IMPROVEMENTS IN COLORIMETRIC DETERMINATIONS


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I. INTRODUCTION

The rapid titration methods of Schollenberger (1927, 1931), modified by Walkley and Black (1934) and Walkley (1935) and of Khanna and Sen (1946) for estimation of organic carbon in soils are based on oxidation of organic matter by potassium dichromate and potassium ferricyanide in acidic and alkaline medium respectively. In the former reaction the change of chormate (Cr\(^{--}\)) to chromic (Cr\(^{+++}\)) and in the latter from ferricyanide (Fe\(^{+++}\)) to ferrocyanide (Fe\(^{++}\)) takes place in the process of oxidation which can be represented stoichiometrically. Both the methods have been compared, the former by Crowther (1935) at the instance of the Organic Carbon Committee and the latter by Khanna and Sen (loc. cit.) with the combustion method and have given fairly reliable results with the help of appropriate correction factors.

During the application of the dichromate method on a large number of soils with high and low content of organic carbon, it was observed that the colour of the titration mixture changed from yellow to green. This colour change was dependent upon the concentration of organic carbon and as the latter increased, there was noticed a gradual increase in the intensity of green colour. At higher concentrations, deep green colours were formed. This led to the standardization of colours in the titration mixtures in relation to the corresponding concentration of organic carbon in soils and the standardized colours were represented on charts for easy reference. The procedure followed in developing this rapid method and results obtained are given below.

II. PROCEDURE AND DISCUSSION OF RESULTS

Six soil samples, air dried and sieved through 100-mesh varying in organic carbon from 100 mgm. to about 800 mgm. % were weighed 5 gm. each into a 150 c.c. conical flask. 10 c.c. NK\(_2\)Cr\(_2\)O\(_7\) were added, followed by a quick addition of 20 c.c. concentrated H\(_2\)SO\(_4\) (Sp. Gr. 1.84). The mixture
was stirred for a few minutes and allowed to cool under the tap for about ten minutes. By this time, a clear supernatant liquid of different shades of yellow, green and their mixture was obtained. 2 c.c. of this supernatant liquid was pipetted into a 100 c.c. flask and made upto volume. 10 c.c. of this solution was taken for colorimetric estimation in a photo-electric (Klett Sommerson) colorimeter using blue light filter and the scale readings noted as standard (Table I).

**Table I**

*Standardisation of colours by photo-electric colorimeter*

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Sample No.</th>
<th>O.G. carbon per 100 gm. of soil (Walkley and Black)</th>
<th>Average scale readings in the colorimeter (Dilution 50 times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>636</td>
<td>106</td>
<td>450</td>
</tr>
<tr>
<td>2</td>
<td>423</td>
<td>257</td>
<td>300</td>
</tr>
<tr>
<td>3</td>
<td>534</td>
<td>391</td>
<td>195</td>
</tr>
<tr>
<td>4</td>
<td>453</td>
<td>446</td>
<td>134</td>
</tr>
<tr>
<td>5</td>
<td>369</td>
<td>553</td>
<td>105</td>
</tr>
<tr>
<td>6</td>
<td>438</td>
<td>778</td>
<td>82</td>
</tr>
</tbody>
</table>

For developing a field method, a simpler micro-technique was followed. 1 gm. of air dried soil was weighed in a test-tube and 2 c.c. of normal dichromate and 4 c.c. of concentrated sulphuric acid added in the manner described above. The mixture was gently shaken or stirred with glass rod and allowed to cool for ten minutes. 2 c.c. of the supernatant liquid was taken in a glass vial and the different colours represented on the chart (Fig. 1) against the known concentration of organic carbon estimated by Walkley and Black's method (Table II).

**Table II**

*Showing standard colour chart*

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Sample No.</th>
<th>Org. carbon in mgm. per 100 gm. of soil (Walkley &amp; Black)</th>
<th>Colour description</th>
<th>Org. carbon mgm. per 100 gm. of soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>636</td>
<td>106</td>
<td>Bright yellow</td>
<td>100-200</td>
</tr>
<tr>
<td>2</td>
<td>423</td>
<td>257</td>
<td>Yellow</td>
<td>100-200</td>
</tr>
<tr>
<td>3</td>
<td>534</td>
<td>391</td>
<td>Greenish yellow</td>
<td>200-300</td>
</tr>
<tr>
<td>4</td>
<td>453</td>
<td>446</td>
<td>Yellowish green</td>
<td>300-400</td>
</tr>
<tr>
<td>5</td>
<td>369</td>
<td>553</td>
<td>Dull green</td>
<td>400-500</td>
</tr>
<tr>
<td>6</td>
<td>438</td>
<td>778</td>
<td>Bright green</td>
<td>500-600</td>
</tr>
</tbody>
</table>

For developing a field method, a simpler micro-technique was followed. 1 gm. of air dried soil was weighed in a test-tube and 2 c.c. of normal dichromate and 4 c.c. of concentrated sulphuric acid added in the manner described above. The mixture was gently shaken or stirred with glass rod and allowed to cool for ten minutes. 2 c.c. of the supernatant liquid was taken in a glass vial and the different colours represented on the chart (Fig. 1) against the known concentration of organic carbon estimated by Walkley and Black's method (Table II).
Improvements in Colorimetric Determinations—II

In view of the encouraging results obtained, a large number of soils varying in organic carbon content were studied (Table III) both by the combustion and the colorimetric methods.

TABLE III

Comparative results of organic carbon estimation by the two methods

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Sample No.</th>
<th>Org. carbon mgm. 100 per gm. soil (Walkley &amp; Black)</th>
<th>Org. carbon mgm. per 100 gm. soil (Colorimetric method)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>401</td>
<td>217</td>
<td>236</td>
</tr>
<tr>
<td>2</td>
<td>403</td>
<td>331</td>
<td>366</td>
</tr>
<tr>
<td>3</td>
<td>404</td>
<td>284</td>
<td>285</td>
</tr>
<tr>
<td>4</td>
<td>405</td>
<td>174</td>
<td>185</td>
</tr>
<tr>
<td>5</td>
<td>406</td>
<td>204</td>
<td>201</td>
</tr>
<tr>
<td>6</td>
<td>407</td>
<td>354</td>
<td>303</td>
</tr>
<tr>
<td>7</td>
<td>408</td>
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<td>8</td>
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<td>377</td>
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<td>370</td>
<td>337</td>
</tr>
<tr>
<td>10</td>
<td>412</td>
<td>234</td>
<td>232</td>
</tr>
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<td>11</td>
<td>413</td>
<td>274</td>
<td>282</td>
</tr>
<tr>
<td>12</td>
<td>416</td>
<td>307</td>
<td>335</td>
</tr>
<tr>
<td>13</td>
<td>417</td>
<td>404</td>
<td>397</td>
</tr>
<tr>
<td>14</td>
<td>418</td>
<td>94</td>
<td>127</td>
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<td>451</td>
<td>335</td>
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<td>452</td>
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<td>816</td>
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<td>404</td>
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<td>457</td>
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<td>468</td>
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<td>348</td>
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<tr>
<td>23</td>
<td>469</td>
<td>202</td>
<td>230</td>
</tr>
<tr>
<td>24</td>
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<td>658</td>
<td>661</td>
</tr>
<tr>
<td>25</td>
<td>461</td>
<td>287</td>
<td>202</td>
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<td>26</td>
<td>462</td>
<td>414</td>
<td>361</td>
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<td>633</td>
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<tr>
<td>40</td>
<td>642</td>
<td>615</td>
<td>577</td>
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<td>7 F</td>
<td>287</td>
<td>286</td>
</tr>
<tr>
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<td>649</td>
<td>217</td>
<td>202</td>
</tr>
<tr>
<td>43</td>
<td>639</td>
<td>628</td>
<td>656</td>
</tr>
<tr>
<td>44</td>
<td>637</td>
<td>244</td>
<td>262</td>
</tr>
</tbody>
</table>

In the above table columns 3 and 4 give for different soils, the value of organic carbon (in mgm. per 100 gm. of soil) estimated by the method due
to Walkley and Black and the colorimetric method. Analysis of variance of these values is given (Table IV) below:

**TABLE IV**

*Showing analysis of variance*

<table>
<thead>
<tr>
<th>Due to</th>
<th>D.F.</th>
<th>S.S.</th>
<th>M.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soils</td>
<td>43</td>
<td>3582771.94</td>
<td>83320.28</td>
</tr>
<tr>
<td>Methods</td>
<td>1</td>
<td>786.01</td>
<td>786.1</td>
</tr>
<tr>
<td>Error (soils x methods)</td>
<td>43</td>
<td>26047.49</td>
<td>605.75</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>87</td>
<td><strong>380905.44</strong></td>
<td></td>
</tr>
</tbody>
</table>

It will be clear from the above analysis that there is no significant difference between the results given by the two methods. The mean values by the two methods over all 44 soils are 344.57 and 338.59 and the difference between them is not larger than its standard error, which is 5.25. There is a very high correlation between the two methods, the correlation coefficient being 0.98. In particular, if one considers the variation between the samples by the colorimetric method, 97% of this variation is accounted for by the regression of the colorimetric value on the standard method. It may be concluded that the colorimetric method reproduces very closely the results given by the standard method.

And the colorimetric method possesses distinct advantages over the Walkley and Black’s method. In the latter, the end point is often overshot because of the solution flashing suddenly to green with little or no warning. Besides the ferrous ammonium sulphate solution needs elaborate arrangements for preservation and use of fluoride in presence of sulphuric acid permanently scratches the glass ware used in these estimations. The procedure may also be applied to all the various oxidation methods where dichromate is used as oxidising agent.

In the study of the field method (R.C.M.), the same soils as were used in Table III, were taken by two independent workers at two different times and organic carbon content determined by comparing against the colour standards (Fig. 1) mentioned above. It will be obvious (Table V) that barring two cases of divergence in results marked with asterisk due to different workers and except at lower ranges, the correspondence between the Walkley and Black’s methods and the field technique was fairly close to provide, for all practical purposes, an extremely easy and simple means for assessing the value of organic carbon in soils.
### TABLE V

Comparison of field technique (R.C.M.) and Walkley and Black's method

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Sample No.</th>
<th>Worker A</th>
<th>Worker B</th>
<th>Organic carbon % (Walkley and Black)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colour</td>
<td>Org. carbon mmg. %</td>
<td>Colour</td>
<td>Org. carbon mmg. %</td>
</tr>
<tr>
<td>1</td>
<td>Greenish yellow</td>
<td>300-400</td>
<td>Greenish yellow</td>
<td>300-400</td>
</tr>
<tr>
<td>2</td>
<td>Bright yellow</td>
<td>100-200</td>
<td>Bright yellow</td>
<td>100-200</td>
</tr>
<tr>
<td>3</td>
<td>Greenish yellow</td>
<td>300-400</td>
<td>Greenish yellow</td>
<td>300-400</td>
</tr>
<tr>
<td>4</td>
<td>Yellow</td>
<td>200-300</td>
<td>Yellow</td>
<td>200-300</td>
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<tr>
<td>5</td>
<td>Greenish yellow</td>
<td>200-400</td>
<td>Greenish yellow</td>
<td>300-400</td>
</tr>
<tr>
<td>6</td>
<td>Bright yellow</td>
<td>100-200</td>
<td>Bright yellow</td>
<td>100-200</td>
</tr>
<tr>
<td>7</td>
<td>Bright green</td>
<td>Above 600</td>
<td>Bright green</td>
<td>Above 600</td>
</tr>
<tr>
<td>8</td>
<td>Bright yellow</td>
<td>100-200</td>
<td>Bright yellow</td>
<td>100-200</td>
</tr>
<tr>
<td>9</td>
<td>Bright green</td>
<td>Above 600</td>
<td>Bright green</td>
<td>Above 600</td>
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<td>10</td>
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<td>100-200</td>
<td>Bright yellow</td>
<td>100-200</td>
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<tr>
<td>11</td>
<td>Dull green</td>
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<td>Dull green</td>
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<td>Yellow</td>
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<td>Yellow</td>
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<td>14</td>
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<td>200-300</td>
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<td>15</td>
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<td>Greenish yellow</td>
<td>300-400</td>
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<td>300-400</td>
</tr>
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<td>Bright yellow</td>
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<td>Bright yellow</td>
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</tr>
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<td>26</td>
<td>Greenish yellow</td>
<td>300-400</td>
<td>Greenish yellow</td>
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<td>Yellow</td>
<td>200-300</td>
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<td>200-300</td>
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<td>28</td>
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<td>29</td>
<td>Yellow</td>
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<td>Greenish yellow</td>
<td>300-400</td>
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<td>32</td>
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<td>Yellow</td>
<td>200-300</td>
</tr>
<tr>
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<td>Dull green</td>
<td>500-600</td>
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<td>Bright yellow</td>
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<td>Bright yellow</td>
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<td>41</td>
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<td>43</td>
<td>Dull green</td>
<td>500-600</td>
<td>Dull green</td>
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<tr>
<td>44</td>
<td>Yellow</td>
<td>200-300</td>
<td>Yellow</td>
<td>200-300</td>
</tr>
</tbody>
</table>

### III. Summary

1. The paper describes a rapid method for estimation of organic carbon by photo-electric colorimeter which has distinct advantages over Walkley and Black's wet combustion method. In the procedure followed,
the change of colour from chromate yellow to chromic green in oxidation of organic matter by dichromate and sulphuric acid, has been made use of for colour standardization and estimation of organic carbon in soils.

2. A simple method suitable as field technique (R.C.M.) has also been evolved on the same principle and tested on a large number of soils of different organic carbon concentration with a fair degree of correspondence with Walkley and Black's method.

IV. ACKNOWLEDGEMENTS

The work was carried out as part of the Sugarcane Research Scheme in Bihar financed jointly by the Bihar Government and the Indian Central Sugarcane Committee to whom grateful thanks are due. The assistance afforded by Mr. K. S. Bandopadhyay, Statistician at the station in analysing data pertaining to Table III and by Mr. Sheodayal Sinha, Junior Research Assistant in analytical work, is also acknowledged.

REFERENCES

4. Ibid., 1931, 31, 483–86.