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HOW TO CITE:
Marais A, Kotzé E, Labuschagne J, Visser L, Morris CD. Proposed adaptation of the $KMnO_4$ oxidation method for determining active carbon for South African soils. S Afr J Sci. 2020;116(1/2), Art. #6443, 2 pages. https://doi.org/10.17159/sajs.2020/6443

ARTICLE INCLUDES:
☐ Peer review
☐ Supplementary material

KEYWORDS:
soil organic carbon, shaking position, soil quality

PUBLISHED:
29 January 2020

Proposed adaptation of the $KMnO_4$ oxidation method for determining active carbon for South African soils

One of the most widely acknowledged indicators of soil quality is soil organic matter and its elemental constituents, like soil organic carbon (SOC) and nitrogen.[1,2] However, due to the fact that soil organic matter has no definite chemical composition, SOC is more commonly estimated and reported in scientific literature.

With an ever-increasing interest in sustainability and the subsequent quality of soils, it is of utmost importance to measure sensitive indicators of soil quality. One of these indicators is active or labile carbon. This portion of the SOC is a small but relatively labile fraction and acts as fuel for the soil food web.[2] Thus the active carbon fraction could be used as an early indicator of changes in soil quality because of the influence of agricultural management practices.[3] Various methods have been published whereby active carbon can be measured. A review[4] on the then current methods was published in 2006, listing the advantages and disadvantages of each method.

A major advantage of the $KMnO_4$ oxidation method is that it is easy to perform and does not use hazardous chemicals in large amounts. There are, however, many different versions of the same method in which aspects like incubation time, amount of soil used, shaking time and manner, differ.[2,3,5-8]

The aim of this contribution was to adapt the $KMnO_4$ oxidation method for soils from different South African localities, because it was found that strictly following the protocol, especially with low carbon soils, resulted in low repeatability. The method described by Culman et al.[7] was used and adapted as deemed necessary.

Four soils from different localities in South Africa were chosen and analysed for particle size as well as organic carbon.[9] The general characteristics of these soils as analysed by the Elsenburg Analytical Laboratories are depicted in Table 1. The organic carbon varied widely between the four soil types, with Clanwilliam having the lowest (0.2%) and George the highest (4.84%).

Table 1: Characteristics of the surface soils (0–10 cm) from four locations in South Africa

Location	GPS co-ordinates	Coarse sand (%)	Medium sand (%)	Fine sand (%)	Clay (%)	Silt (%)	Organic carbon (%)
Bethlehem	-28.1557 28.29095	2	4	68	12	14	2.22
Clanwilliam	-32.114162 18.703532	37	20	19	2	22	0.20
George	-33.978533 22.420484	4	5	67	6	18	4.84
Riviersonderend	-34.16047222 19.90427778	27	8	41	16	8	1.79

Because it was difficult to get comparable and positive results in soils with low carbon content, such as with Clanwilliam, both 5 g of soil, as was originally suggested[2], as well as 2.5 g as suggested by some other authors[7,8], were tested. Most of the methods studied were unclear as to the required positon of the tubes during shaking. The tubes with the soil samples and $KMNO_4$ were therefore shaken either in an upright or flat position on an orbital shaker at 120 rpm for 2 min as suggested by Culman et al.[7] Shaking the centrifuge tube in the flat position should result in better mixing of the $KMnO_4$ with the soil sample, thus potentially extracting more active carbon.

Each soil sample was tested, with five repeats, with both amounts of soil (2.5 vs 5 g) in both positions (flat vs upright).

The data were subjected to a three-way factorial ANOVA (soil type (4) x amount (2) x position (2) x 5 replications) using the software program Genstat 18.[10] The dependent variable (active carbon, mg/kg) was not transformed because residuals were neither skewed nor heteroscedastic.

Soils differed markedly ($p<0.001$) in their average active carbon content (Tables 1 and 2), ranging from almost 800 g/kg for carbon-rich George soil to very low carbon sandy soil from Clanwilliam (7.4 mg/kg), with Riviersonderend (221.4 mg/kg) and Bethlehem (136.8 mg/kg) soils being of intermediate active carbon content. Generally, more active carbon ($p<0.001$) was extracted in the flat than the upright tube shaking position (377.6 vs 204.1 mg/kg) and when using 2.5 g rather than 5 g of soil (301.5 vs 280.2 mg/kg). However, because all two-way interactions as well as the soil x amount x position interaction were significant ($p<0.001$), the most effective combination of position and soil amount depended on soil type (Table 2).

For Bethlehem soil, the flat shaking position produced a similar result for both amounts of soil whereas in the flat position, 2.5 g of soil yielded 435.1 mg/kg more carbon than 5 g of soil for George soil (Table 3). Flat shaking with 5 g of soil gave better results (by 80 mg/kg) than 2.5 g for Riviersonderend soil (Table 3), although this increase was not significant. Clanwilliam's sandy soil produced very low, variable and often negative results for active carbon and no method seemed to achieve acceptable results (Table 3).

Table 2: Results of an ANOVA for active carbon in different soils extracted using different methods (amount of soil and tube shaking position)

Source of variation	d.f.	*F*-value	*p*-value
Soil	3	2075.15	<0.001
Amount	1	7.75	0.007
Position	1	512.10	<0.001
Soil x Amount	3	86.88	<0.001
Soil x Position	3	30.60	<0.001
Amount x Position	1	46.43	<0.001
Soil x Amount x Position	3	46.42	<0.001
Residual	64		
Total	79		

Table 3: Means for active soil carbon content compared with post-hoc Tukey tests (means with letters in common are not different; $p=0.05$)

Location of soil sample	Amount of soil (g)	Shake position	Mean active carbon (mg/kg)	s.d.
Bethlehem	2.5	Flat	245.71^{d}	23.275
Bethlehem	2.5	Upright	2.51^{ab}	50.503
Bethlehem	5.0	Flat	223.21^{d}	19.116
Bethlehem	5.0	Upright	75.79^{bc}	18.549
Clanwilliam	2.5	Flat	-6.64^{a}	21.683
Clanwilliam	2.5	Upright	-48.48^{a}	26.394
Clanwilliam	5.0	Flat	75.46^{bc}	40.074
Clanwilliam	5.0	Upright	9.10^{ab}	2.969
George	2.5	Flat	1142.59^{g}	29.034
George	2.5	Upright	686.88^{f}	65.787
George	5.0	Flat	707.55^{f}	1.798
George	5.0	Upright	654.03^{f}	31.861
Riviersonderend	2.5	Flat	275.79^{d}	12.361
Riviersonderend	2.5	Upright	113.65^{c}	72.41
Riviersonderend	5.0	Flat	356.9^{e}	24.01
Riviersonderend	5.0	Upright	139.20^{c}	7.872

From these results, it is recommended that the tubes should preferably be shaken in the flat position in order to allow the $KMnO_4$ to properly mix with the soil sample, using 2.5 g of soil as most of the published protocols suggested. In case of negative values obtained for a certain soil, the experiment should be redone using 5 g of soil, because it was found that increasing the amount of soil resulted in more detectable values in the low carbon soils, although not significantly so.

The final protocol that gave the best repeatable results is consistent with that of Culman et al.[7], but with the tubes lying flat while being shaken. Additionally, if negative absorbance values are obtained at 550 nm, it is advised that the procedure should be repeated, using 5 g of soil and adapting the equation accordingly.

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