

DILUTION EFFECT ON THE INVESTIGATION OF SOIL ORGANIC MATTER QUALITY IN UV-VIS SPECTRA

A HÍGÍTÁS HATÁSA A TALAJ SZERVESANYAG-MINŐSÉG VIZSGÁLATÁRA AZ UV-VIS SPEKTRUMBAN

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Abstract

Soil organic matter is a factor in many areas. Its knowledge helps to develop more accurate models and to use more effective predictions and estimates. While the quantity of organic matter can be well characterised, determining soil organic matter quality is a major challenge. Several methods (mainly based on spectral analysis of organic matter extracts) have been developed, such as the $E_{4/6}$ [465/665 nm] or $E_{2/3}$ [250/365 nm] ratios. The ultraviolet and visible light (UV-VIS) spectra of the extracts (200-900 nm) form exponential curves, from which the ratio derived from the data measured at selected points provides information on the molecular size distribution of the organic matter (ratio of humic acids to fulvic acids). The conventional $E_{4/6}$ values generally give good results for the qualitative distribution of organic matter. If the samples contain some interfering factor, conventional calculations based on the ratio of the two points can be problematic. However, our EFA (Exponential Fitting Approach) method gives much more reliable and repeatable results by fitting hundreds of points. EFA is less sensitive to dilution differences in samples, which has proven to be more reliable (or at least as good) than the conventional method. Therefore, its use can improve the accuracy and repeatability of organic matter quality determination.

Keywords: soil organic matter, alkaline soil extract, quality, $E_{4/6}$, humic acid

JEL code: Q01

Összefoglalás

A talajok szervesanyag tartalma számos területen jelenik meg, mint tényező. Ismerete segíti a pontosabb modellek kidolgozását, a hatékonyabb előrejelzések, becslések alkalmazását. Míg a szervesanyag mennyisége jól jellemezhető, addig komoly kihívást jelent a talaj szervesanyag-minőségének meghatározása. Erre vonatkozóan (főleg a szervesanyag-kivonatok spektrális elemzésén alapuló) számos módszert fejlesztettek ki, például az $E_{4/6}$ [465/665 nm] vagy az $E_{2/3}$ [250/365 nm] arányt. A kivonatok ultraibolya és látható fény (UV-VIS) spektrumai (200-900 nm) exponenciális görbéket alkotnak, amelyekből a kiválasztott pontokon mért adatokból levezetett arány a szerves anyag molekulaméret-eloszlásáról (a huminsavak és a fulvosavak aránya) ad információt. A hagyományos $E_{4/6}$ értékek általában jó információt adnak a szerves anyagok minőségi eloszlásáról. Ha a minták valamilyen zavaró tényezőt tartalmaznak, a két pont arányán alapuló hagyományos megközelítések számos problémát rejthetnek magukban. A bemutított EFA (Exponenciális illesztés módszere) azonban sokkal megbízhatóbb és

megismételhetőbb eredményeket ad, több száz pont illesztésével. Az EFA kevésbé érzékeny a minták hígítási különbségeire, amelyek esetében megbízhatóbbnak (vagy legalább ugyanolyan jónak) bizonyult, mint a hagyományos módszer. Ezért használata javíthatja a szervesanyag-minőség meghatározásának pontosságát és megismételhetőségét.

Kulcsszavak: talaj szervesanyag, lúgos talajkivonat, minőség, $E_{4/6}$, humin-sav

Introduction

Soil is one of our most important resources. It serves many functions, from agriculture and industry to recreation. Protecting, preserving and understanding it is a priority for many fields of science. The formation of humic substances occurs in the so-called humification process, during which plant substances (carbohydrates, proteins, waxes, etc.) and animal origin matters (fats, oils, proteins, etc.) undergo physical, chemical, enzymatic and microbiological transformations (FEKETE et al. 2011; FEKETE et al. 2023). The changes can be well categorised and grouped into systems. The 'First Hungarian yield cadastre' in Hungary was the Golden Crown system (1875), which was actively used between 1875 and 1981 (VÁGI 1970). In the same year, FÓRIZS et al. (1971) created the so-called '100-point system' based on the estimation of soil fertility, which represented a significant advance on the previous years. Its introduction was greatly assisted by the national overview maps produced in the 1950s and 1960s (STEFANOVITS 1963). This method was more widely used between 1981 and 1990. More modern survey methods rely heavily on measurement-based diagnostic approaches.

Measuring soil organic matter (quantity, in particular its quality, such as the presence and proportion of fulvic acid - FA and humic acid - HA fractions) is a continuous task, the methods of which have been continuously developed over the last century. The organic matter both degrading and developing through the decades, keeping a slow dynamic balance in the soils. Therefore, the proper methods for measuring the quantity and quality is essential to understand and react to these processes during the agriculture or environmental protecting works. The carbon content of soils is quantified based on the property that organic matter is oxidizable. Oxidation is carried out by destructive oxidation with potassium dichromate in sulphuric acid media. Organic carbon reduces Cr^{6+} - ions to Cr^{3+} - ions (TYURIN 1931, MSZ 21470/52:1983, WALKLEY & BLACK 1934, FAO 2019). In Hungary, the two solvent-based humus characterisation methods, also known as the "Hargitai method" (HARGITAI 1955 and 1964; MSZ 21470/52:1983), are known for quality determination. The method consists of separate leaching with NaOH and NaF solutions. The resulting humus extracts are compared with each other by photometric measurement of the light absorption capacity of the extracts, thus obtaining the so-called humus stability number (Q).

Some, also commonly accepted methods (especially in the Eastern European region) are the $E_{4/6}$ [465/665 nm] or $E_{2/3}$ [250/365 nm] ratio (CHEN et al. 1977; NADI et al. 2012; SARLAKI et al. 2020). These measurement methods are based on the spectral analysis of organic matter extracts. The ultraviolet and visible light (UV-VIS) spectra (200-900 nm) of the extracts are exponential curves, from which the ratio derived from the data measured at selected points provides information on the distribution of FA and HA (WANG & HSIEH 2001, CHEN et al. 2002). Many authors mention the confounding effect of aromatic molecules, which can modify the results and give erroneous information about the distribution of molecules (HAYASE & TSUBOTA 1985, YAN et al. 2012). To eliminate these errors, YACOBI et al. 2003 attempted to linearize the exponential spectrum, but this met with many difficulties, mainly due to simplifications caused by linearization. In our investigations here (and in previous ones by SEBŐK et al. 2018), we sought to avoid this problem by fitting a curve to the full exponential curve. This provides good information on the FA/HA ratio, but it is important to note that the

bias caused by dilution should also be considered. For this reason, samples with different organic matter content were tested to determine at what dilution ratio the information becomes uncertain and how effective the EFA (Exponential Fitting Approach) method is in such cases, compared to the results obtained with the conventional $E_{4/6}$ method.

Material and methods

Material

The experiment was carried out on two types of soil and on an organic matter-rich compost sample (the compost also contained 5 % plant stem residues). The first soil (sample A, from Keszthely, upper 0-30 cm layer) is a Hortiic Terric Cambisol (Dystric, Siltic) WRB classified (World Reference Base of Soils, FAO 2015), carbonate-free, organic matter-poor soil (SOM content: 1.45 %). The second soil (sample B, from Kápolnásnyék, upper 0-30 cm layer) is a Calcic Vermic Chernozem (Anthric, Siltic) WRB soil, rich in organic matter (SOM content: 3.70 %), with a high carbonate content (10 %).

Methods

Soil extracts were prepared using the modified STEVENSON method (1982), which contains 0.5 M NaOH and 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ solutions, based on BAGLIERI et al. (2007). 20 ml of extractant was added to each soil sample (5.0 g). Samples were shaken for 4 hours to homogenise. To separate the supernatant extract from the soil, the samples were centrifuged (10 min at 5000 rpm) and filtered (MN 751 filter paper, Reanal) (STEVENSON 1982). The prepared extracts were diluted to the desired concentration with 0.5 M NaOH solution. The pH of all samples was then measured, making sure that their values were strongly alkaline, because literature data (BREMNER&LEES, 1949; OHNO et al. 2019; JAKAB et al. 2022) suggest that precipitation can occur in samples under non-alkaline conditions. Full spectral analysis was performed using a Shimadzu 1900i UV-VIS spectrophotometer in the 450-800 nm range at 1 nm resolution. The exponential curves were fitted to the measured data using Microcal Origin 6.0 (Microcal Software, Inc, 1991-1999).

$E_{4/6}$ calculation: absorbance values at two wavelengths of interest were used to determine the $E_{4/6}$ values. The value measured at 465 nm was divided by the absorbance value measured at 665 nm (HELMS et al. 2008). This result gives the $E_{4/6}$ value.

Exponential Fitting Approach (EFA) method

The $E_{4/6}$ calculation based on two certain read of the spectrum. This implies that if the measurement at these two wavelengths is inaccurate, the result will also be incorrect. This has been demonstrated previously (SEBŐK et al. 2018). To avoid that error we improved a more reliable method, called Exponential Fitting Approach, which based on the original calculation but determine the value in a different mathematical way. Because of its importance, we repeat the previous derivation:

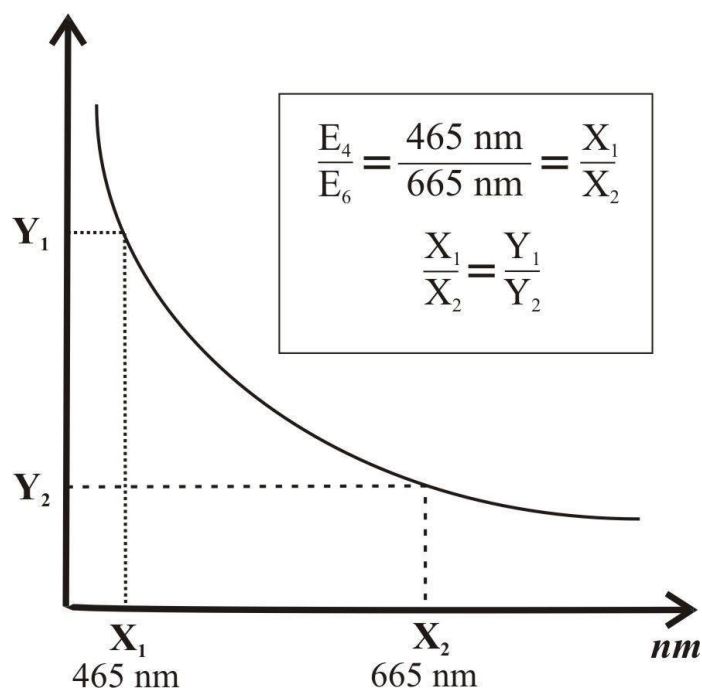


Figure 1: $E_{4/6}$ wavelengths at 465 and 665 nm (x-axis) and their corresponding absorbance values (y-axis) (SEBŐK et al. 2018)

The measured absorbance of the organic matter in the spectrum follows an exponential decrease curve, for which the classical first-order exponential equation can be written:

$$y = A * e^{\frac{-x}{t}}$$

The parameters are: x: the wavelength, y: the absorbance at the given wavelength, A: the multiplication factor, which depends on the dilution, t: the fitting parameter.

Using this relationship, we can express the values of y_1 and y_2 shown in Figure 1 at a given wavelength in our case in terms of the $E_{4/6}$ highlighted values (Figure 1). The result is the two equations below:

$$y_1 = A * e^{\frac{-x_1}{t}} = A * e^{\frac{-465}{t}}$$

$$y_2 = A * e^{\frac{-x_2}{t}} = A * e^{\frac{-665}{t}}$$

$E_{4/6}$ is obtained as the ratio of the absorbance values for the two wavelengths. If we substitute the above relations into the fitted exponential equation, we obtain the following relation:

$$\frac{E_4}{E_6} = \frac{y_1}{y_2} = \frac{A * e^{\frac{-465}{t}}}{A * e^{\frac{-665}{t}}} = \frac{e^{-465/t}}{e^{-665/t}} = e^{-\left(\frac{-465}{t} - \frac{-665}{t}\right)} = e^{\frac{200}{t}}$$

Based on these results, it can be stated that the $E_{4/6}$ value can be derived from the fitting parameter of the exponential equation:

$$\frac{E_4}{E_6} = e^{\frac{200}{t}}$$

It should be noted that the Exponential Fitting Approach (EFA) presented here can be applied not only to $E_{4/6}$, but also to other wavelength pairs (e.g. $E_{2/3}$) (SEBÖK et al. 2018).

Results

Figure 2 shows the result of the exponential fit to the compost samples, performed using Origin 6.0. Similar fits were also made for the two soil samples, but only the results are reported. The parameters obtained and the absorbance values at 465 and 665 nm are presented and given in Tables 1-3. The t values are the fitting parameters (in case of Origin programme, it is marked as t_1), while the SD values are their errors. Using the t values, we calculated the $E_{4/6}$ values (Cal. $E_{4/6}$), as well as the $E_{4/6}$ values calculated conventionally (based on the ratio of two wavelengths) from the unmatched wavelength data for comparison.

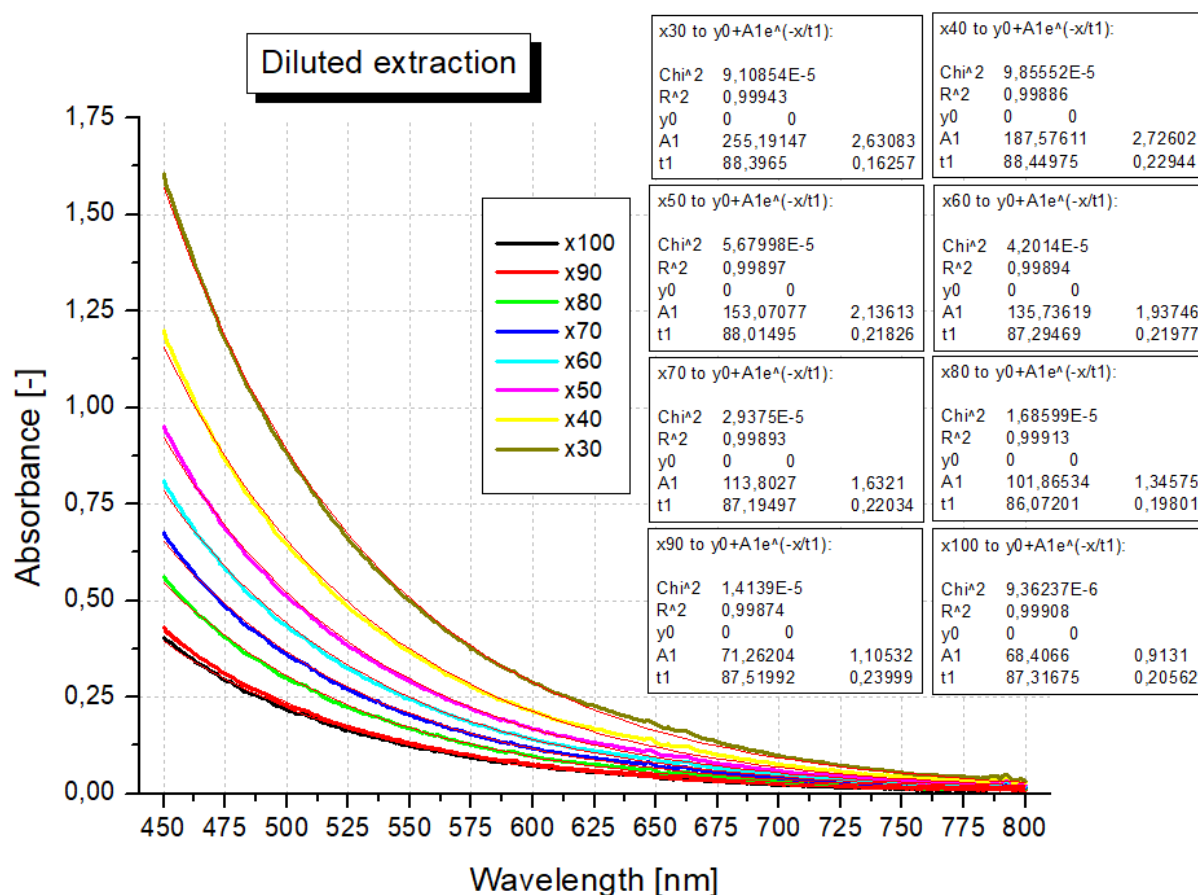


Figure 2: Absorbance results of compost samples, fitted exponential equations and their parameters

Table 1: Compost sample dilution values and corresponding E_{4/6} values, calculated values and their averages with standard deviation

Dilution rate	t ₁	465 nm	665 nm	E _{4/6}	±SD	Calc. E _{4/6}	±SD
30×	88.40	1.333	0.153	8.7	0.33	9.6	0.18
40×	88.45	0.982	0.115	8.5	0.21	9.6	0.24
50×	88.01	0.782	0.091	8.6	0.12	9.7	0.17
60×	87.29	0.663	0.075	8.8	0.30	9.9	0.05
70×	87.19	0.552	0.063	8.8	0.21	9.9	0.16
80×	86.07	0.460	0.050	9.2*	0.38	10.2*	0.36
90×	87.52	0.353	0.040	8.8	0.18	9.8	0.17
100×	87.32	0.334	0.037	9.0	0.26	9.9	0.18
Average				8.8	0.20	9.8	0.19

*Outliers that are not included in the Average

The dilution series was presented starting at 30× dilution because the more concentrated solutions were so concentrated that the spectrophotometer reached its maximum absorbance in the sub-500 nm range. For samples such as compost samples, it is not uncommon to use dilutions of up to 100× for humus quality measured by this method, so we have gradually increased the ratios so far and have made a series (three replicates). The results in Table 1 are significantly correlated for the different dilutions for both the conventional and EFA methods. While the mean value was 8.8 for the conventional method, it was 9.8 for the EFA method, with similar SD values (0.2 and 0.19, respectively). The 80× dilution results are not included in any of the means (marked with asterisk) because they were outliers with high SD values. Although there is a difference of one value between the two results, it can be said that both methods are suitable for the full dilution series with good repeatability.

In the case of sample A (Table 2), a sample with a medium-low organic matter content (SOM content: 1.45 %) was analysed. Knowing the history of the soil (forest soil), the expected quality values are also lower, which was confirmed by the analysis. Both the conventional and the EFA methods gave very close results (mean 6.30 and 6.27, SD: 1.85 and 0.76 respectively). What is immediately apparent from the table is that for the 90× and 100× samples, the two methods measured very low values.

Table 2: Sample A dilution values and corresponding E_{4/6} values, calculated values and their means with standard deviation

Dilution rate	E _{4/6}	±SD	Calc. E _{4/6}	±SD
30×	6.69	0.16	6.28	0.20
40×	7.34	0.36	6.83	0.19
50×	6.92	0.34	6.43	0.15
60×	7.09	0.35	6.49	0.13
70×	8.19	0.56	7.13	0.31
80×	7.40	0.60	6.65	0.37
90×	3.51*	0.15	4.75*	0.53
100×	3.28*	0.15	5.56*	0.46
Average	6.30	1.85	6.27	0.76
Average 2	7.27	0.52	6.64	0.31

*Outliers that are not included in the average

This was due to over-dilution, in which range photometric detection of humic substances is problematic. This is indicated with asterisk in the table and a second average (Average 2) was calculated by omitting these values. These two modified averages are now significantly different. For both values, the standard deviation has been significantly improved, and while the EFA value remains close to the original value, it has increased by almost one value for the conventional calculation. However, the EFA method still gave a much more consistent result across the dilution series.

Table 3: Sample B dilution values and their corresponding $E_{4/6}$ values, calculated values and their averages with standard deviation

Dilution rate	$E_{4/6}$	\pm SD	Calc $E_{4/6}$	\pm SD
30×	6.22	0.12	6.01	0.07
40×	6.37	0.13	6.06	0.17
50×	6.49	0.07	6.19	0.10
60×	6.42	0.26	6.12	0.18
70×	5.59*	1.09	6.97*	0.74
80×	6.71	0.15	6.33	0.22
90×	4.81*	1.32	6.75*	0.20
100×	5.68*	0.21	6.81*	0.93
Average	6.04	0.63	6.41	0.38
Average 2	6.44	0.18	6.14	0.12

*Outliers that are not included in the average

Sample B (Table 3) is a more humus-rich soil type (SOM content: 3.0 %). This is reflected in the results of the dilution series: a more even distribution of values throughout. For the 90×, 100× dilution levels, the conventional calculation also shows a distinct drop here, whereas the EFA method does not show the same drop. There was a dropout for the 70× dilution series, so those values were also removed from the averaging (Average 2). As a result, the final result (Average 2) is much more uniform with a much lower variance.

It is worth highlighting the 90× dilution curves for sample B, the three replicates. This is illustrated in Figure 3. The figure is interesting for two reasons; one is the low absorbance value, which is due to the over-dilution mentioned for sample A.

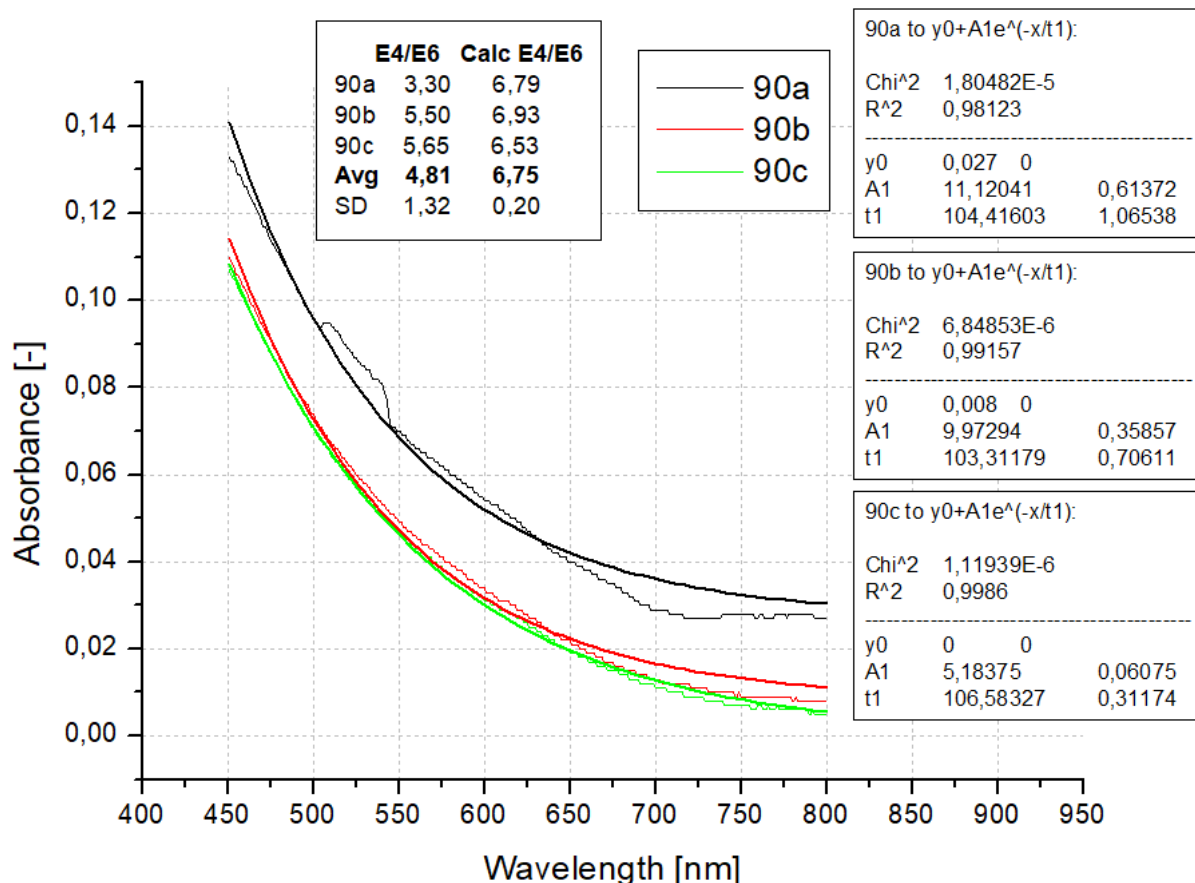


Figure 3: Sample B 90× dilution values and fitted exponential curves

The other is the curve labelled 90a, which gives a rather chunky curved line instead of a nice exponential run. For conventional counting this is a serious problem, since if one of the two points is taken from one of these rising/falling points, it can significantly distort the result. This is due to the outliers of the conventional value (SD: 1.32). The EFA method masks such outliers, clearly shown by the fitted exponential equation and its parameters in the figure, which do not show large outliers compared to the other two measurements. The reason is that the EFA method considers hundreds of points, thus allowing a more stable measurement for local excursions, which is particularly useful in extreme situations.

Conclusions

The investigation method of the quality of soil organic matter give a good overview about the status of the organic matter in the soils. Although, the conventional $E_{4/6}$ values generally give good results for the qualitative distribution of organic matter, in case of dilution, it can lead to false results. The examined situation, where the samples content are in extreme cases or when samples need to be diluted, may show large variations. If the samples contain some kind of interfering factor, the traditional $E_{4/6}$ calculation based on the ratio of the two points can also be problematic. However, the EFA method presented here gives much more reliable and repeatable results by fitting hundreds of points, as it is less sensitive to potential problems in the samples. It is more reliable (or at least as good) than the conventional method for both within-sample repeatability and dilution differences. For this reason, it seems more suitable for future measurements, especially as it can be adapted to existing methods based on photometric measurements or even developed into new ones.

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