

Introduction of a method for estimating organic matter in the field (**Bowman, 1997**)

*Introducción de un método de estimación del contenido de materia orgánica en el campo (**Bowman, 1997**)*

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ABSTRACT: Soil organic matter (SOM) is essential for soil health and agricultural productivity, improving physical, chemical, and biological properties. Its measurement is key for sustainable soil management. Traditional methods (Walkley-Black and loss on ignition) have critical limitations in resource-limited environments: the use of toxic and carcinogenic reagents (sulfuric acid, dichromate), complex waste management, high energy consumption, and the requirement for specialized equipment. Given this, the EDTA (ethylenediaminetetraacetic acid) extraction method proposed by Bowman in 1997 was validated as a safe, rapid, and applicable alternative in situ. The results of both methods were compared in 17 Cuban soil samples with variability in groupings, textures, colors and degree of aggregation, finding i) High linear correlation ($R^2 = 0.76$) between optical densities (OD), with OD (EDTA) 2.3 times higher than OD (Walkley-Black), ii) confirmed validity for soils with SOM < 6%, similar to international studies and iii) Elimination of hazardous reagents and reduction of operating costs. In addition, the influence of texture and aggregation percentages were identified as factors that may affect the reading of the values, and pertinent technical recommendations were made. The results support the development of a standardized field kit with local standards, offering a technically robust, safe and accessible solution for SOM monitoring in Cuba.

Keywords: Technical Validation, In Situ Medition, Edaphic Indicators.

RESUMEN: La materia orgánica del suelo (MOS) es fundamental para la salud edáfica y la productividad agrícola, al mejorar propiedades físicas, químicas y biológicas, siendo su medición clave para un manejo sostenible de los suelos. Los métodos tradicionales (Walkley-Black y pérdida por ignición) presentan limitaciones críticas en entornos con recursos limitados: uso de reactivos tóxicos y cancerígenos (ácido sulfúrico, dicromato), manejo complejo de residuos, alto consumo energético y requerimiento de equipos especializados. Ante esto, se validó el método de extracción con EDTA (ácido etilendiaminotetraacético) propuesto por Bowman en 1997, como alternativa segura, rápida y aplicable in situ. Se compararon los resultados de ambos métodos en 17 muestras de suelos cubanos con variabilidad en agrupamientos, texturas, colores y grado de agregación, encontrándose i) Alta correlación lineal ($R^2 = 0.76$) entre las densidades ópticas (DO), con DO (EDTA) 2.3 veces superiores a DO (Walkley-Black), ii) validez confirmada para suelos con MOS < 6%, similar a estudios internacionales y iii) Eliminación de reactivos peligrosos y reducción de costos operativos. Se identificó además la influencia de la textura, los porcentajes de agregación como factores que pueden afectar la lectura de los valores, realizándose las recomendaciones técnicas pertinentes. Los resultados sustentan el desarrollo de un kit de campo estandarizado con estándares locales, ofreciendo una solución técnicamente robusta, segura y accesible para el monitoreo de la MOS en Cuba.

Palabras clave: validación técnica, medición in situ, indicadores edáficos.

INTRODUCTION

Soil organic matter (SOM) comprises all organic materials of plant or animal origin, in varying degrees of decomposition ([Silva and Mendonça, 2007](#); [Nilo, 2019](#)). In tropical soils, its degradation is faster than in temperate climates due to high temperatures and humidity, resulting from intense rainfall and warm conditions ([Ross, 1993](#); [Craswell and Lefroy, 2001](#); [Castro et al., 2015](#)).

Increased soil organic matter content has multiple effects on soil habitats. These include improved soil particle aggregation, resulting in improved soil structure. This, in turn, optimizes air and water movement in the soil and increases its water retention capacity. A more stable soil structure results in less erosion, which retains nutrients and protects water quality. From a chemical perspective, soil organic carbon contributes to soil cation exchange capacity,

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which is necessary for the retention of nutrients such as calcium, magnesium, and potassium (Van der Wal and de Boer, 2017). Furthermore, increased soil organic carbon increases the biomass and diversity of soil biota, as it constitutes a source of nutrients available to these organisms, which, by transforming them, make them available to plants (Nilo, 2019).

For all these reasons, the determination of organic matter (OM) is essential for the knowledge of soil quality and health, constituting the primary indicator of these (Schloter et al., 2003; Weil & Magdoff, 2004; Obalum et al., 2017), and is essential to evaluate the agricultural and forestry productivity of soils (La Manna et al., 2007) and to define agricultural management conditions related to tillage, fertilization and irrigation (Reeves, 1997; Conceição et al., 2005; Roscoe, 2006; Silva and Mendonça, 2007; Madari et al., 2009; Souza et al., 2016).

Among the most widely used analytical methods for quantifying organic carbon in soils are loss on ignition and wet combustion (Walkley-Black, 1934). Loss on ignition measures the weight loss of a sample when incinerated at 430°C for 24 hours, oxidizing all forms of SOM (Davies, 1974). This inexpensive and simple method does not require chemical reagents, but it is energy-intensive (La Manna et al., 2007).

On the other hand, wet combustion (Walkley-Black, 1934) uses potassium dichromate in sulfuric acid, partially oxidizing organic carbon. Its underestimation is corrected by factors adjusted for soil type and horizon (Rosell et al., 2001; Certini et al., 2002; De Vos et al., 2007). This method, although limited in coverage, is simple, accessible, and standardized by GLOSOLAN (Nilo, 2019).

However, it is recognized that the use of this method entails a high risk to human and environmental health. Sulfuric acid (H_2SO_4 , CAS: 7664-93-9) can cause skin burns and generates corrosive, toxic, and irritating vapors. According to Nilo (2019), it requires that its handling be carried out under an extraction hood and that its waste not be discharged into the drainage system, conditions that can be difficult to meet in Cuban laboratories today. Regarding potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$, CAS: 7778-50-9), it is noted that it is an inorganic, highly corrosive and strongly oxidizing compound, which emits toxic chromium vapors when heated and due to such characteristics and easy reactivity with combustible materials, it presents high risks in its storage. In addition, it is a known human carcinogen and is associated with an increased risk of lung cancer.

Considering all this, visible and near-infrared (VIS/NIR) spectroscopy has recently been proposed as a proven technique as a rapid and relatively accurate alternative to laboratory analysis of soil properties (Stenberg, 2010). The main advantages of these techniques are their speed, relative accuracy, and non-destructive nature (Nduwamungu, 2009;

Volkan, 2010; Stenberg, 2010). The main disadvantage is the high cost of the necessary equipment and the high level of specialization required by the personnel involved in this activity.

Furthermore, the current socioeconomic context requires rapid and efficient responses to a large number of farmers from diverse production systems, emphasizing the rational use of material and human resources. In this regard, the implementation of field methods is preferable. These methods require basic steps that are easy to follow by any operator, regardless of their technical level, under field conditions. Therefore, a certain level of precision is often sacrificed in pursuit of simplicity and ease of implementation, through the use of visual observations or portable instruments, which are not as sensitive as laboratory instruments.

For his part, Bowman (1997) proposes a method for determining organic matter in soil for use in field conditions. This method is based on the extraction capacity of EDTA (ethylenediaminetetraacetic acid, CAS 60-00-4), which produces a liquid whose color is directly proportional to the intensity of this extraction. Thus, the darker the extract, the higher its organic matter content. Subsequently, the color of the "problem" sample is compared with field standards, soils with known organic matter content, in order to make a rapid and inexpensive semi-quantitative estimate of the organic matter content of the same (using ranges).

Bowman (1997) also proposes that the sensitivity of this analysis can be increased by filtering the extract and reading its absorbance at 520 nanometers using a spectrophotometer (if portable equipment is available, this can be done in the field). In this case, a calibration curve would be made using the known standards and the value of the organic matter content would be calculated using the regression equation obtained, being a quantitative determination.

EDTA, on the other hand, has low toxicity upon ingestion and is considered a mild skin irritant. It also poses no storage risks. Sodium hydroxide (NaOH , CAS: 1310-73-2), although recognized as an irritant and corrosive material, is used in this method at low concentrations, which reduces the human and environmental risks.

According to this, the following work has two fundamental objectives: i) the validation of the method proposed by Bowman (1997) in Cuban soils from several groups, comparing the results obtained by this method with those obtained through the Walkley-Black method and ii) preparation of a kit for the estimation of organic matter content in the field, selecting the soils used for standards.

MATERIALS AND METHODS

Seventeen samples were used, the descriptions of which are attached (see Table 1). The selection was based on the greatest possible heterogeneity in terms of the soil groupings represented, textural classes, colors, aggregation percentages, and organic matter content.

Table 1. Selection of samples based on showing the greatest possible heterogeneity in relation to the soil groups represented, textural classes, colors, aggregation percentages and organic matter contents

Sample	Study site	Coordinates	Management	Soil taxonomy (Cuban system)	Soil taxonomy (WRB) ¹	Texture	pH	% aggregation
1 Tbc 010	Viñales, Pinar del Río	N 22° 34.376' W 83° 42.491'	Tobacco crop	Alítico	Rhodic, Xanthic, Aluminic Alisol	Sandy loam	6,72	20,44
2 Cfg 1020	Manicaragua, Cienfuegos	N 22°09.401' W 80°06.266'	Forest	Pardo grisáceo	Dystric Cambisol	Silt loam	6,08	57,43
3 Vin 1020	Viñales, Pinar del Río	N 22° 34.578' W 83° 42.758'	Forest	Alítico	Rhodic, Xanthic, Aluminic Alisol	Sandy clay	4,86	77,93
4 Pul 010	Alquízar, Artemisa	N 22° 46.399' W 82° 36.463'	Common bean crop	Ferralítico	Ferralic, Rhodic, Lixic, Eutric Nitisol	Clayey	6,73	77,79
5 Hbn 010	Hanabaniña, Cienfuegos	N 22°06.904' W 080° 04.845'	Forest	Gley	Eutric, Clayey, Humic Gleysol	Sandy clayey silty	6,30	48,28
6 Tbc 1020	Viñales, Pinar del Río	N 22° 34.376' W 83° 42.491'	Tobacco crop	Alítico	Rhodic, Xanthic, Aluminic Alisol	Sandy loam	6,68	25,82
7 Tbl 010	Manicaragua, Cienfuegos	N 22° 15.772' W 80° 10.651'	Pastures	Pardo grisáceo	Dystric Cambisol	Sandy clayey silty	6,25	22,46
8 Tbl 1020	Manicaragua, Cienfuegos	N 22° 15.772' W 80° 10.651'	Pastures	Pardo grisáceo	Dystric Cambisol	Sandy clayey silty	6,67	32,14
9 CnVC 1020	Santo Domingo, Villa Clara	N 22°38.522' W 80°14.546'	Sugarcane crop	Pardo grisáceo	Dystric Cambisol	Silt loam		79,83
10 Hlg 010	Antilla, Holguín	N 20° 51' 1.76' W 75° 39' 54.29'	Forest	Vertisol crómico	Pellic Vertisol	Silty clay	7,47	86,64
11 Hlg 1020	Antilla, Holguín	N 20° 51' 1.76' W 75° 39' 54.29'	Forest	Vertisol crómico	Pellic Vertisol	Silty clay	7,25	90,59
12 Mq5C2	Empresa Pecuaria Niña Bonita, Artemisa	N 22° 59.618' W 82° 30.903'	Sugarcane	Gley nodular ferruginoso	Petroferric Gleysol	Silty clay	7,04	59,62
13 Mq5C3	Empresa Niña Bonita, Artemisa	N 22° 59.031' W 82° 30.606'	Sugarcane	Gley nodular ferruginoso	Petroferric Gleysol	Silty clay	6,25	61,64
14 Mq6C1	Empresa Niña Bonita, Artemisa	N 22° 59.091' W 82° 30.603'	Pastures	Fersialítico Pardo Rojizo	Eutric Cambisol	Clayey	7,58	72,45
15 Mq6C3	Empresa Niña Bonita, Artemisa	N 22° 59.091' W 82° 30.603'	Pastures	Fersialítico Pardo Rojizo	Eutric Cambisol	Clayey	7,26	77,81
16 Mq6C4	Empresa Niña Bonita, Artemisa	N 22° 59' 5.46' W 82° 30' 36.47'	Pastures	Fersialítico Pardo Rojizo	Eutric Cambisol	Clayey	7,47	75,25
17 PtoB5	Carretera Bauta Baracoa	N 23°1'26.18'' W 82° 32'58.52''	Pastures	Ferralítico Rojo Compactado	Ferralic, Rhodic, Lixic, Eutric Nitisol	Clayey	6,55	76,24

¹ The correlation of the Cuban soil classification system (2015) with the World Reference Base (WRB) system was carried out according to [Hernández-Jiménez, 2021](#).

The percentage of organic matter was determined in all samples using the wet digestion method, developed by Walkey and Black (1934) and standardized according to the Cuban standard (NC 51.1999), and through EDTA extraction (Bowman, 1997). For the latter, 5 to 10 grams of dry soil were pulverized in a mortar, 0.5 grams of which was taken, 20 ml of basic EDTA was added and the mixture was shaken vigorously for 30 seconds. This extract was filtered and its absorbance was measured at 520 nm. Subsequently, the percentage of correlation between the results obtained in both cases was calculated using SPSS V. 20.

RESULTS AND DISCUSSION

Table 2 shows the results obtained from the two methods for determining the percentage of soil organic matter, measuring the optical density of the extracts at 650 and 520 nm, respectively, as indicated in the protocols for each method.

Table 2. Values obtained for optical density and organic matter contents, using the methods of Walkey-Black (1934) and EDTA extraction (Bowman, 1997)

Sample	DO 650 nm (wet digestion)	DO 520 nm (EDTA extraction)	% SOM	Standards
1 Tbc 1020	0.026	0.636	0.99	1%
2 Tbl 1020	0.045	0.689	1.76	
3 Tbl 010	0.050	0.739	1.97	
4 Tbc 010	0.053	0.659	2.05	2%
5 CnVC 1020	0.056	0.667	2.19	
6 Mq6C4	0.070	0.678	2.68	2.5%
7 Pul 010	0.070	0.810	2.70	
8 Hlg 010	0.076	0.651	2.92	
9 Mq6C3	0.085	0.774	3.27	3%
10 Hlg 1020	0.081	0.724	3.44	3.5%
11 Mq6C1	0.095	0.689	3.62	
12 Vin 1020	0.102	0.840	3.88	
13 Mq5C2	0.121	0.791	4.57	4.5%
14 BautaP5	0.133	0.775	5.03	
15 Cfg 1020	0.144	0.978	5.45	5.5%
16 Mq5C3	0.144	0.841	5.46	
17 Hbn 010	0.164	1.004	6.18	≥5%

Using these values, the equation that shows the existence of a linear correlation between both variables was calculated, whose graph is shown in Figure 1. It shows a directly proportional relationship, obtaining values

2.3 times higher in the optical density of the extract with basic EDTA measured at 520 nm in contrast to those determined in the H_2SO_4 - $\text{K}_2\text{Cr}_2\text{O}_7$ extract at 650 nm. The correlation coefficient ($R^2 = 0.76$) was similar to that obtained by Card (2004) when making a similar comparison in 41 soils from Alberta, Canada ($R^2 = 0.67$), being this relationship valid in soils with organic matter contents lower than 6%.

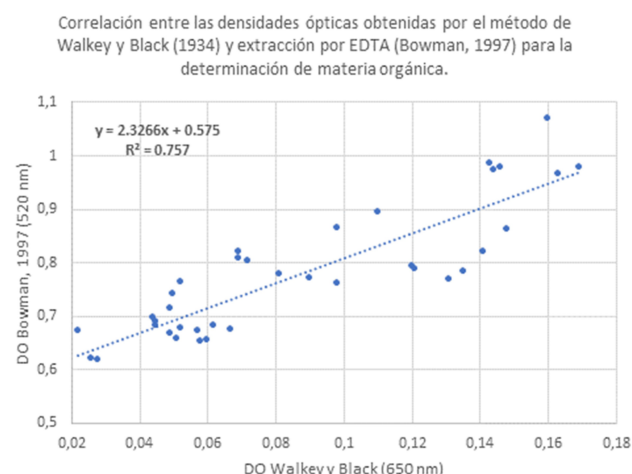


Figure 1. Correlation between optical densities obtained by the Walkey and Black method (1934) and EDTA extraction (Bowman, 1997) for the determination of organic matter. The graph shows the results of 54 determinations with a wide range of organic matter contents.

The high correlation coefficients obtained allow this method to be used as an alternative to wet digestion, avoiding the risks associated with handling highly toxic reagents, as well as the environmental impact resulting from the generation of toxic waste.

Regarding the principle of the method, NaOH works by solubilizing organic carbon and EDTA acts as a chelating agent, increasing the efficiency of this process (Bowman and Moir 1993). However, the texture and the percentage and size of soil aggregates can affect the result of the determination, over or underestimating their contents. This occurs due to the physical protective effect of the aggregates on organic matter (Balesdent *et al.*, 2000; Le Bissonnais, 2023; Even & Cotrufo, 2024) and the greater difficulty of dispersion in clayey soils due to their mineralogical characteristics and the interaction of clays with organic matter (Nelson *et al.*, 1999; Plante *et al.*, 2006; Huang & Hartemink, 2020). Thus, we consider that, in soils with a clayey texture, vertic characteristics or high levels of aggregation, the pulverization in the mortar and the stirring and filtration times should be increased.

On the other hand, although there is a high correlation between soil color and its organic matter content (Shulze *et al.*, 1993; Wu *et al.*, 2018), the evident differences in soil color related to mineralogical characteristics and pedological processes must also be taken into account in the field implementation of this method.

CONCLUSIONS

The study demonstrated that determining soil organic matter content using the EDTA extraction method described by Bowman in 1997 is a simple, economical, and easy-to-implement alternative, both in field and laboratory conditions, enabling rapid and effective responses to this fundamental indicator of soil quality and health. However, there are limitations to its application under specific soil texture, aggregation, and color conditions, which require further analysis in order to propose standardized variations of the methodology.

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ANNEX 1. PROCEDURE FOR DETERMINING CARBON IN THE FIELD.

Equipment.

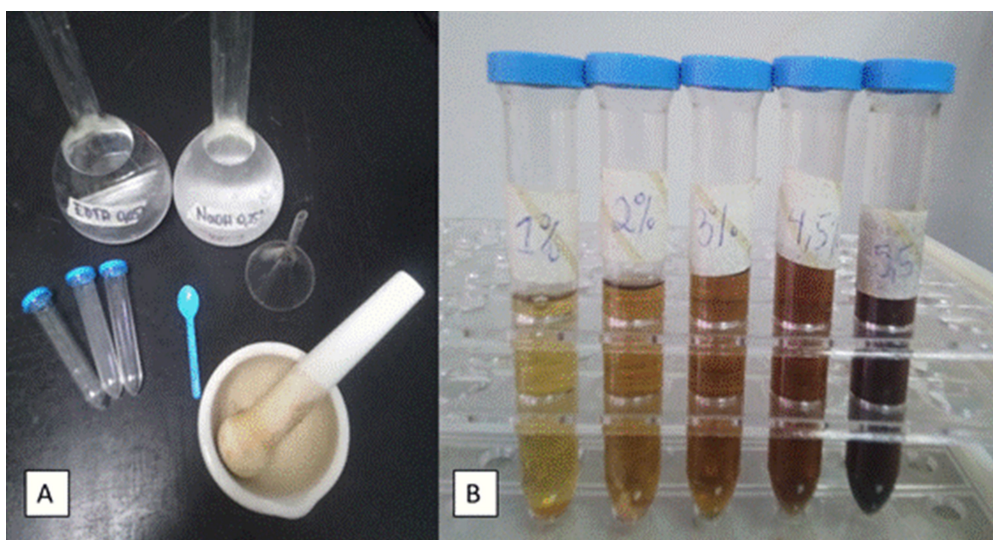
- Scoop with an approximate measurement of 0.5 g
- Mortar and pestle
- Tubes with lids
- Cut filter paper
- Funnels
- 25 ml graduated cylinder

Reagents:

- Distilled water
- 0.25 M sodium hydroxide (NaOH) (10 g/L)
- 0.05 M disodium EDTA (Na₂EDTA) (18.6 g/L)
- Basic EDTA: Mix NaOH and Na₂EDTA in equal proportions (v/v)

Previously, six soils with varying SOM contents (1%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, and greater than 5%) were identified, calculated using the EDTA extraction method with spectrophotometer reading, which will serve as standards (Table 1). Variations from these standards should be easily distinguishable by field workers.

ANNEX 2. (A) EQUIPMENT REQUIRED TO CARRY OUT THE QUALITATIVE METHOD FOR DETERMINING ORGANIC MATTER IN THE FIELD (BOWMAN, 1997). (B) STANDARDS PREPARED FOR USE.



To perform the determination, a dry soil sample will be taken and ground. Then, using a spoon, a sample of approximately 0.5 g will be taken and mixed with 20 ml of basic EDTA, shaking vigorously for 30 seconds, and then filtered. The color of the resulting mixture will be visually compared with the standards to determine the percentage of organic matter by comparison.