

Comparative performance of two extractants in colorimetric determination of soil organic matter in paddy soil

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ABSTRACT

Commonly used soil organic carbon (SOC) determination methods are expensive, time consuming, require equipment and skilled personnel making them impractical to use at field level. Therefore, a laboratory analytical study was carried out to identify a suitable extractant for SOC extraction as part of an ongoing effort to develop a low cost quick method for determining soil organic matter (SOM) at field level. For this purpose, 38 soil samples were collected from different Agro Ecological Zone (AEZ) to represent typical rice based cropping pattern covering a sufficient range in texture, soil organic matter, pH, and type of management. Two extractants e.g. NaOH-Pyrophosphate + HCl and NaOH-EDTA were used to extract organic carbon from soil and two wavelength e.g. 300 and 400 nm were used to measure the absorbance of organic carbon in spectrophotometer. Soil organic carbon measurements by CNS analyzer were used as reference measurement to compare the extracting efficiency of SOC by the selected extractants. Both the extract and wavelength showed different degrees of error in OC measurement. The error sum square of NaOH-Pyrophosphate + HCl and NaOH-EDTA measured at 300 nm wavelength were 15.21 and 13.35 whereas at 400 nm it was 227.37 and 19.95, respectively. Consequently, the average error percentages at 300 nm were -33 and -8 while at 400 nm it was -187 and -16. Based on the error sum square and error percentage, NaOH-Pyrophosphate + HCl performed better than the NaOH-EDTA, and 400 nm wavelength came out better than the 300 nm wavelength. In addition, both the extracts produced coloured solutions which were easily visible or detectable by naked eye and the colour intensity increased with the increase in SOC content. Variations in colour among the soils depending on organic matter content indicate that it would be possible to develop a new low cost method for SOM determination at field level by using NaOH-Pyrophosphate + HCl as the extractant. Further research is warranted to confirm this finding.

Key words: Soil organic matter, Colorimetric determination, extractants, low cost method, rice soils

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INTRODUCTION

Soil organic matter is a mixture of components of different forms and types of biotic origin, and can be broadly classified into living and non-living (Coleman *et al.*, 1989). The living component, which makes up to 4% of the total organic carbon, includes plant roots, macroorganisms and microorganisms. The non-living biomass, which forms up to 98% of the total organic carbon, can be sub-divided into macroorganic matter and humus. Macroorganic matter, commonly referred to as the light fraction, makes up 10–30% of the total organic carbon and consists largely of plant residues at varying stages of decomposition. The dead organic matter, remaining after separation of the light fraction, is called humus.

Physical, chemical and biological processes occurring in soils are greatly influenced by soil organic carbon (SOC) content. Soil organic matter (SOM) is being considered as the most stable and important indicator for evaluating soil fertility. Therefore, soil organic matter has always been in the forefront of soil research. Estimates of SOM are often required for a wide variety of agricultural, environmental, and engineering applications. The amount of SOC is also a good indicator of soil productivity potential. Therefore, estimates of SOC are often required for assessing the productivity of soils. In some cases soil organic carbon analysis are routinely used to evaluate the soils potential to supply N during a cropping season. Different types of quantitative and semi-quantitative methods for SOC determination have been developed and used at this end these are wet oxidation methods (Walkey and Black, 1934), modified wet oxidation method (Springer and Klee, 1954), dry combustion method (Nelson and Sommers, 1996) and more recently developed spectrophotometric method

(Harvey *et al.*, 2009). The first three methods are quantitative while the later one is semi quantitative. Wet oxidation and modified wet oxidation methods work based on the oxidation of organic matter (OM) by potassium dichromate ($K_2Cr_2O_7$) in the presence of sulfuric acid (H_2SO_4) (dilution of H_2SO_4 provides heating for the oxidation reaction) followed by back titration with ferrous sulphate ($FeSO_4$) to determine the unused $K_2Cr_2O_7$. Dry chemistry techniques determine SOC by burning the sample in an oven at elevated temperatures ($850^\circ C$ to $1150^\circ C$) followed by quantification of the end product of combustion, CO_2 through titrimetric, gravimetric, manometric, spectrophotometric, or gas chromatographic techniques. Spectrophotometric method works based on the Beer's law, assuming that the base extractable UV-active soil components/total SOC ratio is relatively constant. Therefore, soil extract absorbance (A_{300}) is used as a proxy for total SOC.

However, those methods are typically more expensive and time consuming (Abella and Zimmer, 2007), required equipment and skilled personnel making them impractical for applications of a large number of samples and field use. Therefore, it is very important to develop a rapid, in-field and easy method for the farmers to measure soil organic matter at field level. Such field level soil organic matter determination method will help in formulating an accurate fertilizer advice by taking into account the inherent fertility of soil contributed by soil organic matter. These will reduce the excessive use of costly chemical fertilizers and environmental pollutions cause by excessive fertilizer use. Such method could be usable for other agricultural ecosystems and may contribute to efficient evaluation of soil productivity in other regions.

As a first step, it is necessary to find out a suitable extractant solution that is able to

extract SOM from soil. It would be better, if the extractant develop color solution with varied intensity of color that is visible by naked eye and get differentiated according to the varied levels of SOM.

MATERIALS AND METHODS

Soils

In total a set of 38 soils (top 15cm) were collected from farmers' fields to represent majority of the typical rice based cropping patterns in Bangladesh covering different combinations of soil terrain types (e.g. non calcareous floodplain soil, calcareous floodplain soil, terrace soil, etc.) and land

types. Among them 25 soils were earlier described by Kader et al, 2015. Fifteen sub soil samples from a whole field in each location were collected by means of an auger (Ø 2.5 cm) and these samples were bulked and left to dry in air. Prior to the physico-chemical analysis of the soil samples, the air dried soils were broken apart and crushed by hand to pass through a 2-mm sieve. Soil series of the collected soil samples were identified in most of the cases, with the help of expert personnel of Soil Resources Development Institute (SRDI). Name of the soil series along with locations, land type and cropping patterns are presented in Table 1.

Table 1. Soil series name with their location, agro-ecological region (AEZ), taxonomic classification, land type and cropping pattern of sampled soils (adapted from Kader *et al*, 2015)

Soil series	Location	AEZ	Taxonomy (USDA)	Land type	Cropping patterns
Amnura	Koichor, Bogura sadar	25	Aeric Albaquepts	MH	P/M-R-R
Atwary	Ponchogor sadar, Ponchogor	1	Typic Haplaquepts	H	W-F-R
Balina	Batipar, Boror char, Mymensingh	9	Mollic Haplaquepts	VL	R-F-F
Damrai	Sirta Nowapara, Mymensingh sadar	9	Typic Haplaquepts	ML	R-F-F
Faridgonj	Larairchar, Faridgonj, Chandpur	17	Aeric Haplaquepts	MH	P/M-R-F- R
Gangachhara-1	Arathinyamat, Burirhat, Gongachora, Rangpur	3	Typic Haplaquepts	H	F-R-R
Gangachhara-2	Dhobadanga, Hazigonj, Nilfamary sadar	3	Typic Haplaquepts	MH	R-F- R
Ghatail	Katlasen, Dapunia, Mymensingh	9	Aeric Haplaquepts	L	R-F/R- R
Gopalpur	Domrakandi, Faridpur sadar	10	Aquic Eutrochrepts	MH	R-F- R
Gorargao	Bausia Bill, Mymensingh sadar	9	Typic Haplaquepts	VL	R-F-F
Ishordi	Domrakandi, Faridpur sadar	12	Aeric Haplaquepts	ML	R-F- R
Jamun	Vatgao, Sundorpur, Kahrul, Dinajpur	1	Aeric Haplaquepts	H	W/Mu-F-R
Kalma	BADC farm, Modhupur, Tangail	28	Aeric Albaquepts	ML	R-F- R
Karail	Borbila, Fulbaria Mymensingh	9	Cumulic Humaquepts	ML	R –F-F
Melandoho	Sirta Nowapara, Mymensingh sadar	9	Aeric Fluvaquents	MH	R-F- R
Noadda 2	BADC farm, Modhupur, Tangail	28	Ultic Ustochrepts	MH	R-F- R
	Bhaluka, Mymensingh	28	Ultic Ustochrepts	H	F-F- R

Noakhali	Sontospur, Faridgonj, Chandpur	17	Arent	MH	R-F- R
Pritompasha	Koroitola, Haluaghat, Mymensingh	22	Typic Haplaquepts	MH	F-F- R
Ramgoti-1	Giarchar ; Raipur, Laxmipur	17	Aeric Fluvaquents	ML	R-F- R
Ramgoti-2	Giarchar ; Raipur, Laxmipur	17	Aeric Fluvaquents	ML	R-F- R
Ranisankail-1	Vatgao, Sundorpur Kahrul, Dinajpur	1	Udic Ustochrepts	H	P-P-Mu
Ranisankail-2	Sonahar, Debigonj, Ponchaghor	1	Udic Ustochrepts	MH	P/F-F/J/R-R
Ruhia	Panchagor sadar , Ponchogor	1	Entic Haplumbrepts	H	W/S/O-F-R
Sherpur	Anondipur, Sirta, Mymensingh	9	Aquic Eutrochrepts	H	V-F- R
Silmondi-1	Katlasen, Dapunia, Mymensingh	9	Aeric Haplaquepts	ML	R-F/R- R
Silmondi-2	RARS, Jamalpur sadar	9	Aeric Haplaquepts	MH	R/F-F- R
Silmondi-3	Boro nondura, Netrkona sadar	9	Aeric Haplaquepts	ML	R-F- R
Silmondi-4	Mograpara, , Narayangonj	19	Aeric Haplaquepts	ML	R-F-F
Sonatola-1	BAU farm, Mymensingh	9	Aeric Haplaquepts	ML	R-F- R
Sonatola-2	BAU farm, Mymensingh	9	Aeric Haplaquepts	MH	R-F- R
Sonatola-3	Katlasen, Dapunia, Mymensingh	9	Aeric Haplaquepts	MH	V-F- R
Sonatola-4	Mograpara, Sonargoan, Narayangonj	19	Aeric Haplaquepts	ML	R-F-F
Sonatola-5	Mograpara, Sonargoan, Narayangonj	19	Aeric Haplaquepts	ML	R-F-F
Sontalola-6	Ragendrapur, Chollisha, Netrokona Sadar	9	Aeric Haplaquepts	MH	R-F- R
Sontalola-7	Boro nondura, Netrkona sadar	9	Aeric Haplaquepts	H	V-V-R
Sulla	Boroitola, Karimgonj, Kishoregonj	9	Typic Haplaquepts	VLL	R-F-F
Tarakanda	Modhupur, Fulphur, Mymensingh	9	Typic Fluvaquents	MH	F-F-R

H=High land, MH=Medium highland, ML=Medium lowland, VL=Very lowland R=Rice, F = Fallow, Mu = Mustard, V=Vegetable, W=Wheat, M=Maize, P=Potato, J=Jute, O=Oil seed, S = Soybean

General soil properties

Mechanical analysis of soil was done by hydrometer method (Buoyoucos, 1926) and the textural class was determined following “Marshall’s Triangular Coordinates” using USDA system. The pH_{KCl} of air-dried soil samples was measured in 1M KCl (1:2.5 soil weight (g): extractant volume (ml)) suspension using a glass electrode. Total N content in soil was determined by Kjeldahl method (Bremner and Mulvaney, 1982).

Soil organic Carbon determination

Reference method

Soil organic carbon (SOC) determined by Varimax CNS analyser (Elementar Analysensysteme, Germany) and Simudzu TOC analyser were used as the reference measure of SOC. Soil samples having no carbonate were analysed with a Variomax CNS-analyzer on 800 mg sub-samples. Carbonate containing soils were analysed

using a Shimadzu TOC analyzer with a 'SSM-5000A' module for solid samples and fed into the 'TOC-V_{CPN}' which determined subsequently the organic carbon contents. The organic carbon concentration was calculated as the difference between the total carbon and the inorganic carbon concentration.

Spectrophotometric measurement

The experiments were geared toward identifying a suitable extractant for SOC quantification. Two extractants on the extraction of SOC were evaluated at a soil/extractant ratio of 1:200. The extractants tested were 0.25 mol L⁻¹ NaOH + 0.05 mol L⁻¹ EDTA (after Bowman *et al.*, 1991, Harvey *et al.*, 2009), and 0.25 mol L⁻¹ NaOH + 0.1 mol L⁻¹ Na₄P₂O₇·10H₂O (pyrophosphate) followed by 1 mol L⁻¹ HCl (after Schnitzer, 1982). NaOH was chosen for its ability to extract particulate SOM (unprotected SOM pool) while EDTA was used to bind Fe & Al and Na₄P₂O₇·10H₂O were chosen for its ability to extract mineral bound SOM pool. An amount of 0.1g of air-dried soil was weighed into a 50-mL polypropylene centrifuge tube followed by addition of 20 mL of NaOH-pyrophosphate extractant solution and shaken by hand for 15 to 20 s at 1-min intervals for 5 min. The extract was filtrated through 0.45 μm polycarbonate syringe filters.

The absorbance of the filtrate was measured at 300 nm and 400 nm with a UV-Vis spectrophotometer (Spectronic GENESYS 5 UV-Vis Spectrophotometer, USA) with a path length of 1 cm. A calibration curve was prepared by using absorbance and SOC content of the selected soil samples (developed using Beer's law and soils of known SOC content) to determine SOC content of all unknown soil samples based on measured absorbance.

Colorimetric measurement

It was a visual observation. The soils were sub grouped into four groups based on the BARC (2005) soil organic matter classification. According to this classification, soil containing >1, 1-1.7, 1.8-3.5 and <3.5% organic matter were classified as very low, low, medium and high organic matter content soil. Based on the visual observation of the extract colour the extracts were then classified in to the above mentioned four groups.

Statistical Analysis

Correlation analysis was used to investigate the relation among soil properties in SPSS service pack 19 (SPSS Lnc, USA). Mean comparison of the sum squares of errors and percentage of errors were also made in SPSS by means of one way ANOVA followed by Dunckan's Multiple Range Test (DMRT).

RESULTS

General Soil Properties

The general physical and chemical properties of soils of some important rice growing soil series of Bangladesh are presented in Table 2 and Figure 1. There was a wide variation in soil texture among the studied soil series (Table 2). The sand, silt and clay content of the collected soils ranged between 78% (in Tarakanda series) and 4% (in sonatala-1 series), 88% (in Gopalpur series) and 14% (in Tarakanda series), 59% (in Sulla series) and 7% (in Gopalpur series), respectively. The textural classes of the studied soils were mostly silt loam with some other classes such as silty clay loam, loam, clay loam, clay, silty clay and sandy loam (Figure 1).

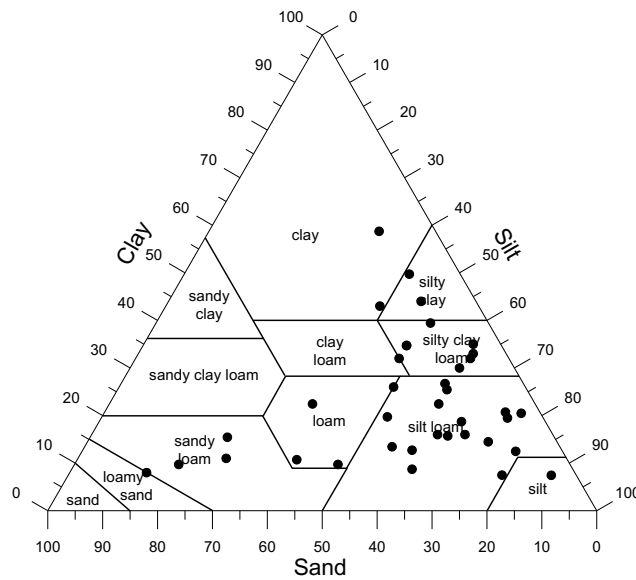


Figure 1. Textural distribution of soil according to USDA Textural Classification

Soil pH (pH_{KCl}) ranged from 5.47 in Dhamrai soil to 6.10 in Gopalpur soil with most being close to neutral (on average

5.86 ± 0.26). There was a wide variation in soil CEC among the studied soil series varied from $58.0 \text{ cmol kg}^{-1}$ soil in Karail series to $16.0 \text{ cmol kg}^{-1}$ in Ranisankail-2 series (Table 2).

The total N content of the soils varied from 0.32% in Gorargao series to 0.036% in Gangachara-2 soil series (Table 2). Soil samples collected from low lying areas having higher clay content showed higher total N content. In contrast, soil samples collected from high land and sandy areas showed lower total N content. Organic carbon content (%) of the studied soils also showed a wide variation and the highest (3.52) was found in Karail series and the lowest (0.391) was found in Tarakanda series. The % OC content of the majority of our studied soil series lies around 1

Table 2. Physico-chemical Properties of the selected soils

Soil series	Granulometric composition			Organic Carbon (% OC)	Total N (% N)	pH_{KCl} (-)	CEC (cmol kg^{-1} soil)
	%Sand	%Silt	%Clay				
Amnura	19±0	65±1	16±1	1.235	0.150	5.88	30.0
Atwary	41±0	37±1	22±1	1.855	0.189	5.85	39.8
Balina	17±3	48±0	35±3	1.526	0.193	5.95	43.1
Dhamrai	15±0	60±0	25±0	1.696	0.185	5.47	34.8
Faridgonj	14±0	79±1	7±1	1.144	0.120	6.10	29.2
Gangachhara-1	27±0	60±0	13±0	0.995	0.096	5.77	33.4
Gangachhara-2	42±1	48±0	10±1	0.418	0.036	6.02	20.9
Ghatail	9±0	41±1	50±1	2.116	0.224	5.90	44.7
Gopalpur	5±0	88±0	7±0	0.759	0.088	6.10	35.3
Gorargao	18±3	39±1	43±4	3.054	0.317	5.95	37.6
Ishordi	11±3	50±3	39±6	2.316	0.220	6.92	54.1
Jamun	31±0	56±0	13±0	1.025	0.102	5.55	30.0
Kalma	10±0	60±0	30±0	1.444	0.149	6.13	36.5
Karail	20±0	48±0	32±0	3.518	0.274	5.75	58.0
Melandoho	21±1	63±1	16±0	0.815	0.093	5.54	25.4
Noadda-1	10±0	46±0	44±0	0.915	0.101	5.49	35.9
Noadda 2	24±0	50±0	26±0	1.053	0.113	5.68	34.6
Noakhali	9±1	79±1	12±0	1.641	0.164	6.63	24.3
Pritompasha	17±0	48±0	35±0	1.320	0.138	5.67	32.3
Ramgoti-1	7±1	74±0	19±1	1.410	0.146	6.55	30.8
Ramgoti-2	6±1	73±1	21±0	1.482	0.155	6.29	38.1
Ranisankail-1	60±1	25±1	15±3	1.119	0.076	5.74	30.4

Ranisankail-2	71±0	19±1	10±1	0.648	0.069	5.80	16.0
Ruhia	62±0	27±1	11±1	1.844	0.161	5.73	28.2
Sherpur	49±0	40±0	11±0	0.932	0.091	5.49	21.5
Silmondi-1	14±1	59±1	27±3	1.643	0.162	6.10	34.3
Silmondi-2	6±0	61±1	33±1	1.240	0.133	5.93	33.1
Silmondi-3	28±1	52±0	20±1	1.072	0.124	5.78	27.7
Silmondi-4	16±0	68±0	16±0	1.307	0.140	6.50	25.4
Sonatola-1	4±0	76±3	20±3	2.136	0.206	5.99	44.2
Sonatola-2	13±1	73±1	14±3	1.628	0.169	5.85	34.9
Sonatola-3	15±0	66±3	19±3	0.944	0.099	5.87	31.5
Sonatola-4	7±4	61±1	32±6	1.745	0.197	6.20	47.0
Sonatola-5	5±1	60±0	35±1	2.890	nd	6.40	35.9
Sontalola-6	18±1	60±0	22±1	1.212	0.127	5.75	28.2
Sontalola-7	29±0	62±3	9±3	0.813	0.076	5.80	28.1
Sulla	10±1	31±1	59±3	1.727	0.239	5.59	42.5
Tarakanda	78±0	14±0	8±0	0.391	0.041	5.66	18.6

Correlation between soil properties

Pearson's correlation coefficients between % sand, % silt, % clay, pH_{KCL}, SOC (%) and total N (%) are listed in Table 3. Among the soil particles, clay was found to be very active and showed significantly strong positive correlation with CEC, % OC and %

total N whereas sand showed a significantly negative relation with them. Unexpectedly, %sand and %silt content showed a significant negative and positive correlation with pH_{KCL}.

Table 3. Pearson's correlation coefficient of sand, silt and clay percentage, pH_{KCL}, soil organic carbon (%) and total N (%)

	%silt	%clay	CEC	pH _{KCL}	%OC	%Total N
%sand	-0.75**	-0.47**	-0.55**	-0.41**	-0.36*	-0.48*
%silt		-0.22	0.12	0.44**	-0.01	0.03
%clay			0.66**	0.02	0.54**	0.66**
CEC				0.22	0.77**	0.76**
pH _{KCL}					0.24	0.23
%OC						0.94**

**Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed).

Spectrophotometric measurement of Soil Organic Carbon

NaOH-Pyrophosphate Extracted Organic Carbon

Organic carbon (%) extracted by NaOH-Pyrophosphate measured at 300 and 400 nm wavelength varied widely in the studied soil samples (Table 4). Organic carbon measured

at 300 nm wavelength ranged between 0.62% in Tarakanda and 4.43% in Karail series with an average of 1.78±0.8% which was slightly higher than our reference CNS measured value (1.45±0.68%) indicating that an over estimation of soil organic carbon by this method. On the other hand, OC measured at 400 nm wavelength ranged between 0.40% in Tarakanda series to 5.45% in Karail series with a mean value of

1.54±0.96% which is very close to our reference mean value (1.45±0.68%) indicating the efficiency of this method though there was slight over estimation of OC.

NaOH-EDTA Extracted Organic Carbon

Variation was also observed in OC% extracted by NaOH-EDTA measured at 300 and 400 nm wavelengths among the soil series (Table 4). The OC (%) measured at 300nm wave length ranged between 1.89 (Gangachhara 2) and 5.22 (Sonatola-4) with an average of 3.86±0.90 which was much

higher than the average value of (1.45±0.68) suggesting a huge overestimation of OC with this method. On the other hand, %OC measured at 400nm wave length varied from 5.80 in Karail series to 0.39 in Sherpur series with a mean value of 1.73±1.07 which was higher than the average value of reference (1.45±0.68), however, it was lower than the % OC measured at 300 nm (3.86±0.90) suggesting the suitability of the OC measurement at 400 nm after extraction with NaOH-EDTA.

Table 4. NaOH-Pyrophosphate + HCl and NaOH-EDTA extracted OC (%) measured at 300 and 400 nm wavelength

Soil series	NaOH + Pyrophosphate + HCl Extracted OC (%)		NaOH + EDTA Extracted OC (%)	
	300nm	400nm	300nm	400nm
Amnura	1.74±0.56	1.23±0.01	3.37±0.06	1.09±0.01
Atwary	3.45±0.80	3.38±0.03	5.22±0.00	3.83±0.00
Balina	1.53±0.51	1.06±0.01	3.41±0.01	0.96±0.02
Damrai	2.52±0.65	1.70±0.12	4.86±0.08	1.87±0.02
Faridgonj	1.31±0.33	1.04±0.19	3.24±0.04	1.11±0.02
Gangachhara1	1.86±0.54	1.47±0.02	3.84±0.14	1.38±0.08
Gangachhara2	0.69±0.32	0.47±0.01	1.89±0.11	0.35±0.07
Ghatail	1.74±0.56	1.59±0.05	4.92±0.09	2.00±0.05
Gopalpur	1.70±0.49	1.13±0.03	3.33±0.35	1.03±0.16
Gorargao	3.51±0.87	3.19±0.34	4.94±0.39	2.99±1.31
Ishordi	1.48±0.50	1.48±0.04	4.51±0.14	1.82±0.15
Jamun	1.77±0.49	1.25±0.04	3.90±0.01	1.39±0.02
Kalma	1.06±0.38	0.96±0.01	2.95±0.21	0.93±0.13
Karail	4.43±1.18	5.45±0.11	5.00±0.03	5.80±0.37
Melandoho	1.40±0.33	0.78±0.05	2.48±0.04	0.60±0.05
Noadda-1	1.40±0.49	1.00±0.01	2.94±0.32	0.80±0.32
Noadda-2	2.01±0.57	1.38±0.02	3.24±0.11	0.95±0.06
Noakhali	1.64±0.66	1.21±0.19	3.15±0.01	1.16±0.01
Pritompasha	1.60±0.54	1.51±0.15	5.22±0.00	2.28±0.02
Ramgoti-1	1.09±0.36	1.03±0.04	3.74±0.14	1.52±0.05
Ramgoti-2	0.94±0.45	0.73±0.09	3.43±0.13	1.12±0.06
Ranisankail-1	1.75±0.57	1.43±0.09	4.05±0.10	1.54±0.03
Ranisankail-2	0.94±0.47	0.77±0.08	2.64±0.06	0.75±0.06
Ruhia	2.96±0.73	3.19±0.02	3.07±3.04	3.47±0.44
Sherpur	1.35±0.33	0.96±0.21	3.04±0.00	0.39±0.76
Silmondi-1	1.74±0.43	1.73±0.19	4.87±0.49	2.36±0.66
Silmondi-2	1.66±0.48	1.54±0.09	4.09±0.13	1.64±0.07
Silmondi-3	1.36±0.44	1.12±0.02	3.46±0.05	1.22±0.01

Silmondi-4	1.81±0.58	1.71±0.18	4.50±0.11	2.06±0.09
Sonatola-1	1.43±0.16	1.37±0.04	3.84±0.23	1.46±0.10
Sonatola-2	1.10±0.53	0.85±0.16	3.21±0.09	1.12±0.04
Sonatola-3	1.40±0.57	0.90±0.01	3.17±0.34	1.04±0.18
Sonatola-4	2.14±0.53	2.18±0.08	5.22±0.00	3.03±0.21
Sonatola-5	2.93±0.70	2.66±0.13	5.22±0.00	2.73±0.16
Sontalola-6	1.35±0.39	1.06±0.06	3.57±0.04	1.26±0.01
Sontalola-7	2.03±0.53	1.52±0.11	4.11±0.00	1.82±0.02
Sulla	2.37±0.74	2.16±0.01	5.22±0.00	2.57±0.17
Tarakanda	0.62±0.35	0.40±0.05	nd	nd

nd= not determined

Error in Spectrophotometric measurement of Soil Organic Carbon

Error in NaOH-Pyrophosphate + HCl Extracted Organic Carbon Measurement

Error of the spectrophotometric measurement of soil organic carbon (SOC) are presented as error square (Table 5) as well as percentage error (Figure 2). During both the error calculation, the CNS measurement of SOC was used as the reference value and compared with other OC measurement methods. It was observed that the error square was not constant for all the soils as well as for all the methods and varied widely in the studied soils and methods. The error square of OC measurement at 300 nm wavelength after extraction with NaOH-Pyrophosphate ranged between 0.00 in Noakhali and Balina series and 2.56 in Atwary series. There was a huge propagation of error in Atwary (2.56), Ruhia (1.52) and Sontalola-7 (1.48) soil series. Likewise error square, there was also a wide variation in error percentage ranging from -150 (Sontalola-7) to 36 (Ramgoti and Ishordi series) with an average of -33 indicating an average 33% overestimation of OC by this method.

The error square of OC measurement at 400nm wavelength ranged between 0.00 in Damrai, Amnura and Tarakanda series and 3.747 in Karail series. In this method, in addition to previously mentioned sandy soil,

the soils of the submerged Karail series also showed a large propagation of error. The error percentage of OC measurement at 400 nm range between -87 (Sonatola 7) and 48 (Sonatola 2) with an average of -8 indicating an average 8% overestimation of OC by this method.

Error in NaOH-EDTA Extracted Organic Carbon Measurement

The error square of OC measurement after extraction with NaOH-EDTA at 300nm wavelength varied widely from soil series to series (Table 5) and ranged from 0.00 (in Tarakanda series) to 12.05 (in Sonatola-4 series). As with the error square, the percentage of error in organic carbon measurement at 300 nm wavelength also varied widely in studied soils. The error percentage in soils ranged between -406 in Sontalola-7 series and 0 in Tarakanda series with an average of -187. There was huge propagation of error in 300 nm wavelength measurements suggesting less suitability of the method. The error square of OC measurement at 400 nm wavelength also varied widely and the highest (5.21) was found in Karail series while the lowest (0.00) was found in Sherpur series (Table 5). The percentage of error in OC measurement in this wavelength ranged between 37 in Balina series and -124 in Sontalola-7 series with an average of -16.

Table 5. Error square in spectrometric Organic Carbon measurement

Soil series	Error square			
	NaOH + Pyrophosphate + HCl Extracted OC		NaOH + EDTA Extracted OC	
	300nm	400nm	300nm	400nm
Amnura	0.259	0.000	4.542	0.021
Atwary	2.555	2.321	11.299	3.895
Balina	0.000	0.219	3.565	0.324
Dhamrai	0.684	0.000	9.989	0.031
Faridgonj	0.027	0.011	4.407	0.001
Gangachhara1	0.744	0.228	8.072	0.151
Gangachhara2	0.074	0.003	2.174	0.005
Ghatail	0.144	0.272	7.838	0.014
Gopalpur	0.880	0.137	6.598	0.071
Gorargao	0.212	0.020	3.546	0.004
Ishordi	0.707	0.704	4.826	0.245
Jamun	0.553	0.051	8.269	0.133
Kalma	0.145	0.235	2.257	0.260
Karail	0.838	3.747	2.201	5.206
Melandoho	0.237	0.001	2.760	0.045
Noadda-1	0.236	0.007	4.087	0.012
Noadda-2	0.923	0.106	4.804	0.010
Noakhali	0.000	0.183	2.265	0.233
Pritompasha	0.076	0.038	15.182	0.930
Ramgoti-1	0.105	0.148	5.427	0.013
Ramgoti-2	0.289	0.567	3.808	0.129
Ranisankail-1	0.394	0.097	8.597	0.176
Ranisankail-2	0.087	0.015	3.982	0.010
Ruhia	1.252	1.825	1.499	2.649
Sherpur	0.179	0.001	4.463	0.000
Silmondi-1	0.009	0.007	10.396	0.508
Silmondi-2	0.177	0.092	8.145	0.159
Silmondi-3	0.084	0.003	5.724	0.022
Silmondi-4	0.249	0.161	10.175	0.566
Sonatola-1	0.497	0.581	2.909	0.456
Sonatola-2	0.275	0.611	2.518	0.255
Sonatola-3	0.209	0.002	4.959	0.009
Sonatola-4	0.152	0.192	12.051	1.651
Sonatola-5	0.001	0.051	5.412	0.025
Sontalola-6	0.018	0.024	5.580	0.003
Sontalola-7	1.483	0.506	10.871	1.013
Sulla	0.407	0.184	12.176	0.714
Tarakanda	0.052	0.000	nd	nd

nd= not determined

Comparison of the Soil Organic Carbon Determination Methods

Significant variations were observed among the error sum squares as well as error percentages of the methods used in this study for the determination of soil organic

carbon. The error sum square of NaOH-Pyrophosphate + HCl and NaOH-EDTA measured at 300 and 400 nm wavelength were 15.21, 13.35, 227.37 and 19.95, respectively (Figure 2). Consequently, the average error percentages were -33, -8, -187 and -16 (Figure 3).

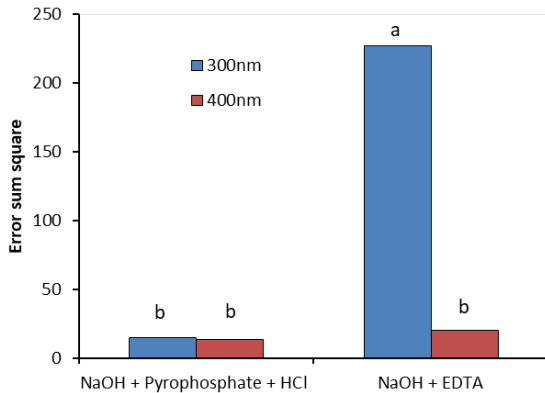


Figure 2. Comparison of error sum square of NaOH-Pyrophosphate + HCl and NaOH -EDTA extracts measured at 300 and 400 nm wavelength.

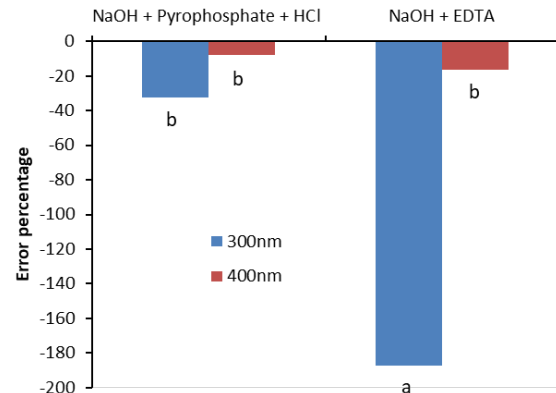


Figure 3. Comparison of error percentage of NaOH-Pyrophosphate + HCl and NaOH-EDTA extracts measured at 300 and 400 nm wavelength.

Comparison of the Colour of the Soil Extracts

During the spectroscopic measurement of OC, it was observed that the NaOH-Pyrophosphate and NaOH-EDTA extracts are practically coloured solution and these colours are easily visible or detectable by naked eyes. More interestingly, the intensity of the colour increased with the increase of SOC content (Figure 4). From the visual observation it was clearly showed that the colour of the both extracts (NaOH-Pyrophosphate and NaOH-EDTA extracts)

were very light yellow for the soils containing very low OM (<1%). On the other hand it was light yellow for the soils containing low OM (1-1.7%). However, the colour was slightly different between the extracts for the soils containing medium OM (1.8-3.5%). NaOH-EDTA produced yellow to light brown colour extract whereas NaOH-Pyrophosphate produced yellow colour extract for this group. Soil containing high OM (3.5-5.5%) produced brown colour solution for both the extracts (Figure 4).

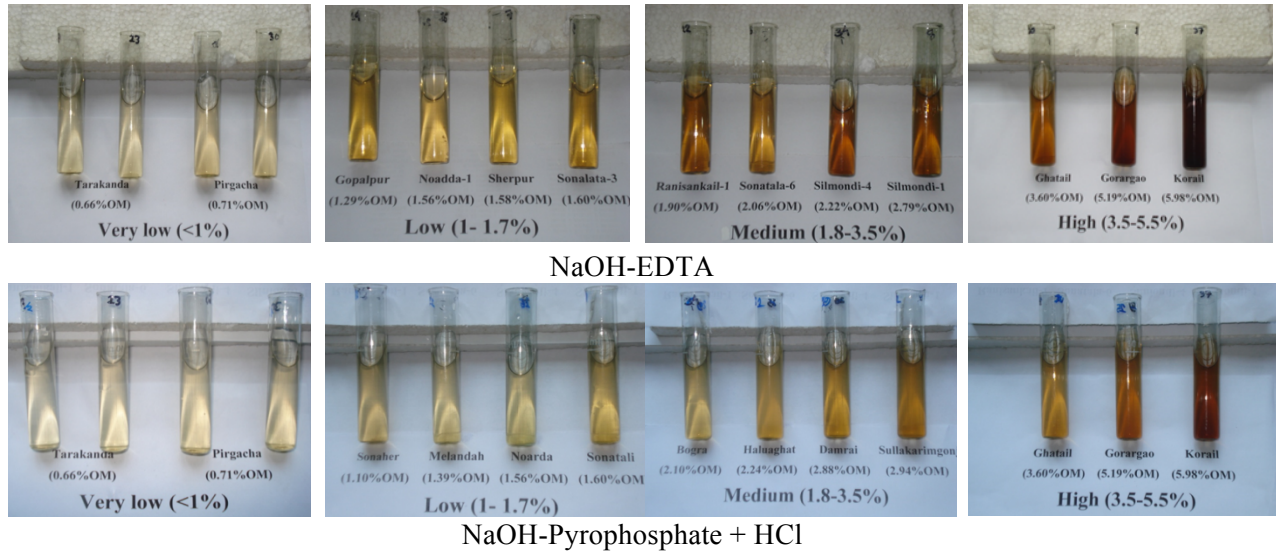


Figure 4. Colour of the NaOH-EDTA and NaOH-Pyrophosphate + HCl extracted from soil containing very low, low, medium and high organic matter

DISCUSSION

General soil properties

The texture of the studied soil set was mostly of medium-textured (mainly silt loam). As the majority of soil samples were collected from the rice growing floodplain areas, therefore, they showed the typical textural characteristics of floodplain soil with high silt content (FAO, 1971, Kader *et al.*, 2015). The variation in soil texture was found to be related with the position of soils or land type. Usually, soils collected from higher elevation (medium highland) were found coarse-textured while soils collected from lower elevation (medium lowland and lowland) were found fine-textured. This is mainly due to the deposition of finer soil particles in the lower position from the surrounding upper elevation through run-off water (Kader *et al.*, 2015). Textural Classes of our studied soils are in the same order of magnitude as previously reported texture of these soil series by Hossain and Satter (2002) and Islam *et al.* (2001).

The highest and the lowest CEC found in Karail and Ranisankail-2 series, respectively might be due to higher and lower clay content of the studied soils. This is also indicated by strong positive correlation between CEC and clay content. Soils having higher pH values were mostly collected from the Calcareous Dark Grey Floodplain Soil (Table 2). On the other hand, soils having lower pH values were collected from highly weathered terrace soil and Piedmont Soil. The values of pH_{KCl} and CEC of studied soils are in line with the previously reported values of rice soils pH_{KCl} and CEC by Alam *et al.*, 1993.

Organic carbon content (%) of the studied soils also showed a wide variation with the highest in Karail series might be due to high clay content and the lowest (0.391% OC) in Tarakanda series might be due to sandy in texture as it is believed that texture has a very strong influence on soil organic carbon content (Six *et al.*, 2002). Organic carbon content of our studied soils are in line with previously measured % OC of these soil series by Alam *et al.*, (1992), Hossain and

Satter (2003) and Islam *et al.*, (2001). Likewise total N content, higher % OC content was also found in those soils which were collected from low lying areas might be due to low lying areas are clayey in texture which favours accumulation of SOC. On the other hand, low lying areas remain submerged major part of the year which also favours accumulation of SOC by lowering microbial decomposition of SOM (Sahrawat, 2008). In addition, wetland soils become deficient in electron acceptors such as easily reducible iron, that may have reduced the rates of OM oxidation and mineralization of soil organic N, leading to net accumulation of SOC and total N (Sahrawat and Narteh, 2001; Sahrawat, 2002; Roden and Wetzel, 2002). Significant positive correlation between SOC and total N with clay content indicated the role of clay in SOM stabilization. It is well documented in literature that the clay particle plays an important role in physical and chemical stabilization of SOM through microaggregate formation and complexation with humus (Six *et al.*, 2002). Wide variation of the studied soil set in soil texture, pH, CEC, total N and SOC indicate that the sampled soil set is suitable for comparison of SOC determination methods.

Comparison of the Soil Organic Carbon Determination Methods

Both the extract and wavelength showed different degrees of error in OC measurement. Huge propagation of error in NaOH-Pyrophosphate + HCl extraction at 300nm for Atwary, Ruhia and Sontalola-7 soil series might be related with their texture and SOM stabilization mechanism. Atwary and Ruhia soil series are sandy in texture and belongs to black terrai soil where soil organic matter (SOM) is considered to be stabilized with sand particles historically with a special mechanism (Sleutel *et al.*, 2010) under waterlogged situation in the

foothill (Uddin *et al.*, 2014). On the other hand, Sonatola-7 soils are also sand dominated (Table 2) therefore similar stabilization mechanism might worked with this soil. The majority of this stabilized SOM might be recalcitrance in nature. Normally, NaOH and Pyrophosphate were used to extract particulate and mineral bound SOM leaving recalcitrant SOM with the extraction residues (Sleutel *et al.*, 2010). However, in those sandy soils, the recalcitrant SOM probably extracted by NaOH-Pyrophosphate as the sand particle does not have the ability to strongly bind the SOM. Therefore, the huge error of these three soils was not a methodological error rather it was related with the quality and stabilization mechanism of SOM. In addition, there was also a large error percentage in Gopalpur and Ishordi series which were a calcareous soil. Calcareousness of these soils could be a reason for this huge error.

The submerged Karail series also showed a large propagation of error in addition to previously discussed sandy soil when extracted with same extractant but measured at 400nm wavelength. This soils may be also contained a large recalcitrant SOM which was relatively young in nature compared to the recalcitrant SOM of black terrai soils. Therefore, this recalcitrant SOM probably showed their peak or detected at 400 nm wavelength resulting an overestimation of SOC and huge error.

The minimum error sum square and error percentage of OC measurement from both the extracts measured at 400nm wavelength suggesting the superiority of OC measurement at 400nm wavelength over 300nm wavelength. This is in contrast with the absorption maxima of humic acid substances (280-300 nm) (Stevenson, 1994) suggesting that the NaOH-Pyrophosphate + HCl and NaOH - EDTA extracted other

organic substances in addition to humic substances. Based on the error sum square and error percentage, it can be concluded that NaOH-Pyrophosphate + HCl performed better than the NaOH-EDTA and also 400 nm wavelength measurements corresponded well than 300 nm wavelength measurement with the reference OC measurement. Higher error in the NaOH-EDTA extracted OC might be due to the presence of colored EDTA-Fe³⁺ complexes, resulting in false positive detection or overestimation of SOC as the experimental soils were all air dried (oxidized soils) and SOC was extracted at room temperature where Fe³⁺ is a common constituent in soil (Bowman *et al.*, 1991; Harvey *et al.*, 2009).

Visual variation in colour of extractant among the soil groups classified depending on organic matter content indicate that a new low cost method could be developed for soil organic matter determination at field level by using the above discussed extractant solutions.

CONCLUSION

NaOH-Pyrophosphate + HCl extract with 400 nm wavelength performed better for determination of soil organic carbon as compare to NaOH-EDTA extract. This extract also preferred to be better than NaOH-EDTA as it seemed to produce intense colour. Intensity of the colour increased with the increase of SOC indicate the potentiality of NaOH-Pyrophosphate + HCl as an extractant to develop a new low cost method for SOM determination at field level. However, further research is needed with fine tuning to finally recommend this extractant as the essential part of the expected low cost method for determination of soil organic matter.

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