Review on Agriculture and Rural Development 2017 vol. 6 (1-2) ISSN 2063-4803

THE EXAMINATION OF HUMIC SUBSTANCES IN SOILS AND COMPOSTS WITH HIGH ORGANIC CONTENT WITH DIFFERENT METHODS

VIKTÓRIA LABANCZ[1], GABRIELLA RÉTHÁTI [1], ANDRÁS MAKÓ[2], TAMÁS SZEGI[1]

[1]Szent István University, Department of Soil Science and Agricultural Chemistry, 2100 Gödöllő, Páter K. u 1.
viktoria.labancz.91@gmail.com, rethati.gabriella@mkk.szie.hu, szegi.tamas@mkk.szie.hu
[2]Institute for Soil Sciences and Agricultural Chemistry, Centre for Agricultural Research, Hungarian Academy of Sciences, 1022 Budapest, Herman Ottó út 15.
mako.andras@agrar.mta.hu

ABSTRACT

The organic materials, especially the complex structural humic substances are acid-characteristic polymers which are key elements of soils. Despite their relatively small quantity, humic substances have beneficial effects on soil nutrient management, development of optimal soil structure, regulation of soil temperature, and proper water management.

The application of the UV-VIS spectrophotometry for describing humic substances in soils and determining of humifical state is nowadays a widespread method. The E4/E6 procedure (which were determined between 465 and 665 nm wavelength) and the Hargitai-method (the extinction of extracts measured between 400 and 750 nm at 9 wavelength) are procedures that have become the general tool for determining the quality of humic matters because of its easy accessibility and smaller need of instrumentations. Nevertheless, their usefulness has been criticized in scientific communities because of the high human error factor and the technical limitations of the simpler spectrophotometric instruments. Nowadays the spreading light scattering photometric examinations using lasers as the Static Light Scattering (SLS) or the Dynamic Light Scattering (DLS) could be a new way of measuring the quality of the humic matters.

This study is based on the examination of different quality soil and compost samples which were extracted from different Hungarian sites like Trizs, Szárítópuszta and Csobánc. Additionally, not only the soils and composts were analyzed but also any soil conditioners (biochar, bone charcoal) which were applied and affected their properties.

The main goal of the research was to measure the soil and compost samples with the E4/E6 and the Hargitai-method and with also using Zetasizer Nano ZS device that could lead to more detailed results about the weight and the size of the humic molecules.

Based on the summarized analytic results the outcomes of the E4/E6 procedure is applicable for drawing relevant conclusion regarding the humic quality of the given sample. On the contrary, the applied Hargitai-method has not proved to be effective. Although the measurement of the molecule's size and weight with the Zetasizer Nano ZS device has brought out exciting results and displayed similarities with the E4/E6 outcomes, only it's tendencies proved to be informative because of its methodological background.

Keywords: E4/E6 ratio, Hargitai-method, Zetasizer Nano ZS, humic substances, charcoal

INTRODUCTION

The nutrient and toxic element buffering capacity of soils are highly dependent on the organic fraction of soil (SPOSITO, 1989), thus the replacement of organic matter (OM) and the promotion of humification is indispensable. The level of OM content in soils can be increased by using composts or pyrolyzed organic materials (biochar). Both materials have high carbon content. The mineralization and transformation of composts in soils are faster (~10 years), while the pyrolyzed organic matter (charcoal, bonechar) are only slightly degrading over a century (LEHMAN ET AL., 2009). Because of the different impacts of the soil conditioners in the soil-system is it essential to parameterize its mode of action in order the efficient soil quality improvement. One of the parametrization tools is the examination of the appearance and quality of the humic material. The most common tool for analyzing the quality of the humic material is the UV-VIS spectrometric examination which includes the our used E_4/E_6 and the Hargitai methods. One of the key results in our research is that we could observe the differences between the two methods during the analyzation of our given samples. The measurement with the Zetasizer Nano Zs device has confirmed our comparative study.

MATERIAL AND METHOD

For our examinations, the following materials were used: brown forest soils from two different regions of Hungary (Gödöllő, Trizs); topsoil of three brown forest soils collected from charcoal burning piles that were used 25, 35, and 80 years ago; compost made of green wastes; bonechar, and charcoal (*Table 1*).

Table 1. Parameters of the samples used in our research

	Soil Gödöllő	Soil Trizs	25-year-old Soil-charcoal	35-year-old Soil-charcoal	80-year-old Soil-charcoal	Compost	Charcoal	Bonechar
pH_{KCl}	5.7	3.9	5.0	4.7	3.9	8.3	8.3	7.6
C (%)*	0.75	4.4	12	10	5.1	20	80	10

* carbon content determined by Loss on ignition method

The soil science literature divides the humic substances to three basic group according to their solubility these are the fulvic acids (FA), humic acids (HA) and the humic materials (HM). The size and weight of the molecules and the nitrogen contents are increasing from the fulvic acids to humic materials, while their oxygen content is decreasing. In the case of the UV-VIS spectrophotometric methods these characteristics can distinguish the differences between the analyzed humic samples.

The detection of aqueous HA and FA solution's optical density or absorbance can be made at 465 and 665 nm (E_4/E_6) and between 400 nm and 750 nm at 9 wavelength (Hargitai-method). These measurements are widely used for the characterization of humic substances. Although the E_4/E_6 ratio and the Hargitai-method have different examination procedure and evaluation they are related to the degree of condensation of the aromatic carbon network, carbon content, and molecular weight of humic substances (KONOVA, 1966; SCHNITZER AND KHAN, 1972; TAN, 2003). FA, with lower molecular weight, have higher (6-8,5); whereas the HA, with higher molecular weight and better quality, have lower (<5) E_4/E_6 ratios (STEVENSON, 1994). Although by the usage of two solvents at the Hargitai-method the denser humic materials are solvable more efficiently than in case of the E_4/E_6 method, however the results can be drawn only by using multiple and complicated calculations (RÉTHÁTI ET AL., 2015).

The weight and size of the molecules in the given sample solution can be measured by laser light scattering where the *Zetasizer Nano* device can measure in wide range of test concentration. In this respect, the it can be an adequate supplementary to the UV-VIS spectrometric procedures.

From the obtained results of the E_4/E_6 the Hargitai-method and the Zetasizer Nano measures, we would like to draw conclusions about the quality of the organic matter.

The preparation E_4/E_6 of the samples was carried out in two steps. First, we centrifuged three grams of the samples with 30 cm^3 2% HCl solution in 50 cm^3 centrifuge tubes (5 minutes, 5000 rpm) in order to dissolve carbonates. This step was repeated one more time after the supernatant was discarded. After discarding the HCl containing supernatant, 30

cm^3 of distilled water was added to the samples and centrifuged again in order to remove the remnant of HCl. The second step was the organic matter extraction, in which after discarding the supernatant of the distilled water, 30 cm^3 0.5 mol NaOH solution was given to the samples, and was put into a rotary shaker for 24 hours. After shaking, the samples were centrifuged (25 minutes, 5000 rpm), then the supernatant was diluted to the required concentration, and the absorbance of the solutions were measured and 465 and 665 nm. The measurements were carried out by Jenway 6405 UV/Vis- Spectrophotometer (RÉTHÁTI ET AL., 2015).

In case of Hargitai-method two solvents were used; 0.5% NaOH and 1% NaF solutions. We gave to every air-dry sample 20-20 cm^3 solvent then for 48 hours we stored them at 5 ^oC temperature cold storage. As the next step, we gave distilled water to the solvent sample to make dilution of 1:5 and 1:10. After that, similarly to the E_4/E_6 procedure, we used the Jenway 6405 UV/VIS spectrophotometry device at 9 wavelength (between 400 and 750 nm) to measure the samples. Then on a given sample we divided the absorbance values of the NaF solution by the values of the NaOH solution which resulted a Q stability number. We divided the Q number by all humic content (according to Tyurin; mark: H) which lead to the humic stability coefficient values (K). According to the values in the table of Hargitai we classified the measured samples by their potential humic content (BUZÁS, 1988).

At the Zetasizer Nano ZS measures we mainly focused on the soil charcoal systems (from charcoal burning piles that were used 25, 35, and 80 years ago). With using the static light scattering (SLS) we defined the average molecular weight of the given solution sample, while with the dynamic light scattering (DLS) we detected the average molecular size. Like at the E_4/E_6 process, we used 0.5 M NaOH solvent, and the whole sample preparation procedure was the same as far as the prior to, detection phase. The measurement was carried out at a constant 25 ± 0.1 °C external temperature, while for illuminating the samples we used 4 mW He-Ne laser. The prepared solutions were dosed at a glass cuvette and were examined in 1 ml quantities. All measurements were repeated three times. At the dynamic light scattering measurements we detected the scattered light in two mode, at 173° (back scattering mode) and at 180° (dual-angle) mode.

The analyses were repeated three times and the results were statistically tested. The analysis of the variance program was used for data assessment in Microsoft Office Excel Macro. The program was developed based on Sváb's algorithm (1981). It was successfully used in several publications (KOVÁCS ET AL., 2013; SZABÓ ET AL., 2013; RÉTHÁTI ET AL., 2015).

RESULTS AND DISCUSSION

The average absorbance values of the NaOH extracted humus substances, that was measured at 465 and 665 nm, can be found in *Table 2*. The color of the NaOH extracted samples was brown, which means that all samples contained some acidic humus substances. The exception is that concerning the charcoal and bonechar samples significant amount of humic substances cannot be dissolved by NaOH from the samples. This phenomenon can be seen from the colorlessness of the dissolved samples, which in case of the charcoal were caused by the pyrolysis generated aromatic ring structure. In the case of the bonechar it was caused by the high calcium and phosphate content level which was diluted during the acidic sample preparation phase.

The rest of the sample E_4/E_6 values of all the samples were below 6, which indicate the presence of humic acids. Since the extraction was carried out by 0.5 M NaOH solution, we do not have to consider great pH-dependence of the E_4/E_6 ratios of the fulvic acids (CHEN

ET AL., 1977). The E_4/E_6 ratio of the brown forest soils (Gödöllő, Trizs) were 4.94 and 5.67, respectively, which indicate high amount of fulvic acid compared to the soils treated with charcoal and compost. The humus substances with complicated structure and lower E_4/E_6 values formed in composts (E_4/E_6 = 3.14) and in soil-charcoal system with different ages (E_4/E_6 = 2.71-3.51) can be due to the intensive microbial activity. The lowest E_4/E_6 value was in case of the 35 year-old soil-charcoal system ($E_4/E_{6(35yr)}$ = 2.71), which might be due to the fact that humification process was the most intensive and the most progressed (RÉTHÁTI ET AL., 2015).

Table 2. The average absorbance of the samples, measured at 465 and 665 nm, and the E_4/E_6 ratios

	Soil Gödöllő	Soil Trizs	25-year-old Soil-charcoal	35-year-old Soil-charcoal	80-year-old Soil-charcoal	Compost	Charcoal	Bonechar
465 nm	0.47	0.99	2.79	2.72	2.16	2.86	0.18	0.02
665 nm	0.10	0.17	0.80	1.00	0.64	0.91	0.04	0.01
E_4/E_6	4.94	5.67	3.51	2.71	3.35	3.14	4.93	1.40

In case of the Hargitai-method the humification state of the given sample can be described by using the calculated K values to classify on a scale from 0.001-10, where the low number indicates primary humic release forms and the higher number means a more complicated structured humic substances. The measured results of the Hargitai method are shown in *Table 3*.

Table 3. Results of the Hargitai-method

	Control	25 years	35 years	80 years	Csobánc settlement
Q	3.483	0.5994	0.6273	0.4785	2.9172
H	4.06	12.63	11.77	6.13	8.7
K	0.86	0.05	0.05	0.08	0.34

The expected results of the Hargitai-method are not displaying a high degree of identity with the E_4 / E_6 ratios, because of the K values results have described the humification state reversely (25-35-80 years sample contains more complex, higher-molecular humic substances) comparing what we have expected. The Hargitai method's test results can be interpreted more precisely if we examine the C / N ratio of the sample to define the state of humification (TAN, 2003).

The static light scattering (SLS) molecular weight measurement results of the Zetasizer Nano ZS device can be read in *Table 4*. Our examination in this case was oriented to the soil charcoal systems, which illustrates well the time dimension of the humification process. The results of the molecular weight measurement by far exceeds the publicized literature's 500-1,360,000 Da values for humic substances; (CHIN ET AL., 1994; STEVENSON, 1994; TAN, 2003; KAWAHIGASHI ET AL. 2011). The static light scattering measured results of molecular weight contrary to the Hargitai-method, comparable with the E_4 / E_6 method's results, and it confirms the experienced tendencies.

Table 4. The results of SLS measures

Name of the sample	T (°C)	MW (kDa)	A2c mL mol/g²	Standard deviation of NaOH (kcps)	Toluol standard deviation (kcps)
Controll	25	**1180**	5.24E-04	67.3	245.7
25 years	25	**3790**	3.54E-04	84.1	246.5
35 years	25	**5070**	3.94E-04	33.1	234
80 years	25	**1.24E+04**	9.02E-04	34.2	292.9
Soil Gödöllő	25	**1050**	1.80E-04	41.1	239.8

Determining the average hydrodynamic diameter related to the dynamic light scattering (DLS) definable weight of the solution was not lead to obvious results. Because the results of the measurements displayed more significant particle presence in the range from 1000 to 10,000 nm, therefore our measured humic substances could be in the range, but they were not being able to be identified. The reason for this fact is the complex content of the samples (sedimenting mineral particles containing soil samples).

CONCLUSIONS

In conclusion, the different types of UV-VIS spectrometry methods (E4/E6 and Hargitai) which we used are not sufficient by themselves for determining perfectly the humic state of a given soil, however they can be used for determining a kind of humification index for soil improving purposes (CHEN ET AL., 1977, KUMADA, 1985, STEVENSON, 1994, TAN, 2008).

In case of the E_4/E_6 method, the values for determining the humification level, which we predicted, were confirmed. The forest soil samples had higher (4.97-5.67) E_4/E_6 ratios, while the soil-charcoal systems and the composts (2.71-3.51), that had easily mobilizable organic matter fractions, had lower E_4/E_6 ratios. Regarding the Hargitai's method for determining the quality of humus the samples with more advanced humification status progress in contrast to the expectations displayed lower K values as the lass advanced samples.

The results of the SLS and DLS measurements, which were made on Zetasizer Nano ZS device, have proved difficult to interpret because of their solutions are showing the properties both the association colloids and polyelectrolytes and with a relatively minor change on circumstances the colloidal dispersion characteristics too.

Regarding the Zetasizer Nano ZS measurements, it caused problems that we tested complex soil solutions which contained both organic as well as inorganic fractions. SLS-molecular weight and DLS-molecular size determinations and the results become unstable due to these facts, however during the SLS measurements of the E_4/E_6 correlating measurements were established trend in the case of the soil as well. The result’s usefulness of the SLS molecular weight and DLS molecular size measurements are questionable because of these factors, however the SLS measurements have shown correlating tendencies with E_4/E_6 in case of soil solutions.

In the soil solutions compared to other organic molecules the humic substances have unique behavior and they are more sensitive for the changes of the solution’s parameters.

REFERENCES

BUZÁS, I. (1988): Talaj és agrokémiai vizsgálati módszerkönyv 2. A talajok fizikai-kémiai és kémiai vizsgálati módszerei. Mezőgazdasági Kiadó- Budapest

CHEN, Y., SENESI, N., SCHNITZER, M. (1977): Information Provided on Humic Substances by E_4/E_6 Ratios. Soil. Sci. Soc. Am. J. 41(2): 352-358.

CHIN, Y. P., AIKEN, G. & LOUGHLIN E. O., (1994): Molecular weight, polydispersity and spectroscopic properties of aquatic humic substances. Environmental Science and Technology 28: 1853–1858.

FILEP, GY. (1988): Talajkémia. Akadémiai Kiadó, Budapest, 293 p.

KAWAHIGASHI, M. ET AL. (2011): Particle Sizes of Standard Humic Substances Calculated as Radii of Gyration, Maximum Diameter and Hydrodynamic Radii, Humic Substances Research 8: 6.

KONOVA, M.M. (1966): Soil organic matter. Pergamon Press, Oxford p. 400-404

KOVÁCS, ZS., TÁLLAI, M., KÁTAI, J. (2013): Examination on the effect of lead and copper heavy metal salts on soil microorganisms under laboratory circumstances. Növénytermelés 62 Suppl.: 261-264.

LEHMANN, J., JOSEPH, S. (ed.)(2009): Biochar for Environmental Management.science and Technology. Earthscan, London. pp. 183-200.

NÉMET, T. (1996): Talajaink szervesanyag-tartalma és nitrogénforgalma, MTA Talajtani és Agrokémiai Kutatóintézete, Budapest, pp. 35-56.

RÉTHÁTI, G., LABANCZ, V., TOLNER, L., SZALAI, Z., ALEXA, L. (2015): Examination of humic substances of composts and differently aged soil-charcoal systems. XIV. Alps-Adria Scientific Workshop Neum, Bosnia and Herzegovina, Növénytermelés / Crop production 64 Suppl.: 103-106.

SCHNITZES, M., KHAN, S.U. (1972): Humic substances in the environment. Marcel Dekker, New York. pp. 57-60.

SPOSITO, G. (1989): Soil organic matter. The chemistry of soils. New York: Oxford University Press, pp. 51-56.

STEFANOVITS, P., FILEP, GY., FÜLEKY, GY. (1999): Talajtan. Mezőgazda Kiadó, Budapest. 470 p.

STEVENSON, F.J. (1994): Humus Chemistry, John Wiley & Sons, 496 p.

SVÁB, J. (1981): Biometriai módszerek a kutatásban. Mezőgazdasági Kiadó, Budapest

SZABÓ, A., BALLA-KOVÁCS, A., KREMPER, R., KINCSES, S-NÉ., VÁGÓ, I. (2013): A tápközeg és az angolperje (Lolium perene L.) jelzőnövény P- és K- tartalmának alakulása különböző komposztdózisok alkalmazásakor. Talajvédelen (Különszám): 459-468.

VELASCO, M.I. (2004): Analysis of humic acid from compost of urban wastes and soil by fluorescence spectroscopy, Agriscientia, 8 p.

TAN, K.H (2003): Humic Matter in Soil and the Environment, Marcel Dekker, Inc., New York, 371 p.