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Full Length Research Paper

Optimization of the analytical method for the determination of organic matter

Zeyede Aregahegn

Ethiopian Institute of Agricultural Researches, P. O. Box 2003, Addis Ababa, Ethiopia.

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Soil organic matter represents the remains of roots, plant materials, dead organisms in varies stages of decomposition and synthesis and is variable in composition. Walkley-Black method for the determination of organic matter is the most common method; however, it requires a lot of reagents and chemicals including potassium dichromate which is toxic especially when it is in a hexavalent form. It is very important to be able to reduce the amount of chemicals used during the analysis of organic carbon and it is also very important to reduce environmental pollution since the chemicals released to the environment becomes toxic. As the weight of sample decreases from 1 g (a Walkley-Black recommendation) proportionally with volume of reagents, there is no significant difference shown on the value of organic matter. Using orthophosphuric acid during organic matter determination did not have an impact on the results; however, it helps to observe a sharp endpoint. When the volume of diphenylamine indicator reduces from 15 to 4 drops, the result of organic matter has no significant difference.

Key words: Organic matter, optimization, analytical method, Walkley-Black, potassium dichromate.

INTRODUCTION

Soil organic matter (OM) is the product of decayed plant and animal tissues, forest floor development, living and dead microbial tissues. microbially synthesized compounds, and derivatives of these materials, produced as the result of microbial decay ((Sikora and Hardy, 2014). The forest floor development is the energy source for heterotrophic organisms, a reservoir for mineral nutrients and influences the forest hydrology (Chapman et al., 1975) and ground vegetation (Bargali, 1994). Decomposition processes play an important role in soil fertility in terms of nutrient cycling and the formation of soil organic matter (Bargali et al., 1993, Bargali, 1996). The ways in which these two processes are accomplished determine to a large extent the structural and functional features of ecosystems (Bargali et al.,

E-mail: zeyede.aregahegn@eiar.gov.et. Tel: +251 9 12743947.

1992a,b).

Organic matter is an important constituent of every soil (Shrestha 2015) which increases the cation exchange capacity of the soil thus, the base saturation increases, the relative amount of acid cations decreases. For plant growth the nutrient storage capacity (high CEC) of the organic matter is important, especially in clay poor soils. In clay rich soils it enables the formation of aggregates with large pores and improves therefore water and air availability. Besides being essential in soil studies, the soil organic matter parameter is the most important one in several soil properties such as structure, coloring, water retention, and cation exchange capacity; as a result it becomes important in guiding soil fertilizing and irrigation (Souza et al., 2016).

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Several methods have been used to determine soil organic carbon, and each of the methods has its own advantages and limitations (Konare et al., 2010; Zbíral et al., 2016). Comparing the experimental results obtained when using these methods is an important thing to choose the method which gives nearly accurate results. However, the most common analytical method for soil organic carbon determination is Walkley and Black (1934) method. This method is routine, relatively accurate, and popular method for the determination of soil organic matter (Salehi et al., 2011) in most soil laboratories. However, it requires a lot of reagents and chemicals including potassium dichromate which is toxic especially when it is in a hexavalent form. Soil organic matter contains decayed plant and animal tissues, living and dead microbial tissues, microbially synthesized compounds, and derivatives of these materials, produced as the result of microbial decay. Organic matter is an important constituent of every soil which increases the cation exchange capacity of the soil, thus, the base saturation increases, the relative amount of acid cations decreases. For plant growth, the nutrient storage capacity (high CEC) of the organic matter is important, especially in clay poor soils. In clay rich soils it enables the formation of aggregates with large pores and improves therefore water and air availability. Besides being essential in soil studies, the soil organic matter parameter is the most important one in several soil properties such as structure, coloring, water retention, and cation exchange capacity as a result it becomes important in auiding soil fertilizing and irrigation (Souza et al., 2016).

Nowadays, determination of soil organic carbon needs initial high cost investment. The aim of this study was to evaluate the effect of weight of soil sample and proportion of reagents for the determination of organic carbon using Walkley and Black (1934) method. Recently, the cost of chemicals increases continuously. It is very important to be able to reduce the amount of chemicals used during the analysis of organic carbon and it is also very important to reduce environmental pollution since the chemicals released to the environment become reduced. Although it is a metal that occurs naturally in the environment in the trivalent state (Cr³⁺), considered essential to living things, when it takes the hexavalent form (Cr⁶⁺), it is considered toxic to humans, and may cause ulceration, irritation, and inflammation; it is also associated with the risk of cancer

The Walkley and Black technique uses a strong oxidizing agent, potassium dichromate, to react with the organic matter in the soil. Chromium (VI) is converted to chromium (III) and unreduced chromium is back titrated with ferrous ammonium sulfate which provides an indication of the amount of organic matter present. This assumes complete oxidation of all organic matter present, which may not be the case for all samples. For soils of high organic carbon, the required amounts of reagents suggested by Walkley and Black technique may

not be enough to oxidize all the oxidizable organic carbon. The most common method in soil laboratories involves the reduction of potassium dichromate ($K_2Cr_2O_7$) by soil organic carbon compounds and subsequent determination of unreduced dichromate by oxidationreduction titration with ferrous ammonium sulphate. While the actual measurement is of oxidizable organic carbon, the data are normally converted to percentage organic matter using a constant factor by assuming that organic matter contains 58% organic carbon. However, as this proportion is not constant, the results are reported as oxidizable organic carbon, or multiplied by 1.334 as organic carbon. The oxidation of carbon can be explained by reaction in Equation 1. A known weight of soil is treated with an excess volume of standard K₂Cr₂O₇, in the presence of concentrated H₂SO₄. The soil is slowly digested by the heat of dilution of H₂SO₄ and the organic C in the soil is thus oxidized to CO₂. The highest temperature attained by the heat of dilution reaction produced on the addition of H_2SO_4 is approximately 120°C, which is sufficient to oxidize the active forms of the soil organic C.

$$\begin{array}{ccc} 3C+2K_2Cr_2O_7+8H_2SO_4 \rightarrow 2Cr_2(SO_4)_3+3CO_2+2K_2SO_4+8H_2O\\ Orange\ colour & Green\ colour \\ \end{array} \tag{1}$$

The excess of $K_2Cr_2O_7$ not reduced by the organic matter is titrated back against a standard solution of ferrous ammonium sulfate in the presence of NaF or phosphoric acid and diphenylamine indicator ($Cr_2O_7^{2-}$) will react with carbon as follows:

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

Similarly, $Cr_2O_7^{2-}$ will react with organic hydrogen as follows:

$$Cr_2O_7^{2^-} + 14H^+ \rightarrow 2Cr^{3^+} + 7H_2O$$
 (3)

And then, ferrous iron reacts with $Cr_2O_7^{2-}$ as follows:

$$6Fe^{2+} + Cr_2O_7^{2-} + 14H^+ \rightarrow 2Cr^{3+} + 6Fe^{3+} + 7H_2O$$
(4)

The action of indicator of diphenylamine indicator:

 $\begin{array}{c} 2C_{6}H_{5}NHC_{6}H_{5}+O \xrightarrow[-H20]{} 2(C_{6}H_{5}.NHC_{6}H_{4})+O \xrightarrow[-H20]{} C_{6}H_{5}N-C_{6}H_{4}C_{6}H_{4}N-C_{6}H_{5}\\ \hline Diphenylamine & Diphenylbenzidine (Violet)\\ \textbf{(5)}\end{array}$

METHODOLOGY

Walkley-Black method for the determination of oxidizable organic carbon, total organic carbon and organic matter was applied for this

No.	Wt. (g)	Rep	%00C	%TOC	%OM
1	1	1	0.51	0.68	1.17
2	1	2	0.54	0.72	1.24
3	1	3	0.59	0.78	1.35
4	0.9	1	0.54	0.72	1.24
5	0.9	2	0.54	0.72	1.24
6	0.9	3	0.56	0.75	1.29
7	0.8	1	0.54	0.73	1.25
8	0.8	2	0.54	0.73	1.25
9	0.8	3	0.54	0.73	1.25
10	0.7	1	0.54	0.71	1.23
11	0.7	2	0.56	0.74	1.28
12	0.7	3	0.55	0.74	1.28
13	0.6	1	0.54	0.72	1.24
14	0.6	2	0.56	0.75	1.29
15	0.6	3	0.55	0.74	1.28
16	0.5	1	0.57	0.76	1.31
17	0.5	2	0.54	0.72	1.24
18	0.5	3	0.54	0.72	1.24
Mean	-	-	0.55	0.73	1.26
CV	-	-	3.48	3.37	3.39
LSD (0.05)	-	-	NS	NS	NS

Table 1. Effect of decreasing sample weight on the result of OM.

Wt = Weight, Rep = replication, OOC = oxidizable organic carbon, TOC = total organic carbon, OM = organic matter, CV = coefficient of variation, LSD = least significant different at p<0.05.

experiment. Total organic carbon and organic matter was derived from oxidizable organic carbon by assuming that OM contains 58% of organic carbon (Estefan et al., 2013). The result reported as oxidizable organic carbon which is derived by multiplying by 1.33 (1.33 is used because 77% of C is oxidized by potassium dichromate) as organic carbon (Estefan et al., 2013). Different sample weight (1, 0.9, 0.7, 0.8, 0.6 and 0.5 g) and different reagents (K₂Cr₂O₇, H₂SO₄ and H₃PO₄) were taken as a treatment with a different proportion.

Data analysis

The data was subjected to SAS 9.3 version analytical software. The collected data were first check for normal distribution and after following the assumptions. Data were analyzed in a completely randomized design using one-way analysis of variance (ANOVA). For multiple comparison tests among the treatments of both experiments, the least significant difference (LSD) test at p < 0.05 was used to detect significant differences among the means.

RESULTS AND DISCUSSION

Effect of sample weight differences on the result of OM

As the weight of sample decreases from 1 g (Walkley and Black recommendation) proportionally with volume of reagents, there is no significant difference shown on the value of organic matter.

This is due to the fact that both the sample and volume of reagents decreases proportionally so that the results have no significant difference. As the amount of sample decreases, the amount of oxidizable organic carbon decrease proportionally and the volume of $K_2Cr_2O_7$ required to oxidize the organic carbon as a result decreases proportionally with the amount of sample. It is possible to save the volume of reagents by 50% and this could have a great economic value (Table 1).

Effect of using orthophosphuric acid on the result of OM

Using orthophosphuric acid did not make a difference on the value of organic matter as compared to the value obtained without using orthophosphuric acid. The values have no significant difference for the highest and the lowest sample without using orthophosphuric acid. However, using orthophosphuric acid has an advantage to observe a sharp endpoint (Figure 1 and Table 2).

Effect of diphenileamine indicator amount on OM analysis result

For 1 g soil sample, 15 drops of diphenileamine indicator



Figure 1. Endpoint of a reaction using orthophosphuric acid (4 at the left) and without using orthophosphuric acid (4 at the right) for similar soil sample.

Table 2. Effect of	using	orthophosphuric	acid	on	the	result	of	OM	compared	with	using	and	without	using
orthophosphuric aci	d.													

No.	Wt. of soil (g)	Rep	V of H₃PO₄ (mL)	%00C	%TOC	%OM
1	1	1	10	0.65	0.86	1.48
2	1	2	10	0.66	0.88	1.52
3	1	1	0	0.66	0.88	1.52
4	1	2	0	0.68	0.90	1.55
5	0.9	1	9	0.67	0.89	1.53
6	0.9	2	9	0.65	0.87	1.49
7	0.9	1	0	0.65	0.87	1.49
8	0.9	2	0	0.67	0.89	1.53
9	0.8	1	8	0.66	0.88	1.51
10	0.8	2	8	0.66	0.88	1.51
11	0.8	1	0	0.68	0.90	1.55
12	0.8	2	0	0.64	0.85	1.47
13	0.7	1	7	0.66	0.89	1.53
14	0.7	2	7	0.64	0.86	1.48
15	0.7	1	0	0.66	0.89	1.53
16	0.7	2	0	0.64	0.86	1.48
17	0.6	1	6	0.65	0.87	1.49
18	0.6	2	6	0.68	0.90	1.55
19	0.6	1	0	0.65	0.87	1.49
20	0.6	2	0	0.68	0.90	1.55
21	0.5	1	5	0.69	0.92	1.59
22	0.5	2	5	0.66	0.88	1.52
23	0.5	1	0	0.66	0.88	1.52
24	0.5	2	0	0.69	0.92	1.59
Mean	-	-	-	0.66	0.88	1.52
CV	-	-	-	2.58	2.58	2.58
LSD (0.05)	-	-	-	NS	NS	NS

Wt = Weight, Rep = replication, OOC = oxidizable organic carbon, TOC = total organic carbon, OM = organic matter, CV = coefficient of variation, LSD = least significant different at p<0.05.



Figure 2. End point of a reaction using 15 drops of diphenylamine indicator (at the right) and using 8 drops of diphenylamine indicator (at the left) for similar soil sample.

and 4 drops of diphenileamine indicator give similar results. For minimum weight of soil sample, that is, 0.5 g soil, 8 drops diphenileamine indicator and 2 drops diphenileamine indicator give similar results. Therefore, using excess volume of indicator has no advantage on the results of soil organic matter. Rather, adding excess amount of diphenylamine indicator results in difficulty for observing the end point of the reaction since it makes the sample very dark (Figure 2).

Conclusion

As the sample of weight and volume of reagents decrease proportionally, the results of soil organic matter are similar and it is possible to reduce the amount of reagents up to 50%. The advantage of using orthophosphuric acid during determination of organic matter is to see the sharp endpoint; however, it has no significant effect on the results. It is possible to reduce the amount of diphenileamine from 15 drops (Walkely and Black, 1934) to 4 drops.

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