

A comparative study of the properties of industrially produced humic substances

M. Klavins^{1,*}, K. Upska^{1,2}, A. Viksna², M. Bertins², L. Ansone-Bertina¹ and J. Krumins¹

¹University of Latvia, Department of Environmental Science, 19 Raina Blvd, LV-1586 Riga, Latvia

²University of Latvia, Department of Analytical Chemistry, 19 Raina Blvd, LV-1586 Riga, Latvia

*Correspondence: maris.klavins@lu.lv

Abstract. Humic substances (HSs) are produced industrially in large quantities from low rank coal, weathered coal, peat, also from soils, composts and other sources. Considering that the applications of industrially produced HSs also include food, pharmaceutical applications and environmental technologies, it is important to evaluate their composition and quality and to identify their sources. The aim of the present study is to compare the properties of industrially produced HS samples. HSs were characterised using spectroscopic and other methods. For the identification of origin of HSs, different methods can be used, such as elemental analysis and ratios of light stable isotopes. The results of the study indicate that many industrially produced HSs are of poor quality (low concentration of basic substance, admixture of undesirable substances, pollutants, no quality indications). In this situation, rigorous quality control should be implemented, providing detailed characteristics of the product. The composition of materials suggested for agricultural applications has not been analysed much. Most of the studied materials were designated as HAs, followed by fulvic acids (FAs) and HSs. However, an analysis of the humic matter types indicates that the majority of substances offered on the market are in fact mixtures of HAs and FAs; so, it would be more appropriate to designate them as HSs or their salts. This study identifies the main quality problems of industrially produced humic substances: 1) lack of strict quality indicators, 2) absence of indication of source materials/origins of HSs.

Key words: peat, humic substances, humic acids, characterisation, spectroscopy.

INTRODUCTION

Humic substances (HSs), being a refractory part of natural organic matter, are compounds with high molecular mass (from < 1,000 up to several millions Da) and acidic nature due to presence of carboxylic and phenolic hydroxyl groups (Tan, 2014). HSs are products of decay of living organic matter formed in its degradation reactions and can provisionally be classified as humic acids (HAs), fulvic acids (FAs) and humin, depending on their solubility (Stevenson, 1994). Both degradation and synthetic processes in decaying organic matter are described as humification. Humification is a process of the transformation of organic molecules of living organic matter into groups of substances with similar properties – humic substances. All these processes are

important for understanding the carbon cycle and transformation processes of originally living matter (Ciais et al., 2013). HSs can be extracted from the source materials using alkaline compounds, ion exchange and other techniques; however, their structure is changed during the isolation and purification process (Tan, 2014). As HSs form from any living organic matter, they can be found not only in any organic fossil but also in any recent organic decay product. Typically, HSs are extracted from low rank coal (lignite, leonardite and others), weathered coal, sediments of waterbodies, peat, also from soils, composts and other organic matter degradation products. Despite a long lasting history of research of humic matter, many questions about humification processes and about structure and properties of HSs are still open, including questions concerning the relation of properties of HSs to their biological precursor material, humification conditions and length (age of HSs).

Nowadays HSs are produced industrially in quantities of hundreds (maybe even thousands and more) of tonnes, first of all for agricultural applications. They are produced worldwide from all types of source materials. Since the major source of HSs is soil, they are an important material in various areas of agriculture, such as soil chemistry, fertility, plant physiology and others. At the same time, HSs can be considered as an important resource, because they can be extracted in industrial amounts from sources where they are abundant and then applied to achieve or increase their positive impacts. The role of HSs in soil environments and their possible use in agriculture have been examined in reviews (Nardi et al., 2002; Peña-Méndez et al., 2005; Calvo et al., 2014; Olk et al., 2018). Considering that the applications of industrially produced HSs also include food, pharmaceuticals and environmental technologies, it is important to evaluate their composition and quality and to identify their sources. Until now, properties of HSs of different origins have been analysed; however, their comparative studies have not been done.

The aim of the present study is to compare the properties of samples of industrially produced HSs with ones isolated from known source materials and with reference humic samples as well as to examine approaches for identification of HS sources.

MATERIALS AND METHODS

The studied humic substances containing samples

In the study, HSs produced in China, USA, Russia, Germany, Belarus, Finland, Switzerland, Mexico, Latvia and Poland were used (to avoid conflicts of interest, the producers and trade names of the studied products were kept anonymous). Humic substances were analysed as obtained (in solid state).

The source materials of industrially produced HSs included low rank coal (such as leonardite and lignite), peat and, in some cases, waste products of cellulose production (lignosulphonates). For comparison, HAs and potassium humate were isolated from well characterised samples of raised bog peat, fen peat, soil and vermicompost. HAs were extracted and purified, using procedures recommended by the International Humic Substances Society (IHSS) (Tan, 2005). IHSS reference materials were also used (<https://humic-substances.org/#products>). In this study industrially produced HSs are classified by their source material and producer country: coal – Czech Republic (HA), Germany (HA), USA (HA), China (FA), Russian Federation (HS), Mexico (FA); peat – Belarus (HA), Latvia (HA), Finland (HA), USA (HA), USA (FA), Finland (FA);

water – USA (FA), Latvia (FA), Estonia (FA), Lithuania (FA), Russia (FA), Latvia (HS); lignite – Russia (HS); soil – USA (HA), Latvia (HS); unknown – Switzerland (HA).

Most of the studied materials were designated as HAs, followed by FAs and HSs. Altogether, 32 industrially produced HS samples of different types and origins and 18 reference samples were studied.

Characterisation of humic substances

Elemental analysis (C, H, N, S and O) was carried out using an Elemental Analyzer Model EA–1108 (Carlo Erba Instruments), and the found values were normalised with respect to ash content. Ash content was measured after heating 50 mg of each humic sample at 750 °C for 8 h.

Atomic ratios were calculated from elemental analysis using the Eqs (1) and (2),

$$O/C = \frac{(M_c \cdot O\%)}{(M_o \cdot C\%)} \quad (1)$$

$$H/C = \frac{(M_c \cdot H\%)}{(M_H \cdot C\%)} \quad (2)$$

where MX is the element molecular mass, and X% is the percentage of the element (C, O, H) in the sample.

UV–Vis Spectroscopy

UV–Vis spectra were recorded on a Thermospectronic Helios γ UV (Thermo Electron Co) spectrophotometer in a 1 cm quartz cuvette. The E4/E6 ratio (Chen et al., 1977), i.e. ratio of absorbances at 465 and 665 nm, was determined for 5 mg of a humic sample solution in 10 mL of 0.05 N NaHCO₃.

Fluorescence Spectroscopy

Fluorescence spectra were recorded using a Perkin Elmer LS 55 fluorescence spectrometer on the aqueous solutions of each sample at a concentration of 25 mg L⁻¹, adjusted to pH 7 with 0.5 M HCl. Emission spectra were recorded at a fixed excitation wavelength of 350 nm (scan speed 500 nm min⁻¹, with 10.0 nm slit over the wavelength range of 380 to 650 nm). The ratio of fluorescence intensity of 460 to 510 nm (I₄₆₀/I₅₁₀) was used as a humification indicator, as previously suggested by Milori et al. (2002).

Excitation-Emission Matrix (EEM) Fluorescence Spectroscopy

Excitation-emission matrices were recorded for humic substances (as obtained from producer, reference samples or isolated) aqueous solutions using an Aqualog total luminescence spectrometer. Matrices were recorded in the excitation range of 250–600 nm and emission range of 250–600 nm with 5 nm increments to reflect major fluorescent components in HAs. Data were processed with parallel factor analysis.

Determination of inorganic element content

1.00 g humic matter samples were weighed into Teflon tubes, adding 8 mL of 69% HNO₃ and 2 mL of H₂O₂. The tubes were closed to provide high pressure. The prepared samples were mineralised using a microwave sample preparation system (Milestone Advanced Microwave digestion system, Ethos Easy) at 200 °C for 20 min. The resulting

samples were filtered through filter paper and diluted to 50 mL with deionised water. The concentrations of inorganic elements in HSs were determined by inductively coupled plasma spectrometry with optical emission detection (ICP-OES), using a Thermo Scientific iCAP 700 series ICP spectrometer.

FT-IR absorption spectra of humic substances

A Fourier Transform Infrared Spectrophotometer Shimadzu IR-Tracer 100 was used with the following settings: wave number: 4,000–400 cm^{-1} , resolution: 2 cm^{-1} , the number of scans: 20. 3 mg of the sample of the studied humic substance and 200 mg of KBr powder were mixed, and 30 mg of the mixture was weighed and compressed into a tablet. Spectra were taken in the infrared spectrum of 400 cm^{-1} to 4,000 cm^{-1} .

Determination of stable isotope ratios of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$

For determination of the ratio of C and N stable isotopes, samples were weighed into tin capsules on an analytical balance with an accuracy of ± 0.01 mg and within a weight range of 0.80 to 1.20 mg. Glutamic acid standards were used for calibration (0.20, 0.50, 0.80, 1.00 and 1.50 mg). For determination of the ratio of O stable isotopes, silver capsules were used, and sucrose was used for calibration. The measuring system consisted of a EuroVector Euro EA3000 element analyser (a quartz reaction tube filled with chromium (III) oxide and silvered cobaltous oxide (1,030 $^{\circ}\text{C}$), a quartz reduction tube filled with copper shards (650 $^{\circ}\text{C}$) for determination of C and N, and an HT-PyrOH block with a ceramic outer tube and a glassy carbon inner tube filled with glassy carbon chips (1,450 $^{\circ}\text{C}$) for O analysis. Measurements were taken using a Nu HORIZON mass spectrometer by Nu Instruments (acceleration voltage: 5 kV, mass range: 2–100 Da, mass dispersion: > 30 cm). The results were processed by the Nu Stable Control Software v1.69.

Statistical analysis of data

One-way analysis of variance (ANOVA) with post hoc Tukeys HSD test ($p < 0.05$) was performed using SAS JMP®, Version 15 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Most of the studied materials were designated as HAs, followed by FAs and HSs (data not shown). However, the analysis of the humic matter types (HAs or FAs, based on the solubility at pH 2) indicated that the majority of the substances offered on the market are in fact a mixture of HAs and FAs; so, it would be more appropriate to designate them as HSs or their salts.

The analysis of the amounts of organic matter in the compositions of humic materials indicated that only few of the industrially produced HSs indeed predominantly consist of organic matter, while the majority contain significant amounts of inorganic substances, as indicated by the ash content in the compositions: the ash content in some compounds reached even 35%; on average it was 6% (Fig. 1). So high concentrations of inorganic substances in industrial humic products is an indicator of poor quality of prepared products as in pure humic substances only traces of inorganic substances has been found. The highest ash content was characteristic of the HSs isolated from lignite, leonardite and weathered coal.

An important quality indicator of HSs for agricultural applications can be the presence of inorganic elements. The inorganic components of HSs mostly are major elements. For instance, Na (up to 44 mg g⁻¹) and K (up to 100 mg g⁻¹) in highest concentrations can be found in such preparations as potassium and sodium humates. Notably, these elements in high concentrations can even be found in products sold under such trade names as ‘Humic Substance’ and ‘Humic Acid’. In humic products, Al, Ca and Fe can also be found in high concentrations (up to 33 mg g⁻¹, 20 mg g⁻¹ and 5 mg g⁻¹ respectively). Further, high concentrations (up to 70 mg g⁻¹) of S were found in products obtained from cellulose production waste materials (lignohumates). High concentrations of P (up to 5 mg g⁻¹) were found in several products, indicating amendments of phosphorus fertilizers. Next, toxic trace elements can be found in relatively high concentrations in some humic products: for example, 197 µg g⁻¹ Sr in an industrially produced FA obtained from lignite, 50.8 µg g⁻¹ Cr and 30.7 µg g⁻¹ Cu in a HA from leonardite, 27 µg g⁻¹ As and 51 µg g⁻¹ Ba in a lignohumate preparation, as well as high concentrations in other products.

Comparatively, in the reference samples and in the samples isolated in laboratory, trace elements were found in trace concentrations (for elements such as Na, Ca, Mg, Fe < 0.1 mg g⁻¹, but for trace elements, such as Pb, As, Co, Ni < 0.2 µg g⁻¹).

To characterise elemental composition, atomic ratios (Van Krevelen graphs) were used. H/C versus O/C ratios (Fig. 3) illustrate the relative percentage of aromaticity in HS structures. By far the most important

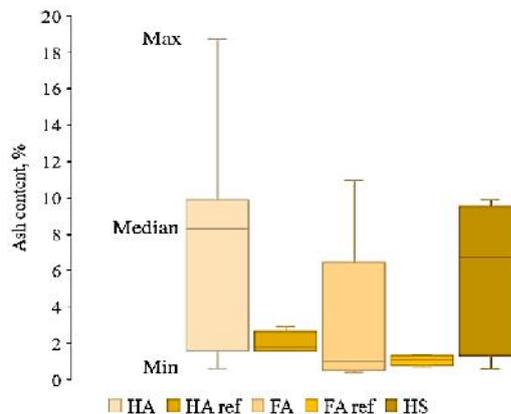


Figure 1. The box plot of ash content in the studied industrially produced (n = 32) and reference (n = 18) HAs, FAs and HSs (Max – the highest value; Min – the lowest value; Median – the mid-point of the data).

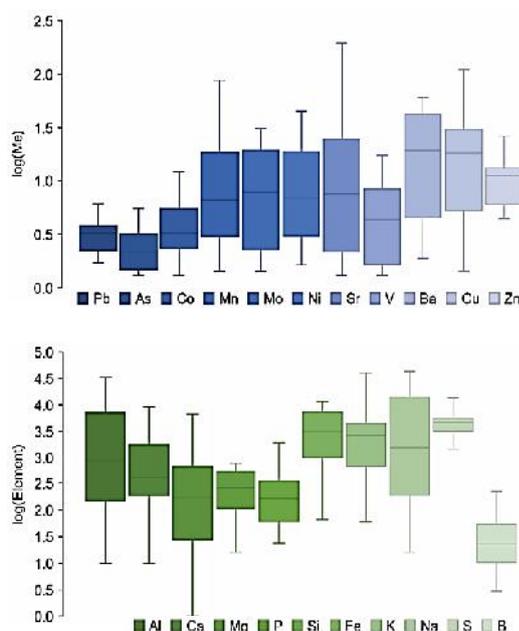


Figure 2. Box plot of major and trace element concentration (µg g⁻¹) in the studied industrially produced (n = 32) humic substances. Error bars represent the highest and lowest values. The median of data is presented. (log(Me) – the logarithm of metal concentration; log(Element) – the logarithm of element concentration) (results are represented in logarithmic values on account of large concentration of element presented in sample).

reactions influencing the HS structure are demethylation (split off of $-CH_3$ groups), dehydration and decarboxylation (split off of $-COOH$ groups). Van Krevelen graph characterises the depth of the humification process (Klavins et al., 2019). The demethylation, dehydration and decarboxylation processes result in an increased aromaticity and an increased humification degree and is characteristic of HSs isolated from deeply humified materials, such as lignite and leonardite. Soil, peat and aquatic HSs demonstrate a lower humification degree and their application for agricultural purposes (as biostimulant, soil amendment, fertiliser) is more beneficial, than of material which has undergone deep transformation process (Nardi et al., 2002).

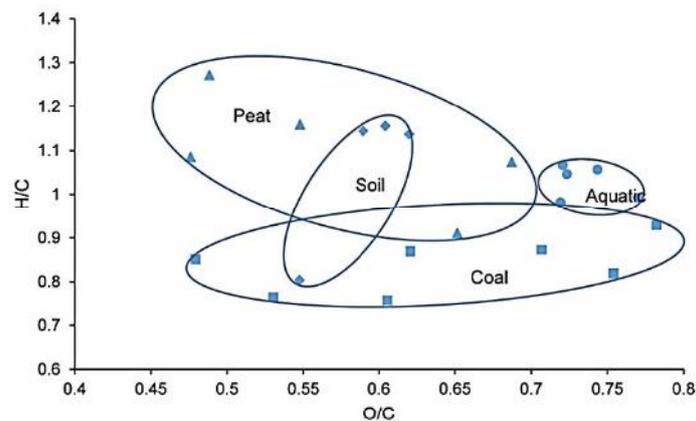


Figure 3. Van Krevelen graph of industrial and reference HS composition element H/C ratio versus O/C ratio (Peat (\blacktriangle), Coal (\blacksquare), Soil (\blacklozenge), Water (\bullet), Lignite (-)).

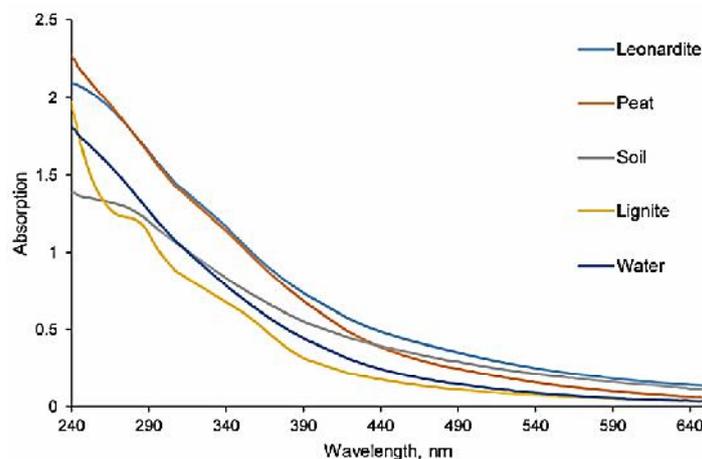


Figure 4. UV-Vis spectra of the studied HSs depending on their origin.

The UV-Vis spectra of the FAs and HAs obtained (Fig. 4) do not have well expressed sorption lines and decrease with increasing wavelength. However, the HSs isolated from highly transformed organic material demonstrate a shoulder at 280 nm characteristic of aromatic structures (not so expressed for HSs of other origins). The UV

spectra of both HAs and FAs are relatively similar. The UV sorption ratios at 465 and 665 nm are used as condensation of aromatic groups measure (aromaticity), but they also reflect the particle size and molecular weight of HS (Chen et al., 1977). The E4/E6 ratio for the studied HSs varied from 3.46 to 16.35, demonstrating high variability of the studied HSs. The highest E4/E6 ratios were found for the aquatic FA as well as for HSs from soil as described also in literature (Stankevica et al., 2019). The E4/E6 ratio for FAs is higher than for HAs (Zalba et al., 2016), thus indicating lower molecular weight, higher acidity and thus also better accessibility for plants.

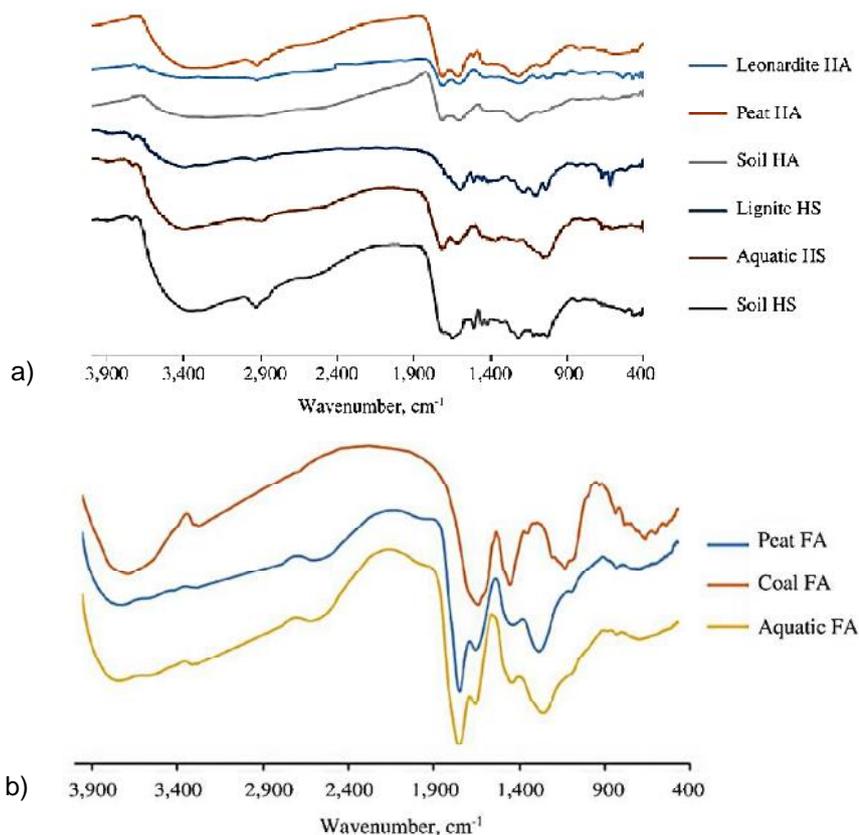


Figure 5. Fourier transform infrared (FTIR) spectra of the studied HAs, HSs (a) and FAs (b) depending on their origin.

The IR spectra of the FAs (Fig. 5, b) and HAs (Fig. 5, a) obtained have general similarity. The FTIR spectra of the analysed HSs can be split by regions, depending on the informativity and the presence of important functional groups. The Fourier transform infrared spectra (Fig. 5, a, b) can also be used to discriminate between FAs and HAs and to identify the origin and quality of HSs. However, the presence of mineral substances and, possibly, other mixtures delimit the possibilities to obtain good quality spectra. Absorption bands in the spectral region of 3,600–2,800 cm⁻¹ are broad. Absorbance in this spectral region is determined by the presence of -OH groups. Sorption at the wavelengths of 2920 and 2,860–2,850 cm⁻¹ characterise the presence of the CH₃-

and CH₂- groups. The FTIR spectra data of soil HSs show that methylene groups - (CH₂)_n- exist as short alkane chains (n < 4). Intensive sorption at 1,700 cm⁻¹ (1,725–1,700 cm⁻¹), is common for carbonyl groups in aldehydes, ketones and carbonic acids.

Fig. 6 shows a separation between the four origin of HSs. ANOVA results represent that the values of δ¹⁸O and δ¹⁵N of the soil and water origin of HSs relate to the one group (C). According to statistical analyses the values of δ¹³C of peat origin of HSs relate to two groups – A (coal) and B (soil).

The isotope ratio measurements show a difference of up to 5‰ for each HS group (Fig. 6), which allows to distinguish them one from another. The C, N and O isotopic patterns of water and soil HSs are very similar. The reason for that most probably is their common source of origin, i.e. water. HSs leach into water bodies from soil. Also, the C and N isotopic patterns of coal and peat HSs are very similar, except for δ¹⁸O, which is dependent mostly on temperature changes and, in this case, is indicative of different environmental temperatures at the time of coal and peat HS formation time. For all sample groups, δ¹⁵C changes within the range from -25‰ to -30‰. Since C is accumulated in plants mostly from air by means of photosynthesis, as the δ¹³C values show, all humic substance groups are formed from the C₃ type plants. The δ¹⁵N values depend on many factors, including the isotopic composition of soil in different geographical locations. If we assume that the isotope ratio is fixed at the time of formation of a humic substance, then, taking into account the N₁₅ discrimination processes in plants, larger δ¹⁵N values, as in the soil and water HS, are indicative of the lower amounts of N available for plants at the time of formation of the humic substance.

The excitation-emission matrix fluorescence spectra illustrate the fluorescent components in HSs, HAs and FAs (Figs 7, 8, 9). In a mixture of HAs and FAs, the most intensive fluorescence signal comes from FAs (approximately 350 nm excitation and 450 nm emission), while the fluorescence intensity of HAs (present at approximately

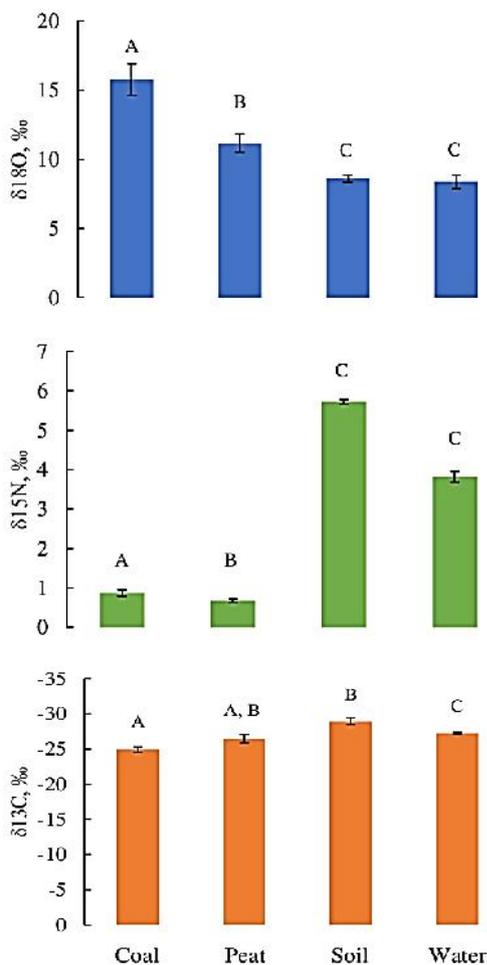


Figure 6. Isotope ratios of the studied HSs depending on their origin. Error bars represent standard deviation ($n = 5$). Letters above bars represent significant differences among different groups (Tukeys HSD $p < 0.05$).

470 nm excitation and 540 nm emission) is comparatively less expressed. FAs have a stronger fluorescence signal due to higher phenolic group content, while HAs have more condensed structures. EEM spectra can be used as a tool to identify the type of an HS: whether it is an HA, or an FA, or a mixture of both. Moreover, EEM spectra can be used for the identification of other groups of substances with fluorescent characteristics, for instance, if there is chlorophyll in vermicompost, and the like. In this study, the excitation-emission fluorescence spectra were recorded at the excitation and emission range between 250 and 650 nm, and the data show evident differences between the fluorescence characteristics in HAs, FAs and mixtures of both.

Lignite HA has a typically weak fluorescence peak at 470 nm excitation and 540 nm emission (Fig. 7). At the same time, the EEM spectra results also show the presence of low quantities of FA in this industrial HA. Additionally, there is a signal of some plant remnants, which shows that lignite is originally formed from peat/plants.

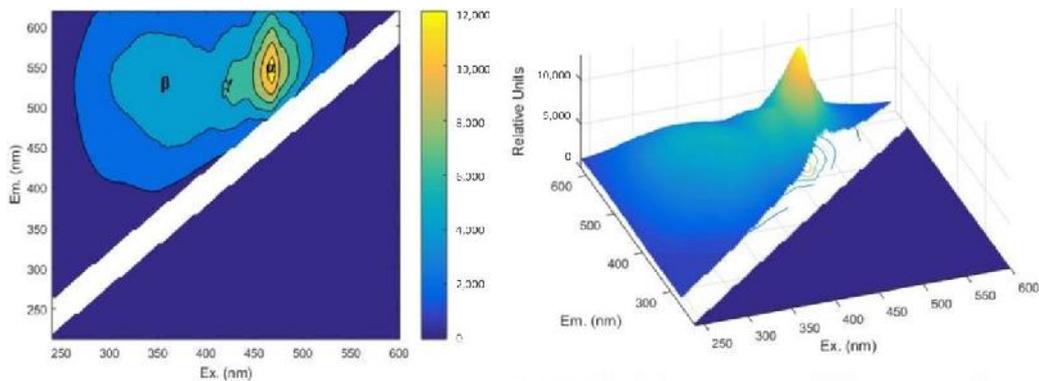


Figure 7. Lignite HA 2D (left) and 3D (right) EEM spectra.

Lignite FA (Fig. 8) has a typical, intensive and complex fluorescence signal at around 350 nm excitation and 450 nm emission. This is due to high phenolic concentration. However, the data also show a weak HA signal. HA could have got into the FA solution during the isolation procedure.

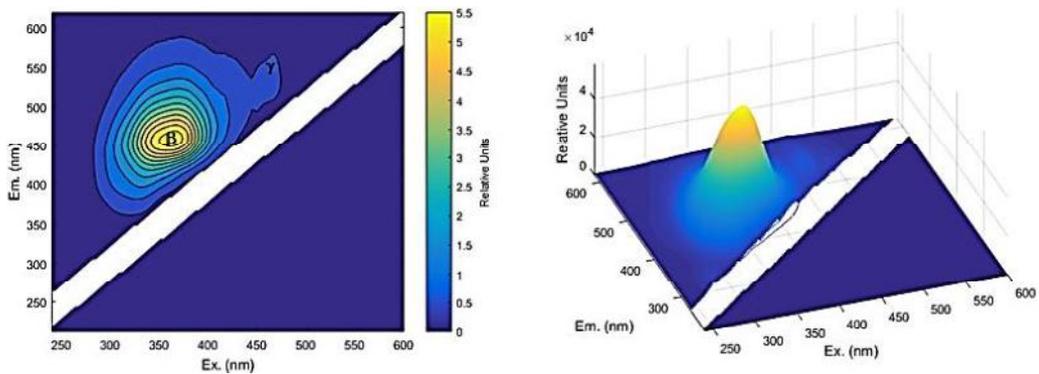


Figure 8. Lignite FA 2D (left) and 3D (right) EEM spectra.

The excitation-emission matrix of soil HSs shows a mixture of HA and FA fluorescence signals and indicates small amounts of FA in the soil (Fig. 9).

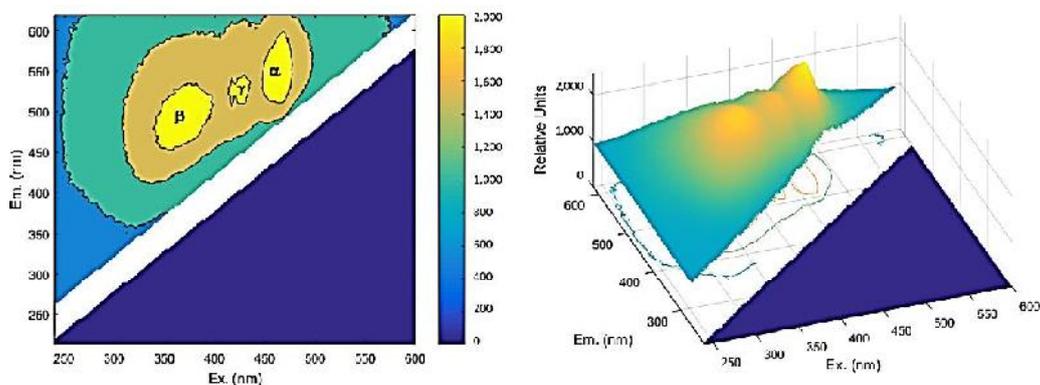


Figure 9. HS (isolated from soil) 2D (left) and 3D (right) EEM spectra.

CONCLUSIONS

As HSs increasingly become an industrially produced material for agricultural applications, their quality analysis and control is of utmost importance. The quality assessment of HSs is yet more important in view of the fact that there are no strict definitions of HSs and regulations of their quality control. The properties of HSs depend on their origin, and significant differences have been observed depending on the source materials. Major differences are common for freshly humified humic substances (isolated from soil, vermicompost, but also peat) on one hand, but on the other – for humic substances isolated from highly humified materials, such as lignite, leonardite and others. In addition, the comparison with the IHSS reference materials can be used for research purposes and not so much for describing the properties of industrially produced products. Most industrially produced HSs are very poorly characterised, sometimes chemical characteristics are absent, including, for example, the amount of the claimed product (HS), ash content and other parameters. This study reveals the main quality problems of industrially produced HSs: 1) lack of strict quality indicators; 2) absence of indication of a source material/origin of the HS.

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