

Standard and modified methods for soil organic carbon determination in agricultural soils

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Abstract. The presented study demonstrates the benefits of using modified vertical photometry methods for rapid, reliable and accurate soil organic carbon determination in agricultural soils. For tests we selected and analysed 25 arable (Ap horizon) topsoil (*Cambisol*) samples, so that the determined values cover as wide a range as possible. In the proposed method soil organic carbon is determined using 0.2 M potassium dichromate solution in diluted (1:1) sulphuric acid. The data obtained using the proposed method correlated strongly with the data obtained by *Vario EL* and titration methods. The new modification safeguards the objectivity of results, since it gives strong correlation coefficients ($r = 0.989\text{--}0.990$, $P < 0.001$, $n = 25$) with the dry combustion method data. The findings suggest that different photometers did not significantly affect results. However, comparison of conventional photometry and photometric determination using *Multiskan MS* equipped with the *Genesis Lite* microplate software revealed the following advantages of the latter technique: higher measurement accuracy, the facility to edit and save calibration graphs, the possibility of optimized and multiple replications, higher labour efficiency; improved safety for research personnel and lower reagent consumption. Strong and highly significant ($P < 0.001$) correlations were determined between the data obtained using different methods, and respective linear regression equations for the re-calculation of SOC values were developed.

Key words: soil organic carbon, agricultural soils, determination methods

INTRODUCTION

The Earth's soil cover is one of the main reservoirs of organic carbon in the biosphere. In accordance with long-term international strategies (IPPC, 2001), it is essential that agricultural land management policies include detailed information about soil C status, C-sequestration and storage in soils and its potential impacts on global climate change (Smith et al., 1997; Follet, 2001; Kremar et al., 2001; Lal, 1999; Shuman et al., 2002; West and Marland, 2003). For the assessment of the carbon dioxide emission to the atmosphere influencing climate change, it is necessary to assess the global reserves of organic carbon in soils (Abakumov & Popov, 2005). The actual content of soil organic carbon (SOC) must be periodically measured for soil classification and nutrient management purposes. Traditional SOC tests are relatively

time-consuming and expensive. Accurate and rapid quantification of SOC concentrations are essential (Tóth et al., 2006). The speed and accuracy of methods are largely dependent on the technical facilities available (Aleksandrova and Naidenova, 1976; Jankauskas et al., 2006(a); Jankauskas et al., 2006(b); Kogut & Frid, 1993). Attempts to apply multichannel photometer *Multiskan MS* for the determination of various parameters are described in previous publications (Slepetiene & Butkute, 2003; Slepetiene & Liaudanskiene, 2005). The *Multiskan MS* is widely used in scientific and practical medical research, molecular biology, and for evaluation of pharmaceutical, cosmetic and agricultural products. This photometer offers superior photometric measurement performance for 96-well plates. The multichannel photometer utilizes the original concept of vertical photometry in which the light beam passes through the whole sample. In vertical photometry, light absorption is proportional to the amount of light absorbing substance in the well. To expand the application range of the *Multiskan MS* instrument since 2001 we used the Windows- based microplate software *Genesis Lite (Labsystems, Finland)*. The software is designed to give the user maximum flexibility in data manipulation with the ease of use of the Windows environment.

Various methodologies are used for SOC determination (Aleksandrova & Naidenova, 1976; Kogut, 1998). ‘Wet chemistry’ methods constitute a separate group and are widely used, especially in Russia and East European countries (Aleksandrova & Naidenova, 1976, Kogut, 1998). The essence of the method lies in the determination of organic carbon by oxidation with a mixture of potassium dichromate and sulphuric acid. The organic carbon values obtained can be re-calculated into humus or organic matter using the mean coefficient (1.724) or experimentally determined coefficients (Aleksandrova & Naidenova, 1976; Nelson & Sommers, 1982; Orlov & Grishina, 1981).

The aim of this work is to investigate the use of the program *Genesis Lite Windows*-based microplate software installed in *Multiskan MS* for the rapid, accurate and inexpensive photometric determination of SOC in agricultural soils. This is based on new experimental data, which is used to describe and evaluate the method. Then the correlation between the data obtained by different methods was calculated and the feasibility of mathematical data recalculation from one method into another was assessed.

MATERIALS AND METHODS

The study used 25 Ap horizon topsoil samples taken from arable land in Central Lithuania at 0–30 cm depth during the period 2002–2005. The test soils were non-calcareous, in which soil carbon was only in the organic form. Prior to analyses, all visible plant and animal residues were removed from soil samples and then the remaining soil samples sieved using a 0.25 mm sieve.

The procedures used are specified in Table 1. The modified method of wet combustion with photometric carbon determination measures organic carbon content according to the dichromate approach, using combustion at 160°C for 30 minutes (Nikitin, 1999; Slepetiene & Butkute, 2003). The sample was weighed and placed in 100 mL Erlenmeyer flasks; 5 mL of chromous mixture (using 0.2 M potassium dichromate solution in diluted (1:1) sulphuric acid) was added (Aleksandrova & Naidenova, 1976). Wet combustion was performed according to the Nikitin

modification in a thermostat (Table 1, 1a, 1b, 1c). Combustion time was measured from the moment when the temperature in the thermostat rose to 160°C. Combustion procedure in the thermostat allowed us to minimize temperature variations. Titrimetric (volumetric) determination is a classical method and is generally used for routine analyses, since it is sufficiently accurate, straightforward and rapid, and does not require special equipment. In the volumetric method, excess dichromate is titrated with standardized Mohr's salt solution (0.1–0.2 M). After cooling, the content of the flasks was transferred to 50 mL measuring flasks and diluted; afterwards it was shaken and left overnight to settle. The next day measurements were performed using the photometer *Multiskan MS* and *Genesis Lite* software. SOC was measured photometrically using glucose standards. For the preparation of the standard solutions 2.5 g of glucose was dissolved in the measuring flask in 1 L of distilled water, to obtain a standard solution (1 mg C mL⁻¹). The standard series was prepared from the main standard solution (0.0–10 mL) and the standards were poured into Erlenmeyer flasks. Both the standard and test solutions were evaporated on an electric hot plate. Drying was completed in a thermostat at ≤ 70°C. It is important that the contents of the flasks do not overheat and turn brown. While preparing a series of standards, different amounts of standard glucose solution (1 mg C mL⁻¹) were evaporated and combusted as per the test samples, adding the same volume of chromium mixture. The obtained solutions were diluted to 50 mL in volumetric flasks. They were then shaken, left overnight to settle and used for photometry.

Photometric determination after combustion procedure

The final procedure in SOC determination is photometry or titration using standardized Mohr's salt solution. The day after wet combustion, 5 mL of the test solutions were taken with an automatic pipette for photometry and poured into a special 8-channel reservoir. Microplates were filled with the test and standard solutions (volume: 300 µL) by an 8-channel automatic micropipette (Finnpipette multichannel, *Labsystems*). The 96-well flat-bottom microplates (*Microtiter*) are made from clear plastic and they have high resistance to many organic solvents and acids.

Two instruments were used for photometric determination. *Spectrophotometer M-107* (*Camspec, UK*) (Tables 1: 1b, 3b) is a general purpose instrument. The wavelength range of light was 325-1000 nm (accuracy ±2 nm). Measurement readings are shown on a digital screen; the operation of the instrument is straightforward and user-friendly. Quartz glass cuvettes of 1 cm diameter were used for the work and 590 nm wavelength was used.

Photometer *Multiskan MS* (*Labsystems*) is a new generation 8-channel vertical light filter automatic photometer (Table 1: 1c, 3c) (*Multiskan MS. Labsystems operating instructions, 1998; Slepiciene & Butkute, 2003*) and is extensively used in scientific research. The light source is a quartz-halogen lamp (Osram 64607, 8V/50W), which is equipped with an aluminium-coated elliptical reflector. Light wavelength is chosen using eight high-quality interference filters. In our work, we used a 590 nm light filter. When measuring by the vertical photometry method, light absorption is proportional to the amount of light absorbing substance and is expressed by the equation: $A = m * a/S$, where A = absorption; a = molar absorptivity of the substance; m = mass of absorbing substance; S = cross-sectional area, perpendicular to the light

path (Multiskan MS. Labsystems operating instructions, 1998; Slepeliene and Butkute, 2003).

Advantages of the vertical measurement include the fact that neither inaccurate filling of non-absorbing liquids or evaporation of non-absorbing liquids during the reaction affect absorption values. The use of multi-channel photometry (96 simultaneous measurements) secures rapid measurement, replication, economical use of reagents and less environmental pollution. The *Genesis Lite* microplate software was installed in the computer interfaced with the photometer.

The dry combustion (DC) method belongs to the suite of direct carbon determination methods, by which carbon is determined according to the amount of CO₂ released during combustion. The DC method was invented with the development of analytical techniques associated with the new-generation of automatic analysers. The analyser *Vario EL III* (Germany) (Vario EL operating instructions 2002) automatically analyses and computes sample SOC. We compared two chemical SOC determination methods (1 and 3), six determination treatments, and one dry combustion method (Table 1).

Statistical analysis was performed using computer programs ANOVA and STAT from the SELEKCIJA package (Tarakanovas & Raudonius, 2003).

RESULTS AND DISCUSSION

Refinement of organic carbon determination methods is aimed to make all analytical procedures user-friendly, safe, time-efficient and economical. During the combustion procedure on an electrical hot plate, combustion temperature is variable (Table 1).

Table 1. Description of the method.

No	Combustion procedure	Final determination procedure		
		Titration	M 107 technique	<i>Multiskan MS</i> equipped with <i>Genesis Lite</i>
		a)	b)	c)
1	160°C in a thermostat	+	+	+
2	1150°C using automatic dry combustion analyser	-	-	-
3	Boiling on electrical hot plate for 5 minutes.	+	+	+

Note: 1. Chemical dichromate method (a, b; c - proposed method); 2. Dry combustion method, analyser Vario EL III; 3. Chemical dichromate method, standard GOST 26213-84.

The variable temperature regime during organic matter oxidation is the main drawback, as reported by Aleksandrova and Naidenova, (1976). Replacement of titration by the photometric procedure improved the method. Thus, there is no need to prepare solutions for titration and constantly check their concentrations, the amount of chromous mixture (*see: Materials and Methods*) does not necessarily have to be very precise, and instead of titration, optical density is measured.

Table 2. SOC concentrations and statistical parameters determined using different analytical methods. Soil samples are from *Cambisols* (0–30 cm depth); Number of samples: $n = 25$; with 2 replicates.

No	Method	SOC concentration (%)		SD	$V_x\%$
		Min	Max		
1	Dichromate oxidation modification				
	a) titration	0.94	3.19	0.00-0.10	0.0-4.6
	b) <i>M 107</i> technique	0.95	3.22	0.01-0.03	0.2-1.3
	c) <i>Multiskan MS</i> technique	0.93	3.15	0.00-0.01	0.0-0.6
2	DC	1.01	3.63	0.00-0.07	0.1-2.7
3	GOST				
	a) titration	0.84	2.92	0.00-0.02	0.0-1.7
	b) <i>M 107</i> technique	0.90	3.23	0.00-0.02	0.0-1.0
	c) <i>Multiskan MS</i> technique	0.93	3.26	0.00-0.01	0.0-0.7

Note: 1. Chemical oxidation modification (a, b; c - proposed method); 2. Dry combustion (DC) using analyser Vario EL III; 3. Standard dichromate oxidation method according to GOST 26213-84. $V_x\%$ = Coefficient of variation.

To operate the photometer Multiskan MS equipped with the Genesis Lite software it is necessary to create an operating protocol, enter the values of standard solutions in the program, select measurement parameters, perform measurements and record and print the data analyses.

The operation of the up-to-date automatic photometer using the developed technology is rapid, user-friendly and highly sensitive. Microplates can be used both as reaction vessels and optical cuvettes and variations in the volumes of non-absorbing liquids do not affect absorbances. To improve the work with the Multiskan MS instrument we purchased a 590 nm light filter and Genesis Lite (Labsystems) software. Genesis Lite is a fully Windows-compatible software package supplied and specified by Labsystems and Thermo Life Sciences. Genesis Lite is designed to expand the operational possibilities of automatic photometers.

Measuring type is chosen (single, dual or kinetic single wavelength). The kinetic mode of reading also requires the user to input: lag time (i.e. the delay before the first reading), reaction time (i.e. the total time over which the reaction is to take place) and interval time (i.e. time between readings). If the plate is used as a reaction vessel, one can use a shaking function. Mixer parameters are also set (mixer speed, mix on time, mix off time, inter-reading mix on time).

Table 3. Linear correlation and regression (using different analytical methods).

Methods ^(m)		Linear correlation		Linear regression		Mean (X)	
X	Y	r	s	Y = A +	BX	\bar{x}	S_x^-
1a	1b	0.994***	±0.016	0.023	1.003	1.73	±0.072
1a	1c	0.996***	±0.013	0.010	0.995		
1a	2	0.988***	±0.022	0.058	1.136		
1a	3a	0.992***	±0.019	-0.002	0.941		
1a	3b	0.986***	±0.024	-0.043	1.054		
1a	3c	0.993***	±0.017	-0.054	1.064		
1b	1a	0.994***	±0.016	-0.002	0.986	1.76	±0.073
1b	1c	0.999***	±0.007	-0.007	0.990		
1b	2	0.990***	±0.021	0.040	1.128		
1b	3a	0.989***	±0.021	-0.010	0.930		
1b	3b	0.983***	±0.026	-0.052	1.043		
1b	3c	0.992***	±0.018	-0.066	1.054		
1c	1a	0.996***	±0.013	0.005	0.996	1.73	±0.072
1c	1b	0.999***	±0.007	0.012	1.008		
1c	2	0.989***	±0.021	0.052	1.138		
1c	3a	0.992***	±0.019	-0.005	0.941		
1c	3b	0.986***	±0.024	-0.047	1.055		
1c	3c	0.994***	±0.016	-0.059	1.066		
2	1a	0.988***	±0.022	-0.008	0.860	2.02	±0.083
2	1b	0.990***	±0.021	0.002	0.868		
2	1c	0.989***	±0.021	-0.007	0.860		
2	3a	0.985***	±0.025	-0.019	0.813		
2	3b	0.977***	±0.031	-0.058	0.909		
2	3c	0.987***	±0.023	-0.074	0.920		
3a	1a	0.992***	±0.019	0.031	1.046	1.63	±0.068
3a	1b	0.989***	±0.021	0.048	1.052		
3a	1c	0.992***	±0.019	0.034	1.045		
3a	2	0.985***	±0.025	0.082	1.194		
3a	3b	0.995***	±0.015	-0.043	1.122		
3a	3c	0.999***	±0.007	-0.048	1.129		
3b	1a	0.986***	±0.024	0.090	0.922	1.78	±0.077
3b	1b	0.983***	±0.026	0.107	0.927		
3b	1c	0.986***	±0.024	0.092	0.922		
3b	2	0.977***	±0.031	0.153	1.050		
3b	3a	0.995***	±0.015	0.054	0.882		
3b	3c	0.996***	±0.013	0.009	0.998		
3c	1a	0.993***	±0.017	0.075	0.926	1.79	±0.080
3c	1b	0.992***	±0.018	0.090	0.933		
3c	1c	0.994***	±0.016	0.076	0.927		
3c	2	0.987***	±0.023	0.132	1.058		
3c	3a	0.999***	±0.007	0.046	0.884		
3c	3b	0.996***	±0.013	0.004	0.994		

Note: Methods^m as in Table 2; *** $P < 0.001$, $n = 25$.

The following are also specified: the type of curve fit algorithm used, data transformation (prior to plotting), outlier rejection criteria, weighting, X and Y axis scales, units and whether a cut off calculation is to be employed. *Genesis Lite* will extrapolate to give results for values beyond the top and bottom of the curve. If a user has several plates to run, but only one set of standards on the first microplate, the standards curve is saved and can be used for analysing all selected samples in different microplates. SOC concentration values and statistical indicators obtained using different analytical methods are presented in Table 2.

Especially low data variation was achieved when *Multiskan MS* was used in the final procedure of SOC determination (1c and 3c treatments). The lowest standard deviation (0.00–0.01) was also identified for these treatments. The SOC values depended on the method used. When analysing by two dichromate methods, using three different final determination procedures (a, b, c), the SOC values obtained were: 0.84–3.26% using GOST and 0.94–3.22% using the new methodology (Table 3). When in the final determination procedure titration was replaced by photometry, higher values were obtained: in Nikitin modification: 0.00–0.03% of SOC and GOST: 0.15–0.16% of SOC.

The data presented in Table 3 suggest a strong linear correlation [$P < 0.001$ (***)] between the data of all assays (experimental treatments 1–3). Correlation coefficients of the data obtained by different methods ranged from 0.977–0.999*** ($n = 25$). The data reported in Table 3 shows the potential for recalculation of data using regression equations. Although the correlation of the data obtained by different methods was strong, absolute SOC data values differed (Table 3, means according to X): from 1.63%, obtained by applying GOST methodology with titration, to 2.02% obtained by dry combustion (DC).

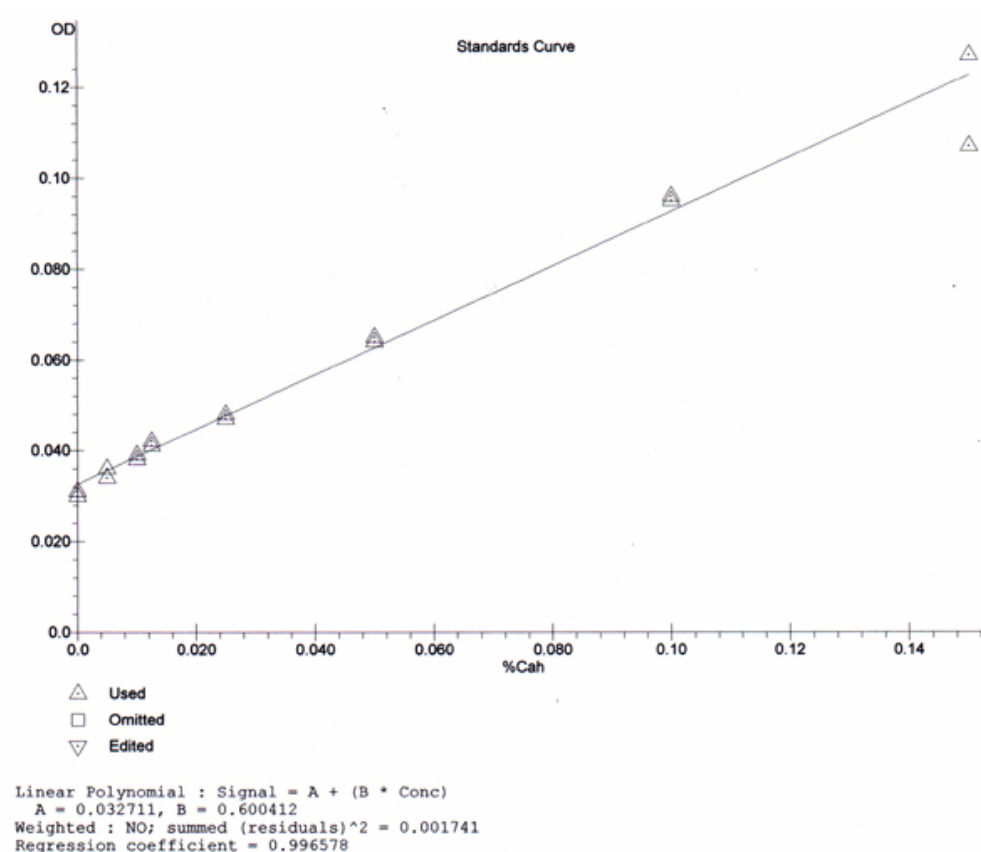


Fig. 1a. The regression coefficient ($R^2 = 0.996^{***}$) without using the editing or omission functions of the calibration graph. Curve edit: LIN/LIN Linear Polynomial method.

The use of *Genesis Lite* expands the possibilities of photometric determination using *Multiskan MS*. In our experiments we tested the use of the standards curve editing function for the improvement of statistical parameters of the equation. It was found that having eliminated 1 of 24 calibration points (8 standards x 3 replicates in the plate) and having edited 1, R^2 increased from 0.996 to 0.999 (Fig. 1a, 1b). Having studied kinetic reading of the instrument when performing automatic measurement set in the program every 15 minutes, it was found that within 300 mins. measurements of the whole plate showed that optical density remained stable (Fig. 2a). A detailed view of one individual sample's kinetic reading function showing stability of absorption (abs) measurement is presented in Fig. 2b.

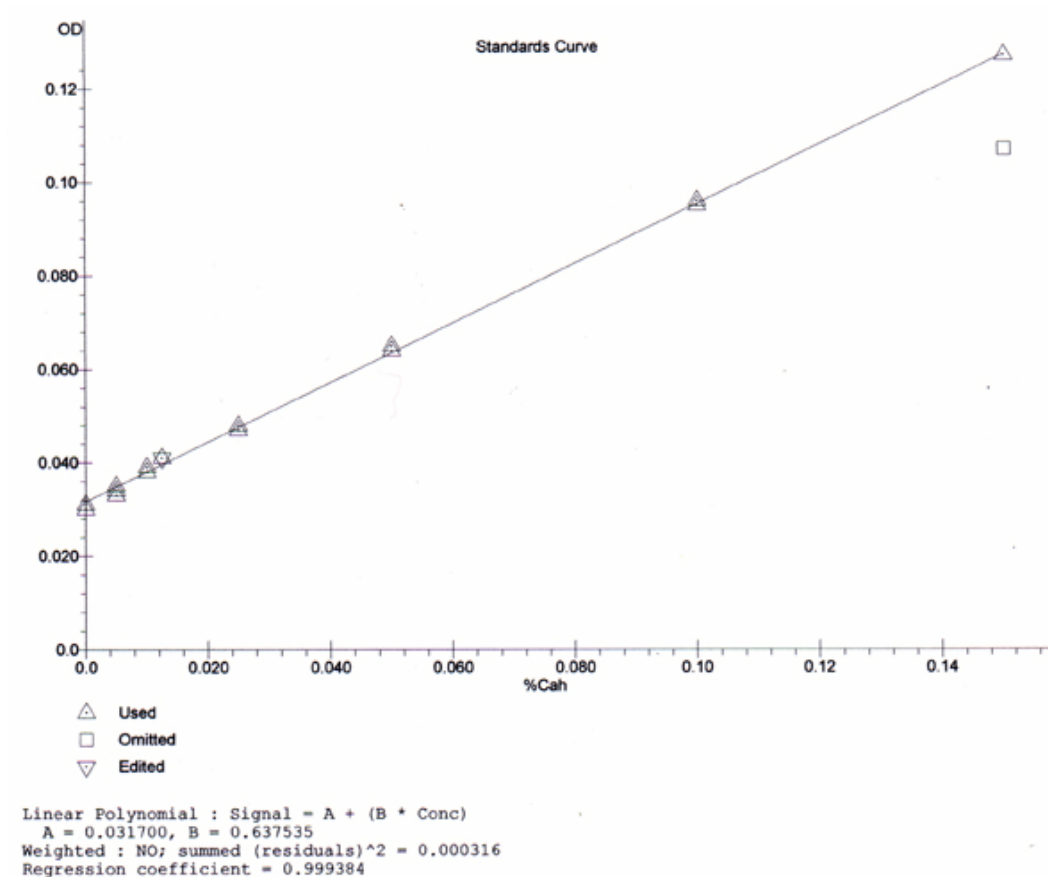


Fig. 1b. The regression coefficient ($R^2 = 0.999^{***}$) using the editing and omission functions of the calibration graph. Curve edit: LIN/LIN Linear Polynomial method.

In order to precisely assess soil fertility, it is necessary to have an accurate and objective SOC determination method. Having compared two oxidation modifications it was identified that the modification using photometric determination with the photometer *M 107* and *Multiskan MS* with *Genesis Lite* microplate software (1b, 1c) secures high result integrity. The data received using the proposed method has a strong correlation coefficient ($r = 0.989^{***}$, $n = 25$) with the dry combustion method. Our experimental data show that the deviation from the mean for the parallel determinations of all methods, while analysing separate samples, was lower than that indicated by Nikitin (1999). Having introduced *Multiskan MS* and *Genesis Lite* microplate software for photometry, reliable results were obtained. Therefore, we recommend using this methodology for SOC determination, which is suitable for the accurate determination of even small changes in SOC.

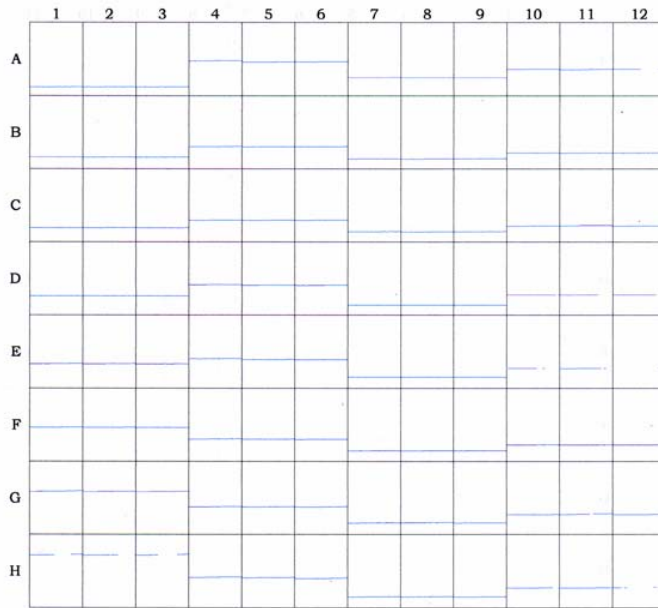


Fig. 2a. Absorption stability of individual measured soil samples (3 replicates per plate). *Note:* From A-H (axis y) and from 1–12 (axis x) show position of each microchannel in the microplate.

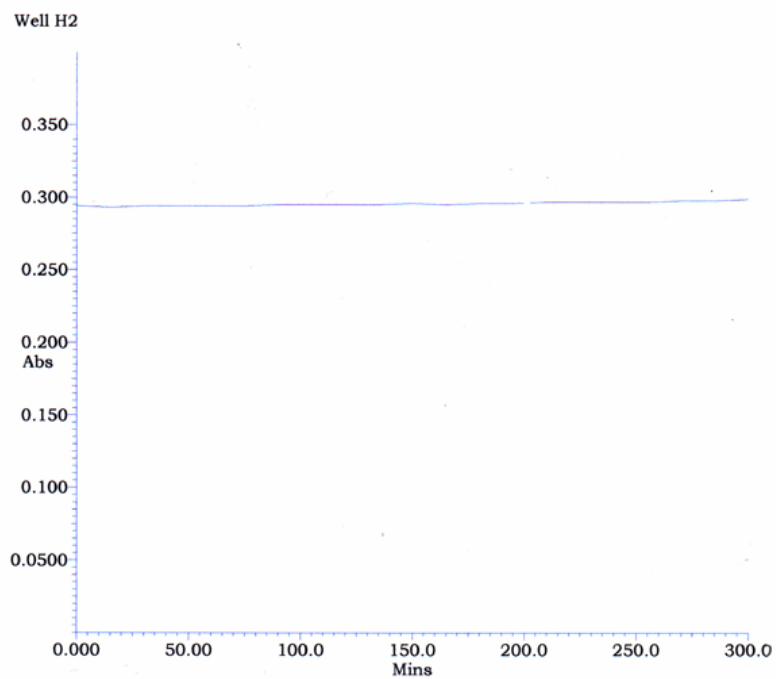


Fig. 2b. Absorption stability of measured soil samples (1 individual sample measured during 300 mins.).

CONCLUSIONS

A methodology for the photometric determination of SOC content using *Genesis Lite* Windows microplate software installed in Multiskan MS has been developed. Assessment of the statistical parameters of the data obtained suggested that for the making of operating protocols the recommended optimum number of replications for the analysis is three. We investigated the operating protocols, editing and saving calibration graphs, using kinetic reading functions and other possibilities of the program. The proposed modification of the method gives strong correlation coefficients ($r = 0.989\text{--}0.990^{***}$, $n = 25$) with the dry combustion method data and least variation in parallel determinations. The use of MS equipped with *Genesis Lite* software, compared with conventional photometry, had several advantages. These include higher measurement accuracy, the facility to edit and save calibration graphs, the possibility of having more replications in a short time, higher labour efficiency, improved personnel safety and lower reagent consumption. A strong correlation ($P < 0.001$) was determined between the data obtained by different methods, and linear regression equations were proposed, which enable re-calculation of SOC values obtained by different methods.

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