

Article: IHSS18 Special Paper**Changes in Several Properties of Humic Acids after Application of Exogenous Organic Materials****Lubica Pospisilova^{1*}, Magdalena Habova¹, Eva Doleželová¹,
Tomas Simon², and Mikulas Madaras²**¹ Mendel University in Brno, Faculty of Agri Science, Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Zemedelska 1, 612 00 Brno, Czech Republic² Crop Research Institute, Drnovska 507/73, 161 06 Prague, Czech Republic**Abstract**

Exogenous organic materials (biochar, compost, digestate, lignite, and lignohumate) were applied to a Haplic Cambisol with the aim to improve soil chemical properties and soil organic matter quality. Ultraviolet-visible spectroscopy was used for comparing alkali-extracted humic substances. Elemental composition and specific peaks in Fourier transformed infrared (FT-IR) spectra were used for comparison of the isolated HAs. All the HA samples contained alkyl, aromatic, carboxyl, amido, amino, and carbonyl groups. The highest content of aliphatic C–H groups in the HAs after compost and lignohumate applications and the prevalence of aromatic groups in the HAs after biochar and lignite applications were estimated. Based on the differences in FT-IR spectra, stability indexes of HAs were calculated.

Keywords: Exogenous organic materials, humic acids, UV-VIS spectroscopy, IR spectroscopy**Introduction**

Exogenous organic materials, such as lignohumate, digestate, lignite, biochar, and compost, are applied to agricultural fields with aim to improve the quantity and quality of humic substances (HS) and plant nutrition regime in soil. Lignohumate is a commercial product (Amagro, s.r.o., Czech Republic) rich in HS and micro nutrients and has been reported to show the plant growth stimulation effect (Amagro, 2008). It can also be applied to a wide range of crops (Ložek and Varga, 2005). Digestate represents a residue after anaerobic fermentation process in biogas plant production. Its composition is associated with primary products and digestion processes, with usually high content of $\text{NH}_4^+\text{-N}$ (Ciganek et al., 2010). Lignite, a low-rank coal, containing a significant amount of humic acids (HAs) has been studied for the removal of heavy metals (Kučerík et al., 2003). An application of biochar, the pyrogenic products from biomass, has been expected to increase the amount of carbon (C) in

the stable form and the nutrient sorption capacity in soil (Sohl et al., 2010). Compost is an organic fertilizer made of various kinds of organic residues and waste biomass. Controlled composting process produces a valuable organic material rich in nutrients including microelements (Klučáková et al., 2011). Application all of them directly affects the quantity and quality of HS. While the effects of the above amendments on soil properties have been documented, less is known about the associated alterations of soil HAs.

Determination of functional groups using infrared (IR) spectroscopy can help to indicate the chemical composition of HAs (Griffiths and de Haseth, 2007; Viscarra-Rossel et al., 2008; Tinti et al., 2015). The specific peaks obtained by Fourier transformed (FT)-IR spectroscopy can be used for characterization and comparison of HAs isolated from soils after applying exogenous organic materials. Their stability is possible to evaluate according to Demyan et al. (2012) as a ratio of the sum of integrated area of $1660\text{--}1580\text{ cm}^{-1}$ and $1546\text{--}1520\text{ cm}^{-1}$, both derived mainly from aromatic C=C, to integrated area of $3010\text{--}2800\text{ cm}^{-1}$

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associated with aliphatic C–H. Ultraviolet and visible (UV-VIS) spectroscopy has also frequently been used for obtaining optical curves and indexes of degree of darkening (Chen et al., 1977; Kumada, 1987; Del Vecchio and Blough, 2004).

The aim of this study was to evaluate the changes in the composition of HS (percentages of HAs and fulvic acids (FAs)) and chemical composition of HAs after exogenous organic materials application, using elemental analysis and UV-VIS and FT-IR spectroscopies.

Materials and Methods

Sample preparation

Surface soil (0–15 cm) of a Haplic Cambisol was collected from locality Vatin (Mendel University School Research Stationary, Brno, Czech Republic), air-dried, and sieved (< 1 mm). Soil pH (KCl) was 4.7 and electric conductivity was 200 $\mu\text{S cm}^{-1}$. Total organic C content was 16 mg g^{-1} . Detailed characterization of the Haplic Cambisol used in pot experiment is given in Pospisilova et al. (2011).

For pot experiments we used 835 g of the mineral soil and 50 g of the selected soil conditioner, except lignohumate. Lignohumate was applied in dose 5 g to 835 g of soil, because of high contents of soluble salts. All variants were performed in triplicate. Lettuce (*Lactuca sativa*) was chosen as the tested plant. Experiments were carried out in phytotron CLF PlantMaster (Wertingen, Bavaria, Germany), with the following regime: daytime temperature, 20°C; night time temperature, 18°C; air moisture, 65%; duration of daytime, 12 hours; and intensity of lighting, 300 $\mu\text{m}^{-1} \text{s}^{-1}$. Drinking water was supplied to plant every 3–5 days. After two months, lettuce was harvested and soil was used for the following analyses.

Fractionation and analyses of HS

Fractionation of humus was conducted according to Kononova-Belchikova method (1963). In brief, HAs and FAs were extracted from 5 g soil sample with 100 mL of 0.1 M NaOH-0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ (1:1, v/v) solution by standing at room temperature for 24 h. The soil residue was separated by centrifugation at 2800 g for 10 min and washed with 50 mL of extractant three times. The washings obtained by centrifugation were combined with the extract and refilled to 250 mL with the extractant. The sum of HS (= HAs and

FAs, indicating alkali-soluble organic materials in the extract here) was determined using an aliquot (25 mL) after evaporating by wet digestion (Nelson and Sommers, 1982). Another 50 mL was acidified with 1 mL of conc. H_2SO_4 , warmed up to 50–60 °C for 30 min and left overnight to precipitate HAs. HAs were separated by centrifugation (2800 g for 10 min) and determined using the wet digestion method. All samples were analysed in triplicate (Pospisilova et al., 2011).

Powder sample of HAs was prepared as follows: 100 g of soil sample was washed with 1000 mL of 1.2 M HCl by stirring for 1–2 h (decalcination process). After confirmation of negative reaction for CO_2 (detected by seeing no bubbles), the soil was rinsed with 0.05 M HCl until no more Ca^{2+} was detected when ammonium oxalate was added to the extract. The soil residue was further rinsed with distilled water until no more Cl⁻ was detected by AgNO_3 and shaken with 600 mL of 0.1 M NaOH at room temperature for 7–8 h. The suspended solution was allowed to stand overnight and centrifuged at 5000 g for 15 min. Humic acids in the supernatant solution was precipitated by acidifying it to pH = 1 with conc. HCl and standing overnight. Coagulated HAs were centrifuged, dissolved in 600 mL of 2 M NaOH, and allowed to stand overnight. Dissolved HAs were precipitated by adding conc. HCl to pH = 1 and standing overnight. Coagulated HAs were centrifuged, dissolved in 600 mL of 0.1 M NaOH, allowed to stand overnight, and reprecipitated by the same manner as above. Coagulated HAs were centrifuged (3500 g for 15 min), rinsed with distilled water several times, and centrifuged again. Then HAs were shaken with mixture HCl + HF (950 mL H_2O , 5 mL conc. HCl, and 5 mL conc. HF), dialyzed against distilled water until chloride-free, and freeze-dried (Pospisilova et al., 2011).

C, H, and N contents in HAs were determined by CHNS elemental analyser PE2400 (Perkin Elmer, Waltham, MA, USA). The oxygen content was calculated as the difference: $\text{O}\% = 100 - (\text{C} + \text{H} + \text{N})\%$, and the data obtained were corrected for the moisture and ash content. Sulphur content was not determined.

UV-VIS spectra of the alkali-extracted HS were measured without dilution using Varian Cary 50 Probe spectrometer within the range of 300–700 nm (Varian, Mulgrave, Victoria, Australia).

FT-IR spectra of isolated HAs were recorded over the range of 4000–500 cm^{-1} using homogenous

mixture of 100 mg of samples and 300 mg dried KBr (spectroscopy grade) without any pressure. Spectra were recorded on Nicolet Avatar 320 FT-IR spectrometer (Thermo Nicolet Corporation, Madison, WI, USA) operating with Smart Diffuse Reflectance accessory (Nicolet, Madison, USA) under the following operating conditions: absorption mode, KBr background, peak resolution of 4 cm⁻¹, 256 scans, and data spacing of 1.929 cm⁻¹. The areas of absorption bands were integrated with spectrometer software (Omnic, version 6a, Thermo Fisher, Waltham, USA) and defined as intensities according to Demyan et al. (2012).

Metal contents in bulk soil, isolated HAs, and lettuce harvested from all the treatments were analysed using atomic absorption spectroscopy (AAS; ContrAA 700 Analytik, Jena AG, Jena, Germany).

Results and Discussion

Plants from each variants, as we mentioned before, were very similar in shape, color and size, when compared with control. Composition of alkali-

extracted HS after exogenous organic materials application is given in Table 1. All the organic amendments increased total organic carbon and HS (sum of HAs and FAs) contents in soil and the lignite applied soil showed the maximum values. These results were also suggested by UV-VIS spectroscopy (Fig. 1). The highest absorbance of alkaline-extracted HS in UV-VIS spectral range was observed for the lignite applied soil.

Elemental composition of the isolated HAs is given in Table 2. The highest C content and lowest O content was observed in the HAs from the biochar applied soil. On the other hand, the HAs from the digestate or compost applied soils showed the lowest C and highest O contents.

Figure 2 shows that the FT-IR spectra of the isolated HAs have the following absorption bands derived from: aromatic C=C groups at 1620–1546 cm⁻¹; amido C=O groups at 1655–1654 cm⁻¹; carboxyl C=O groups at 1720–1690 cm⁻¹; aliphatic CH, CH₂, and CH₃ groups at 2942–2920 cm⁻¹; and O–H groups at 3500–3200 cm⁻¹. Differences in the HA samples were observed in the peak intensities of aliphatic C–H

Table 1 Average fractional composition of soil humic substances after exogenous organic materials application.

Treatment	TOC* (mg kg ⁻¹)	Alkaline-extracted HS* (mg kg ⁻¹)	HAs* (mg kg ⁻¹)	FAs* (mg kg ⁻¹)	HAs/FAs
Control	14.5	4.5	1.2	3.3	0.4
+ Biochar **	21.0	4.7	1.5	3.2	0.5
+ Compost**	20.0	5.0	2.1	2.9	0.7
+ Digestate**	22.5	6.1	1.8	4.3	0.4
+ Lignite**	35.0	8.7	5.3	3.4	1.6
+ Lignohumate***	21.0	6.0	3.3	2.7	1.2

*TOC, Total organic carbon content; HS, Sum of HAs and FAs; HAs, Humic acids; FAs, Fulvic acids. **Mixture ratio was 835 g of bulk soil and 50 g of amendment. ***Mixture ratio was 835 g of bulk soil and 5 g of lignohumate).

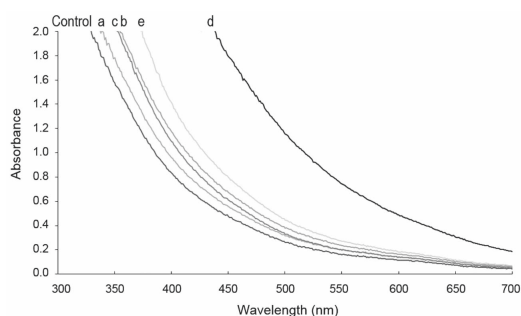


Figure 1. UV-VIS spectra of alkali-extracted humus samples from (a) biochar, (b) compost, (c) digestate, (d) lignite, and (e) lignohumate applied soil.

Table 2 Elemental composition of isolated humic acids (atomic percentage).

Treatment	%C	%H	%N	%O
Control	35.5	41.7	2.4	19.4
+ Biochar	46.2	36.7	2.2	14.5
+ Digestate	37.5	40.4	2.7	19.5
+ Lignite	39.3	39.2	7.0	14.5
+ Lignohumate	39.1	42.0	0.9	18.1
+ Compost	37.5	41.0	2.5	19.1

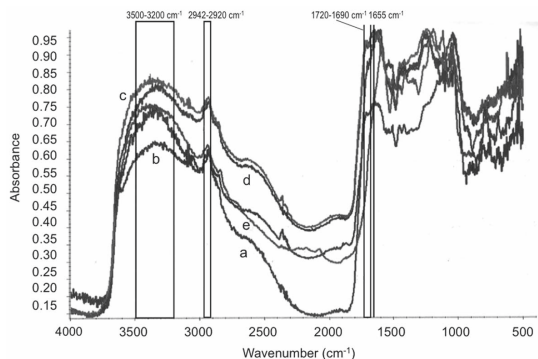


Figure 2. FT-IR spectra of isolated humic acid samples from (a) biochar, (b) compost, (c) digestate, (d) lignite, and (e) lignohumate applied soils.

and O–H groups, which were larger in the HAs from compost- and lignohumate-applied soils (Table 3; integral limits A). Prevalence of aromatic groups was observed in the HAs from biochar- and lignite-applied soils (Table 3; integral limits B and C). Index of HA stability was calculated according to Demyan et al. (2012) as a ratio of the sum of the integrated areas of 1660–1580 cm^{-1} and 1546–1520 cm^{-1} to the integrated area of 3010–2800 cm^{-1} (Table 3). The stability index of HAs from the soils with exogenous organic materials was larger in order: compost > digestate > lignohumate > lignite > biochar. Hydrophobicity index was also calculated according to Demyan et al. (2012) as a ratio of the integrated area of 3010–2800 cm^{-1} to that of 1740–1600 cm^{-1} . The results indicated that the hydrophobicity index of HAs was lower in the compost and digestate amended soils. These results suggested that HAs after compost or lignohumate application are more labile and thus nutrients in them can be easily delivered to plant roots and soil microorganisms. On the contrary, HAs after lignite or biochar application were more stable.

Table 3 Integral limits in FT-IR spectra and stability index* of isolated humic acids.

Treatment	Integral limit A (3010–2800 cm^{-1})	Integral limit B (1660–1580 cm^{-1})	Integral limit C (1546–1520 cm^{-1})	Index stability [(B+C)/A]
Control	6.0	0.8	0.1	0.15
+ Biochar	5.2	2.0	0.2	0.42
+ Compost	6.2	0.9	0.01	0.15
+ Digestate	5.6	1.3	0.02	0.24
+ Lignohumate	6.2	2.0	0.2	0.35
+ Lignite	5.6	2.2	0.2	0.43

*Demyan et al. (2012).

Metallic element contents in soil, isolated HAs, and lettuce were also compared (data not shown). Haplic Cambisol (without amendments) had originally low content of micro and macro elements. Although the contents of micro and macro elements in soil, lettuce, and HAs increased after exogenous organic materials were applied, none of toxic elements concentration in soil or lettuce was overstepped.

Conclusions

We can conclude that the application of exogenous organic materials directly influenced soil HS composition and chemical characteristics of HAs. Stability and hydrophobicity of HAs were lower in the soil amended with compost or lignohumates, which means more labile and nutrients for plant roots and soil microorganisms. Soil amended with digestate, lignite or biochar has less labile aliphatic groups and were more stable.

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