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# Comparison Between Walkley-Black and Loss On Ignition Methods for Organic Matter Estimation in Different Moroccan Soils

Zineb El Mouridi[1,2*], Rabea Ziri[2], Ahmed Douaik[1], Sahar Bennani[1], Ibtissame Lembaid[1], Loubna Bouharou[3], Najiba Brhadda[2], Rachid Moussadek[1,4]

[1] National Institute of Agricultural Research (INRA), BP 6356, CRRA Rabat, Av. Al Irfane, 10100 Rabat, Morocco

[2] Ibn Tofail University, Faculty of Sciences, Plant and Animal Productions and Agro-Industry Laboratory, Department of Biology, PO Box 133, Kénitra, Morocco

[3] Ibn Tofail University, Faculty of Sciences, Geoscience Laboratory, Department of Geology, PO Box 133, Kénitra, Morocco

[4] International Center for Agricultural Research in the Dry Areas (ICARDA), North-Africa Platform, BP: 6443, Rabat Institutes, Av. Al Irfane, 10100 Rabat, Morocco

* Corresponding author's e-mail: zineb.elmouridi@inra.ma

## ABSTRACT

The present study aims to compare two different methods for the determination of soil organic matter (SOM); the wet acidified dichromate oxidation methods Walkley-Black (WB) and the combustion's one (mass loss on ignition – LOI); in order to find out the best one in terms of suitability, environmentally-friendly, time-efficiency and economical benefit. Forty-five Moroccan soil samples (El Guich, Koudia and Merchouche area) from 0–20 cm depth with different classes, textures and organic matter contents were evaluated. According to the results, a strong linear relationship was observed between LOI-OM and WB-OM methods for all the sites. However, El Guich and Koudia recorded much larger regression coefficients ($0.745^{***}$ and $0.611^{***}$ respectively) compared to Merchouche site ($0.452^{**}$). Therefore, the SOM by walkley-Black method can be easily calculated from the LOI method at 500±25 ºC for 2 hours using linear equation in order to minimize the analysis time and consumption of chemicals for routine laboratory. In addition, it was noticed that the SOM contents calculated by the LOI method are higher in Merchouche soils compared to that of WB due to the presence of a large amount of clay minerals in the Merchouche soils (43.9 < %clay < 56.1) in comparison with those of El Guich (5 < %clay < 7.5) and Koudia (16.1 < %clay < 20.4). This study revealed that both methods were reliable but W.B method was more accurate and suitable for calcareous soils and those with high clay contents. However, LOI method is an accurate and beneficial for non-calcareous soils with low clay content.

**Keywords:** Clay mineralogy, Mass Loss on Ignition, Non-calcareous soil, Soil organic matter, Walkley-Black.

## INTRODUCTION

Soil organic matter (SOM) is a major parameter in assessing soil quality and is considered as a key indicator of soil health (Franzluebbers, 2002). Despite its presence in relatively small amounts in soils (1–6%), it has an overwhelming effect on many soil physical, chemical and biological properties, especially at the upper layer of the soil ( Brady and Weil, 2008; Magdoff and Van Es, 2009; Laghrour et al., 2016). Several studies demonstrated that SOM has an important influence on the improvement of soil structural stability and the reduction of erosion through strengthening bonds between aggregates able to resist to the stress caused by rapid wetting (Tisdall and Oades, 1982; Boyle et al., 1989; Lembaid et al., 2021). The SOM also enhances soil water infiltration and storage within soil by simulating microbial activity and increasing stable aggregates (Franzluebbers, 2002; Bessam and Mrabet, 2006; Nakamura and Touch, 2020; Lembaid et al., 2022). Furthermore, it improves plant growth by acting as a slow nutrient reservoir (Brady and Weil, 2008; Bessam and Mrabet, 2006), increases and preserves soil biodiversity (Mrabet, 2006), and mitigates climate change effects through carbon sequestration (Lal, 2009; Wang et al, 2013).

Several methods of measuring SOM have been developed and studied during the last decades. Walkley and Black (WB) method is based on wet oxidation (also known as wet combustion), where the organic carbon in the soil is determined via oxidation with a mixture of potassium dichromate and sulfuric acid (Slepetiene et al., 2008; El Mekkaoui et al., 2021). The organic matter consists of 58% carbon, therefore, the organic carbon values obtained are conventionally converted to SOM using a correction factor of 1.724 (Grewal et al., 1991; Paramananthana et al., 2018;). This classical method is widely used and highly adequate for calcareous soils (Grewal et al, 1991; Fernandes et al, 2015;). However, recent studies revealed many disadvantages related to the use of this method and concluded that it is expensive, much time-consuming, which uses hazardous of chemical products during the reaction for the manipulator and may lead to erroneous results due to incomplete oxidation of organic carbon where the average proportion of oxidized soil organic carbon is about 77% (Konare et al, 2010; Wang et al, 2013; Shamrikova et al., 2022). Therefore, due to these economic, environmental and human health concerns of the WB method, several laboratories are transitioning to the Loss On Ignition (LOI) method (Oscar, 2011). This technique is a promising approach for quantifying SOM. It is based on the measurement of the weight loss from a dry soil sample after calcination under a very high temperature. The weight loss measured is proportional to the amount of the organic matter present in this soil sample (Adolfo Campos, 2010; Wang et al., 2013; Vahil et al., 2017). The protocol of the experimentation varies according to the laboratory, and consequently gives different results for the same sample, in particular according to the calcination temperature which range from 350 to 1100 °C and the duration that range from 2 to 32 hours (Konare et al, 2010; Fernandes et al, 2015). The LOI technique has several advantages. It is simple, fast, inexpensive and less labor intensive, which makes it more suitable for soil laboratories routines compared to the chemical methods (Konare et al, 2010; Wang et al, 2013; Vahil et al, 2017; Alan et al, 2008). Moreover, this method requires simple standard equipment available at all laboratories such as a muffle furnace, an oven and an analytical balance.

Considering these advantages and the proven reliability of its results (Howard and Howard, 1990; Dean, 1999; Abella and Zimmer, 2006; Brunetto et al., 2006; Escosteguy et al., 2007, Salehi et al., 2011), the LOI method should be integrated as a standard method in the national soil laboratories for the determination of the SOM. In this perspective, the present study aims to compare the two methods for determining soil organic matter, Walkley-Black and loss on ignition, by analyzing 45 samples considering three types of Moroccan soil covering a range of textures and organic matter contents. The objectives were: 1) to establish the equations between WB and LOI methods, and 2) to compare the spatial variability of SOM determined using both methods in three major experimental stations of different soil features.

## MATERIAL AND METHODS

A laboratory experiment was conducted on 45 soils samples of varying organic matter content at three study sites (15 in Merchouche site (X: 00377931; Y: 00335472; Z: 410 m), 15 in Koudia site (X: 356 133; Y: 355 314; Z: 206 m) and 15 in El Guich site (X: 00364958; Y: 00376561; Z: 51 m)) from Rabat region, Morocco (Figure 1).

Soil samples were collected from both cultivated and uncultivated areas using a hand auger at 0–20 cm depth, then air dried, crushed, sieved to 0.2 mm and 2 mm and put into boxes before being analyzed.

The particle size distribution was carried out by the Bouyoucos method using sodium pyrophosphate as a dispersing agent after the destruction of the total limestone by a strong acid (3N HCl) and the degradation of the organic matter by hydrogen pyroxide ($H_2O_2$, 30%) followed by heating on a hot plate over several hours. The total limestone is determined by volume calcimetry using the Bernard's calcimeter, which is based on the decomposition of calcium carbonate present in the soil by the effect of hydrochloric acid (HCl) and the measurement of the volume of $CO_2$ released.

The analysis of organic matter was carried out by two recognized methods:

1) Walkley and Black method (WB) where the

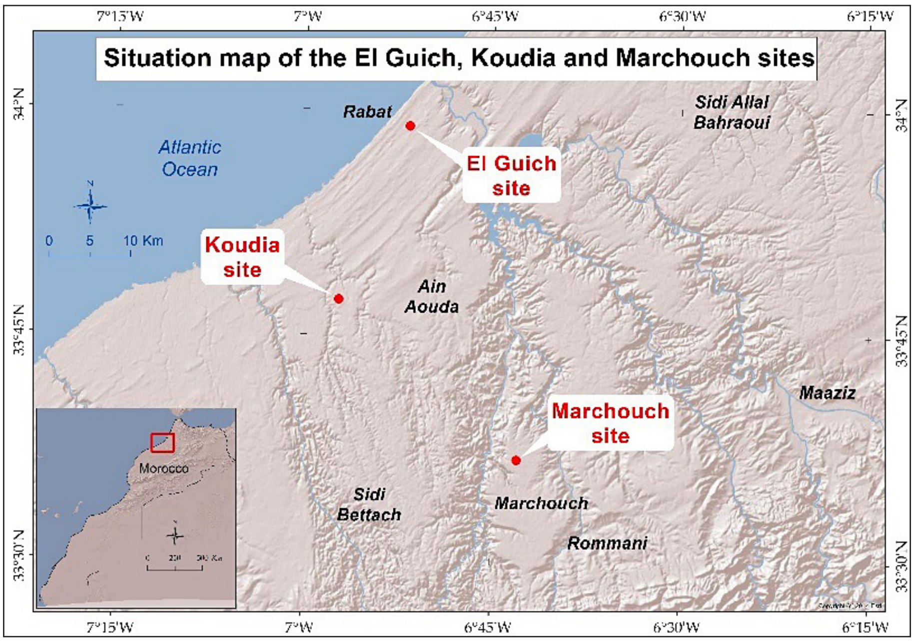


**Fig. 1.** Situation map of the El Guich, Koudia and Marchouche study sites

oxidation of organic carbon is carried out cold under wet conditions with potassium dichromate ($K_2Cr_2O_7$) in a sulfuric environment ($H_2SO_4$) until the release of $CO_2$. The excess dichromate unused in the reaction is determined by 0.5N Mohr's salt in the presence of diphenylamine and sodium fluoride (NaF) (Dabin and Brion, 1967; El Mekkaoui et al, 2021). The organic matter (OM) content is obtained by multiplying the percentage of total carbon (C) by the Bemmelen factor 1.724 (conversion factor) according to equation (1):

$$OM\ (\%) = C\ (\%) \cdot 1.724 \quad (1)$$

2) Loss on ignition method (LOI), which is a weight determination, based on the calcination of total organic matter in dry conditions. Soil samples (8g) were placed in porcelain crucibles of specific mass, dried at 105 °C for 2 hours to remove moisture and then weighed. Then, they were placed in the muffle furnace at 500 °C for 2 hours, cooled in a desiccator and reweighed. The percentage of mass lost to ignition is calculated as the weight loss between 105 °C and 500 °C according to equation (2) (Moebius-Clune et al, 2016):

$$LOI\ (\%) = \frac{Weight\ 105℃ - Weight\ 500℃}{Weight\ 105℃} \times 100 \quad (2)$$

where: weight 105 °C is the mass of the soil sample dried at 105 °C, and weight 500 °C is the mass of the calcined soil sample at 500 °C.

The LOI (%) is converted to OM (%) using equation (3):

$$OM\ (\%) = [LOI\ (\%) \cdot 0.7] - 0.23 \quad (3)$$

### Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics 25. Descriptive statistics of soil samples includes Maximum, Minimum, Mean and standard deviation data. Spearman rank and Pearson correlation coefficient were calculated to assess the degree of linear correlation between the two methods. The relationships between WB-OM and LOI-OM were further investigated by simple linear regression.

## RESULTS AND DISCUSSION

### Descriptive statistics of soil samples

The results of the 45 samples were used to measure the soil organic matter (SOM) considering two methods, Walkley and Black method (WB) and Loss on ignition method (LOI). Table 1 illustrates descriptive statistics (classes, particle size composition, total limestone and organic matter contents) of soil samples collected from the three study sites (El Guich, Koudia and Merchouche). The results showed that these soils are not calcareous, and belong to different classes covering a wide range of soil textures: El Guich (sandy,

**Table 1.** Descriptive analyses of organic matter contents determined by two methods, soil classes, limestone and particle size composition in different soils of studied soil sites

| Site | | N | Minimum | Maximum | Mean | Standard deviation |
|---|---|---|---|---|---|---|
| El Guich | LOI_OM | 15 | 0.39 | 1.36 | 0.956 | 0.265 |
| | WB_OM | 15 | 0.51 | 1.89 | 1.335 | 0.402 |
| | Differences | | 0.12 | 0.53 | | |
| | Limestone | 15 | 0.0 | 0.0 | 0.000 | 0.00 |
| | Clay | 15 | 5.0 | 7.5 | 5.967 | 1.157 |
| | Silt | 15 | 2.4 | 10.8 | 5.847 | 2.275 |
| | Sand | 15 | 81.7 | 90.1 | 88.187 | 2.180 |
| | Soil Class [(1)] | 15 | Arenosol | | | |
| Koudia | LOI_OM | 15 | 1.36 | 2.48 | 1.692 | 0.278 |
| | WB_OM | 15 | 2.80 | 4.60 | 3.520 | 0.544 |
| | Differences | | 1.44 | 2.12 | | |
| | Limestone | 15 | 0.0 | 4.0 | 1.180 | 1.595 |
| | Clay | 15 | 16.1 | 20.4 | 18.393 | 1.315 |
| | Silt | 15 | 19.1 | 24.9 | 21.767 | 1.899 |
| | Sand | 15 | 57.6 | 62.3 | 59.840 | 1.329 |
| | Soil Class [(1)] | 15 | Chromic Luvisol | | | |
| Merchouche | LOI_OM | 15 | 2.18 | 4.08 | 2.864 | 0.514 |
| | WB_OM | 15 | 1.50 | 3.30 | 2.520 | 0.512 |
| | Differences | | 0.68 | 0.78 | | |
| | Limestone | 15 | 0.0 | 0.3 | 0.020 | 0.078 |
| | Clay | 15 | 43.9 | 56.1 | 48.70 | 3.0488 |
| | Silt | 15 | 28.3 | 34.4 | 31.813 | 1.791 |
| | Sand | 15 | 15.4 | 22.8 | 19.460 | 2.413 |
| | Soil Class [(1)] | 15 | Vertisol | | | |

**Note:** [(1)] According soil taxonomy.

Arenosol); Koudia (sandy loam, chromic-Luvisol); Merchouche (clayey, vertisol).

Figure 2 showed that El Guich has the lowest values of SOM compared to the other sites based on the two methods, which can be explained by the sandy texture of its soil. The highest values were noted for Merchouche site using LOI method, while Koudia values were the highest using WB method. When comparing the estimation of SOM based on the two methods, it was noticed that the SOM contents calculated by the LOI method are higher in merchouche soils compared to those of WB (Figure 2). This is due to the presence of a large clay mineralogy in the merchouche soils (43.9 < %clay < 56.1) in comparison with El Guich (5 < %clay < 7.5) and koudia (16.1 < %clay < 20.4). Our results are in agreement with other studies (Abella et al., 2007; Vahel and al., 2017; Jensen et al., 2018), which support the fact that the LOI method often overestimates the organic matter content in soils with high clay content.

In fact, the clay soil retains more moisture which is lost as water during the heating process, and given that the LOI method is based on the difference in weights, this moisture will be taken into account in the calculation of SOM and may give slightly higher results compared to the WB method.

## Analysis of variance and means comparison

The analysis of variance showed a very highly significant difference between the two methods of determining SOM, WB and LOI ($p < 0.001$). Duncan's mean comparison test showed that, for both methods, each site is different from the other. This result was confirmed by Kruskal-Wallis test where the differences were significant between the sites.

## Statistical relationship between the two methods

The Pearson coefficient was used to estimate the relationship between the two methods for each site.

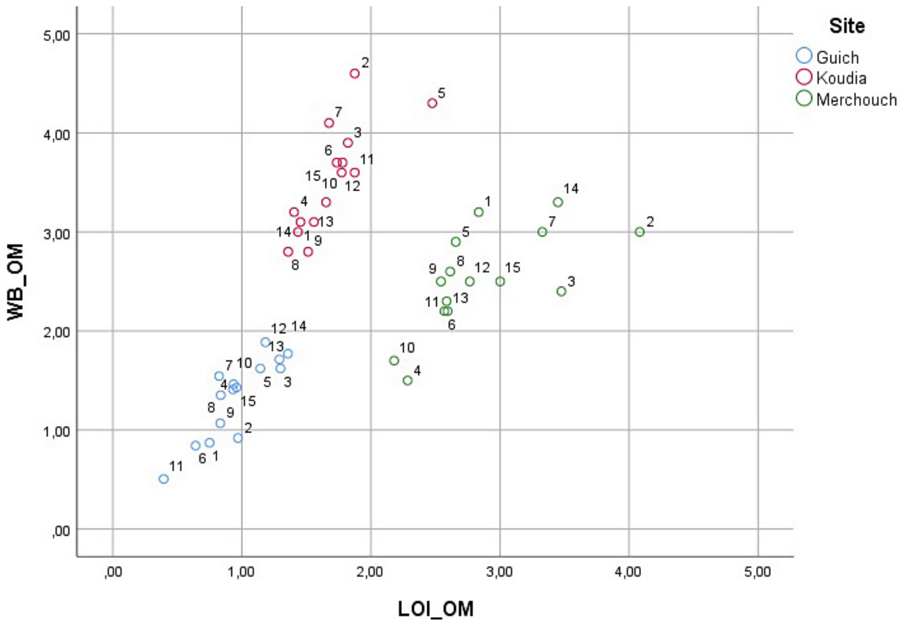


**Fig. 2.** Distribution of SOM (%) in the three sites based on the two methods WB and LOI

The results showed that the correlations between the two SOM measurement methods, WB and LOI, were highly significant at each site. The value of the highest correlation coefficient has been recorded for El Guich site (0.86) followed by Koudia site (0.78) and finally Merchouche site (0.67).

The ranking-based Spearman correlations between the two methods still remain highly significant with larger coefficients compared to the Pearson coefficients. The Spearman coefficient between the two methods varies between (0.84 in El Guich, 0.83 in Koudia and 0.73 in Merchouche).

The regression models of the LOI-OM method on WB-OM were highly significant for the Merchouche site, noting the lowest regression coefficient (adjusted $R^2 = 0.452^{**}$) (Figure 5). However, those of the El Guich and Koudia sites presented a very strong significant relationship by recording much larger regression coefficients (adjusted $R^2 = 0.745^{***}$ and adjusted $R^2 = 0.611^{***}$ respectively) (Figure 3 and 4). Therefore, depending on the soil samples types, the SOM by walkley-Black method can be easily calculated from the LOI method at 500 ± 25 ºC for 2 hours, using the following simple linear equation for each site (Figure 3). These equations will help us to calculate the SOM of one method from the other in order to minimize the analysis time and the consumption of chemicals in the laboratory routine.

## CONCLUSIONS

By comparing the traditional method for the determination of the SOM (the Walkley-Black

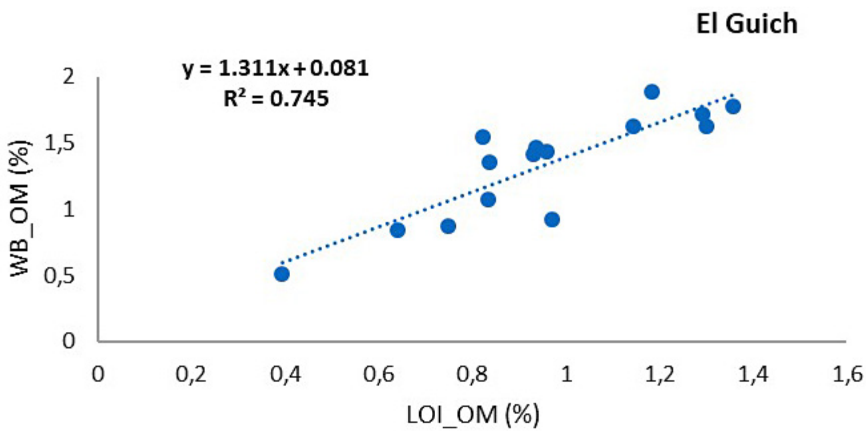


**Fig. 3.** Regression coefficient $R^2$ and the relationship between the two methods of estimating organic matter (WB-OM =Y, LOI-OM =X) for El Guich site

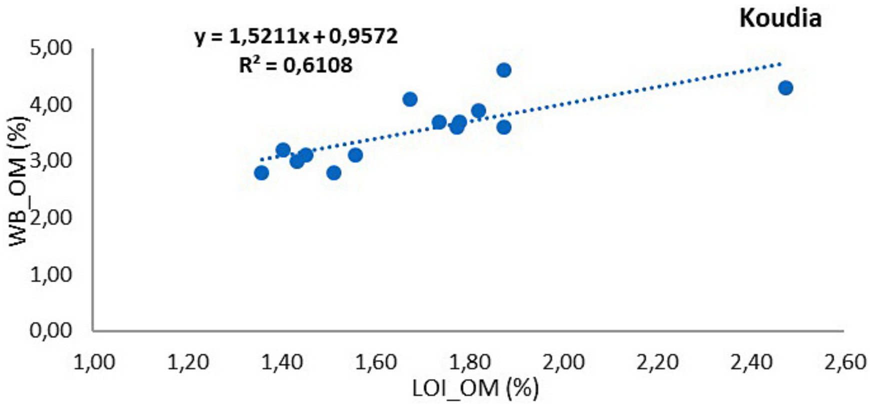


**Fig. 4.** Regression coefficient $R^2$ and the relationship between the two methods of estimating organic matter (WB-OM =Y, LOI-OM =X) for Koudia site

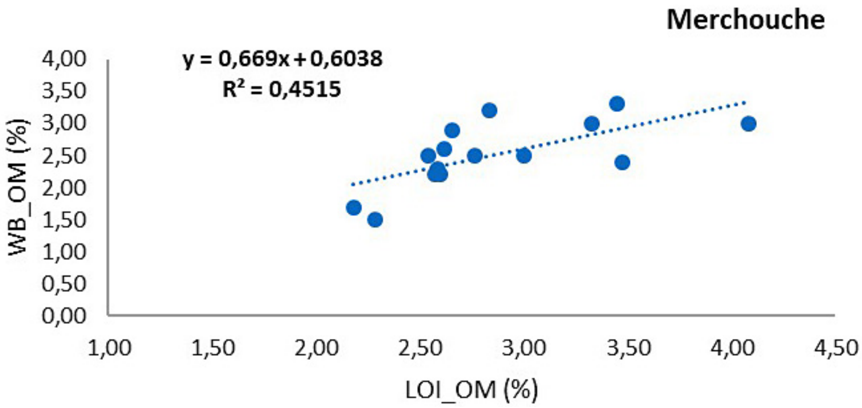


**Fig. 5.** Regression coefficient $R^2$ and the relationship between the two methods of estimating organic matter (WB-OM =Y, LOI-OM =X) for Merchouch site

method) with the combustion's one (LOI method), a strong significant correlation between the two methods was noted in each study site, recording the highest correlation coefficient for the El Guich site (0.838**) followed by Koudia (0.828**) and finally Merchouche (0.727**). Our study demonstrated that the LOI method was reliable and can be used to accurately predict organic matter contents in Moroccan soils with low clay (El Guich (sandy soil), Koudia (loamy sand)) and limestone contents. However, the WB method can be always suggested for all types of soils (more precisely in calcareous soils and high clay content). Considering that the LOI is a fast and less expensive method and presents several advantages, namely analysis of huge samples simultaneously, low cost of equipment, safety of personnel and reduced consumption of reagents, it will be more practical to calculate the SOM-WB from the LOI method, while thinking later about conducting future studies to adjust the LOI method for clay soils.

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