

Full Length Research Paper

Optimization of the analytical method for the determination of organic matter

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Soil organic matter represents the remains of roots, plant materials, dead organisms in various 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 orthophosphoric 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.,

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

No.	Wt. (g)	Rep	%OOC	%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

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 ($K_2Cr_2O_7$, H_2SO_4 and H_3PO_4) 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 orthophosphoric acid on the result of OM

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

Effect of diphenylamine indicator amount on OM analysis result

For 1 g soil sample, 15 drops of diphenylamine indicator



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

Table 2. Effect of using orthophosphoric acid on the result of OM compared with using and without using orthophosphoric acid.

No.	Wt. of soil (g)	Rep	V of H ₃ PO ₄ (mL)	%OOC	%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 diphenylamine indicator give similar results. For minimum weight of soil sample, that is, 0.5 g soil, 8 drops diphenylamine indicator and 2 drops diphenylamine 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 orthophosphoric 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 diphenylamine from 15 drops (Walkley and Black, 1934) to 4 drops.

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