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## Multivariate analysis of images in spectrophotometric methods: Quantification of soil organic matter

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### RESEARCH ARTICLE

### ABSTRACT



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Soil organic matter (SOM) is usually quantified by Walkley-Black titration method or using a spectrophotometric method. This study proposes an alternative method for quantification of SOM using digital image from scanner and mathematical algorithms to replace titration and spectrophotometry procedures. For this, after SOM oxidation by potassium dichromate, digital images were acquired. Posteriorly, extraction of RGB color histograms from images have occurred, followed by the use of multivariate calibration method: partial least squares (PLS). Six soil samples were analyzed. We used the Walkley-Black method as reference. SOM was estimated by images using the PLS tool. The new method, besides being a fast, low cost, and more operational alternative, presented statistically equal results in relation to the reference method, as assessed by the Student t-test and F-test at 95 % confidence.

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### 1. Introduction

The soil organic matter comprises vegetables and animal residues at different states of decomposition, as well as living organisms [1]. The SOM influences different soil properties, such as water retention [2], structure [3], and nutrient cycling [4]. Therefore, the SOM is an important parameter of soil quality, especially for agricultural purposes [5].

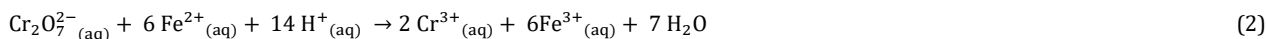
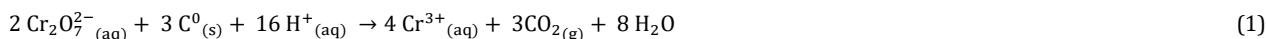
Generally, SOM is quantified by Walkley-Black method [6]. In this method, firstly the organic carbon is oxidized by dichromate ions in acid conditions, Equation (1). Then, it is possible to determine the dichromate excess by applying titration with Fe(II), Equation (2).

The organic carbon determined by Walkley-Black method can be converted into SOM by the van Bemmelen factor, 1.724. The determination of this factor was based on the average composition of organic carbon found in the SOM for different soil types, estimated at 58 % [7].

Although widely used, the Walkley-Black method has environmental and operational disadvantages. Besides being a laborious and time-consuming method, sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) (PA), a strong oxidizing agent, and potassium dichromate, a carcinogenic substance, were required [8,9]. Moreover, for each

soil sample, a considerable volume of sulfochromic residue is produced. In addition, if not accomplishing these containment actions, leakage of this type of residue will likely contaminate nearby soil and water. In order to overcome the operational difficulties and reduce the amount of residue, Souza *et al.* (2016) [10] proposed spectrophotometric adaptations for Walkley-Black method. This proposition increases the daily productivity in more than 100 % and reduces the residue production by 91 %, without any quality loss for the analyses results. Despite these efforts, the oxidizing and toxic residues continue being produced, although in smaller quantities. In addition, the cost of a spectrophotometer is reasonably high, making these adapted methods to spectrophotometry harder to implement in laboratories with scarce resources.

Another alternative for determining the SOM is instrumental elemental analysis, which is considered a technique of reference for the determination of total contents of different elements in a sample. The most common analyzers determine C, H, N, S, and O in solid samples. The elemental analysis consists of the combustion of the sample at a high temperature, >600 °C, followed by the conversion of the obtained products to the elemental forms or to simple inorganic substances (CO<sub>2</sub>, N<sub>2</sub>, H<sub>2</sub>O,



SO<sub>2</sub>), depending on the oxidation and reduction reagents contained in the reactive distillation columns of the equipment. Then, these gases are conducted through the system by an inert carrier gas, usually helium, separated by chromatography. Finally, their concentration measurement happens through different types of detectors, such as thermal conductivity detectors and infrared spectroscopy. The concentration of each gas is directly related to the sample elements. For example, the amount of CO<sub>2</sub> is directly proportional to the C concentration of the sample [11-13].

The elemental analysis has several advantages over the Walkley-Black method, among which we can highlight: high precision and recovery of the analytes, simultaneous analysis of some elements, generation of residues, satisfactory operability, relatively short analysis time, and the possibility of automation through automatic samplers [14]. On the other hand, the elemental analysis presents a series of disadvantages, especially the high investment cost, associated with its implementation and the acquisition of analytical gases and reagents for the oxidation and reduction columns. Due to the low budget, many soil fertility laboratories still do not have elemental analyzers, therefore, using classical wet methods, such as Walkley-Black, for SOM determination.

An alternative to the use of the spectrophotometer and elemental analysis is the adoption of digital images. Digital images can be defined as representations of real objects or situations [15]. Several devices can capture these images, such as cameras, scanners and smartphones.

Several areas already apply digital images, such as in biology, for bacterial classification [16], in medicine, for cancer prognosis [17], in the analysis of beverage analyses, in the quantification of *Lactobacillus* in fermented milk [18], for the determination of adulterants in milk [19], in the analysis and classification of unprocessed teas [20] and in the classification of soft drinks [21] and beers [22].

Also, digital images have been used in agricultural product analysis, for the estimation of moisture content in coffee [23], for the evaluation of mechanical damages in soybean [24], for the inspection of citrus fruits [25] and for the classification of castor bean seeds [26]. In chemistry, digital images have already been used as analytical signals, for the determination of ascorbic acid in medicaments [27], for the quantification of mercury in fish [28], for the identification of commercial tannin extracts [29], for determination of ascorbic acid in commercial vitamin C tablets [30], for characterization and quantification of organic dyes [31], for the determination of nitrite and nitrate in natural waters [32], for the characterization of extra virgin olive oil [33] and for the analysis of pH [34] and drinking water hardness [35]. Digital images were also used to soil analysis, for the quantification of organic carbon [36,37], and soil texture [38,39].

The applicability of digital images in various scientific fields has been intensifying thanks to its simplicity, low cost, high efficiency, low generation of residues and high operability in relation to the traditional methods as molecular absorption spectrophotometry and instrumental elemental analysis, for example. In the industrial field, this application also stands out, mainly due to the possibility of in-line process monitoring [40]. The general increasing use of images is being made possible due to the recent technological development, especially of the computational resources, responsible for driving the simultaneous treatment of a large amount of data obtained from multivariate phenomena.

Therefore, this study proposes a method for quantification of organic matter in soil samples by means of digital images obtained using a commercial flatbed scanner. The proposed method can be used instead of titration or the spectrophotometric reading. The results of this new method were compared, along with its viability, with the Walkley-Black method, a consolidated methodology in the literature.

## 2. Experimental

### 2.1. Soil samples

Six soil samples were considered for this study. The collection of samples took place in four Brazilian cities: Santo Antônio de Goiás, in the state of Goiás; Campo Verde, in the state of Mato Grosso, Palmas, in the state of Tocantins and Boa Vista, in the state of Roraima. Subsequently, the soil samples were air dried, ground and sieved through 2 mm mesh size.

These samples present contrasting physical-chemical properties, providing increased representativity when comparing methods. The organic matter contents of these samples are diverse, ranging from 4.5 to 49.8 g/kg. Therefore, it is possible to compare the two methodologies, both based on colorimetric methods, containing different concentrations. It is important to note that the application of multivariate image analysis in analytical solutions is as reliable as spectrophotometry, presenting low standard deviation values [27,41,42]. Therefore, it is possible to state that a small number of solutions, although with a wide concentration range, is sufficient to obtain satisfactory analytical calibration and good generalization capacity.

The determination of the organic matter in the soil employed the Walkley-Black spectrophotometric methodology proposed by Souza *et al.* (2016) [10]. This method consists of an adaptation of the traditional Walkley-Black methodology, by replacing the titration with the spectrophotometric reading.

Firstly, there was the weighing of 0.2 g of the soil sample, then the addition of 4.0 mL of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 0.167 mol/L and 8.0 mL of H<sub>2</sub>SO<sub>4</sub> (PA). After the cooling process, there was the addition of 12 mL of deionized water. At the end of two days of decantation, there was the transference of 5 mL of the supernatant to a cylindrical cuvette of 16 mm in diameter and the spectrophotometric reading was performed at 590 nm.

The construction of the calibration curve used ten standard solutions of [Cr<sup>3+</sup>] concentrations between 0.000 and 0.052 mol/L. To this end, there was the addition of 8.0 mL of H<sub>2</sub>SO<sub>4</sub> (PA) in 4.0 mL of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 0.167 mol/L. After cooling, different volumes of Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O 0.5 mol/L solution were added to pH = 0.3, followed by the addition of complementary volumes of deionized water in order to obtain a final volume of 24 mL, using an electronic repeater pipette with detachable tips, which are adjustable according to the desired volume. The processes of decanting, aliquot collection and spectrophotometric reading were the same as those performed for the samples. The preparation of all samples and standard solutions were in triplicate, totaling 48 aliquots.

### 2.2. Digital images acquisition

After the spectrophotometric reading, the standard solutions and samples were transferred quantitatively to a microplate made of 24-well transparent polystyrene. Figure 1 shows the two microplates used, containing the standard solutions and samples.

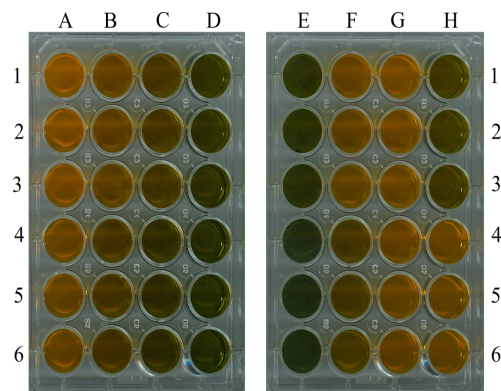


Figure 1. Digital image of the two microplates containing the standard solutions (columns A to E) and samples (columns F to H).

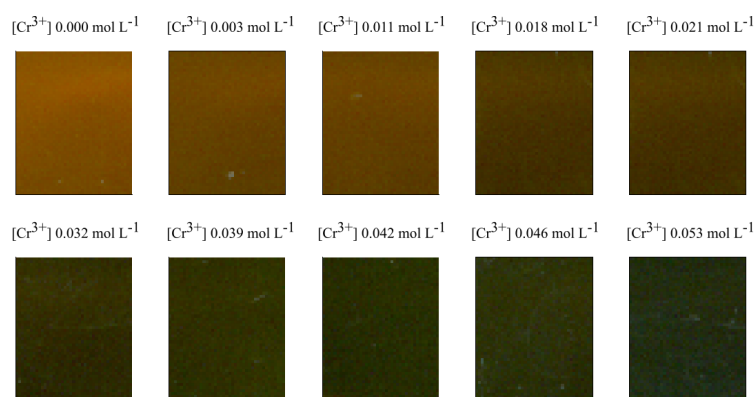


Figure 2. Digital images of the standard solutions.

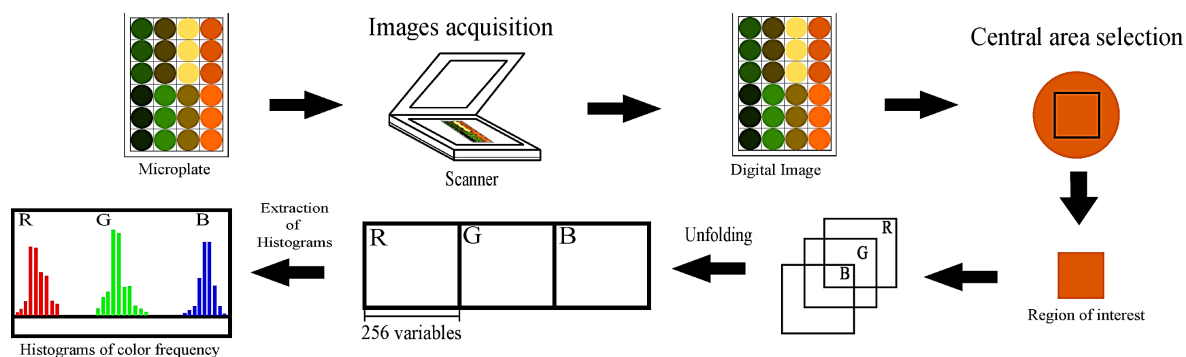


Figure 3. Schematic containing all steps for acquiring the color frequency histograms.

The positioning of the microplate was in the center of an HP® Scanjet G4050 flatbed scanner. Four images were obtained from each plate in the RGB (Red, Green, Blue) color system, with a resolution of 200 dpi (Dots per Inch) and in TIFF format.

### 2.3. Histograms acquisition

The process obtained eight images (four for each microplate of Figure 1) in total. There was the selection of the samples and central regions (region of interest) of the standard solutions from each image. Figure 2 shows an example of the selected areas for standard solutions. After setting the central areas, they were decomposed into occurrence histograms for the R (red), G (green), and B (blue) color channels.

Figure 3 shows the flowchart for obtaining the color histograms. After obtaining the histograms, there was the calculation of the mean for each quadruplicate image of the samples and standard solutions.

### 2.4. Data analysis

RGB histograms from digital images of standard solutions were organized as a data matrix  $X$ , which each row and column corresponds, respectively, to a standard solution and RGB color index. The  $\text{Cr}^{3+}$  concentrations of standard solutions were organized as a vector  $y$ .

The PLS [43] method was used to correlate occurrence histograms of the digital images with  $\text{Cr}^{3+}$  contents. This technique implements the decomposition of both the matrix  $X$ , containing the histograms of images, and the vector  $y$ , containing the quantified  $\text{Cr}^{3+}$  contents in latent variables (LVs). In this study, a cross-validation (CV) was used to determine the ideal number of LVs. This technique consists of the removal of one or more samples from the total set, followed by the construction of a calibration model, which is able to predict the removed samples.

**Table 1.** Results obtained from soil organic matter of the samples by the PLS and Walkley-Black spectrophotometric.

Sample	SOM <sup>a</sup> (g/kg)	
	PLS <sup>b</sup>	Walkley-Black spectrophotometric
1	15.8±0.4	15.2±0.9
2	38.2±1.1	37.7±1.2
3	4.9±0.8	4.5±0.5
4	6.5±0.4	6.2±0.2
5	49.7±0.4	49.8±0.9
6	4.9±0.7	5.2 ±0.5

<sup>a</sup>Soil organic matter.<sup>b</sup>Partial least squares.

The repetition of the procedure happens until the model estimation occurs for all samples from the total set. For each added LV, there is the calculation of the root mean squared error cross-validation (RMSECV).

Similarly, to standard solutions, the RGB histograms from digital images of samples were also organized as a data matrix. Posteriorly, the organic matter contents of soil samples were estimated by PLS model.

Digital image processing, data dimensionality reduction and multivariate calibration were all performed using MATLAB™ software, version 8.4.0 (Mathworks, Natick, USA).

### 3. Results and discussion

The standard solutions used in this study vary in coloration range from orange to green. As the amount of Cr<sup>6+</sup> orange colored compound reduces, there is the generation of Cr<sup>3+</sup>, which provides the greenish coloration to the solutions. Thus, the higher the Cr<sup>3+</sup> concentration, the more intense the greenish coloration of the solution. On the other hand, low concentrations of Cr<sup>3+</sup> present a more intense orange coloration due to the high concentration of Cr<sup>6+</sup>.

The extraction of the color occurrence histograms for the R, G and B channels followed the acquisition of the images of the standard solutions and samples. After the obtention of histograms, the correlation of these with Cr<sup>3+</sup> concentrations used the multivariate calibration method, partial least squares (PLS).

In this study, the selection of the number of LVs for the PLS calibration model occurred according to the lowest RMSECV. The PLS model was built using six latent variables.

#### 3.1. Quantification of soil organic matter contents

The PLS model was used to quantify soil organic matter in six samples collected from different regions. After the estimation of the concentrations of Cr<sup>3+</sup>, there was the determination of organic carbon contents by means of equation 1. Subsequently, the contents of SOM were quantified using the van Bemmelen factor, 1.724 [7].

In order to compare the proposed methodology, which uses digital images, the six samples and ten standard solutions were also analyzed by the Walkley Black spectrophotometric method. Table 1 shows the results obtained for the six samples by the two methods, PLS and Walkley-Black spectrophotometric.

According to Table 1, there was no statistical difference between the variances and the mean organic matter content of the soil obtained by PLS and Walkley-Black spectrophotometric methods, which used the  $F(p=0,05,2,2)$  test and  $t(\alpha=0,05)$  test.

The Walkley-Black spectrophotometric method (used as reference) and the image-based method followed by PLS calibration, presented statistically equivalent precision and accuracy. In other words, it is possible to use the estimation of SOM using images in the laboratory routine without compromising the quality of the results. This technique will also reduce the analysis time and lower its cost of implementation since it does not require the acquisition of a spectrophotometer,

a costly piece of equipment for many laboratories. The use of a scanner would also reduce the quantity of the reagents used, minimizing the amount of toxic residue produced and the cost per test. Furthermore, the calibration could be transferred to other scanners from soil analysis laboratories or other users interested in SOM determination.

### 4. Conclusions

This study proposed a new methodology for the quantification of soil organic matter by means of digital images obtained by a scanner. This new approach is faster, of lower cost, and more operational than the traditional Walkley-Black titration or spectrophotometric method. The multivariate calibration method, partial least squares was used in order to correlate the histograms of the images with the contents of [Cr<sup>3+</sup>].

Regarding the Walkley-Black spectrophotometric method and the PLS calibration and image method, for all evaluated samples, there was no statistical difference for neither the mean nor the variance values. Therefore, the new scanning image method proposed can be used instead of the Walkley-Black method without any loss of quality regarding the results and also with a lower implementation and operational cost and improved analytical speed. This proposed alternative can be used to democratize SOM quantification, because a scanner is multiple of times cheaper than a spectrophotometer. In this way, a lot of soil analysis laboratories can offer this determination at a low cost.

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### Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.

Ethical approval: All ethical guidelines have been adhered.

Sample availability: Samples of the compounds are available from the author.

### CRedit authorship contribution statement

Conceptualization: Pedro Augusto de Oliveira Morais, Diego Mendes de Souza; Methodology: Pedro Augusto de Oliveira Morais; Software: Pedro Augusto de Oliveira Morais; Validation: Pedro Augusto de Oliveira Morais, Diego Mendes de Souza; Formal Analysis: Pedro Augusto de Oliveira Morais, Diego Mendes de Souza; Investigation: Pedro Augusto de Oliveira Morais, Diego Mendes de Souza; Resources: Beata Eموke Madari; Data Curation: Pedro Augusto de Oliveira Morais; Writing - Original Draft: Pedro Augusto de Oliveira Morais, Diego Mendes de Souza; Writing - Review and Editing: Pedro Augusto de Oliveira Morais, Diego Mendes de Souza, Beata



Emoke Madari; Visualization: Diego Mendes de Souza; Funding acquisition: Beata Eموke Madari; Supervision: Diego Mendes de Souza, Beata Eموke Madari Project Administration: Beata Eموke Madari.

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