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Carbon and nitrogen estimation in soils : Standardizing methods and internal standards for C/N analyzer

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Abstract : It has been found that soil organic carbon (SOC) is very easily oxidized in the oven during analysis through C/N analyzer. There is no literatures on the relative effects of CaCO₃ in the determination of total C in soils. To avoid, effects, if any, we have developed separate methods for calcareous and non-calcareous soils. It is hoped that, with a prior knowledge of soil-site, a suitable method can be chosen for both these types of soils to determine C and N in soils.

Keywords : Soil organic carbon (SOC), calcareous soils, non-calcareous soils, C/N analyzer.

Introduction

The importance of the level of carbon in soils is important from the ecological and agricultural point of view. Since organic form of carbon forms the major source of nitrogen in soils for plant growth, the efforts to measure carbon in soils also necessitates knowledge of nitrogen in soils. Quantitative understanding of a scientific principle that governs carbon and nitrogen balance in a given ecosystem is essential for development of strategy to increase the carbon sequestration. So far as soils are concerned such attempts evolve accumulation of quantified carbon and nitrogen data set for a host of soils. Carbon occurs in soils in both the forms of mineral and organic matter viz. (i) carbonate mineral forms, chiefly as CaCO₃ and (MgCO₃, CaCO₃); small amount of carbon also occurs as CO₂ and HCO₃⁻ and CO₃²⁻ ions of more soluble salts, (ii) highly condensed, nearly elemental organic carbon (charcoal, graphite, coal), (iii) altered and resistant organic residues of plants, animals and microorganisms such as humus and (iv) slightly altered organic residues of plant and animals and living and dead microorganisms. The total carbon of soils includes all the above four forms while the organic carbon includes the last three forms.

Organic carbon in soils has been traditionally deter-

mined in laboratory using the method of Walkley and Black¹. Inorganic form of carbon in soils contributed largely by CaCO₃ is determined by acid-base titration^{2,3}. In spite of serious efforts made by soil researchers complete recovery of both organic and inorganic forms of carbon remains difficult; and more so for organic carbon. Organic carbon determination in soils may be carried out (after removal of carbonate, usually by acid) by (a) dry combustion in a furnace and/or, (b) chromic acid oxidation, followed by measurement of CO₂ evolved. Organic carbon content of soil may be reported directly as percentage C or calculated as organic matter. The conventional organic carbon to organic matter factor is 1.724 based on the assumption that soil organic matter consists of 58 percent C. However, such factor for a conversion of the carbon content of many surface soils to organic matter content has been found to be 1.9 and the factor for many types of subsoil is about 2.5⁴⁻⁶. The total organic matter content of soils is determined by (a) oxidation with H₂O₂, (b) ignition at moderate temperature, or (c) ignition after decomposition of silicates with HF-HCl. Soil organic matter exhibits correlation with (a) total nitrogen content, (b) climate and (c) clay content. Multiplication of total soil nitrogen content by 20 roughly ap-

proximates the organic matter content in soils. This assumes 5 percent N in the organic matter of soil with C : N ratio of 11.6 since the organic matter is conventionally assumed to contain 58% carbon. The determination of total nitrogen in soils and other complex heterogeneous materials containing several forms of N presents many difficulties. These difficulties are increased by the inadequacy of knowledge with different forms of soil N and by low N content of the materials (soil) analyzed. The total soil N ranges from <0.02% in subsoils to >2.5% in peat; the surface layer of the cultivated soils contains between 0.06 and 0.5% N. Two methods have gained general acceptance for determination of total N; the Kjeldahl method, which is essentially a wet-oxidation procedure, and the Dumas method which is fundamentally a dry oxidation (i.e. combustion) technique. There are reports that N content measured through C/N analyzer (modified Dumas method) of the subsoil are much more than the surface soils. The reason may be due to relatively more content of difficultly decomposable N (obtained through organic matter) in the subsoils than the surface soils. This observation finds support from the deviation of commonly accepted 'Bemmelon' factor of 1.724 to 1.9 for several surface soils to 2.5 for the subsoils^{2,3}.

Automated laboratory instruments : In view of the availability of the latest technological know-how in determining soil C and N⁷⁻⁹, the present work aims at standardizing methods after selecting appropriate standards. Moreover, tropical soils vary in both inorganic and organic C content¹⁰. Since most of the N in soils is dependent on organic C, the knowledge of SOC and SIC (soil inorganic carbon) in a particular type of soil (used as a standard, for combustion machine procedure) vary widely and therefore, the standards need to be method-specific. In other words, for different ranges of soil C and N to be analyzed, different standards as well as different methods are necessary. Keeping these points in view the present study was undertaken to define standards to adopt method (s) for determining C and N in soils to develop internal (soil) standards to determine C and N in calcareous and non-calcareous soils. We have also made an attempt for comparing two sets of results from laboratory (chemical) and C/N analyzer to validate the methods developed.

Materials and methods :

Materials : Two types of standards were used during the analytical work. The first group of standards belongs to chemical compounds with known composition which shows very rigid carbon and nitrogen content (Fig. 1; Table 1). The other group of standards is soil. These standard soil samples show variable amount of carbon and nitrogen. The C and N in these soil standards were further confirmed as detailed later. The organic matter is the source of organic form of carbon and nitrogen. The calcium carbonate nodules are the source of inorganic form of carbon.

Methods : Fig. 2 details the procedures and various experimental steps to run a method and to run the standards.

Results and discussion

The residual portion of salt standards (Table 1) (particularly Li_2CO_3) chokes the wall of graphite crucible after burning and thereby create problem in the analyses of samples. The other disadvantages of Li_2CO_3 and PbCO_3 are that these chemicals are harmful and might be dangerous during handling. We tried three other standards (Leco) with unknown chemical composition since these had an advantage of known C and N values (0.84 to 3.0% and 0.097 to 0.195% N). Moreover the C, and N contents of these standards fall within the commonly found ranges of C and N values of soils. These standards are, therefore, acceptable for developing method for routine soil analyses in the C/N analyzer and also for developing internal (soil) standards for future study.

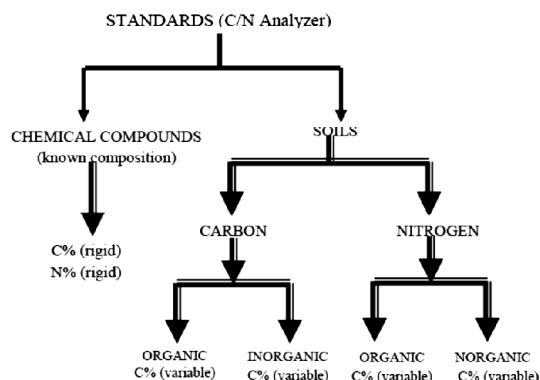
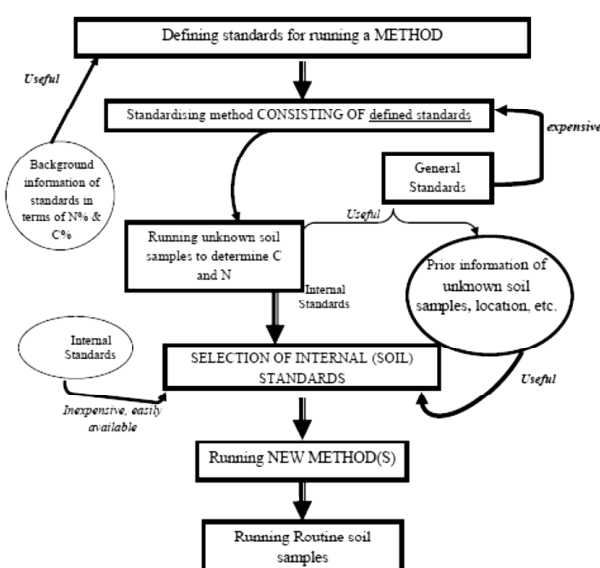


Fig. 1. Flow chart showing types of standards used for C/N analyzer (Please also see Table 1).

Table 1. Standards used for C/N analyzer

Sl. No.	Name of the compound ^a	Chemical formula	C (%)	N (%)
1.	Ethylene diamine tetraacetic acid (EDTA)	[CH ₂ N(CH ₂ .COOH)CH ₂ .COONa] ₂ .2H ₂ O	41.3 ± 0.12	9.57 ± 0.04
2.	Potassium hydrogen phthalate	COOH.C ₆ H ₄ .COOK	47.01	Nil
3.	Lead carbonate	PbCO ₃	4.491	Nil
4.	Calcium carbonate	CaCO ₃	12.03	Nil
5.	Oxalic acid	C ₂ H ₂ O ₄ .2H ₂ O	19.03	Nil
6.	Lithium carbonate	Li ₂ CO ₃	18.18	Nil
7.	EDTA + calcium carbonate ^b	[CH ₂ N(CH ₂ .COOH)CH ₂ .COONa] ₂ .2H ₂ O + CaCO ₃	23.66	9.57

^aGR Grade (Merck, Germany) EDTA, ^bC 40% of EDTA and 60% C of CaCO₃.


Fig. 2. Steps followed to run a method in C/N analyzer instrument.

Defining standards : Before developing internal (soil) standards, we chose (through various trials) the known standards which should fall within the range of total carbon and nitrogen. It should be worthwhile to mention here that soils are generally of two types in terms of total C content, viz. (i) calcareous and (ii) non-calcareous. Non-calcareous soils are composed of organic form of carbon and we found that these soils may contain organic C to the extent of 2–3%¹¹. Presuming C : N ratio of 10 : 1 and understanding the fact that N is sourced by organic matter only, these soils can safely measure N to the tune of 0.2 to 0.3%. So far as calcareous soils are concerned, the sources of C in both organic and inorganic^{10,12}. Inorganic C is usually contributed by CaCO₃ or *calcrete*. These are pedogenically formed and often referred to as

pedogenic carbonates (PC)¹². Soils containing high PC represent areas with relatively low rainfall¹³ and these areas contain less organic matter. The relation between SOC and SIC is inverse. Our experience shows that soils may contain about 45% CaCO₃ at maximum level. This indicates a value of $45 \times 0.12 = 5.4\%$ C. Usually such highly calcareous soils contain very low SOC (~0.2–0.4%) which make the total C in these soils as 5.8 or ~6.0%. Standard chemicals contain high C (Table 1). We used fillers (viz. CaCO₃) to reduce the C content to the level of 4.5% to mimic C levels of soils. We tried C neutral salts also; but those chemicals were rejected since they created side-effects (Table 2). With these chemicals the methods developed were used to standardize the internal (soil) standards and ultimately to develop methods for fine-tuning acceptable procedure as discussed below.

Developing soil standards : Using chemicals of known composition we developed four methods out of which one was recommended (Table 2) for measuring both C and N and the other for only C. These latter three methods are useful for samples with low (4.5–22%), medium (12–47%) and high level of C (41–47%) (Table 2). For brevity the calibration curves of EDTA are shown in Fig. 3 for nitrogen (N) and carbon (C).

Using soil standards of known C and N, analytical methods for soils were developed which may be recommended for different types of soils containing various ranges of these two elements (Table 3; Figs. 4 and 5). As mentioned earlier the methods shown in Tables 2 and 3 helped establishing the C and N content of a few selected soil samples from the Indo-Gangetic Plains (IGP)^{13,14} and the black soil regions (BSR) of the semi-arid tropics¹⁵. After this step was over, a series of soil samples from

Table 2. Methods developed using standards of known chemical composition

Sl. No.	Methods developed	Standards selected	Remarks	
			For C ^a	For N ^a
1.	EDTA	EDTA	Recommended (0.13–2.0)	Recommended (0.029–0.46%)
2.	High	EDTA and phthalate	Recommended (41–47)	Not recommended ^b
3.	Medium	PbCO ₃ , CaCO ₃ , COOHCOOH	Recommended (4.5–22)	Not recommended ^b
4.	Calcareous high IGP	CaCO ₃ , Li ₂ CO ₃ , Pthalate EDTA + CaCO ₃ (40+60)	Recommended (12–47)	Not recommended ^b

^aParentheses indicate range of C and N in percent. ^bReasons : measures only C. Not recommended for N samples analyses, because most of the standards used do not contain N.

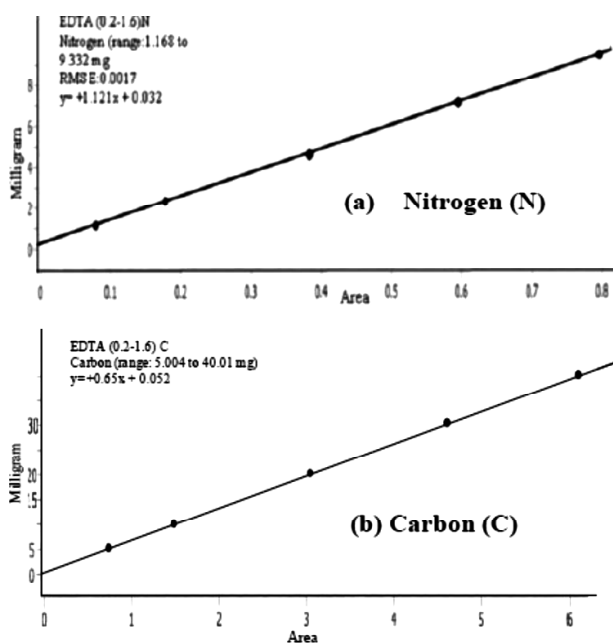


Fig. 3. Calibration curve for EDTA as standard for (a) nitrogen (N) and (b) carbon (C).

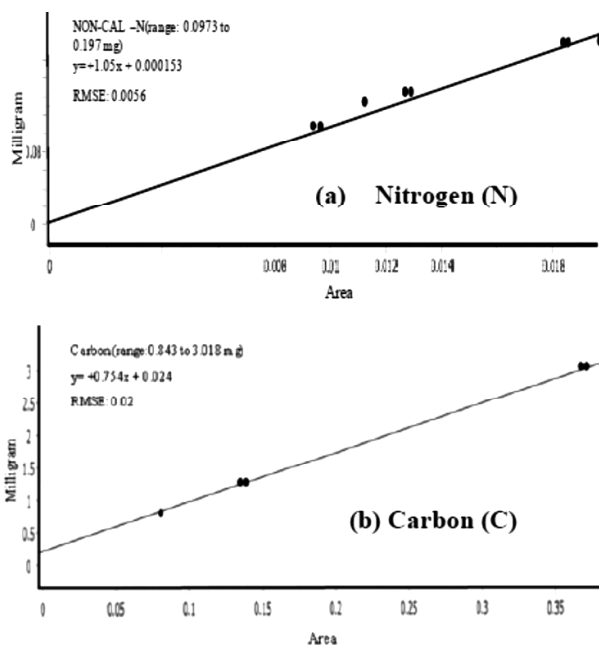


Fig. 4. Calibration curve for non-calcareous soils for (a) nitrogen (N) and (b) carbon (C) using Non-Cal method (Please also see Table 3).

Table 3. Analytical methods for soils developed for C/N analyzer using soil standards of known carbon and nitrogen content

Sl. No.	Methods	Remarks	
		C ^a	N ^b
1	Non-Cal	Recommended (0.84–3.0)	Recommended (0.097–0.195)
2	Nbss	Recommended (2–3)	Recommended (0.13–0.19)
3	High range	Recommended (1.5–4.5)	Recommended (0.097–0.292)

these two regions (IGP and BSR) with varying range of soil organic carbon (SOC) and soil inorganic carbon (SIC)

were selected as standards. Using these soil standards and keeping in view the range of C (both organic and inorganic carbon) and N (mainly organic matter content as the source of N), five standard methods were developed (Table 4). Figs. 6 and 7 show the calibration curves for two representative methods (Non-Cal and Cal High) in terms of nitrogen (N) and carbon (C).

Comparison of data sets : The representative benchmark soils were analysed using both C/N analyzer and the chemical methods (wet digestion method of Walkley and Black¹). A close look at Table 5 indicates that be-

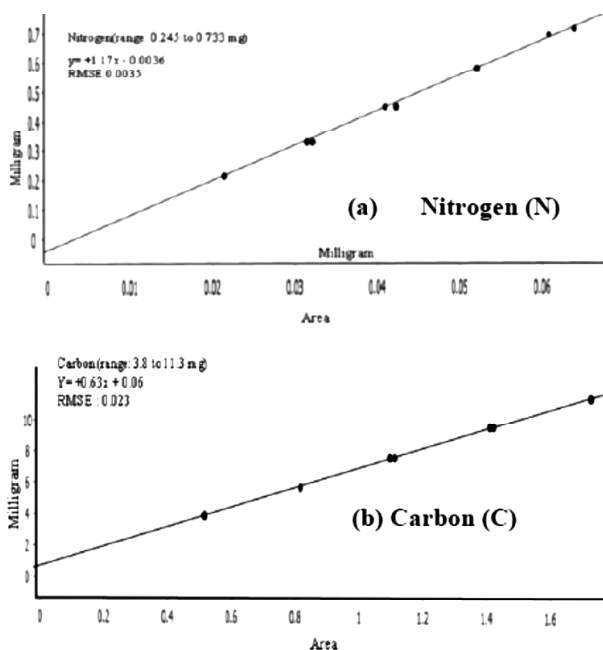


Fig. 5. Calibration curve of soils containing higher levels of organic carbon for (a) nitrogen (N) and (b) carbon (C) (Please also see Table 3).

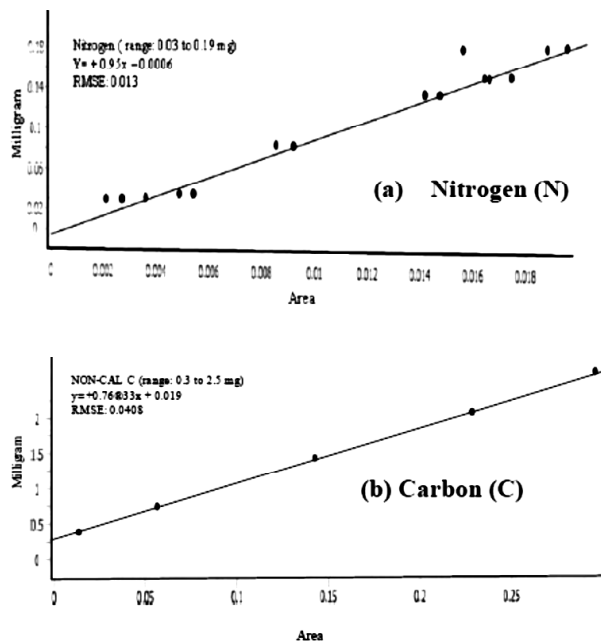


Fig. 6. Calibration curve for non-calcareous soils for (a) nitrogen (N) and (b) carbon (C).

Table 4. Methods developed for C/N analyzer using internal standards

Sl. No.	Methods	Remarks	
		C ^a	N ^b
1.	Non-Cal	Recommended ^b (0.35–2.44)	Recommended ^b (0.03–0.18)
2.	Cal Low	Recommended ^c (0.48–2.22)	Not recommended (0.045–0.12)
3.	Cal High	Recommended ^d (2.32–6.10)	Recommended (0.041–0.071)
4.	Cal Low	Recommended ^e (0.90–2.60)	Recommended (0.002–0.05)
5.	Cal High	Recommended ^f (2.60–4.60)	Recommended (0.01–0.04)

^aParentheses indicate range of C and N in percent; ^bRecommended for all soils either from IGP or BSR; ^cRecommended for IGP soils containing 20% CaCO₃; ^dRecommended for IGP soils containing 20–50% CaCO₃; ^eRecommended for BSR soils containing 20% CaCO₃; ^fRecommended for BSR soils containing 20–40% CaCO₃.

tween the methods used for C/N analyzer, the C data are reproducible in most of the cases. All the methods appear good and produce consistently similar values of carbon. The study indicates that the data obtained through method Non-Cal is acceptable for N. The reason is that the range selected for this standard covers the general range of N

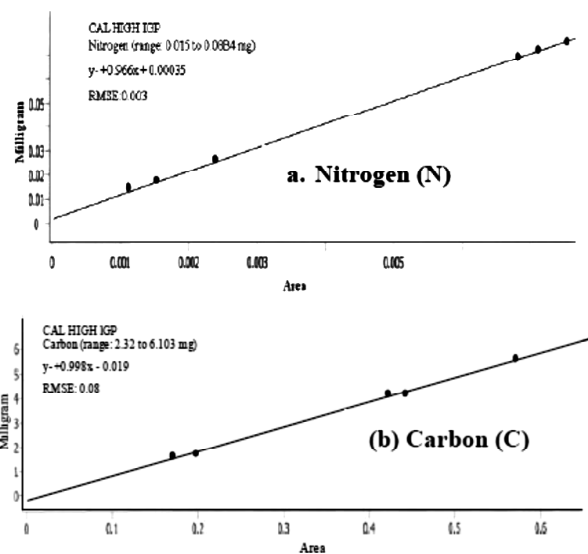


Fig. 7. Calibration curve for soils in Indo-Gangetic Plains (IGP) for (a) nitrogen (N) and (b) carbon (C).

found in Indian soils. Other methods show that for Cal Low (IGP) method, the lower range of N (standards) is too low to accommodate N level of Indian soils. This is in spite of the fact that the method produces reproducible data. For Cal High (BSR) method, the N values could be acceptable in spite of the fact that it overestimates if com-

Table 5. Comparison of soil carbon and nitrogen values (%) obtained by different methods

Soil series	Soil depth (cm)	C/N Analyzer : Methods					Chemical method (wet digestion)
		For all soils	For IGP soils		For BSR soils		
			Non-Cal	Cal Low	Cal High	Cal Low	
Carbon (C)							
Kovilpatti (Organic Farming)	0-6	1.025	1.095	1.120	1.035	1.020	0.985
	6-20	1.030	1.055	1.100	1.045	1.010	0.891
	20-41	1.100	1.220	1.115	1.060	1.080	0.985
	41-74	1.145	1.025	1.155	1.005	0.962	1.291
	74-104	1.045	1.225	1.255	1.050	1.040	1.799
	104-118	1.025	1.060	-	1.015	1.000	1.813
	118-128	1.390	1.400	-	1.365	1.360	2.151
	128-140	4.565	4.485	-	4.465	-	2.211
Kovilpatti (Wasteland)	11-31	0.910	0.882	-	0.891	-	1.078
	31-55	0.884	0.880	-	0.888	-	2.235
	55-79	1.325	1.330	-	1.320	-	1.677
	79-91	3.095	3.055	-	3.020	-	1.643
	91-105	1.655	1.675	-	-	-	1.001
Nitrogen (N)							
Kovilpatti (Organic Farming)	0-6	0.0173	-	0.0907	0.0199	0.0195	-
	6-20	0.0189	-	0.0213	0.0122	0.0263	-
	20-41	0.0157	0.0036	0.0139	-	0.0099	-
	41-74	0.0180	0.0122	0.0180	-	0.0143	-
	74-104	0.0103	-	0.020	0.0128	0.004	-
	104-118	0.0256	-	-	-	0.0111	-
	118-128	0.0281	0.004	-	-	0.0097	-
	128-140	0.0173	-	-	-	-	-

pared with N data from Non-Cal method. Similar observation is made when N data obtained through Cal High (BSR) method. This happens since the Non-Cal method permits analyses of relatively high range (0.03-0.185) of N as compared to other four methods. We made an attempt to compare data of soil carbon derived from both C/N analyzer and chemical method. Total C was estimated two ways viz. (i) directly from C/N analyzer, and (ii) adding SOC and SIC values obtained by wet digestion and laboratory methods^{1,3}. General inference on the N data obtained through C/N analyzer may be that if N content is >0.01%, the machine, perhaps, recognizes it well and accordingly can measure N with a fair degree of accuracy. This fact assumes importance in view of the fact that the Indian soils are, by and large, impoverished with N. It is seen that total carbon (TC) values obtained from chemical¹ method are always more than those from the C/N analyzer (Table 5). The problem may lie in as-

suming 77% recovery and multiplying values of C% by Walkley-Black Recovery Factor (WBRF) of 1.298 (100/77) to arrive at exact organic carbon content in soils. If we presume that tropical soils are highly weathered and the organic matter is well decomposed, the assumption of uniform 77% recovery of organic matter may be revisited.

The tropical soils are deficient in organic carbon¹⁶. While mapping organic carbon in Indian soils 1.0 percent organic carbon was considered as sufficient. The C/N analyzer is good to estimate total carbon in soils, even if carbon content is low. As pointed out earlier total carbon will be more in soils if the soil contains high amount of both SOC and SIC. As a matter of fact SOC and SIC are inversely related with mean annual rainfall. This is important when we try to judge the performance of C/N analyzer for total N in Indian soils. On an average, N content in soils is 1/10th of organic carbon and again

tropical soils contain <1.0% organic carbon. Estimation of soil N is, therefore, a great challenge in this part of the globe. Logically, if the soils are non-calcareous containing fairly high amount of organic carbon, soil N estimation would be more rewarding. Such soils are mostly found in forest¹¹. This is the reason we got a fairly good N curve for standards (internal) developed using forest soils (Non-Cal method). Again N in soils is related to the quality of organic matter (carbon). In general, C (organic) and N maintain a ratio in soil which depends on the degree of humification of organic matter and its content. Now since most of the biological systems of soils are more active in the surface horizons, the content and rate of humification decreases with soil depth. Accordingly, C/N ratio will change with soil depth. Depending on the climate, crops and other land use and management the level of C and its degree of humification would differ. To get more realistic organic C and N estimates the Walkley-Black Recovery Factor (WBRF) stands for revision for tropical soils.

Conclusions

In view of a close relation between SOC and SIC with other soil and climatic parameters, exact estimation is of utmost necessity. This assumes importance since nearly 60% area of the country has been prioritized areas for SOC sequestration. These areas are mostly rainfed and have very high inorganic C content in soils. The methods in the present study developed for calcareous and non-calcareous soils shall help researchers to fine-tune their estimation. Introduction of C/N analyser has an edge over classical analytical techniques; but it requires careful selection of standards and developing appropriate methods keeping in view of various agro-ecoregions of the country.

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