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Comparison of methods for analyzing organic matter in organic materials

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Magaly Ruiz-Dager
Doctor in Soil Science from the Central University of Venezuela.
Academic institution: Universidad Nacional Experimental de los Llanos Centrales Rómulo Gallegos (UNERG). Center for Research and Extension in Soils and Waters (CIESA).
E-mail: magaruizdager@gmail.com

Carmen E. Carrillo de Cori
Doctor in Soil Science from the Central University of Venezuela.
Academic institution: Universidad Central de Venezuela. Faculty of Agronomy.
E-mail: estercori@gmail.com

Francisca Sosa
Master's Degree in Agronomy from the Central University of Venezuela.
Academic institution: Universidad Central de Venezuela. Faculty of Agronomy.
E-mail: franciscasosac@gmail.com

Linda M. Aular
Industrial Engineer from the Santiago Mariño University (Venezuela).
Academic institution: Universidad Central de Venezuela. Faculty of Agronomy.
E-mail: linda.mari2010@hotmail.com

Rosalba Mora
Industrial Engineer from the Santiago Mariño University (Venezuela).
Academic institution: Universidad Central de Venezuela. Faculty of Agronomy.
E-mail: virangel2005@gmail.com

Luis Castillo Castillo
Higher University Technician from the Farmer's Service Foundation (FUSAGRI)
Institution: EDAFOFINCA, C.A. Laboratory of Soil and Water Analysis for Agricultural Purposes.
E-mail: edafofinca@gmail.com

Bethsaida Ortega
Higher Agricultural Technician from the Universidad de los Andes (Venezuela).
Academic institution: National Institute of Agricultural Research-Maracay.
E-mail: betsaidacoromotoortegapulido@gmail.com

Egli Castillo
Master's Degree in Soil Science from the Central University of Venezuela.
Academic institution: Universidad Central de Venezuela. Faculty of Agronomy.
E-mail: castilloegli820@gmail.com

Elizabeth Ramirez-Iglesias
Doctor of Science, Ecology from the Central University of Venezuela
Academic Institution: Amazon State University
E-mail: eliza2030@gmail.com

Jesus Rodriguez
Master's Degree in Soil Science from the Central University of Venezuela.
Academic institution: Universidad Central de Venezuela. Faculty of Agronomy.
E-mail: rodriguezb.jesus@gmail.com

Marianela León
Chemical Engineer from the University of Carabobo (Venezuela)
Academic institution: National Institute of Agricultural Research-Yaracuy.
E-mail: marianela_leon@hotmail.com

Carmen Silva Sequera
Higher university technician in quality control from the Andrés Eloy Blanco Institute of Technology. Barquisimeto. Edo. Lara. Venezuela.
Academic institution: National Institute of Agricultural Research-Yaracuy.
E-mail: carmens186@hotmail.com

María R. Tovar
Doctor in Education Sciences from the Universidad Nacional Experimental de los Llanos Centrales Rómulo Gallegos.
Academic institution: Universidad Nacional Experimental de los Llanos Centrales Rómulo Gallegos (UNERG). Center for Research and Extension in Soils and Waters (CIESA).
E-mail: mariatovar_70@hotmail.com

Ayuramy Martinez
Chemical Engineer from the University of Carabobo (Venezuela).

Academic institution: National Institute of Agricultural Research-Guárico.
E-mail: ayuramy76@gmail.com

Aura M. Reverón
Agricultural Engineer from the Ezequiel Zamora National Experimental University of the Western Plains (UNELLEZ).
 Academic institution: National Institute of Agricultural Research-Guárico.
E-mail: revemer_62@hotmail.com

Florencio Gámez
Agricultural Engineer from the Central University of Venezuela.
Academic institution: Universidad Central de Venezuela. Faculty of Agronomy.
E-mail: florenciogamez@gmail.com

ABSTRACT
Two methods (wet combustion and calcination at 550 ºC) for the determination of organic matter in organic materials were compared. Nine laboratories participated in the assay, using five organic materials (three composts and two substrates), analyzed in quadruplicate. The precision was evaluated by calculating the repeatability and reproducibility, according to the COVENIN 2972-92 (ISO 5725-86) standard. No significant differences were found between methods, laboratories, or replicates, so it is assumed that the compared methods generate statistically equal results. The precision evaluated through repeatability and reproducibility was not dependent on the level of organic matter. The lower repeatability and reproducibility values obtained with the calcination method indicate that this analytical modality is more precise. The application of the calcination method is recommended because it is based on a fast, simple procedure, does not consume chemical reagents and does not generate toxic waste for the environment and health.

Keywords: Compost, Interlaboratory study, Oxidizable organic carbon.

1 INTRODUCTION

Organic matter (OM) represents a fundamental edaphic component, as it influences many chemical, physical and biological properties of the soil (Diovisalvi et al., 2008). That is why the addition of organic waste has been a successful practice since ancient times to improve and maintain soil fertility. This waste can vary in its state of decomposition, from fresh plant and animal waste, to material with a stable composition, such as that achieved through the composting process. One of the most important parameters for determining the quality of composts and monitoring their production process is the C:N ratio. That is why it is important to use easy-to-implement methodologies that determine organic carbon (CO) correctly. Traditionally, the most widely used method for determining soil organic carbon has been wet combustion (WB) (Walkley & Black, 1934). This analytical modality has also been applied to samples of organic waste and compost. The WB method determines the easily oxidizable C. Despite being a fast and inexpensive methodology, it uses large amounts of sulfuric acid and the products generated are carcinogenic and toxic (Abella & Zimmer, 2007; Eyherabide et al., 2014). In addition to the use of hazardous reagents, the final estimation of C requires a correction factor that is variable, because OM oxidation is incomplete and differs according to soil type (Nelson & Sommers, 1996). Martínez *et al*. consider that the dry combustion (CS) method, which converts all carbon into CO2 and is then determined by infrared spectroscopy (Pribyl, 2010), can be considered as a reference method. However, this modality requires very expensive equipment, highly trained personnel and finely processed samples. Given the importance of these determinations, it is desirable

to develop quick, simple methodologies that do not generate toxic waste for the environment and health. The calcination method (CM) promises to be an alternative that can be used quite successfully and meets the conditions indicated. It only requires an analytical balance and a muffle to be applied and directly determines the OM. The only important requirement is to use a suitable conversion factor to obtain organic carbon. In this regard, Martínez *et al*., (2017), using seventy molysols from Argentina, compared the method of calcination at various temperatures with that of dry combustion, or elemental analyzer, finding that the best temperature (360, 430, 550 and 600) was 550 ºC and obtained a factor of 2.2 to transform OM by calcination into CO. On the other hand, Iglesias-Jiménez and Pérez-García (1992), working with compost from urban waste, found that despite the heterogeneity of the organic matter of the samples analyzed, the CO content was always around 54%, recommending the calcination method and a factor of 1.8 to obtain the CO content for this type of material. In Venezuela, all soil laboratories use WB's wet combustion method, with a factor of 1.724 for soils and for organic materials, its use is becoming widespread, but using a factor of 1.8. However, in recent years, the economic crisis that the country is going through has also affected laboratories and the application of methods that consume large amounts of acid is becoming increasingly expensive, both because of its scarcity in the market and because of its high cost. The aim of this study was to compare the results obtained by applying the WB method with the calcination method when analyzing different types of organic materials.

2 MATERIALS AND METHODS

Five organic materials were used to carry out the tests, which correspond to the following generic name: Combined compost of worm castings and plant materials (Worm Humus 1), Worm Humus 2, Organic Substrate, Organic Fertilizer and Humified Cane Substrate. The characteristics of these materials are summarized in Table 1.

2.1 THE SAMPLES WERE ANALYZED BY TWO METHODS, THE PROCEDURES OF WHICH ARE BRIEFLY DESCRIBED BELOW:

Wet combustion method (Walkley and Black, 1934, modified): Weigh 0.05 g $\pm$ 0.005 g of each sample (previously ground and dried at 70 °C to constant weight) in a 50 mL volumetric balloon, add 5 mL of 1N potassium dichromate solution, gently rotate the volumetric balloon to mix well, Add 10 mL of concentrated sulfuric acid 96%-98% w/w, and shake for 5 to 10 seconds. Add a volume of distilled water close to capacity. Leave to rest for 3 or 4 hours and flush at 50 mL with distilled water, mix and leave to rest overnight. The next day, transfer the transparent supernatant to the cells and read the absorbance at the wavelength of 590 nm in UV-Visible spectrophotometer. For the determination of organic carbon contents, develop a calibration curve by preparing standards in 50 mL balloons

containing 0.0000; 1,0876; 2,1753; 4,3506; 6,5259; 8,7012; 10,8765; 13.0518 and 17.4024 mg C; from a standard sucrose or glucose solution containing 2.1753 mg C/mL. Add 5 mL of 1N potassium dichromate, 10 mL of sulfuric acid to each standard, measure at 50 mL with distilled water and follow the same procedure applied to the samples simultaneously with them.

Table 1. Characteristics of the organic materials analyzed in the interlaboratory study.

Characteristics	ORGANIC MATERIAL				
	Hummus from Worm 1	Hummus from Worm 2	Substrate Organic	Fertilizer Organic	Substr. Cane Humidified
Source Material	Worm Humus Compost and Plant Materials: Cattle Manure, Cachaça and sugarcane bagasse	Worm castings made from: bovine manure, newspaper, cardboard and coffee pulp	Organic substrate made with rice husks, worm castings, coconut sawdust and cachaça	Compost of manure, leaf litter and plant materials.	A by-product of sugarcane processing with humification treatment.
Aspect	Heterogeneous	Heterogeneous	Heterogeneous	Heterogeneous	Heterogeneous
Colour	Dark brown	Dark brown	Dark brown	Brown dark	Dark brown
% Total Nitrogen	ND	ND	ND	ND	0,55
Fósforo (% P2O5)	3,65	4,20	2,24	2,75	1,81
Knights (F2O)	ND	ND	ND	ND	0,74
% Organic Matter	15-16	31-32	46-47	41-42	15-16
% Humidity	5	8	10	12	30
Densidad (g.mL^{-1})	0,8582	0,7914	0,7110	0,9498	0,7658
Presence of carbonates.	REFUSAL	REFUSAL	REFUSAL	REFUSAL	REFUSAL

The percentage of organic matter in the sample is calculated according to the equation:

$$\% \, MO = \frac{(Absorbance\ reading - Intercept\ calibration\ curve) \times 1{,}3333 \times 1{,}8 \times 100}{(Calibration\ curve\ slope\ x\ Pm \times 1000)}$$

Where:

% OM = percentage of organic matter in the sample.

Pm = Sample weight, (g)

1.3333 = Factor resulting from the consideration that the average oxidation of carbon by this method is 75%

1.8 = Factor for converting organic carbon content into organic matter in compost and other organic materials (Iglesia-Jiménez and Pérez-García, 1992).

Calcination method (TMECC Method 05.07. 2001 and Chilean Standard, protocols, Sadzawaka et al., 2005). Weigh 5 grams (accuracy 0.0001 g) of air-dried, inert-free, ground and

homogenized sample in a crucible. If the sample contains carbonates, remove them by adding 0.05 mol/L HCl until bubbling stops. Dry in the oven at 70±5 °C to constant weight. Record the dry sample weight. Place on the muffle and slowly raise the temperature to 550 °C. Hold the temperature for 2 h and then slowly decrease it to around 200 °C. Place the sample in the desiccator and let cool to room temperature. Weigh and record the weight with an accuracy of 0.0001 g. The organic matter content, expressed as a percentage on a dry basis at 70±5 ºC, is calculated according to the following equation:

$$Organic\ matter\ (\%) = \frac{(a - b)\ x\ 100}{a}$$

Where:

a = weight, in g, of the dried sample at 70±5°C, before calcination

b = weight, in g, of the sample calcined at 550°C

The analyses were carried out by the technical staff of nine laboratories attached to the following institutions: EDAFOFINCA, National Institute of Agricultural Research-Aragua, National Institute of Agricultural Research-Guárico, National Institute of Agricultural Research-Yaracuy; Central University of Venezuela-Agronomy, Rómulo Gallegos National Experimental University-Center for Research and Extension in Soils and Waters, Simón Rodríguez University-IDECYT.

The assay was established as an unrestricted sampling for fixed-effect factor, including all nine laboratories, two methods, and five organic materials. The wet combustion method was applied by all the laboratories participating in the experimental round, resulting in forty-five (45) extractions, which repeated four times gave a total of one hundred and eighty (180) analyses. The calcination method was developed by six laboratories, as three of the nine laboratories did not have the required equipment. In the latter case, there were 30 analyses with four repetitions, for a total of 120. Altogether, 300 analyses were conducted.

The data obtained were tested with a Wilk-Shapiro normality test. The analysis of variance was performed using the non-parametric Kruskall-Wallis test. Differences between methods and between laboratories were analyzed. For the correlation analysis between the methods, only the data generated by the six laboratories that performed the two procedures were included.

Statistical processing was performed using Statistix for Windows, Version 8 (Statistix 2003). The accuracy of the method was determined by applying the COVENIN 2972-92 (ISO 5725-86) standard (COVENIN, 1992), to evaluate the results in terms of repeatability (r) and reproducibility (R). Repeatability allowed us to know the variability of the assay in each laboratory under constant conditions (the same laboratory, the same operator, and the same equipment) at short intervals of time.

Reproducibility measures variability between laboratories in tests conducted under widely varying conditions (different laboratories with different operators and equipment).

3 RESULTS AND DISCUSSION

The results of the analyses of five organic materials by nine laboratories using the wet combustion and calcination methods are presented in Table 2.

Table 2. Organic matter content (%)* in five organic materials, analysed by nine laboratories, applying two methods.

LAB	MATERIAL ORGANICO									
	Humus Lomb. 1		Humus Lomb. 2		Substr. Organic		Fertilizer		Sust Caña Hum.	
	COM HUM	CALCIN	WITH HUMPH	CALCIN	WITH HUMPH	CALCIN	WITH HUMPH	CALCIN	WITH HUMPH	CALCIN
1	16,48	18,80	31,80	31,15	45,26	42,22	41,74	42,90	18,74	15,08
2	15,71	20,01	32,38	32,99	ND	41,38	32,07	42,85	17,80	16,68
3	16,55	17,43	30,61	28,67	46,34	40,58	42,72	44,13	16,77	14,69
4	16,72	18,05	30,65	30,32	45,23	40,60	36,40	42,36	14,49	15,50
5	17,22	ND	32,28	ND	43,69	ND	42,50	ND	16,48	ND
6	17,18	20,17	30,67	30,96	43,74	41,99	38,86	43,84	17,28	17,56
7	16,74	ND	29,93	ND	42,14	ND	40,53	ND	14,56	ND
8	18,42	18,67	33,95	30,61	45,05	42,18	46,09	43,82	15,40	14,91
9	18,26	ND	33,26	ND	49,76	ND	49,50	ND	17,33	ND
AVERAGE	**17,03**	**18,86**	**31,72**	**30,68**	**45,15**	**41,49**	**41,16**	**43,32**	**16,54**	**15,74**

*Average of four repetitions. COM HUM = wet combustion method; CALCIN = calcination method; ND = not determined. Humus Lomb. = worm castings.

The Wilk-Shapiro normality test applied to the set of 296 data yielded a coefficient of 0.8718, with a $p = 0.0000$. It is possible that the lack of normality of the data is due to the fact that the five materials actually represent three (3) levels of organic matter as will be explained later. Consequently, it was decided to use the non-parametric Kruskall-Wallis pathway to perform the analysis of variance. The results are presented in Table 3.

Table 3. Result of the analysis of variance when applying the Kruskall-Wallis test

| Source of Variation	Probability	Interpretation
Method	0,9383	NS
Laboratory	0,3541	NS
Organic material	0,0000	**
Replica	0,9880	NS

No significant differences were found between methods, which means that the two methods under study generate statistically equal results. This is important to emphasize, since the objective of this work is to evaluate the possibility of replacing the wet combustion method with the calcination method and to recommend it for all national laboratories that analyze fertilizers and other organic materials. There are also no statistically significant differences between laboratories or between

replicates. The lack of significance among the results of the laboratories indicates that despite the different conditions in terms of equipment, operators and general conditions, the Group continues to work with uniformity

in their analytical skills. The highly significant differences found between materials are obvious, and are due to the fact that the samples analyzed correspond to three levels of organic matter, as revealed by the Kruskal-Wallis comparison of means, which classifies the analyzed materials into three groups: A, B and C. Group A, which includes organic substrate and organic fertilizer with average organic matter contents of approximately 41 to 45%; B, which includes only worm castings 2 with levels of about 32 % organic matter, and C, which includes humified cane and worm castings 1, with approximate average values between 16 and 19 % OM (Table 2). This can be seen in the scatter plot in Figure 1, which is prepared with the data generated by the application of the two methods.

Figure 1. Correlation between organic matter values obtained by wet combustion and calcination methods
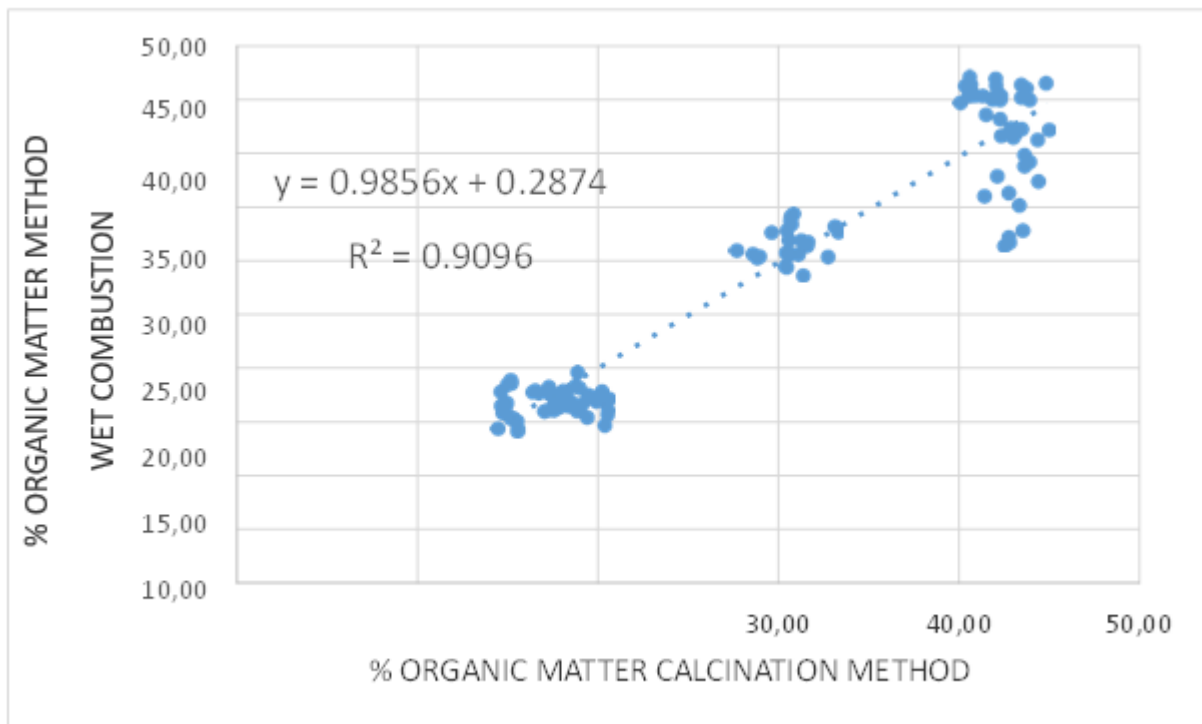
To reinforce the interpretation indicated above, the data were grouped by levels, resulting in three groups (A, B and C). The Wilk-Shapiro test was applied, and normality was found in groups B and C, as shown in Table 4.

Table 4. Normality test result applied to the three levels of organic matter

Approximate average level of Organic matter (%)	Number of data points	Wilk-Shapiro coefficient	Probability	Interpretation
A (41-45)	116	0,9283	0,0000	Not Significant at 0.05
B (~32)	60	0,9765	0,2973	Significant at 0.05
C (16-19)	120	0,9872	0,3187	Significant at 0.05

On the other hand, the Wilk Shapiro normality test was also performed, for the two sets of 116 data corresponding to the Wet Combustion and Calcination methods respectively performed by six of the nine laboratories, as explained in the section on materials and methods. The results are shown in Table 5.

Table **5**. Result of the Wilk-Shapiro Normality tests applied to the data corresponding to each method.

METHOD	DATA NUMBER	COEFFICIENT OF WILK-SHAPIRO	PROBABILITY
Wet combustion	116	0,8622	0,0000
Calcination	116	0,8412	0,0000

It is accepted that although the Wilk-Shapiro coefficient is less than 0.9, due to its proximity to this figure and the large number of observations, it could be assumed that the distribution is normal. To determine the relationship between the values found when applying both methods, Pearson's correlation coefficient was determined, having 116 pairs of data, resulting in a correlation coefficient of 0.9537 (P < 0.0000). From the above, it can be confirmed that the results of both methods are equivalent.

Accuracy of methods. The results of the application of the COVENIN 2972-92 Standard (Ministry of Public Works, 1992) to the data obtained are presented in Table 6**.** In both methods, no relationship was found between the values of "r" and "R" with the levels of OM, so the averages of these parameters were calculated, as can be seen in Table 6.

Table 6. Pearson's correlation coefficients, probability, significance, and mean values of "r" and "R" for the methods studied.

Parameter	WET COMBUSTION METHOD				CALCINATION METHOD			
	C. C1 Pearson	P2	S3	Average Value	C. C1 Pearson	P2	S3	Average Value
"r"	0,8279	0,0835	NS	2,63	0,6014	0,2834	NS	1,12
"R"	0,7395	0,1532	NS	6,93	0,7394	0,3752	NS	3,07

1Pearson's correlation coefficient, 2Probability, 3Significance. NS = not significant.

The lower repeatability and reproducibility values obtained with the calcination method indicate that this analytical modality is more accurate.

4 CONCLUSION

The two methods under study generate statistically equal results. This is important to emphasize, since the objective of this work is to evaluate the possibility of replacing the wet combustion method with the calcination method and to recommend it for all national laboratories that analyze fertilizers and other organic materials. No statistically significant differences were found between laboratories. No relationship was found between the values of "r" and "R" and the levels of OM. However, the lower repeatability and reproducibility values obtained with the calcination method indicate that this analytical modality is more accurate. The application of the calcination method is recommended because it is based on a quick, simple procedure, does not consume chemical reagents and does not generate toxic waste for the environment and health.

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