

Relationships between Sulfochromic and Permanganate Oxidizable Carbons in Some Soils of North and North-West of Iran

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ABSTRACT

Original Walkley-Black (OWB) method has been extensively used for measuring Soil Organic Carbon (SOC), mainly because of its convenience. However, the reliability of this method is still under speculation. In recent years, Permanganate Oxidizable Carbon (POXC) has been suggested to be more useful than the total SOC. In the present study, SOC contents of the 20 non-calcareous soil samples (0-20 cm) were determined by the OWB method and its modified versions (WB with external heating and WB using colorimetric determination) to understand their relationships with easy-to-find Soil Organic Matter (SOM) determined by the method of Loss-On-Ignition (LOI) at two temperatures (400 and 550°C). The POXC was also determined in the sampled soils and applicable relationships were specified between these methods. Eight of the 20 soils were selected to determine the accuracy of different WB methods using total organic carbon by CHN elemental analysis. Results showed strong power relationships between LOI and OWB methods. The WB method with external heating exhibited the highest recovery (95.3%) among the tested methods. This could be attributed to the finer soil particles used in the proposed method (less than 0.15 mm) than what has been used in the conventional method (less than 0.5 mm). The POXC method showed a high correlation with OWB method and, on average, accounted for only 4.1% of the Total Organic Carbon (TOC). This would likely reduce the value of POXC as an independent parameter to derive the labile fraction of SOC.

Keywords: Colorimetric method, Loss-on-ignition, Non-calcareous soils, Soil organic carbon.

INTRODUCTION

Soil Organic Matter (SOM) is a key factor in determining soil quality. SOM content can be estimated directly by the Loss-On-Ignition (LOI) method or indirectly by measuring the content of Soil Organic Carbon (SOC). The former is a convenient method for analyzing a large number of soil samples, but its accuracy is debatable (De Vos *et al.*, 2005). Additionally, there are several methods to determine SOC that, in turn, can be converted to SOM.

Determination of SOC by dry combustion method (using, for instance, an automated elemental analyzer such as CHN or LECO)

is assumed to be a reference method due to its high speed, complete carbon recovery, and no use of toxic chemicals (Ramamoorthi and Meena, 2018). The use of this method is limited because of its high cost.

On the other hand, the Walkley-Black (WB) method (Walkley and Black, 1934) and its generalized versions (Nelson and Sommers, 1996 and 1982; Allison and Moodie, 1965) are widely used due to their simplicity and low cost. However, the accuracy and precision of this method is still an issue of research (Gelman *et al.*, 2012). The main disadvantage of this method is the incomplete oxidation of organic carbon and variability in the efficiency of oxidation in

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different soils. Lettens *et al.* (2007) found evidences against the validity of the universal 1.3 conversion factor. For instance, Enang *et al.* (2018) reported that original WB method severely underestimates the SOC content in some soils of Cameroon. Therefore, providing specific correction factors for different soils based on inherent and managed soil characteristics is a way to increase the accuracy of this method (Bahadori and Tofighi, 2017). Inaccuracies will lead to wrong estimations of SOC storage and, therefore, CO₂ emission, especially in large-scale assessments (FAO, 2019).

Complete recovery of organic carbon by external heating (Heanes, 1984) and increasing precision of the method (Raveh and Avnimelech, 1972) along with reducing the volume of toxic waste (Bowman, 1998) by the use of colorimetric analysis instead of back titration (Bahadori and Tofighi, 2016) are among the modifications of the original WB method.

In addition, the WB method uses the toxic hexavalent Chromium (Cr^{VI}) (Bowman, 1998). An alternative oxidant should have at least the same or higher redox potential than Cr^{VI} ($E^0 = 1.36V$) and to be environmentally friendly such as MnO₄⁻ ($E^0 = 1.51V$). However, the reaction kinetics limits the use of these oxidants. For example, many organic substances such as phthalic acids are not oxidized by permanganate (MnO₄⁻) due to kinetic limitations (Kolb *et al.*, 2017). Measurement of Permanganate Oxidizable Carbon (POXC) in soil is a simple method for estimating Labile Organic Carbon (LOC) instead of total organic carbon, which is not dynamic at least in short-terms. This method involves the reaction of the soil LOC upon the addition of potassium permanganate (KMnO₄) and subsequent measurement of the KMnO₄ concentration change (Weil *et al.*, 2003). This method is relatively simple, inexpensive and safe under both field and laboratory conditions (Culman *et al.*, 2012).

The main purposes of this study were to: (1) Provide estimates of the strength of relationships between LOI and OWB

methods; (2) Compare the results of the modified and original WB methods in order to find their relationships; (3) Compare the accuracy of the modified and original WB methods in order to find specific conversion factors; and (4) Detect the possibility of correlation between POXC and WB methods in different soils.

MATERIALS AND METHODS

Soil Sampling and Analyses

A total of 20 non-calcareous surface soil samples (0-20 cm) from the north and north-west provinces of Iran were selected based on the 0.1N HCl test. We chose the carbonate-free soils to avoid those possible errors that came from the removal of this type of carbon before Total Organic Carbon (TOC) determination by the CHN analyzer. The soils were air-dried and grounded to pass through a 2 mm sieve for routine physicochemical analyses and then passed through 0.5 mm and 0.15 mm sieves for SOM and SOC determination, respectively. Soil texture was determined by the hydrometer method (Gee and Bauder, 1986). In addition, soil pH and EC values were measured in 1:2 soil: water ratio (Rhoades, 1996; Thomas, 1996). In eight of the 20 aforementioned soil samples used for testing validity of the methods, soil Cation Exchange Capacity (CEC) was also determined (Chapman, 1965). Then, SOM content was measured by the method of LOI at two temperatures of 400 (Nelson and Sommers, 1996) and 550 °C (Hoogsteen *et al.*, 2015). Moreover, SOC by the methods of Original WB (OWB) (Nelson and Sommers, 1996) and its Modified forms (MWBs) including the WB method under external heating (MWB-1) (Heanes, 1984) and WB method with colorimetric detection of Cr³⁺ and Cr⁶⁺ ions (Nelson and Sommers, 1996), named as MWB-2 and MWB-3, respectively, were measured. Also, the POXC content of the soils was determined (Culman *et al.*, 2012). The correlations of

MWBs methods with the OWB method (the most common method of measuring organic carbon in soil science laboratories) and the POXC method (a relatively new method) were evaluated by regression analysis. More details of SOM and SOC determination methods are given below.

LOI Method

A total of 10 g of the soil sample (passed through a 35 mesh sieve) was heated in an oven at 105°C for 24 hours. The sample was weighed again after cooling down to room temperature in a silica gel desiccator (W_{105}). Then, the oven-dried sample was ignited in a furnace at 400°C for 4 hours and weighed after cooling (W_{400}) (Nelson and Sommers, 1996). The same experiment was performed at 550°C (W_{550}) (Hoogsteen *et al.*, 2015). The SOM was obtained by Eq. (1):

$$\text{SOM}(\%) = \frac{W_{105} - W_{(400/550)}}{W_{105}} \times 100 \quad (1)$$

Where, W_{105} is the Weight of the soil sample after heating at 105°C and W_{400} and W_{550} are the Weights of soil samples after ignition at 400 and 550°C, respectively.

OWB Method

A total of 0.5 g of the soil sample (passed through a 100 mesh sieve) was transferred to a 500-mL Erlenmeyer flask. Then, 10 mL of 1N potassium dichromate solution and 20 mL of concentrated sulfuric acid were added and slightly stirred. The homogenous digestion mixture was allowed to stand at room temperature for 30 minutes. Thereafter, 200 mL of distilled water was added and the mixture was diluted with distilled water to a volume of 250 mL in a volumetric flask. A few drops of the o-phenanthroline indicator were added to 50 mL of the diluted solution and titrated with standardized 0.5N ferrous ammonium sulfate solution. The SOC was obtained by Equation (2) (Nelson and Sommers, 1996):

$$\text{SOC}_{\text{OWB}}(\%) = \frac{N_1 V_1 - 5N_2 V_2}{m} \times 0.003 \times 1.3 \times 100 \quad (2)$$

Where, N_1 and N_2 are the Normality of potassium dichromate and ferrous ammonium sulfate, respectively; V_1 and V_2 are the Volume of potassium dichromate solution consumed in the reaction and the Volume of ferrous ammonium sulfate solution consumed in the titration (mL), respectively; m is the mass of oven-dried soil sample (g), and 0.003 is the milli equivalent weight of C (g). A general correction factor of 1.3 was used to compensate for the incomplete oxidation (76%) of SOC.

MWB-1 Method

This method was the same as the OWB method only with the heating of the digestion mixture on a sand bath at 135°C for 30 min. The SOC was obtained by Equation (3) (Heanes, 1984):

$$\text{SOC}_{\text{MWB-1}}(\%) = \frac{N_1 V_1 - 5N_2 V_2}{m} \times 0.003 \times 100 \quad (3)$$

Where, the general correction factor (1.3) was not used, since it was assumed that the recovery of SOC was complete.

Colorimetric Methods (MWB-2 and MWB-3)

In these methods, the digestion mixture of the OWB method was left undisturbed for 30 minutes, then, centrifuged at 3,000 rpm for 5 minutes to remove the suspended particles. The intensity of Cr^{III} green (MWB-2 method) and/or Cr^{VI} orange (MWB-3 method) colors was proportional to oxidized and/or not oxidized SOC, respectively. In the MWB-2 method, the digestion mixture was diluted twice by adding distilled water and the intensity of the green color was measured at 600 nm wavelength (Heanes, 1984) using a spectrophotometer (UV-9200, BFL Co., China). However, in the MWB-3 method, the digestion mixture was diluted



22 times and the intensity of the orange color was measured at 430 nm wavelength (Carolan, 1948). The calibration curves were prepared using 5 mL of solutions containing different concentrations (2.8, 5.6, 8.4, 11.2, 14 and 15.4 g L⁻¹) of glucose. Diluted sulfuric acid was used as a blank to zero the absorbance.

POXC

A specified amount of air-dried soil sample (< 0.2 mm) was transferred into a polypropylene 50-mL centrifuge tube (1, 2.5, and 5 g soil for SOC contents greater than 3%, 1 to 3%, and less than 1%, respectively). To each tube, 20 mL of 0.02 M KMnO₄ solution prepared in 0.1 M CaCl₂ (pH= 7.2) was added. To adjust the pH, 0.1 M NaOH solution was used. The capped tubes were vigorously shaken by hand for a few seconds, then, shaken on a horizontal shaker at 240 cycles per minute for 2 minutes. After which, the tubes were settled for 10 minutes. Then, 1 mL of the supernatant was transferred into a 100 mL volumetric flask and mixed with 99 mL of distilled water. The absorbance was recorded at 550 nm wavelength using a spectrophotometer (UV-9200, BFRL Co., China). Standard solutions (0.05, 0.10, 0.15 and 0.20 mmol L⁻¹ KMnO₄) were used to prepare the calibration curves. The samples POXC contents were obtained by Equation (4) (Culman *et al.*, 2012):

$$\text{POXC (mg kg}^{-1}\text{)} = [0.02 \text{ mol L}^{-1} - (a + bz)] \times (9000 \text{ mg C mol}^{-1}) \times [0.02 \text{ L m}^{-1}] \quad (4)$$

Where, 0.02 mol L⁻¹ is the concentration of the added KMnO₄ solution, a is the intercept and b is the slope of the calibration curve, z is the absorbance of the soil sample, 9000 mg C mol⁻¹ is the amount of C oxidized by 1 mol of KMnO₄ reduced from Mn (VII) to Mn(IV), 0.02 L is the volume of KMnO₄ solution that reacted with soil, and m is the mass of oven-dried soil (kg) used in the analysis.

Accuracy of Different SOC Determination Methods

Eight out of the 20 soil samples with a wide SOM range from 14 to 123 g kg⁻¹, analyzed by the LOI method at 400°C, were selected to assess the accuracy of the OWB method and its three Modified versions (MWB₁₋₃). For this purpose, the TOC content of the selected soils was determined by the CHN analyzer (Eager 300 for EA 1112). The percent recovery of carbon for different methods (R) can be calculated by the findings of Bahadori and Tofighi (2017) (Equation 5).

$$R (\%) = \frac{\text{SOC measured by a given method}}{\text{TOC measured by CHN analysis method}} \times 100 \quad (5)$$

Statistical Analysis

All measurements, in duplicate, were subjected to Analysis Of Variance (ANOVA) using a General Linear Model (GLM) of the SPSS software package (Version 12.0; SPSS Inc., Chicago, IL, USA) and means were compared by the Duncan test at 5%.

RESULTS

Soil Characteristics

Characterization of the 20 studied non-calcareous soil samples showed a wide range of soil texture classes from sand to clay loam. Soil pH_{1,2} values were from 4.54 to 8.07 (7.16, on average) and the soils were not salt-affected with EC_{1,2} values covering a range of 182 to 1,333 μS cm⁻¹ (568 μS cm⁻¹, on average). The SOC content of the studied soils (OWB method) ranged from 1.47 to 61.57 g kg⁻¹ (23.04 g kg⁻¹, on average). Table 1 shows the general characteristics of the eight soil samples.

Table 1. The physical and chemical properties of the eight selected soil samples.^a

No	Clay (g kg ⁻¹)	Silt (g kg ⁻¹)	pH _{1:2}	EC _{1:2} (μS cm ⁻¹)	CEC (cmol _c kg ⁻¹)	SOC _{OWB} (g kg ⁻¹)	TOC (g kg ⁻¹)
1	60.5	301.5	6.20	287	13.5	10.01	11.50
2	94.4	511.4	7.10	746	21.6	20.41	19.00
3	333.6	581.6	7.58	602	42.8	35.12	37.90
4	367.9	594.5	7.67	844	43.6	24.72	26.20
5	252.2	158.8	4.54	182	22.0	40.61	44.60
6	92.9	244.7	7.94	247	12.7	7.00	12.10
7	18.4	448.5	7.34	672	32.4	47.36	55.20
8	9.2	1.6	8.07	445	4.4	1.51	5.00

^a CEC: Cation Exchange Capacity; SOC_{OWB}: Soil Organic Carbon by Original Walkley and Black method without applying a correction factor of 1.3; TOC: Total Organic Carbon.

Correlations between Methods of Measuring SOM, SOC, and POXC

There was a significant, but not perfect, correlation between the content of SOM measured by the LOI method at two temperatures of 400 and 550°C (Table 2). The slope of this relationship suggests that a 150°C rise in the ignition temperature resulted in an overestimation of 25% by the LOI method. The LOI method at 400°C had a stronger relationship with the OWB method than the LOI method at 550°C. Based on these linear relationships, the conversion factors of 1.566 and 1.832 were obtained to convert SOC (OWB method) to SOM at ignition temperatures of 400 and

550°C, respectively. However, a power equation (6 and 7) can better describe the data:

$$\text{SOM}_{400} = 5.30 \text{SOC}^{0.713} \quad r^2 = 0.940 \quad (6)$$

$$\text{SOM}_{550} = 7.74 \text{SOC}^{0.697} \quad r^2 = 0.923 \quad (7)$$

The OWB method had the highest and lowest correlations with MWB-1 ($r^2 = 0.994$) and MWB-3 ($r^2 = 0.755$) methods, respectively. The POXC method showed the highest correlation with OWB method ($r^2 = 0.968$) (Figure 1) and the lowest with MWB-3 method ($r^2 = 0.721$).

Precision Evaluation of LOI Methods for SOM Determination

The linear relationships between SOM by LOI method and TOC by CHN analysis method were slightly stronger at 550°C than at 400°C (Figures 2-a and -b). The intercept values of these two relationships were non-significant.

Comparing Recovery of Different Methods for SOC Determination

The order of mean recovery values of SOC for the four determination methods in eight selected soil samples was: MWB-1 (95.3%) ≈ MWB-2 (94.2%) > OWB (80.8%) > MWB-3 (66.5%). As can be seen, the SOC recovery rate by the OWB method was on average 14% lower than both MWB-1 and

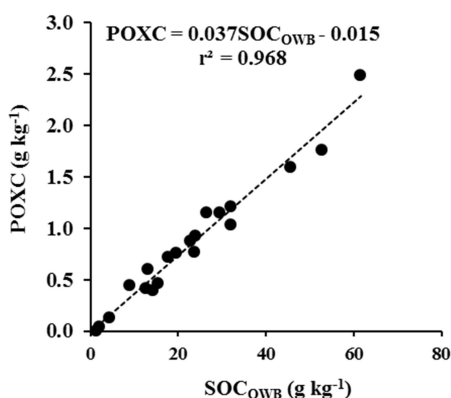
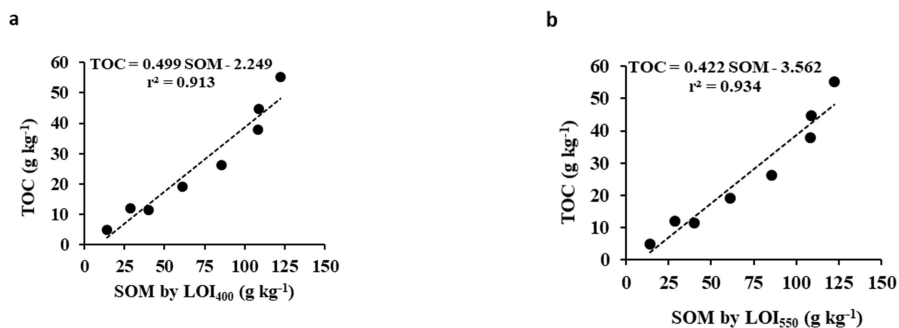


Figure 1. Relationship between Permanganate Oxidizable Carbon (POXC) and Soil Organic Carbon (SOC) measured by Original Walkley-Black method (SOC_{OWB}) in 20 non-calcareous soil samples.

**Table 2.** Regression parameters of the linear relationships between different determination methods of SOM, SOC and POXC in 20 non-calcareous soil samples.

Method ^a	Parameters of the linear regression		r ²	
	x/y	Slope		Intercept
LOI ₅₅₀ / LOI ₄₀₀		0.753 ^{**}	-1.510 ^{ns}	0.837 ^{**}
OWB / LOI ₄₀₀		1.566 ^{**}	11.744 ^{**}	0.910 ^{**}
OWB / LOI ₅₅₀		1.832 ^{**}	23.293 ^{**}	0.843 ^{**}
OWB / MWB-1		0.791 ^{**}	2.628 ^{**}	0.994^{**}
OWB / MWB-2		0.781 ^{**}	0.679 ^{ns}	0.798 ^{**}
OWB / MWB-3		0.594 ^{**}	-2.422 ^{ns}	0.755 ^{**}
POXC / LOI ₄₀₀		39.401 ^{**}	14.539 ^{**}	0.829 ^{**}
POXC / LOI ₅₅₀		46.285 ^{**}	26.390 ^{**}	0.775 ^{**}
POXC / OWB		26.930 ^{**}	1.128 ^{ns}	0.968^{**}
POXC / MWB-1		20.439 ^{**}	3.584 ^{**}	0.955 ^{**}
POXC / MWB-2		20.160 ^{**}	1.685 ^{ns}	0.763 ^{**}
POXC / MWB-3		15.308 ^{**}	-1.666 ^{ns}	0.721 ^{**}

^a LOI₅₅₀: Loss-On-Ignition at 550°C; LOI₄₀₀: Loss-On-Ignition at 400°C; OWB: Original Walkley-Black method by applying a correction factor of 1.32; MWB-1: Walkley-Black method with external heating; MWB-2: Walkley-Black method using colorimetric determination of Cr(III); MWB-3: Walkley-Black method using colorimetric determination of Cr(VI) and POXC: Permanganate oxidizable carbon. ** Indicates significant relationship at P < 0.01.

**Figure 2.** Relationships between Total Organic Carbon (TOC) determined by the CHN analyzer and Soil Organic Matter (SOM) determined by the LOI method at temperatures of (a) 400°C and (b) 550°C; LOI₄₀₀: SOM measured by the LOI method at 400°C; LOI₅₅₀: SOM measured by the LOI method at 550°C.

MWB-2 methods and the MWB-3 method showed the lowest recovery values (Table 3).

Accuracy and Precision Evaluation of Different Methods for SOC Determination

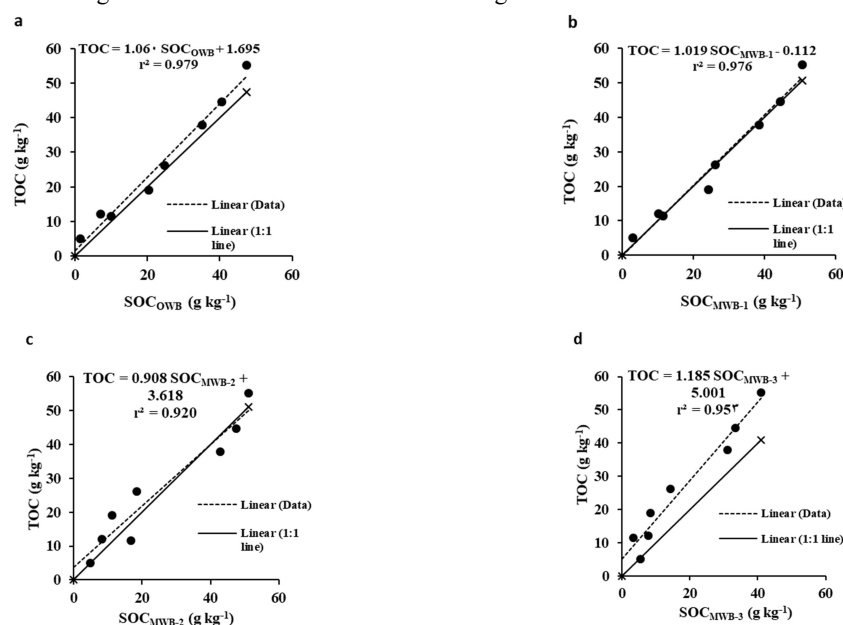
Figure 3-a illustrates the linear relationships between SOC by the OWB method (without correction factor) and TOC by the CHN analysis method. Figure 3-b,

also shows the very good agreement of the MWB-1 method with the CHN analysis method. Both methods of OWB and MWB-1 yielded almost similar precision. Figures 3-c and -d show lower precision of the two colorimetric methods (MWB-2 and MWB-3) compared to OWB and MWB-1 methods. Although the precision of the MWB-3 method was slightly higher than that of the MWB-2 method, the latter method provided more accurate estimates than the former due to the closer proximity of the regression line to the 1:1 line. After dropping the non-

Table 3. The SOC recovery values for different determination methods in eight soil samples selected.

Method ^a	SOC content (g kg ⁻¹)		SOC recovery value (%)	
	Range	Mean (SD)	Range	Mean (SD)
OWB	1.51-47.36	23.34 (±16.67)	30.2-107.4	80.8 (±23.9) b
MWB-1	2.98-50.65	26.04 (±17.31)	59.5-127.1	95.3 (±18.4) a
MWB-2	4.78-51.45	25.12 (±18.22)	103.2-195.1	94.2 (±27.6) a
MWB-3	3.39-40.91	18.08 (±14.70)	29.5-108.8	66.5 (±23.7) c
TOC	5.00-55.20	26.44 (±17.86)	-	-

^a OWB: Original Walkley-Black method without applying a correction factor of 1.3; MWB-1: Walkley-Black method with external heating; MWB-2: Walkley-Black method using colorimetric determination of Cr(III) and MWB-3: Walkley-Black method using colorimetric determination of Cr(VI). (a-c) Different letters indicate a significant difference at $P < 0.05$ according to the Duncan test.

**Figure 3.** Relationships between Total Organic Carbon (TOC) determined by the CHN analyzer and methods of (a) Original Walkley-Black (OWB) without conversion factor, (b) MWB-1, (c) MWB-2, and (d) MWB-3.

significant intercepts in Figure 3 (a-d), the slopes are changed as follows:

$$\text{TOC} = 1.110 \text{ SOC}_{\text{OWB}} / \text{without CF} \quad (8)$$

$$r^2 = 0.993 \quad \text{SE} = 2.8$$

$$\text{TOC} = 1.016 \text{ SOC}_{\text{MWB-1}} \quad (9)$$

$$r^2 = 0.993 \quad \text{SE} = 2.8$$

$$\text{TOC} = 1.005 \text{ SOC}_{\text{MWB-2}} \quad (10)$$

$$r^2 = 0.928 \quad \text{SE} = 5.5$$

$$\text{TOC} = 1.361 \text{ SOC}_{\text{MWB-3}} \quad (11)$$

$$r^2 = 0.977 \quad \text{SE} = 5.1$$

In the present study, a strong relationship between POXC and TOC by CHN analysis ($r^2 = 0.954$) was obtained (Figure 4). In the

eight soil samples studied, POXC contribution to the TOC was in the range of 0.8-6% (4.1%, on average) (Figure 5).

Correlation between Soil Properties and Organic Carbon Content Determined by Different Methods

Among soil properties, only Cation Exchange Capacity (CEC) had a significant positive correlation with SOC determined by the methods of CHN analysis ($r = 0.655^*$),

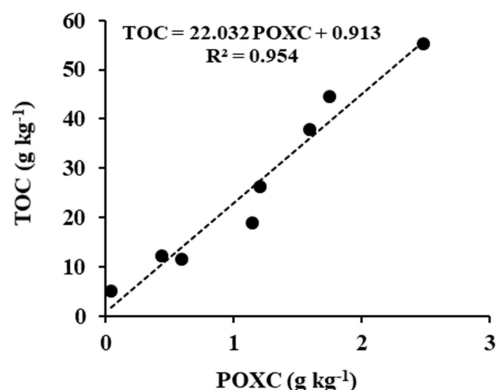


Figure 4. Relationship between Total Organic Carbon (TOC) obtained by the CHN analyzer and Permanganate Oxidizable Carbon (POXC).

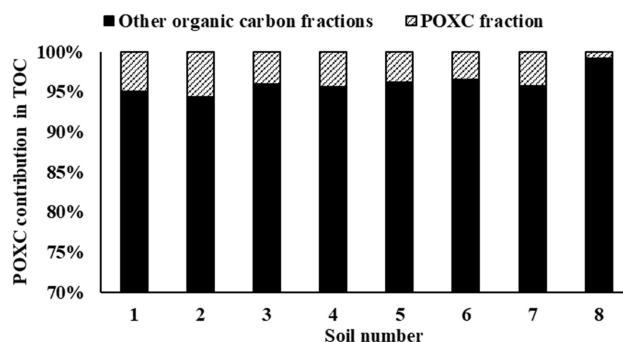


Figure 5. The contribution of POXC in TOC in 8 selected soil samples.

OWB ($r = 0.702^*$), MWB-1 ($r = 0.688^*$), and POXC ($r = 0.693^*$) at $P < 0.05$.

DISCUSSION

Evaluating Loss-on-Ignition Method

An excess weight loss of 25% observed in the LOI method at 550°C compared to 400°C (Table 2) may be due to the loss of hygroscopic, structural, or interlayer water molecules from clay minerals (Grewal *et al.*, 1991). However, John (2004) reported that different parts of SOM are oxidized by ignition at different temperature ranges. According to this assumption, more stable humified organics can be oxidized at higher

temperatures. Thus, the observed excess weight loss may or may not be an overestimation. However, regarding the stronger relationship between the OWB method and the LOI method at 400°C than at 550°C, the overestimation hypothesis seems to be more appropriate. On the other hand, the reverse was true for the relationship between TOC and LOI methods (Figure 2), which strengthens John (2004) hypothesis. Additionally, according to the findings of Grewal *et al.* (1991), when the intercept is non-significant, the loss of hygroscopic, structural, or interlayer water on ignition is unlikely. The intercept and slope of linear relationships between LOI and OWB methods obtained in the present study were different from those of other studies (Jia-

Ping *et al.*, 2013; Salehi *et al.*, 2011). Conversion factors for converting SOC to SOM were lower than those reported by Jia-Ping *et al.* (2013), i.e. 2.2 (ignition at 375°C for 17 hours) and similar to those obtained by Salehi *et al.* (2011), i.e. 1.6 (ignition at 400°C for 2 hours). Other factors such as soil type, SOM range, and the nature of organic compounds comprising the SOC can be the origin of the differences. Therefore, the estimation of SOM from SOC is not highly accurate and varies from soil to soil (Nelson and Sommers, 1996). There was a much stronger correlation between LOI and OWB in this study compared with those of Sato *et al.* (2014) and Rowell and Coetzee (2003). This reveals the importance of the LOI method in the studied soils because of no use of toxic chemicals. However, a large number of soil samples is required to verify the soundness of the relationships found in this research.

Correlations between Methods for Measuring SOC and POXC

The strong correlations observed between various wet combustion methods for SOC determination (Table 2) suggest that these methods digest the same or almost similar sources of SOC. This finding is inconsistent with that of other researchers (Grewal *et al.*, 1991; Ramamoorthi and Meena, 2018; Sato *et al.*, 2014). However, the strongest correlation was found between OWB and MWB-1 ($r^2 = 0.994$). This has been already reported by Schmidt *et al.*, (2012) and means that the applied external heating only increased the amount of SOC oxidized without being influenced by the nature of SOC.

POXC showed significant correlations with all SOM and SOC determination methods, but the strongest correlation was with the OWB method ($r^2 = 0.968$). The obtained relationship was much stronger than those reported by Sepahvand and Feizian (2016) and Ramírez *et al.* (2020) who reported determination coefficients of

0.86 and 0.70, respectively. The weaker relationship of POXC with the oxidized carbon by the MWB-1 method may be attributed to the fraction of SOC that is recalcitrant and cannot be oxidized by potassium permanganate. Despite the greater redox potential of permanganate than dichromate, kinetic limitations can hinder oxidation of many organic compounds by permanganate (Kolb *et al.*, 2017). For this reason, POXC is assumed to consist of simple organic compounds with a rapid turnover. However, some reports suggest that potassium permanganate can digest some of the most recalcitrant forms of SOC found in some soil microorganisms (Bongiorno *et al.*, 2019). It should be noted that POXC is measured at room temperature and increasing above 60°C (for enhancing recovery) leads to permanganate decomposition and overestimation (Geerdink *et al.*, 2017). A strong relationship between OWB carbon and POXC is crucial to understand that easily decomposable fraction of SOM or POXC is a steady feature of soil fertility and its transformation is of paramount interest. Therefore, measuring the kinetics of SOC oxidation with KMnO_4 may be an alternative for determining the real quality of labile SOC (Kolář *et al.*, 2011).

The colorimetric methods, particularly the MWB-3 method, had the weakest correlations with all other methods. However, the findings of Sato *et al.* (2014) and Rowell and Coetzee (2003) indicated a stronger correlation between MWB-2 and OWB ($r^2 = 0.85$).

Comparing Recovery of Different Methods for SOC Determination

The highest recoveries of the investigated SOC determining methods (95.3 and 94.2%) were obtained by, respectively, MWB-1 and MWB-2 methods. In the MWB-1 method, some protected SOC in micro-aggregates can be oxidized via application of external heating (Sato *et al.*, 2014), while in the



OWB method, not enough heat is generated through the dilution of concentrated H_2SO_4 . According to Heanes (1984), in 14 Australian soils, between 85-95% (92%, on average) of the SOC was recovered by the MWB-1 method in which external heating was used. Therefore, our recoveries stand at the upper limit of the aforementioned range. It should be noted that the soil samples used in the present study contained less clay than those of Heanes (1984) study. Bornemisza *et al.* (1979) reported that the SOC recovery rate decreases as soil clay content increases. This may be the reason for high recoveries found in the present research. However, soil sample 8 with sandy texture showed a very low recovery by OWB method (30.2%), implying the complexities involved and hence the contribution of other factors. The average recovery rate relevant to MWB-1 method was found to be only 1.1% higher than MWB-2 method. This observation is consistent with the findings of Ramamoorthi and Meena (2018) who reported that the recovery rate of the MWB-1 method was only 4.1% higher than the MWB-2 method in 50 soils of Tamil Nadu, India.

Accuracy and Precision Evaluation of Different Methods for SOC Determination

Both accuracy and precision of the MWB-1 method were the highest among all other methods. Our results indicate that, through the application of external heating, almost complete recovery was achieved. This result disagrees with the findings of Abraham (2013). A possible reason may be the absence of charcoal-like recalcitrant organic materials in our soils. Conyers *et al.* (2011) are also opposed to converting SOC obtained from the OWB method to TOC due to the large variation in land attributes e.g., soil depth, land use, etc. Sato *et al.* (2014) reported the MWB-2 method as the most accurate one. Nevertheless, they attained the same precision for the tested methods. Some poor results on the precision of the MWB-2

method in our study can be explained by the impact of soil sample size. Bowman (1998) suggested that different soil sample sizes are required to minimize uncertainties in SOC determination by the MWB-2 method. However, we used the same sample size for all soils covering a wide range of SOC content.

The MWB-3 method originally proposed by Carolan (1948) showed the weakest results in this study. Metson (1956) also doubted the reliability of this method and preferred the use of the MWB-2 method. This observation may be due to overlapping of the absorption peak of Cr^{VI} at 430 nm.

Some researchers emphasize the need for introducing specific conversion factors to estimate TOC content from OC_{OWB} content for each group of soils (Bahadori and Tofghi, 2017; Kerven *et al.*, 2000). Our conversion factor (1.11) was close to that reported by Bhattacharyya *et al.* (2015) for some alluvial soils of India with OC_{OWB} content up to 10 g kg^{-1} (1.15). Richardson and Bigler (1982) also proposed a similar equation (12) (Figure 3-a) as follows:

$$OC = 1.130 \text{ SOC}_{OWB/\text{without CF}} + 0.42 \quad (12)$$

Recently, Shamrikova *et al.* (2022) recommended a conversion factor of 1.15, which was lower than that conventionally used (1.3) (Walkley and Black, 1934).

Polyakov *et al.* (2017) also demonstrated a significant overestimation of the OWB method. In contrast, some researchers found conversion factors substantially greater than 1.3 (De Vos *et al.*, 2007; Jha *et al.*, 2014). High recovery values recorded in the present research can be attributed to the mesh size used in the soil sieving step (100 mesh). We followed the comment of Nelson and Sommers (1996), who recommended grinding samples to $< 0.2 \text{ mm}$ to achieve more precision in the SOC analysis. A similar procedure was performed by Bhattacharyya *et al.* (2015), who reported results that were close to those in the present study. Heanes (1984) has previously reported such a result. Furthermore, easily oxidizable organic matter can provide

recovery values near 100% in some west African soils (Rhodes *et al.*, 1981).

The MWB-1 method showed a conversion factor of near unity (1.016). Heanes (1984), Kerven *et al.* (2000), and Ramamoorthi and Meena (2018) proposed correction factors of 1.09, 1.08 and 1.33 for this method, respectively.

In this research, a strong relationship was found between POXC and TOC (Figure 4). Accordingly, POXC accounted for only a small portion of the TOC (4.1%). Awale *et al.* (2017) reported that POXC accounted for 0.15-3.9% of the TOC. However, higher proportions were observed by other workers (Yan *et al.*, 2017). This means that our soils were in the lower range of those reported in the literature, which is expected for soils with not so much organic carbon content.

Correlation between Soil Properties and Organic Carbon Content Determined by Different Methods

A significant correlation of organic carbon with CEC suggests that SOM had the main contribution to soil CEC (Papini *et al.*, 2011). However, the same was not true for clay content. Sharma *et al.* (2014) also reported the significant correlation of POXC with CEC ($r=0.445^*$). Both significant (Culman *et al.*, 2012) and non-significant (Hurisso, *et al.*, 2016) correlations between POXC and clay content were observed in the literature.

CONCLUSIONS

In this research, a power equation described the relationship between Soil Organic Matter (SOM) by the Loss-On-Ignition (LOI method) and Soil Organic Carbon (SOC) by the Original Walkley-Black (OWB method) better than a linear equation. Furthermore, the obtained conversion factors for incomplete oxidation of SOC (1.1, on average) were considerably lower than the general standard conversion

factor of 1.3. We assign this observation to the use of soil sieving mesh size lower than conventionally used (100 instead of 35 mesh), as recommended by Nelson and Sommers (1996). Our results indicate that external heating can be considered as an option to achieve a recovery of about 100%. Therefore, it seems that a combination of both soil sieving at < 0.15 mm and using external heating is sufficient to make unnecessary the use of the standard conversion factor of 1.3. Another modification to achieve complete oxidation of SOC may be increasing oxidation duration from 30 minutes to 24 hours. These make it possible to avoid errors that result from incomplete oxidation of SOC. Lower Permanganate Oxidizable Carbon (POXC)/Total Organic Carbon (TOC) ratios found for the studied soils indicate positive responses to application of organic matter. However, a strong relationship between POXC and SOC_{OWB} found in this research may put under question the value of POXC as an independent parameter in evaluation of SOC, but it can be still useful for evaluating changes in labile SOC. However, large data from various locations is required to support this statement.

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روابط بین کربن‌های قابل اکسایش با سولفو کرومیک و پرمنگنات در برخی خاک‌های شمال و شمال غرب ایران

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چکیده

روش والکلی بلک اولیه (OWB)، اساساً به دلیل راحتی، به طور گسترده‌ای برای اندازه‌گیری کربن آلی خاک (SOC) مورد استفاده قرار گرفته است. با این حال، قابلیت اعتماد به این روش هنوز مورد تردید می‌باشد. در سال‌های اخیر، پیشنهاد شده است که کربن قابل اکسایش با پرمنگنات پتاسیم (POXC) مفیدتر از SOC کل است. در مطالعه حاضر، مقادیر SOC در ۲۰ نمونه خاک غیرآهکی (۰-۲۰ cm)، با استفاده از روش اصلی WB (OWB) و نسخه‌های تغییر یافته آن (WB با حرارت دادن خارجی و WB با تعیین رنگ سنجی) تعیین شده و سپس تلاش شد تا روابط آن‌ها با مواد آلی خاک (SOM) که سهل‌یافت‌تر است با اندازه‌گیری اتلاف احتراقی (LOI) در دو دما (۴۰۰ و ۵۵۰ درجه سلسیوس) مشخص شود. به علاوه، کربن قابل اکسایش با پرمنگنات (POXC) در این نمونه‌ها تعیین گردید. در نهایت، روابط کاربردی بین این روش‌ها ارائه شد. سپس، در هشت خاک منتخب، صحت روش‌های مختلف WB با استفاده از آنالیز عنصری CHN برای اندازه‌گیری کربن آلی کل تعیین گردید. نتایج روابط نمایی قوی را بین LOI و OWB نشان داد. روش WB با حرارت دادن خارجی بیشترین بازایی (۳/۹۵ درصد) را در بین روش‌های مورد آزمون نشان داد. بازایی‌های بالای به دست آمده را می‌توان به واسطه استفاده از ذرات ریزتر خاک در روش پیشنهادی (کوچکتر از ۰/۱۵ میلی‌متر) در مقایسه با روش متداول (کوچکتر از ۰/۵ میلی‌متر) دانست. روش POXC همبستگی بالایی را با روش OWB نشان داد و به طور میانگین تنها ۴/۱ درصد از کربن آلی کل خاک را تشکیل داد. این امر می‌تواند از ارزش POXC به عنوان یک پارامتر مستقل برای دستیابی به بخش لبایل SOC بکاهد.