

ORGANIC CARBON ESTIMATIONS IN SOILS: ANALYTICAL PROTOCOLS AND THEIR IMPLICATIONS

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Several protocols for the estimation of soil organic carbon (SOC) are in practice by different laboratories. Quantitative to semi quantitative methods are followed for different objectives. Dry combustion protocols are quantitative in nature and are utilized to standardise other protocols. Walkley-Black (WB) protocol for organic carbon is widely followed in many laboratories because it is rapid and affordable. However, it is semi-quantitative in nature and do not completely recover the organic carbon in soils. Also considerable sample to sample variations in the recovery of carbon are reported. Through the application of external heat in WB protocol (modified-WB) more recovery, though not complete, of SOC is reported. If WB or modified-WB protocols are followed, standardization with an accurate method is necessary and correction factors need to be generated to account for the non recovered portion of organic carbon in soil. The soils of different rubber growing regions were analysed by WB, modified-WB and dry combustion (DC) methods and the values were compared. It is found that, sample to sample, the recovery percentage of SOC widely varied when WB or modified-WB protocol was followed compared to the values generated by DC protocol. On an average 71 per cent recovery of SOC by WB method and 92 per cent recovery by modified-WB method were observed. Correction factors of 1.3850 for WB and 1.1299 for modified-WB were derived to account the non recovered portion of soil organic carbon. After applying the derived correction factor, the values obtained by WB and modified-WB methods were not significantly differing from the values generated by DC method, suggesting that the correction factors hold good when a group of samples are considered.

Keywords: Dry-combustion method, Soil organic carbon, Soil organic matter, Walkley-Black method

INTRODUCTION

Soil organic matter (SOM) is an important soil component, which can directly or indirectly affect almost all the soil properties (Weil and Magdoff, 2004). Numerous organic compounds are present in soil with widely varying chemical nature and structure. There are direct methods to

estimate SOM, in which the organic compounds present in a specific weight of soil is destructed either thermally or chemically and the weight difference is calculated. Loss on ignition or oxidation by H_2O_2 are such methods, however, these methods are subject to errors (Christensen and Malmros, 1982; Howard and Howard,

1990; Nelson and Sommers, 1996). Another method to estimate SOM is by estimating the organic carbon (OC) in soil and then multiplying with a constant factor. The factor most commonly used is 1.724, assuming that SOM in any soil invariably contains 58 per cent carbon (Kerven, *et al.*, 2000). However, there are several reports that this factor is not universal and varies among different soil types (Broadbent, 1953; Howard, 1966; Christensen and Malmros, 1982; Jain *et al.*, 1997; Westman *et al.*, 2006; Sleutal *et al.*, 2007). Thus neither the direct nor indirect methods of estimation of soil organic matter content are completely accurate. Nelson and Sommers (1996) recommended that because of the problems associated with organic matter estimation, investigators may estimate and report organic carbon concentration as a measure of organic matter in soils.

The soil organic carbon (SOC) is widely used as an index of SOM and is estimated as a part of routine soil test for soil quality assessment (Gregorich *et al.*, 1994; Larson and Pierce, 1994; Pe'rie' and Munson, 2000; Shukla *et al.*, 2006). Based on the assumption that C to N ratio in soil is fairly constant, SOC values are utilized as N index and many soil testing laboratories utilize it for soil fertility evaluation and for fixing N fertilizer doses. The soil organic carbon estimations are basically for the purpose of soil fertility or health assessment. However, in the context of carbon sequestration and green house gas emission studies, carbon stock in soil need to be quantified for which precise estimation of soil carbon is required. The terrestrial C pool is of great significance as it is one among the largest carbon pool holding about 1500 pg (1 pg = 10^{15} g), which is about three times the amount that in the

whole world's vegetation or twice the amount that in the atmosphere (Batjes, 1996; IPCC WGI, 2001).

Thus the objectives of SOC estimations are many and the level of accuracy required also may vary. There are several protocols reported and followed in SOC estimations. Each method has its own advantages and disadvantages. The major criteria behind adoption of any specific analytical method by a laboratory are convenience, cost effectiveness and the level of accuracy. The selection of carbon assessment protocol is critical to the accurate quantification of SOC concentration, content and change over time (Perie and Ouimet, 2008).

The idea of using dichromate-sulphuric acid mixture for the wet oxidation of organic materials was proposed by Rogers and Rogers (1848). This method was later utilized for wet oxidation of SOC, successfully by Schollenberger (1927), Walkley and Black (1934) and Tiurin (1935). All of the presently followed dichromate oxidation methods for SOC determination fall in two groups *viz.* one which is not employing external heat and the other which is heating externally during the oxidation process. Among these two methods, without external heating is the most popular and widely followed method (Walkley and Black, 1934; Nelson and Sommers, 1996). This method is convenient, rapid and cheap. However, by following the 'no external heating' method, complete oxidation of SOC do not takes place and variable level of carbon recoveries had been reported and it ranged from 60 to 86 per cent (Nelson and Sommers, 1996). As a result of the incomplete oxidation, a correction factor of 1.33 is commonly applied to the soil organic carbon values by WB method to account the un-recovered carbon in soil. However, site

specific correction factor is necessary and it had been generated and reported for several soil types from all over the world, where WB protocol is followed (Nelson and Sommers, 1982; Soon and Abboud, 1991; Jolivet *et al.*, 1998; Diaz-Zorita, 1999; Hussain and Olson, 2000; Krishnan *et al.*, 2009). The problem of incomplete digestion of the organic matter in the Walkley-Black procedure was partially solved by supplying external heat during sample digestion in the modified-WB protocol which had been standardised by Nelson and Sommers (1996). In all the wet digestion protocols of soil organic carbon, where external heat is supplied or not, the un-reacted dichromate is back titrated with ferrous ammonium sulphate. Fe^{2+} and Cl^- ions present in soil, may interfere with the dichromate oxidation reaction positively while MnO_2 will interfere negatively (Nelson and Sommers, 1996). Thus the wet digestion protocols are subjected to chemical interferences and also generate hazardous chromium by-products.

Another method for SOC determination is the dry combustion protocol followed in automated elemental analyzers. This protocol is recognized as a standard technique, as SOC is quantitatively oxidized to, and determined as, CO_2 . The protocol is used to standardize all other existing methods for SOC determination (Nelson and Sommers, 1996). Many laboratories now use automated elemental analyzers for SOC estimations and in many studies comparison of SOC values generated out of dry combustion and wet oxidation methods becomes necessary. Establishment of a relationship between SOC estimated through dry combustion and wet digestion methods is very much required to handle data bank comprising results generated out of these different protocols.

The specific objective of this study was to generate the basic information regarding the level of recovery of carbon from rubber growing soils through standard WB protocol and to find out whether the carbon recovery can be improved by increasing the reaction temperature by supplying external heat. Such types of standardisation works for these soils are not reported, hence this study. Also the correlation between carbon values generated through wet digestion methods and dry combustion protocols need to be worked out and correction factor for these soils are to be evolved.

MATERIALS AND METHODS

The soil samples utilized for this study were from traditionally rubber (*Hevea brasiliensis*) growing regions in the state of Kerala, India. The soils of the sampled area were developed under tropical humid climate. These low activity clay soils which are slightly to medium acidic in nature and having low base saturation belong to the soil order 'ultisols' (NBSS and LUP, 1999).

Among the soil samples received from the farmers in the laboratories located at Nedumangad, Kottayam, Moovattupuzha, Kozhikode and Taliparamba, thirty samples each were randomly selected. The samples were sieved (2mm) and air dried. Sub samples of about 10 g were pulverized manually to completely pass through 0.15 mm sieve and used for subsequent analyses. The pH of the soil samples were less than 5.50, hence no inorganic carbon was present and the carbon estimated was considered as organic carbon.

Each sample was analysed for organic carbon following WB, modified-WB (tube digestion) and dry combustion (DC) methods.

Walkley-Black (WB) method

Organic carbon (OC) in the pulverized soil samples was determined following the rapid wet oxidation method. The Walkley and Black (1934) protocol as modified and described by Nelson and Sommers (1996) was followed. In brief, 0.5 g of the pulverized sample was weighed out into a 500 mL conical flask. Ten millilitre of 1 N $K_2Cr_2O_7$ was added and slightly swirled the flask to disperse the soil. Con. H_2SO_4 (20 mL) was rapidly transferred and the flask was again swirled for a minute until the soil and the reagents were mixed. The flask was allowed to stand on an insulating (asbestos) sheet for 30 minutes. Deionised water (200 mL) was added to the flask followed by three to four drops of o-phenanthroline indicator solution and titrated with $Fe(NH_4)_2(SO_4)_2$ (0.5 M) solution. A blank was also run in the same manner without soil to standardize $K_2Cr_2O_7$. In all the cases where more than 75 per cent of the dichromate was consumed, the analysis was repeated with less quantity of soil sample. Organic carbon content in soil was calculated using the following equation.

Organic carbon (%) =

$$\frac{(mL_{\text{blank}} - mL_{\text{sample}})(M Fe^{2+})(0.3)}{\text{Wt of dry soil}}$$

Where 'M' is molarity

Tube digestion method (Modified-WB method)

OC in the same set of soil was determined following the tube digestion method, popularly known as modified Walkley-Black (modified-WB) method as described by Nelson and Sommers (1996).

The protocol is similar to the WB procedure except that external heat is applied to the reaction mixture. The pulverized soil (0.5 g) was weighed out into digestion tubes (100 mL size) and 20 mL con. H_2SO_4 was added and swirled for a minute. The tubes were kept at 150 °C, for exactly 30 minutes in a pre heated block digester (40 tube Kjeldahl block digester supplied by Tecator Inc). The tubes were taken out and the solution was brought to room temperature and the contents were quantitatively transferred to a conical flask (500 mL) and titrated with $Fe(NH_4)_2(SO_4)_2$ (0.5 M) solution. Each set of soil samples were analysed with two unheated and two heated blanks (without soil). Organic carbon content was computed as follows.

$$A = \frac{(mL_{bb} - mL_{soil})(mL_{ub} - mL_{bb}) + (mL_{bb} - mL_{soil})}{mL_{ub}}$$

(where, A - factor; ub - unboiled blank;
bb - boiled blank)

$$\text{Organic carbon (\%)} = \frac{(A) (M Fe^{2+}) (0.3)}{\text{Wt of dry soil}}$$

Where 'M' is molarity

Dry combustion (DC) method

The same set of pulverized soil samples were analysed using an automated elemental analyser to determine the total C content. As the soil contains no inorganic carbon, the total carbon estimated is OC itself. The soil samples were weighed and encapsulated in tin foils and were introduced to the furnace at 950 °C of the elemental analyser (Leco Corporation, USA, Truspec CN) and flushed with oxygen for complete combustion. The carbon containing compounds were oxidized to CO_2 and separated from all other oxides and lead to the infra red gas analyser for estimation. The instrument system was

calibrated with soil standards supplied by Leco Corporation, USA each time the estimations was carried out.

Statistical analysis

Paired 't' test was performed to find out the significant difference between the SOC values obtained through WB and DC methods as well as modified-WB and DC methods. The SOC values obtained through WB and DC methods as well as modified-WB and DC methods were linearly regressed and correction factors for the WB and modified-WB methods were computed from the slopes of the respective regression lines. To find out the variation between correction factor applied WB and DC organic carbon values, paired 't' test was employed. Similarly variation between correction factor applied in modified-WB and DC values were also analysed statistically. SPSS (version 11) package was utilized for all the analyses.

RESULTS AND DISCUSSIONS

The SOC values obtained following WB, modified-WB and DC methods of samples are given in Table 1. It could be noticed that

when WB or modified-WB protocols were followed, SOC was under-estimated and the SOC recovery percentage was varying among different samples. SOC values varied from 0.53 to 3.88 per cent in WB method and 0.70 to 4.90 per cent in modified-WB method and 0.81 to 5.69 per cent in DC method. The recovery percentage of SOC by WB method with respect to the DC method, ranged from 46 to 90 per cent. The corresponding recovery values when modified-WB method was employed ranged from 66 to 101 per cent. Recovery of SOC was invariably more when modified-WB method was employed compared to WB method, however, complete recovery of SOC could not be obtained.

The lower recovery of SOC by WB method was as expected and similar observations were reported by many groups. Nelson and Sommers (1996) had reported that the recovery varied from 60 to 95 per cent when WB method was followed in different soil types. In case of Himalayan region soils of India the recovery was 41.6 per cent while for central Indian region soils, it was 51.2 per cent (Krishnan *et al.*, 2009). Bremner and Jenkinson (1960), Kalembasa and Jenkinson (1973) Nelson and Sommers

Table 1. SOC content (%) - range and recovery percentages in different regions obtained through WB, modified-WB and DC methods

Location	WB method			Modified-WB method			DC method	
	SOC (%) Range	Mean \pm SD	SOC Recovery range (%)	SOC (%) Range	Mean \pm SD	SOC Recovery range (%)	SOC (%) Range	Mean \pm SD
Nedumangadu	0.68-3.31	1.42 \pm 0.75	50.0 - 84.0	0.70-3.95	1.83 \pm 0.79	81.0 - 98.0	0.81-4.41	1.94 \pm 0.94
Kottayam	0.84-1.95	1.19 \pm 0.34	46.0 - 86.0	1.15-2.55	1.53 \pm 0.36	66.0 - 99.0	1.21-2.59	1.72 \pm 0.34
Moovattupuzha	0.53-3.01	1.72 \pm 0.51	66.0 - 3.0	0.83-3.22	2.12 \pm 0.58	91.0 - 101	0.88-3.83	2.34 \pm 0.69
Kozhikode	0.73-3.88	2.10 \pm 0.75	52.0 - 90.0	1.07-4.90	2.56 \pm 0.92	81.0 - 97.0	1.15-5.69	2.84 \pm 1.08
Taliparamba	1.20-2.90	1.86 \pm 0.40	63.0 - 80.0	1.52-3.42	2.26 \pm 0.45	74.0 - 95.0	1.89-3.84	2.63 \pm 0.56
All locations	0.53-3.88	1.60 \pm 0.64	46.0 - 90.0	0.70-4.90	2.03 \pm 0.71	66.0 - 101.0	0.81-5.69	2.24 \pm 0.85

(1975), Rhodes *et al.* (1981) and De Vos *et al.* (2007) also had reported on the widely varying recovery rates of organic carbon in soils of different regions when WB method was followed. Walkley and Black (1934) had reported that on the average 76 per cent of the organic carbon in 20 soils was recovered by their method, however, the variation among different soils were 60 to 86 per cent.

In WB method, heat of dilution of H_2SO_4 (120 °C) is utilized for oxidation which is not sufficient enough for the complete oxidation of the organic matter present in soil (Nelson and Sommers, 1996). The most active forms of SOC only get oxidized by WB method and certain portion of SOC remains unreacted. Walkley and Black (1934) suggested to use a correction factor of 1.33 to account for the unreacted portion of carbon in soil based on the assumption that about 76 per cent of SOC

is oxidised at 120 °C which is generated by H_2SO_4 – dichromate reaction mixture in the WB protocol. Based on reports, it is certain that there exist wide variation in the SOC recovery among different soil samples when WB protocol was followed. Hence, the correction factor also may vary for different soil samples. Nelson and Sommers (1996) gave an account of the correction factors generated for individual soil in a group as well as collectively for a group. The correction factor varied from 1.0 to 2.86 for individual soil samples among different groups while the average correction factor of different group of soils varied from 1.03 to 1.41. Nelson and Sommers (1996) also reported that the correction factor applied WB values may not be highly accurate for many individual soils in the group, but for a group of soils, comparable results could

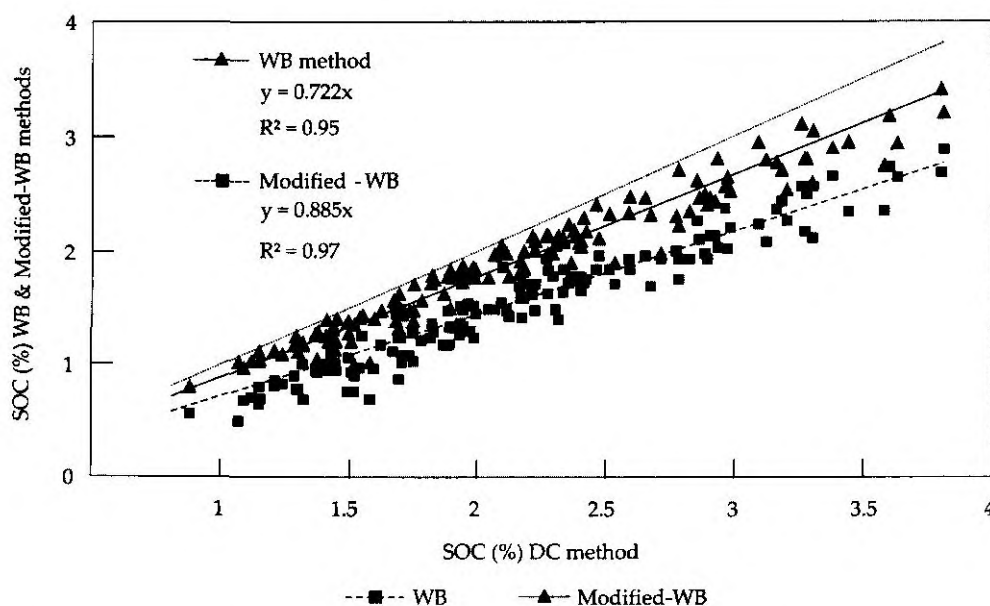


Fig. 1. Soil organic carbon by WB, modified-WB and DC method in rubber growing soils

be obtained with values obtained through DC method.

The SOC values obtained through WB ($SOC_{(WB)}$) and DC ($SOC_{(DC)}$) methods were strongly correlated (Fig. 1). The best fit using a linear regression relationship was:

$$SOC_{(WB)} = 0.722 \times SOC_{(DC)} \quad (\text{where, } R^2 = 0.95, n = 150).$$

Paired 't' test indicated that there was significant difference between the values obtained by the two methods *viz.*, $SOC_{(WB)}$ and $SOC_{(DC)}$ at 99 per cent confidence level. The WB method under-estimates the carbon content in comparison to the DC method and the correction factor was found to be 1.385 which is the inverse of the slope of the linear regression line (0.722). The soil organic carbon values after applying the correction factor on $SOC_{(WB)}$ values were plotted against $SOC_{(DC)}$ (Fig. 2). Paired 't' test

indicated that the values obtained by the two methods were not significantly differing.

As in the case of WB and DC methods, the SOC values obtained through modified-WB ($SOC_{(M-WB)}$) and DC ($SOC_{(DC)}$) methods were also strongly correlated (Fig. 1). The best fit using a linear regression relationship was:

$$SOC_{(M-WB)} = 0.885 \times SOC_{(DC)} \quad (\text{where, } R^2 = 0.97, n = 150).$$

There was significant difference between the values obtained by the two methods *viz.*, $SOC_{(M-WB)}$ and $SOC_{(DC)}$ at 99 per cent confidence level. Though modified-WB under-estimated the organic carbon when compared to DC method, the recovery was more than WB method. This indicated the positive effect of temperature in the oxidation of organic carbon in soil. The correction factor for modified-WB was found to be 1.1299 which

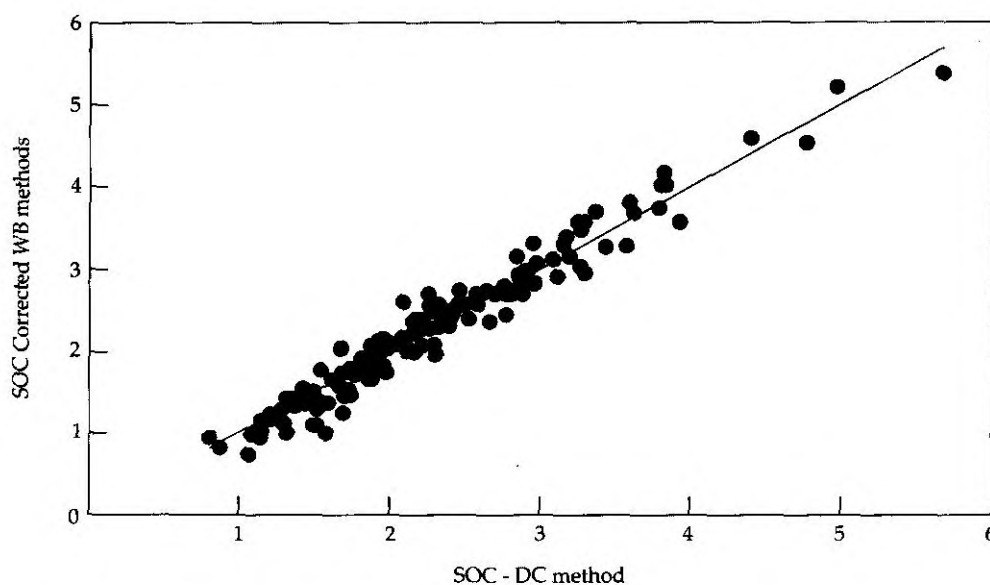


Fig. 2. Soil organic carbon values obtained by DC and correction factor applied WB- methods

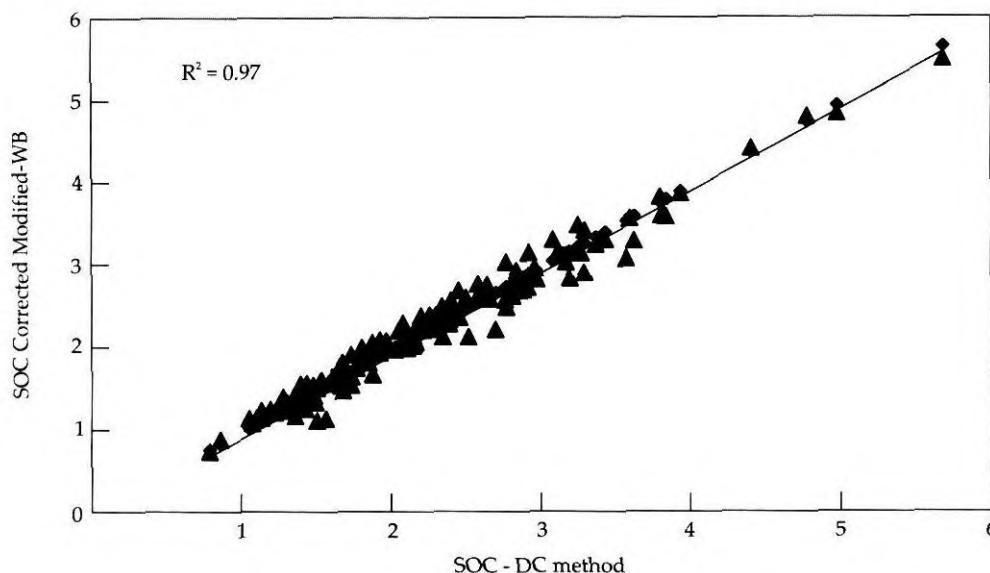


Fig. 3. Soil organic carbon values obtained using DC and correction factor applied modified-WB methods

is the inverse of the slope of regression line (0.885) $\text{SOC}_{(\text{M-WB})}$. The soil organic carbon values after applying the correction factor on $\text{SOC}_{(\text{WB})}$ values were plotted against $\text{SOC}_{(\text{DC})}$ (Fig. 3). Paired 't' test indicated that the values obtained by the two methods were not significantly differing as in the case of correction factor applied WB values.

For obtaining satisfactory results in WB protocol, two important steps in the WB protocol need to be followed rigorously and are emphasized here. Temperature of the reaction media plays an important role in the oxidation process of SOC (Jolivet *et al.*, 1998). The heat generated due to the dilution of the sulphuric acid (120 °C) in the WB protocol is an important condition which needs to be conserved or maintained by keeping the reaction vessel on top of an insulating sheet at least for half an hour.

Another important step in WB protocol is the sample homogenization step. SOC recovery percentage had been reported to be significantly increased by grinding the samples to less than 0.2 mm size (Nelson and Sommers, 1996). Care to be taken that all the sub sample taken for pulverization should pass through the sieve of required size and properly mixed so that a truly representative sample is subjected to analysis and no discrimination of sample takes place.

The mean value of SOC (%) by DC method and corrected WB and modified-WB methods were 2.24, 2.23 and 2.25 respectively for the 150 soil samples analyzed. The correction factors generated for the WB and modified-WB methods holds extremely good for the group of 150 soil samples.

CONCLUSIONS

Through WB and modified-WB protocols, complete recovery of SOC is not possible in the case of soil samples of rubber growing areas. The recovery percentage of SOC widely varied among the analysed samples, when WB or modified-WB protocol was followed compared to the values generated by DC protocol. Significant correlations were noticed between values by $SOC_{(WB)}$ and $SOC_{(DC)}$ as well as between $SOC_{(M-WB)}$ and $SOC_{(DC)}$. On

an average 71 per cent recovery of SOC by WB method and 92 per cent recovery by modified-WB method were noted. Correction factors of 1.3850 for WB and 1.1299 for modified-WB are derived for rubber growing soils. After applying the correction factors, the values obtained by WB and modified-WB methods were not significantly differing from the values generated by DC method suggesting that the correction factors hold good when a group of samples are considered.

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