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Comparison of organic matter determination methods in soil by loss on ignition and potassium dichromate method

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Abstract
Soil organic carbon (SOC) is an important property in regard to soil and environmental quality. There are many methods to determine SOC. In this study, Walkley Black and Loss on Ignition methods were chosen and sixty sub-samples representing were collected from three different parts of Arsi zone areas of Ethiopia, and then nine a composite samples were used for SOC determination methods. The variables were equipment, accuracy, efficiency, complexity, cost and environmental effect. Walkley–Black and loss on ignition (LOI) were determined on whole soils mesh size of 2 mm. From the two selected methods Loss on Ignition was the accurate and simple method for determining soil organic carbon that do not involve the use of chemicals that damage the environment are required. Walkley-Black method is a wet oxidation procedure which is relatively accurate and popular method for the determination of soil organic matter but it is time-consuming, costly and also has a high potential to cause environmental pollution because of disposal of chromium and strong acids. Therefore, loss-on-ignition procedure is a simple and cheap method for SOM estimation, which also avoids chromic acid wastes, deserves more attention. The aims of this research were to study the statistical relationships between SOC determined with the LOI and WB methods to compare the spatial variability of SOC in the selected study area. One way ANOVA was used for the significance test between the two methods and there was a significant difference between the values determined by the Walkley Black and Loss on Ignition methods, at a confidence level of 95%.

Keywords: Loss on ignition, combustion, walkley and black, ignition temperature, environmental effect, wet oxidation

1. Introduction
Soil organic matter (SOM) implies the disintegration of organic residues in soil and creation of some humus from decomposition tissues of soil organisms and well-decomposed substances (Brady and Weil, 1999) [2]. The content of organic matter in soil and its quality affects not only the possibility of plant growth, but also the whole process of soil formation. The amount of organic matter in soil is small compared to other parts of soil surface, but it still has a crucial role for soil quality. In terms of chemical properties, organic matter contains all the necessary plant nutrients. It affects favorable soil conditions and biological properties of soil by being a source of carbon essential for plant life and reproduction of microorganisms. Soil organic carbon, as one of the most influential of the agricultural soil parameters, is a useful indicator of soil fertility and crucial factor in the soil dynamics of various agrochemicals (Pitts *et al*., 1986) [10].
The content of organic matter in the soil can be determined by various methods of wet or dry combustion. Most common wet combustion method is the dichromate method by Walkley and Black (1934) [13] with potassium dichromate and sulfuric acid. The content of organic carbon and thus organic matter in soil samples, after the combustion, is determined by titration with ferrous sulphate solution followed by diphenyl amine indicator. Dry combustion method refers to the loss on ignition (LOI) method where samples are burned at high temperatures in muffled oven and the organic matter content is calculated from the weight loss. The loss on ignition is determined as a percentage of dry matter content and should be corrected for clay content to give an indication of the organic matter content in the soil. Such approach is widely adopted in soil science as it is easy to use, inexpensive, rapid, requires no specialized training, and it has shown to have strong statistical relationships with organic carbon (OC) estimated by standard dry combustion procedures (Sutherland, 1998).

However, its accuracy and precision for predicting organic matter is questioned, it is a rough estimate of organic matter content (Sato *et al*., 2014). Hereafter dry combustion may under- or overestimate the organic matter content depending on what else is present in the soil sample. Structural water in clay lattices and carbonates in calcareous soils can potentially be volatilized and contribute to the LOI result. The method may also give variable results due to errors introduced through sample handling and the conditions within the muffle furnace (Goldin, 1987). There are many methods of determining SOM content. This study included comparisons between Walkley-Black and loss on ignition. Walkley-Black is a classical wet analytical method using the dichromate oxidation-reduction technique (Walkley, 1947; Walkley and Black, 1934) [13, 12]. Loss on ignition is a widely applied method based on weight loss at a certain temperature and duration of ignition (Kamara *et al*., 2007) [4]. Several comparisons (Meersmans *et al*., 2009; McCarty *et al*., 2010) [7, 6] have been made between methods mentioned above and other organic carbon determining methods. However, no comprehensive comparison for these methods has been reported to date. In addition, soils from only one region or one specific type of soils were used for previous methods comparisons. Further, only the accuracy of the methods was assessed and compared in existing literature, whereas equipment, price, availability, efficiency, complexity and environmental effects all contribute to the method chosen for a particular study or laboratory. The objective of this study were; to evaluate a loss on ignition (LOI) method as an alternative to combustion methods and dichromate methods in soils and to compare the spatial variability of SOM determined using both mentioned methods. Walkley-Black and loss on ignition were the methods chosen for comparison. Along with accuracy of a method, other variables including equipment, efficiency, complexity, cost and environmental effects were assessed and compared.

2. Materials and Methods

2.1 Study area and Soil Sampling

Study was conducted at agricultural field of kulumsa's sub centers: Kofele, Asasa and Meraro. Each locations are the most important agricultural lands in the area. Sixty composite surface (0-20cm) soil samples were randomly collected from three different locations with nine composite soil samples from each locations. Geographically, the study area at Kofele is located between 07^0 04'28"N and $38^0$47'11"E with an elevation of 2660 meters above sea levels; Aasasa is located between 07^0 7' 09" and 039^0 11' 56" E with an elevation 2380 meters above sea level and Meraro is located between $07^0$24'27''N and $039^0$14'56''E with an elevation of 2990 meters above sea levels.

2.2 Sample and Reagent preparation

For both methods, the soil sample were air dried, grinded by using mortar and pestle and sieved through a 2-mm sieve. All the chemicals (Potassium dichromate ($K_2Cr_2O_7$) 1.0N standard solution, Ferrous sulphate 0.5N solution, Concentrated Sulfuric acid (H_2SO_4) d= 1.84) used for the determination of SOM by walkey and black method were analytical grade.

2.3 Soil Sample Analyses

2.3.1 Loss on Ignition Analysis

The content of organic matter was determined by two methods, the potassium dichromate method and loss on ignition method. For the LOI analyses, the soil samples were air dried, grinded by mortar and pestle and sieved through a 2-mm sieve. The samples were weighted, then oven-dried at 105 ^{0}C overnight, cooled in a desiccator, and weighed before they were combusted at 360 and 550 ^{0}C for 2 h in a muffle furnace (Carbolite-5/00/1185). After combustion, the samples were cooled in a desiccator and weighed again. An estimation of SOM percentage from the loss on-ignition method (SOM_{LOI}) was calculated by the following equation (Schulte and Hopkins, 1996):

SOM_{LOI} = [(soil weight after combustion−oven-dry soil weight)/oven-dry soil weight] × 100 (1)

2.3.2 Walkey and Black Analysis

The dichromate method is wet combustion method for determination of organic carbon with potassium dichromate and concentrated sulfuric acid where organic carbon is later measured by titration method. The measured OC is then multiplied by correction factor 1.72 to obtain the organic matter content. The most common procedure involves reduction of potassium dichromate ($K_2Cr_2O_7$) by OC compound and subsequent determination of the unreduced dichromate by oxidation redaction titration with ferrous sulfate (Walkley and black, 1934; Walkley 1947; FAO, 1974) [13, 12]. Soil organic matter is oxidized under standard conditions with potassium dichromate in sulfuric acid solution. A measured amount of $K_2Cr_2O_7$ is used in excess of that needed to destroy the organic matter and the excess is determined by titration with ferrous ammonium sulfate or ferrous sulfate solution, using diphenylamine indicator to detect the first appearance of unoxidized ferrous iron. During the oxidation of $K_2Cr_2O_7$ with organic matter the $Cr_2O_7^{-2}$ reacts with carbon as follows:

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

The excess $Cr_2O_7^{-2}$ is back titrated with standard Fe^{++} solution to determine the amount that has reacted. Ferrous ion (Fe^{++}) reacts with $Cr_2O_7^{-2}$ as follows:

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

The walkey and black procedure is based on the principle that the organic matter is oxidized by treating soil with concentrated sulfuric acid. Based on this, 0.5g of soil were weighted in to 500ml conical flask. 10ml of 1.0N $K_2Cr_2O_7$ solution were added and the flask was swirled gently to disperse the soil in the solution. 20ml of concentrated H_2SO_4 was added and immediately swirl the flask gently until soil and reagents were mixed. The mixture were heated on the hot plate at 150 ^{0}C for 1 minute and allowed to stand for 30 minute, then 200ml of distilled water were added in to the cooled solution. Finally, 5-6 drops of ferroin indicator added and titrated with 0.5N ferrous solutions. As the end point was approached, the solution takes on a greenish cast and then changes to dark green. At this point, the ferrous sulphate added drop by drop until the color changes sharply from blue to red.

The Carbon Content of the Soil is:

$$\% C = \frac{N \times V1 - V2 \times 0.39 \times mcf}{s}$$

Where:

N = normality of ferrous sulphate solution (from blank titration).

V1 = ml ferrous sulphate solution used for blank.

V2 = ml ferrous sulphate solution used for sample.
s = weight of air-dry sample in gram
0.39 = 3 x 10^{-3} x 100% x 1.3 (3 = equivalent weight of carbon)
mcf = moisture correction factor.
Conversion of% carbon to% organic matter is done with the following empirical factor 1.724:

% Organic matter = 1.724 x% carbon

2.4 Methods Evaluation

To evaluate the methods, data accuracy was compared and the apparatus, equipment and reagents were listed for each method. The coefficient of determination (r2) between methods was used to assess the accuracy. The number of samples run in the laboratory in an 8-hour day was considered as efficiency of the procedure. The time it takes to train a technician or student to perform a test was regarded as complexity. The cost includes initial set-up cost, cost/sample with and without labor. The environmental effect was considered if hazardous wastes were produced by a method.

3. Statistical analyses

One way ANOVA was used for the significance test between the two methods and it was indicated that there was a significant difference between the values determined by the Walkley Black and Loss on Ignition methods, at a confidence level of 95%. F-Test was also used to test the significance variation of the samples results collected from the three sampling sites. The F-test indicated that there is were no significant difference on the variance values of Loss on Ignition at 360 ℃ and at 550 ℃ but there was a significant difference between the values determined by Walkley Black Method and Loos on Ignition Methods, at 95% confidence level.

4. Results and Discussion

Descriptive analyses of the measured properties for the both methods are given in Tables 1. The SOM values calculated by the walkley-Black method ranged from 4.33 to 4.70% and SOM by LOI method at 360 ^{0}C and 550 ^{0}C 3.17 to 3.70%, 2.87 to 3.26% respectively. The coefficient of determination decreases with increasing ignition temperatures, as was also reported by (Abella and Zimmer, 2007, Salehi *et al*, 2011) [1, 11]. It seemed that this decrease occurred to prevent the overestimation of SOM at higher temperatures. At 550 ^{0}C, R2 decreases with similar RMSE to WB method increasing ignition temperature were more noticeable. As shown on Table 1 the organic matter values determined by the two methods indicated that the results obtained by the Walkley Black Method were higher than that of the results obtained by the Loss on Ignition methods at the specified temperatures. Similarly organic matter values determined by the Loss on Ignition at 360 ℃ were higher than that of Loss on Ignition results obtained at 550 ℃.

Table 1: Descriptive statistics soil samples determined by Walkley Black and Loss on Ignition methods (%)

Determining methods			
Sample code	**Walkley Black (%)**	**Loss on Ignition(%)**	
		At 360 ℃	**At 550 ℃**
k—01	4.55	3.53	3.09
k—02	4.65	3.53	2.93
k—03	4.70	3.45	3.21
AS—4	4.45	3.45	3.26
AS—5	4.33	3.17	3.07
AS—6	4.56	3.25	2.87
ME—7	4.33	3.70	3.20
ME—8	4.58	3.60	3.21
ME—9	4.47	3.35	3.09
mean	4.51	3.45	3.10
Sd.error	0.04	0.06	0.04
sdeviation	0.13	0.17	0.13
variance	0.02	0.03	0.02
minimum	4.33	3.17	2.87
Maximum	4.70	3.70	3.26
R^2	0.167	0.033	0.021
Intercept	4.610	3.456	3.067
Slope	0.019	-0.0035	0.0205

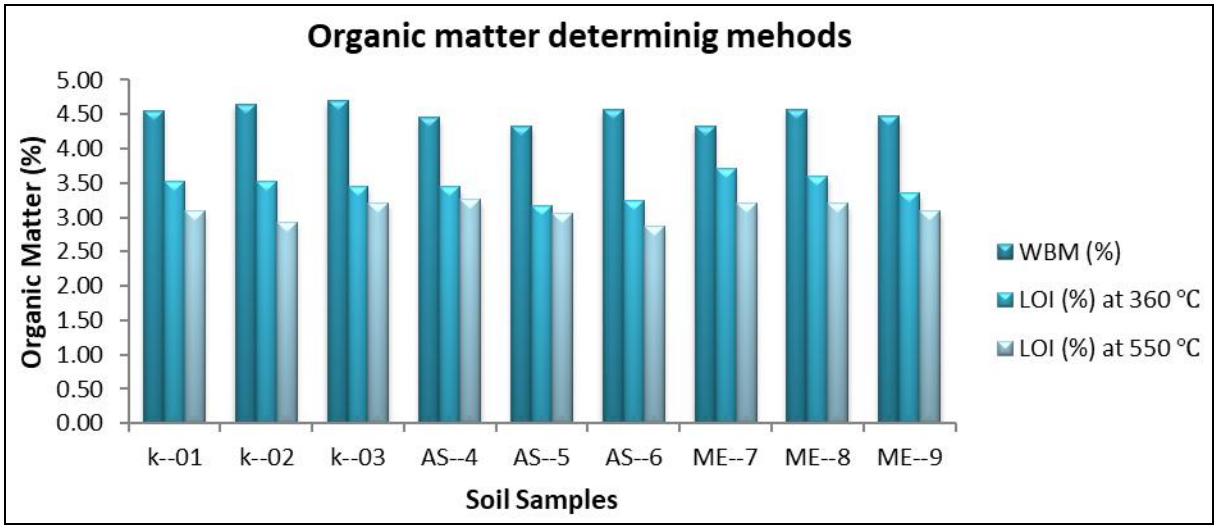

Fig 1: Organic matter determining methods by walkley black and loos on ignition methods in soil samples

As shown on Fig 1. The organic matter results obtained by the Walkley Black and Loss on Ignition methods are displayed and the results showed that there is a difference between the values obtained by the Walkley Black method and Loss on Ignition methods. On the other hand the results obtained by the Loss on Ignition methods at 360 ℃ and 550 ℃ temperatures are almost similar and there was no significance difference at the 95% confidence level. The

correlation analysis of the samples indicated that, almost there was a strong and positive correlation between the samples collected from three different places and has a correlation coefficient values ranged from + 0.9275 - 0.9999 as shown on Table 2.

Table 2: Correlation coefficient of the samples

Sample	k--01	k—02	k—03	AS--4	AS--5	AS—6	ME--7	ME--8	ME—9
k—01	1.0000								
k—02	0.9986	1.0000							
k—03	0.9891	0.9801	1.0000						
AS—4	0.9887	0.9795	1.0000	1.0000					
AS—5	0.9745	0.9615	0.9969	0.9971	1.0000				
AS—6	0.9964	0.9906	0.9981	0.9979	0.9901	1.0000			
ME—7	0.9877	0.9945	0.9540	0.9531	0.9275	0.9708	1.0000		
ME—8	0.9999	0.9978	0.9912	0.9908	0.9778	0.9975	0.9853	1.0000	
ME—9	0.9926	0.9850	0.9997	0.9996	0.9945	0.9993	0.9615	0.9943	1.0000

5. Methods Comparison

In this study Walkley-Black and Loss on Ignition methods were used for comparison by considering the accuracy, efficiency, complexity, equipment, cost and environmental effects of the SOC determination methods. From these two methods, loss on ignition is the simplest, meaning it take the least time. The method with the cheapest initial set-up is Walkley-Black. There are no reagents or additional costs for loss on ignition. When labor is taken into consideration, based on the efficiency and minimum wage for cost of labor, loss on ignition is the least expensive method. Loss on Ignition has no or minimal environmental effect other than Walkley-Black, which need proper disposal for some hazardous reagents used in the procedures. Considering all the factors, loss on ignition is an approach combining accuracy, rapidity, simplicity, relatively cheap set-up, low cost/sample and no hazardous wastes, which may pave the way for a standard approach of routine analyses for soil organic carbon in soil testing laboratories. Wet oxidation procedure, Walkley-Black (WB) method, is a routine, relatively accurate, and popular method for the determination of soil organic matter (SOM) but it is time-consuming, costly and also has a high potential to cause environmental pollution because of disposal of chromium and strong acids used in this analysis. Therefore, loss-on-ignition (LOI) procedure, a simple and cheap method for SOM estimation, which also avoids chromic acid wastes, deserves more attention (Konare *et al*, 2010, Salehi *et al*,2011) [5, 11]. The general linear form of the regression equation was calculated to estimate SOMLOI from SOM obtained by the WB method for both overall samples and individual plains. Forty soil samples were also randomly selected to compare the SOM and CCE before and after ignition at each temperature. Overall accuracy of the continuous maps generated for the LOI and WB methods was considered to determine the accordance of two procedures. Results showed a significant positive linear relationship between SOMLOI and SOMWB. The summary of Walkley-Black and Loss on Ignition methods comparison were discussed in Table 2 below.

Table 2: General description of Walkley Black and Loss on Ignition Methods

Items	Walkley Black Method (WB)	Loss on Ignition (LOI)	Reference
Description	• Quantifies the amount of oxidizable organic matter with a known amount of chromate in the presence of sulfuric acid	• Direct but approximate measurement of organic matter by determination of weight loss following combustion at moderate temperatures	(Chatterjee *et al*., 2009) [3]
Speed	• Time consuming and dichromate oxidation-reduction technique	• Rapid, simple, low-cost and environmentally friendly method	(Chatterjee *et al*., 2009) [3]
Advantages	• Low cost, minimum equipment requirement, simplicity and availability	• An approach combining accuracy, rapidity, simplicity, relatively cheap set-up, low cost/sample and no hazardous wastes • Chemical reagents are not used	(Nelson and Sommers, 1996) [8]
Drawback	• Production of hazardous chemicals, such as dichromate which can cause carcinogen. • Cr a heavy metal waste by the USEPA. It is a hazardous chemical	• Overestimation of organic matter can occur because minerals in soil lose structural water at a certain temperature • Less suitable for low organic matter soils, since errors from inorganic constituents are amplified	(Nelson and Sommers, 1996) [8]

6. Conclusion

A significant linear relationship was observed between SOM_{LOI} at the temperature rates and SOM_{WB}. A temperature around 360 ^{0}C was identified as an optimum for LOI method which almost no significant difference with WB method. Determination of organic carbon through the LOI method is suitable for exploratory soil surveys where rough estimation of organic matter is required. If a laboratory already has a muffle furnace, an analytical balance and an oven or purchasing the equipment is no issue, loss on ignition is the cheapest method with a desirable accuracy (r2=0.86). If there is a cost issue with initial set-up, but high accuracy is required, Walkley-Black (r2=0.89) is a reliable choice with $300 for basic set-up of chemical supplies.

Considering all the factors, loss on ignition is an approach combining accuracy, rapidity, simplicity, relatively cheap set-up, low cost/sample and no hazardous wastes, which may pave the way for a standard approach of routine analyses for SOC in soil testing laboratories as well as research laboratories in universities. The recommendation for methods depends on different situations encountered. In this research, loss on ignition appears to be a good choice for routine soil organic carbon analyses due to its combination of merits such as easy set-up, high accuracy, efficiency, simplicity, low cost and no hazardous wastes. Walkley–Black carbon and loss on ignition (LOI) were determined on whole soils mesh size of 2 mm. From the two selected methods Loss on Ignition was the accurate and simple method for determining soil organic carbon that do not involve the use of chemicals that damage the environment are required. Although our results indicated that selected temperatures were not accurate enough to show the spatial variability of organic matter in different locations, the efficiency of LOI method should be investigated for asasa soils with lower organic matter. We also recommended such a study to be to be done at temperatures from 300 to 400 0C with different duration of ignition.

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