenanthroline monohydrate (AR) and 0.695g ferrous sulphate heptahydrate (AR) in distilled water and dilute to a volume of 100ml. Keep in dark bottle away from light (end point green to brown)

7.5.2 Barium diphenylamine sulphonate indicator - 0.16% aqueous solution

alternatively: Weigh 0.5g diphenylamine in 20ml distilled water then add 120ml conc sulphuric acid (green cast – dark green – blue and finally to red/maroon)

7.6 Titrant Choose either 6.1 or 6.2 (either solutions to be standardized daily)

7.6.1 0.5 M Ferrous Ammonium Sulphate - Dissolve 196g Ferrous Ammonium Sulphate (AR) hexahydrate in 700ml distilled water, add 20ml sulphuric acid, cool dilute to 1000 ml and thoroughly mix..

7.6.2 0.5 M Ferrous Sulphate - Dissolve 140g Ferrous Sulphate (AR) in 500ml distilled water, add 15 ml H₂SO₄, cool and dilute to 1000ml.

8.0 Health and Safety

8.1 Chromium: While chromium as a pure metal has no adverse effects on Health, Chromium largely exists as Cr^{6+} and Cr^{3+}

Cr^{6+} is toxic as well as carcinogenic by inhalation ; if using ordinary pipette use a pipette filler (do not waste dispose in sinks before treating it). Even assuming dichromate ions Cr^{3+} a safer oxidation state, it can without due diligence, end up sooner in the environment where it can be oxidized to Cr^{6+} once again (Check with MSDS) for disposal requirements.

8.2 Sulphuric acid – Corrosive always wear gloves and safety goggles and mask and work under the fumes hood. Remember when diluting add acid to the water

8.3 Always have all the containers clearly labelled (GLP) to start due diligence and prepare quantities envisaged as necessary.

WHENEVER UNSURE ON HOW BEST TO HANDLE REAGENTS REFER TO MSDS BEFORE PROCEEDING

8.4 Diphenylamine is corrosive handle with caution

9. Soil Color Based Weighing guidelines

This is based on observations forwarded that generally dark colored (brown to black) show appreciable higher OM

In this prac being visitors... In absence of prior records we shall be guided by Soil Color (Slide 9) item 9.0 or alternatively by our GLOSOLAN SOP.

Color	% OC	Recommended weight (g)		
black, dark gray, dark brown	> 2	0.1		
brown - dark brown, gray - dark gray	<2	0.25		
brown	< 0.6	0.5		

10.0 PROCEDURE

- 10.1 Weigh 1.0g or as conceptualised in duplicate (in view of weighing guidelines given) air dried soil sieved to pass at most through a 2mm sieve into a 500ml numbered wide mouthed Erlenmeyer flask (record weight down).**
- 10.2 Add quantitatively 10 ml of 0.167 M $K_2Cr_2O_7$ and swirl the flask gently to uniformly wet the soil.**
- 10.3 With care rapidly add 20ml H_2SO_4 directing the stream into the suspension and immediately swirl the flask gently until the soil and reagents are mixed and thereafter more vigorously for 1 minute carefully taking care to keep the suspension to the base as much as is practicable.**
- 10.4 Allow flask to stand on an asbestos pad or insulated surface to maintain the heat for 30 min**
- 10.5 Weigh at least 2 sample replicates for moisture correction factor (mcf)**
- 10.6 Have 2 blanks that are run through the whole procedure like samples**

10.0 Procedure continued

10.5 Add 200ml water and allow to cool further and if diphenylamine indicator is to be used add 10ml H_3PO_4 .

10.6 Add 3-4 drops of the indicator and titrate the solution to the end point. Take care to add titrant drop by drop as the end point approaches. If end point is overshoot, carefully add 1 ml dichromate solution quantitatively using a micropipette and this time carefully add drop wise the titrant.

10.7 Record the volume of titrant for each sample and run at least 2 blanks.

10.8 Include at least 2 replicates for each batch and QC sample as well as and controls CRM and or intralab sample

❖ Calculation

❖ From equation 1, 1 ml of 1 N dichromate is equivalent to 3 mg C

❖ After reaction of back titration

❖ $\% \text{Organic carbon} = (V_{\text{blank}} - V_{\text{sample}}) \times M \text{ Fe}^{2+} \times 0.003 \times 100 \times f \times \text{mcf}/w$

❖ Where V = volume of titrant of blank, V_{sample} = volume of sample, $M \text{ Fe}^{2+}$ = Molarity of standardized Ferrous Ammonium Sulphate or Ferrous Sulphate (used), f = recovery factor and mcf = moisture correction factor.

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11.0 Acknowledgements / Literature Review

- 1. GLOSOLAN - SOP 2019 Walkley - Black Titration and Colometric Method***
- 2. J.R. Okalebo, K.W. Gathua P.L. Woomer Methods of Soil and Plant Analysis: A Working Manual, 2nd edition 2002***
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- 5. Estafan G., Sommer R., and Ryan J. 2013 Methods of Soil Plant and Water Analysis: A Manual for the West Asia and North Africa region, 3rd edition ICARDA : Beirut, Lebanon***



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Thank you for Active Participation

