

NATURAL ORGANIC MATTER: CHEMISTRY, FUNCTION AND FATE IN THE ENVIRONMENT

Source indicator of commercial humic products: UV-Vis and fluorescence proxies

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Abstract

Purpose Over the last decades, commercial humic products (HPs) from various resources found multiple applications in agriculture and environmental technologies. The key factor defining chemical properties and biological activity of HPs is their organic matter origin. Thus, there is a need to find diagnostic criteria for differentiating organic sources of HPs. The objective was to determine indicators using absorption and fluorescence spectra in combination with derivative spectroscopy that might serve as tools to compare HPs from various environments, both as bulk materials and their humic acid (HA) fractions.

Materials and methods HA-like fractions were isolated from commercially available humates, industrially manufactured from a number of raw source materials, such as: fossils (brown coal and leonardite), peat, lake-bottom sediment, and organic waste material (lignosulphonate). They were analyzed using chemical, fluorescence, and UV-Vis measurements. Elemental composition and ash content were determined. The "blue shift" of fluorescence band was defined with excitations at 310 and 270 nm or 355 and 310 nm. The following indices have been calculated from absorption spectra: specific absorbances normalized by the HA content, absorbance ratios $E_2:E_3, E_4:E_6, E_{270/400} E_{280/472}, E_{280/664}, \Delta \log K$, and the spectral slope ratio Sr. In addition, we implement new indices:

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Olga Yakimenko iakim@soil.msu.ru the ratio of amplitudes in the first-order (FDR_{280/240}) and in the second-order derivative spectra (SDR_{267/280}).

Results and discussion Absorption spectra of HA-like fractions isolated from HPs varying in organic matter origin demonstrated similar wavelength-dependent character. However, some HA-like fractions demonstrate weak maxima in the derivative spectra with enhanced spectral resolution at 230 and 280 nm. This effect is most probably due to the presence of low molecular weight phenolic compounds, products of lignin decomposition. HA-like fractions isolated from peat- and lignosulphonate-derived HPs show fluorescence maximum at shorter wavelengths than HA-like fractions from coal- and sapropel-derived HPs. Under excitation at 310 nm, certain peat- and lignosulphonate- originated samples manifest significant "blue shift" of emission band. In contrast, HA-like fractions from coal- and sapropel-derived HPs exhibit excitationindependent fluorescence band position or its small shift to longer wavelengths.

Conclusions Information extracted from absorption and fluorescence spectra can be useful to discriminate organic matter source for HPs from coalified materials (fossils), peat, and lignosulphobate. Indices with the highest descriptive ability, showing strong loadings in principal component analysis, are as follows: specific absorbance SUVA₂₅₄, absorbance ratios $E_{270/400}$, $E_{280/472}$, derivative indices FDR_{280/240} and SDR_{267/280}, wavelength of fluorescence maximum, and the presence of "blue shift".

Keywords Absorbance \cdot Derivative spectroscopy \cdot Fluorescence spectroscopy \cdot Humic products \cdot Humus acids

1 Introduction

The benefits of humic substances (HS) for soils and possibilities for their multiple technological and environmental

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applications are widely studied (Stevenson 1994; Tan 2003; Perminova and Hertkorn 2005; Perminova et al. 2006). This fact provided a trigger for a world-wide production of industrial humic products (HPs). HPs are being manufactured from organic raw materials, mainly lignite, peat, lake bottom sediments, and organic waste materials. Currently, a huge variety of HPs is offered as organic fertilizers, plant growth stimulants, sorbents of toxic substances, or conditioners for degraded soils (Lobartini et al. 1992; Yakimenko et al. 2011; Sorkina et al. 2014; Pukalchik et al. 2015).

Industrial technologies of HPs' production differ from research analytical techniques used for HS isolation. Since the isolation procedure and the extractants used affect significantly the yield and properties of the obtained HS (Zaccone et al. 2007), both chemical characteristics (Malcolm and McCarthy 1986; Tan 2003; Lamar et al. 2014) and spectral-luminescent behavior of commercial HPs (Hemmingsen and McGown 1997; Fu et al. 2004) differ from HS from corresponding environments.

Organic matter (OM) genesis is a key factor that influences chemical properties and biological activity of HS. For HPs, it is especially important because to a large degree determines their potential for agricultural and environmental applications. This creates a demand for diagnostic criteria to differentiate HPs according to their origin and for screening of analytical tools, which could allow such a differentiation.

Chemical proxies have been widely applied to distinguish OM from peat, leonardite, and lignite fertilizers. Cavani et al. (2003) showed that humification parameters (degree of humification, humification rate, and humification index) and electrofocusing may be used to discriminate peat and lignite products, other successfully used ¹H-NMR, functional groups (Francioso et al. 2003), elemental composition, ¹³C CP/MAS NMR and Py-GC/MS data (Lu et al. 2000).

Another powerful tool to characterize and differentiate HS as function of their origin is spectroscopic analysis (Miano et al. 1988; Miano and Senesi 1992; Mobed et al. 1996; Senesi and D'Orazio 2005). Spectroscopic proxies have the advantages of high sensitivity, speed, and relatively low cost. Spectral properties of HS from different environments have been widely studied using UV-Vis absorption and fluorescence spectroscopy (Patsayeva et al. 1991, 1992; Trubetskoj et al. 2002; Alberts and Takacs 2004). Transformations of HS caused by microscopic fungi were monitored using spectroscopic data (Khundzhua et al. 2013). Many investigations show that the fluorescence spectra of humic acids (HA) excited by the UV light have maximum emission in the 500-520 nm, whether the one of fulvic acids is located at shorter wavelength, 445-465 nm (Senesi et al. 1991; Chen et al. 2003; Senesi and D'Orazio 2005; Sierra et al. 2005; Bertoncini et al. 2005). The wavelength of the emission maximum depends on the excitation wavelength (Donard et al. 1989; Patsayeva et al. 1995; Shubina et al. 2010; Gosteva et al. 2012). It was also showed that in some cases, the change of the excitation wavelength from 270 to 310 nm shifts the emission band maximum towards to the shorter wavelengths. Such effect of blue shift was first described by Sierra et al. (1994), and later, the blue shift of emission maximum was investigated in details for various samples of HS of aquatic or soil origin (Shubina et al. 2010).

Derivative spectroscopy is yet another helpful tool to analyze spectral bands and to resolve and discriminate scattering components. For instance, it was used to resolve components in absorption spectra of commercial HPs (Gosteva et al. 2012) or vibrational (IR absorption and Raman scattering) spectra of aqueous ethanol solutions (Burikov et al. 2012).

Unlike numerous studies on fluorescent properties of natural OM, spectral-luminescence properties of commercial HPs of various origins are very scantily investigated. Spectral properties of a number of HPs produced from different humic resources (fossils, peat, and lake-bottom sediment) have been studied in our previous research and revealed certain differences depending on OM sources. It was showed that both fluorescence and absorbance spectra of HPs can reflect their genesis and can be treated as a primary approach to monitor their structure and potential quality (Gosteva et al. 2012). Thus, at excitation at 355 nm, fossil-originated HPs showed the emission maximum at wavelengths about 470-500 nm and typical character of the spectral curves similar to ones of coal HA. At the same time, in plant residuederived HPs (from peat and bottom sediments), the emission maximum demonstrated a 20-30 nm shift towards shorter wavelengths, most probably due to the presence of certain proportion of non-humic compounds (Gosteva et al. 2012).

In the present study, we further investigate spectral properties of HPs in order to find an algorithm to differentiate them according to their origin. Complicated composition of industrial HPs, which contain not only HA-like but also fulvic-like fractions and non-humic organic substances (free carbohydrate, amino acids, carboxylic acids), significantly influences their spectral characteristics. Therefore, HA-like fraction, isolated from initial HPs, may provide more precise information about their nature and origin. It was reported (Senesi et al., 1991; Mobed et al. 1996; Albrect et al. 2011; Horst 2013) that distinct fluorescence properties of HS can provide useful diagnostic criteria for distinguishing between humic or fulvic acids from various sources. Development of such criteria based on absorption or fluorescence spectra for industrial HPs is important for their classification. This approach could not only contribute to understanding the chemical nature of HPs but might also be useful for the development of theoretical considerations on their application in agriculture or environmental technologies.

The objective of this study was to determine indicators using absorption and fluorescence spectroscopy in combination with derivative spectroscopy that might serve as enhanced, fast, and relatively inexpensive tools to compare aqueous solutions of industrial HPs from different environments in bulk and their HA-like fractions.

2 Materials and methods

2.1 Humic substances

We examined HA-like fractions isolated from commercially available HPs. These HPs were produced by industrial companies from a number of raw source materials differing in their OM origin and humification conditions: fossils (brown coal and leonardite), peat, lake-bottom sediment (sapropel), and organic waste material (processed lignosulphonate). Selection of HPs generally reflects the current situation on the global humic market: most of HPs are produced from fossil sources, less are from peat, and few brands offer products from organic waste materials.

Initial HPs are soluble sodium or potassium humates, which have been manufactured by producers basically using alkaline treatment of raw materials. For this study, we extracted HA-like fractions from initial HPs; in other words, commercial humates were purified and converted to H-form. Initial HPs were treated with 0.1 M NaOH at 1:100 ratio. Solutions stayed overnight until complete dissolution of HPs then were filtered against mineral precipitates. The filtrate was acidified with 6 M HCl to pH 1, and the precipitated HA-like fractions were separated from supernatant by centrifuging. Acid-soluble fulvic-like fraction (supernatant) was discharged. The HA-like fractions were suspended in deionized water and purified by dialysis until negative reaction on chloride-ion, dried, powdered in agate mortar, and kept in airtight vial.

The elemental composition of isolated HA-like fractions was determined using Elementar Vario EL 3 Analyzer. Ash content was measured after combustion in muffle furnace at 950 °C for 4 h until constant weight. Moisture content was determined after vacuum drying. Identification, origin, and elemental composition of HAlike fractions under investigation are summarized in Table 1. Properties of initial HPs have been described earlier (Gosteva et al. 2012). Brand names of HPs used are not provided.

2.2 UV-Vis spectroscopy

Both absorption and fluorescence measurements were made under room temperature for the solutions of HAlike fractions in aqueous phosphate buffer with pH 6.9 placed in quartz cuvettes with 1-cm optical path length and 5-mL volume. The UV-Vis absorption spectra of HA-like fractions in the range from 200 to 900 nm were recorded by Unico double-beam spectrophotometer at concentrations of HA in solution equal to 10 and 20 mg L^{-1} versus an aqueous phosphate buffer. The spectral shape of absorption curves for both concentrations was the same for each pair of samples while absorbances differed two times.

To characterize spectral properties, a number of indices have been used (Table 2). Some of them are widely used in NOMstudies (specific absorbances, E_4/E_6); others are mostly used for investigations of DOM (SUVA, $E_{270/400} E_{280/472}$, $E_{280/664}$, $\Delta \log K$, and spectral slope ratio Sr). But since the theoretical considerations behind all of these indices are similar for natural OM of various genesis, we used them to evaluate their descriptive ability in respect to HA-like fractions, derived from HPs.

In addition to indices calculated from original absorption spectra (dependences of absorbance values versus wavelength), we computed the first-order and second-order derivative spectra using differentiation of absorbance values with respect to wavelength. The first-order derivative manifests the wavelength intervals with bigger spectral slopes in initial absorption spectra. The second-order derivative is useful to separate the individual peaks of overlapping bands. The most characteristic feature of a second-order derivative is a negative band with minimum at the same wavelength as the nonresolved peak hidden in the initial absorption band (primary absorption spectrum used for analysis). The second-order derivatives are given in Fig. 2 as negative values along ordinate to show resolved spectral peaks as maxima not minima.

Using derivative spectroscopy, we suggested new indices derived from the first-order and second-order derivative spectra which are calculated as ratios of amplitudes taken at certain wavelengths. Thus, FDR280/240 is the ratio of amplitudes in the first-order derivative spectrum taken at 280 and 240 nm, and SDR267/280 is the ratio of amplitudes taken in the second-order derivative spectrum at 267 and 280 nm.

The FDR_{280/240} index is analogous to the spectral slope ratio (Sr; the ratio of spectral slopes for the intervals of 275-295 nm and 350-400 nm) but calculated in much more narrow spectral intervals (few nm instead of 20-50 nm). The meaning of another derivative index, SDR_{267/280}, is the ratio of resolved absorption peaks located at 267 and 280 nm. According to Zbytniewski and Buszewski (2005), the absorbance at 260-280 nm is due to lignin and quinone moieties, i.e., the material at the very beginning of transformation. The presence of shoulders between 250 and 300 nm in absorption spectra (Domeizel et al. 2004) explained as characteristic to aromatic or unsaturated compounds and their absorption by their double bonds C=C, C=O, and N=N. Therefore, the ratio of resolved peaks at 267 and 280 nm characterizes the samples from the standpoint of the prevalence of aromatic or unsaturated compounds in respect to lignin and quinone moieties. To check the steadiness of FDR_{280/240} and SDR_{267/280} indices, we computed them for both

Sample identity ^a	Source organic material	Ash (%)	Elemental composition ^b				Atomic ratio			
			С	Ν	Н	S	0	H/C	O/C	N/C
BC-EnK-HA	Brown coal	1.3	57.0	2.1	6.3	0.6	34.0	1.31	0.45	0.03
BC-EnNa-HA	Brown coal	3.4	61.2	2.2	4.1	1.6	31.0	0.80	0.38	0.03
BC-HumNa-HA	Brown coal	3.6	58.5	1.0	4.7	1.4	34.3	0.95	0.44	0.02
Le-PhK-HA	Leonardite	10.6	59.3	2.1	4.7	1.6	32.3	0.94	0.41	0.03
Pe-EcoK-HA	Peat	1.7	55.1	2.6	4.5	0.9	36.8	0.98	0.50	0.04
Pe-EdaNa-HA	Peat	3.6	48.7	2.7	5.2	1.6	41.9	1.27	0.65	0.05
Pe-FlexK-HA	Peat	1.3	58.4	1.6	4.5	0.9	34.5	0.92	0.44	0.02
Sa-BigK-HA	Sapropel	4.0	55.6	3.4	5.4	2.1	33.4	1.16	0.45	0.05
OW-LhK-HA	Lignosulphonate	4.2	57.9	0.9	5.5	7.6	28.0	1.14	0.36	0.01

Table 1 Identification, origin, and elemental composition of HA-like fractions

^a HA-like fractions, isolated from humic products, produced from brown coal (*BC*), leonardite (*Le*), peat (*Pe*), sapropel (*Sa*), and lignosulphonate (*OW*) ^b wt% on moist-free and ash-free basis

concentrations of HA-like fractions in solution, 10 and 20 mg L^{-1} , for all the samples.

2.3 Fluorescence spectroscopy

Fluorescence measurements were made under room temperature for HA-like fractions solutions in aqueous phosphate buffer with pH 6.9. Fluorescence emission spectra were recorded by luminescence spectrometer Solar CM 2203 under excitation at 270, 310, and 355 nm using spectral slit widths of 5 nm both for excitation and emission for the HA-like fractions in diluted solutions of aqueous phosphate buffer at concentration of 2 mg L⁻¹ to avoid inner-filtering and quenching effects. The choice of excitation wavelengths was based on our previous reports on HS fluorescence (Gosteva et al. 2012).

The shift of emission maximum, or "blue shift" value, was calculated as difference between fluorescence maximum positions λ max measured with excitation at 270 nm and 310, or 355 and 310 nm, respectively:

 $\Delta 1 = \lambda \max(270) - \lambda \max(310)$ and $\Delta 2$

 $= \lambda \max(355) - \lambda \max(310).$

2.4 Statistics

Principal component analysis (PCA) was carried out using Statistica 10 Software. For PCA, we considered two sets of data: eight variables describing elemental content and nine variables for the most descriptive spectroscopic characteristics (wavelength of emission maximum at $\lambda ex = 310$, value of "blue shift, SUVA, $E_2:E_3$, $E_{270/400}$, $E_{280/472}$, $E_4:E_6$, SDR_{267/280}, and FDR_{240/280}).

3 Results and discussion

3.1 UV-Vis spectroscopic analysis

Absorption spectra for HA-like fractions in visible region are flat curves without extremes with optical density decreasing monotonically with wavelength increase, as it is typical for HS. Absorbances normalized by sample weight are highest for HAlike fractions from fossil-derived HPs and about 2–2.5 times lower for HA-like fractions from other HPs over all the spectral range (Fig. 1). Increase of HA-like fraction specific absorption in a row peat-soil-coal due to increase of molecular weight, degree of condensation and ratio C aromatic to C aliphatic moieties is known for HA from corresponding environments (Peuravuori and Pihlaja 1997; Kumke et al. 2001), and HA-like fractions from HPs follow the same trend.

HA-like fractions derived from processed lignosulphonate showed higher absorption in UV area, drastically decreasing at wavelengths above 280 nm. This can be attributed to the presence of phenolic structures but low degree of aromatic condensation (Domeizel et al. 2004).

In UV-region spectra, some of HA-like fractions give weak maxima or shoulder around 230 and 280 nm (Fig. 2a), which can be seen much more distinct as extrema in the second-order derivative of the optical density (Fig. 2b). The most distinct maxima are observed for the HA-like fractions from lignosulphonate-derived HP at 230 and 285 nm. The reason for this is most probably the presence of low-molecular-weight phenolic compounds, released from source organic material (lignosulphonate) during its oxidation at HP-manufacturing process.

For HA-like fractions from peat- and sapropel-originated HPs, those maxima at 230 and 280 nm are more pronounceable than for HA-like fractions of fossil origin. Since they are observed better for HA-like fractions from the non-fossil organic

Table 2 The indices for HS characterization calculated from	the primary absorption spectrum and its	derivatives
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Indices	Determination or calculation	Explanation	References
Specific absorbances	Absorbance coefficients taken at 240, 254, 267, 270, 280, 400, 465, 472, 600, 650, and 665 nm divided by the content of HA in solution, mg L^{-1}	General characteristics	Stevenson 1994
SUVA ₂₅₄	UV absorbance coefficient at 254 nm divided by C concentration in solution, mg C L^{-1} (A_{254} /DOC)	Indicator of DOM aromaticity; positively correlates with aromatic C content of DOM as measured by ¹³ C-NMR and other studies	Kumke et al. 2001; Weishaar et al. 2003; Cory et al. 2011
$E_2:E_3$	Ratio of absorbances at 250 and 365 nm, (A_{250}/A_{365})	Showed to correlate with molecular size and aromaticity of HS	Peuravuori and Pihlaja 1997
<i>E</i> ₄ : <i>E</i> ₆	Ratio of absorbances at 465 and 665 nm (A_{465}/A_{665})	Believed to be related to age and the degree of aromatic C condensation in HA or OM: could be used as humification index	Kononova 1966; Stevenson 1994
E _{270/400}	UV-Vis absorbance ratio at appropriate wavelengths	Characterizes the degradation of phenolic/ quinoid core of HA to simpler carboxylic aromatic compounds	Uyguner and Bekbolet, 2005
E _{280/472}	UV-Vis absorbance ratio at appropriate wavelengths	Reflects the proportion between the lignins and other materials at the beginning of humification	Domeizel et al. 2004; Albrecht et al. 2011
E _{280/665}	UV-Vis absorbance ratio at appropriate wavelengths	Denotes the relation between non-humified and strongly humified material	Zbytniewski and Buszewski 2005
$\Delta \log K$	$\Delta \log K = \log A_{400} - \log A_{600}$	Reflects the slope of absorption spectra in certain spectral intervals; suggested to describe the degree of OM humification	Fong et al., 2006
Spectral slope ratio, Sr	Dimensionless ratio of the slope of the shorter wavelength region (275–295 nm) divided by the slope of the longer wavelength region (350–400 nm)	Describes the shape of the absorbance curve; independent of DOM concentration and treated as a measure of the average source, quality, and diagenesis of DOM; considered to be related to the molecular weight of HS	Stedmon and Markager 2001 Helms et al. (2008)
FDR _{240/280}	The ratio of amplitudes taken at 280 and 240 nm in the first-order derivative spectrum; analogous to Sr but computed in narrower spectral intervals	Characterizes the change of slope in the absorption spectra at certain wavelengths chosen as shoulders in the first-order derivative spectrum	The current paper
SDR _{267/280}	The ratio of amplitudes taken at 267 and 280 nm in the second-order derivative spectrum	The second-order derivative is used to separate the individual peaks of overlapping or shown as a shoulder in the primary absorption spectrum. The ratio of amplitudes taken at wavelength of resolved peaks located at 267 and 280 nm	The current paper

materials, this suggests that in a technological process of HP production from raw materials, some fragments of lignin structure may be retained and incorporate into corresponding HA-like fractions. In fossils that passed through the deeper transformation and coalification stages, these fragments are not retained and the absorption spectra of HA-like fractions isolated from them do not demonstrate pronounced maxima over the entire wavelength range.

The same phenomenon was early observed for the solutions of initial HPs, containing besides HA-like also a certain proportion of fulvic-like acid-soluble fraction. It consists mostly of free non-humic compounds (phenolic acids, aminoacids, carboxylic acids) (Gosteva et al. 2012), especially for the ones originated from non-fossil organic materials (peat, sapropel, and lignosulphonate).

More spectral proxies are presented in Table 3. Specific ultraviolet absorption (SUVA) at 254 nm is often reported as an indicator of aromaticity, and the highest values are observed for HA-fractions from fossil-derived HPs (6.2–8.8), whether they are lower for HA-like fractions from other peat- and sapropelderived HPs (3.5–5.3). $E_2:E_3$ ratios, also considered to correlate with molecular size and aromaticity, are less variable and range Fig. 1 Specific absorbance of HA-like fractions isolated from brown coal (*BC*-), leonardite (*Le*-), peat (*Pe*-), sapropel (*Sa*-), and lignosulphonate (*OW*-)-derived humic products, in UV-Vis spectral range (absorbance normalized by the content of HA in solution, mg L^{-1})



within 2.4–3.1, except for the HA-like fractions from lignosulphonate-originated HP.

The $E_{280/472}$ and $E_{270/400}$ decrease for HA-like fractions from source materials in a row as lignosulphonate > sapropel = peat > coal. The $E_{280/665}$ is the highest for the HA-like fractions from lignosulphonate-originated HP (47) and lower for other HA-like fractions (30–42). Thus, this index, indicating the proportions of non-humified and humified material, does not allow discriminating HA-like fractions between fossils- and non-fossils-source organic materials, except of lignosulphonate-derived HP.

The E_4/E_6 ratio is traditionally considered to be related to the degree of aromatic condensation, molecular weight, and residence time of HA, being lower (about 3–5) for highmolecular HA with developed aromatic carbon network and higher (6–10) for low-molecular, young, and more aliphatic humic substances, fulvic acids (Stevenson 1994). But many researchers reported that there was no significant correlation between E_4/E_6 and aromaticity, suggesting that a low E_4/E_6 value does not mean an advanced humification degree (Chen et al. 1977; Orlov 1995; González Pérez et al. 2004; Saab and Martin-Neto 2007). This study supports this assumption: the E_4/E_6 ratio for HA-like fractions from peat- and sapropelderived HPs achieves 4.5–5.6 and even higher for the most aromatic fossil-derived HA-like fractions (5.8–6.7).

The value of $\Delta \log K$ coefficient was suggested for describing the degree of organic matter humification (Fong et al. 2006). In this study, this parameter did not show a satisfactory descriptive ability since all the obtained values are very similar, around 0.7.

Slope ratio varied in a range 0.31–0.37 for HA-like fractions from coal- and peat-derived HPs, being remarkably lower only for HA-like fractions derived from lignosulphonate-derived HP. Helms et al. (2008) reported that Sr varied by over a factor of 13 between DOM-rich wetland waters and surface waters. We supposed that this proxy could also provide a diagnostic criterion for distinguishing between HA-like fractions, derived from fossil-, peat-, and lignosulphonate-derived HPs. But for the examined samples, Sr did not provide a good discrimination.

Among information extracted from absorption spectra, the most promising proxy seems to be the indices determined using derivative spectroscopy which can more readily distinguish HA-source (Fig. 3). According to values of FDR_{280/240} and SDR_{267/280} calculated using first- and second-order derivatives of absorption spectra, all the studied HA-like fractions can be classified in three groups: (a) from fossil-derived HPs, (b) from peat- and







Table 3 Calculated specific absorbance A_{254} , UV-Vis absorbance ratios, $\Delta \log K$ and spectral slope ratio (Sr) of HA-like fractions isolated from parent humic products

Sample ^a	SUVA ₂₅₄	$E_2:E_3$	$E_{270/400}$	$E_{280/472}$	$E_{280/665}$	$E_4:E_6$	$\Delta \log K$	Sr
BC-EnK-HA	8.83	2.4	3.2	5.8	31.9	5.8	0.76	0.32
BC-EnNa-HA	6.36	2.6	3.4	6.3	36.5	6.2	0.79	0.33
BC-HumNa-HA	6.22	2.9	3.8	6.6	42.1	6.7	0.79	0.36
Le-PhK-HA	7.50	2.7	3.6	6.2	34.6	5.9	0.77	0.34
Pe-EcoK-HA	5.28	2.8	3.7	6.6	33.6	5.4	0.73	0.32
Pe-EdaNa-HA	3.47	3.0	3.9	7.0	34.1	5.1	0.74	0.33
Pe-FlexK-HA	5.50	2.9	3.8	6.9	36.4	5.6	0.76	0.31
Sa-BigK-HA	4.22	3.1	4.0	7.0	29.7	4.5	0.69	0.37
OW-LhK-HA	4.72	4.1	5.4	10.2	47.4	4.9	0.70	0.12

^a Samples identity is the same as in Table 1

Fig. 3 Indices $FDR_{280/240}$ and $SDR_{267/280}$ calculated using firstand second-order derivatives of absorption spectra for solutions of HA-like fractions (C = 10 and 20 mg L⁻¹) isolated from coal (*BC*-), leonardite (*Le*-), peat (*Pe*-), sapropel (*Sa*-), and lignosulphonate (*OW*-)-derived humic products



FDR_{280/240}



sapropel-derived HPs, (c) from lignosulphonate-derived HP. These indices show good ability to classify the samples independently of HA-like fraction concentration in solution.

3.2 Fluorescence spectra

HA-like fractions obtained from different HPs upon excitation at $\lambda_{ex} = 270$, 310 or 355 nm exhibit fluorescence spectra similar to HS from the corresponding environments: soil, peat, and coal. Fluorescence emission spectrum is a broad band from 300 to 600 nm with a maximum in the region 390–520 nm and bandwidth about 200 nm, depending on the organic matter origin and wavelength of excitation (Fig. 4). On average in HA-like fractions, isolated from peat- and lignosulphonate-derived HPs, fluorescence maximum was accounted at shorter wavelengths than for HA-like fractions from fossil- and sapropel-derived HPs. Similar phenomenon of shorter λ max for peat and compost HA comparably to leonardite HA was reported by Senesi et al. (1991).

Except for lignosulphonate sample OW-LhK-HA with emission maximum around 400 nm, solutions of all other HA-like fractions demonstrated emission maximum position within 490– 517 nm excited at 270 or 355 nm. However, under excitation at 310 nm, most of peat-originated HA-like fractions (except for Pe-FlexK-HA) and HA-like fractions from lignosulphonatederived HP manifested a hypsochromic shift of maximum emission with increasing excitation wavelength ("blue shift"), achieving up to 17–80 nm (Table 4). In contrast, HA-like fractions from coal- and sapropel-derived HPs exhibited practically constant fluorescence band position or a small shift to longer wavelengths (negative values of "blue shift"). We note also that the "blue shift" of fluorescence peak is observed for those HA-like fractions (from peat- and lignosulphonate-derived HPs) for which λ max is located in the spectral range 430–450 nm.

Position of the emission maximum depends on the excitation wavelength, which indicates the presence of multiple fluorophores, fluorescence of which is excited multifariously at different wavelengths (Senesi and D'Orazio 2005). The presence of the blue shift is often reported in the fluorescence spectrum of natural HS of marine, river, and soil origin, indicating a heterogeneous composition of fluorophores (Shubina et al. 2010). The fact that the blue shift was observed also in HA-like fractions from peat- and lignosulphonate-derived HPs, but not in fossils-derived HA-like fractions, can denote that the HP with higher degree of humification is more homogeneous in respect to its fluorescent behavior.

A similar trend has been earlier observed for the initial HPs, when in humates from fossils (brown coal, lignite,



Fig. 4 Fluorescence emission spectra of HA-like fractions isolated from coal (*BC*-), leonardite (*Le*-), peat (*Pe*-), sapropel (*Sa*-), and lignosulphonate (*OW*-)-derived humic products with excitation at 270, 310, and 355 nm

leonardite, and humalite) fluorescence maximum was located around 500 nm and did not vary upon changing excitation wavelength in the range of 270–355 nm (Gosteva et al. 2012). In contrast, for HPs produced from peat and sapropel, λ max was located in the spectral range 430–450 nm and the blue shift of fluorescence emission in 20–40 nm was observed.

3.3 Identification of OM sources using principal component analysis

Table 5 presents the factor loadings, eigenvalues, and the variance percentages corresponding to each principal component separately for chemical and spectroscopic datasets. In chemical dataset, PCA yielded three principle components (PC) with

 Table 4
 Position of fluorescence

 maximum and the "blue shift" of
 emission maximum for excitation

 at three wavelengths for HA-like
 fractions isolated from humic

 products, nanometer
 products, nanometer

Sample ^a	λ max		"Blue shift" of emission maximum		
	$\lambda ex = 270 \text{ nm}$	$\lambda ex = 310 \text{ nm}$	$\lambda ex = 355 \text{ nm}$	$\Delta 1$	Δ2
BC-EnNa-HA	490	504	493	-14	-11
BC-EnK-HA	504	513	503	-9	-10
BC-HumNa-HA	512	517	507	-5	-10
Le-PhK-HA	505	514	502	-9	-12
Pe-EcoK-HA	491	417	497	74	80
Pe-EdaNa-HA	489	418	489	71	71
Pe-FlexK-HA	506	507	500	-1	-7
Sa-BigK-HA	508	506	501	2	-5
OW-LhK-HA	407	390	440	17	50

^a Samples identity is the same as in Table 1

 Table 5
 Eigenvalues and variance percentages corresponding to the principal components for chemical and spectral proxies

Variables	PC1	PC2	PC3
Chemical parameters			
C (%)	0.899	0.232	0.277
N (%)	-0.741	0.273	0.599
Н (%)	-0.302	-0.869	0.201
S (%)	0.441	-0.664	-0.031
O (%)	-0.878	0.252	-0.399
H/C	-0.613	-0.775	0.048
O/C	-0.917	0.073	-0.383
N/C	-0.839	0.193	0.479
Eigenvalue	4.34	2.03	1.01
Total variance (%)	54.22	25.39	12.68
Cumulative variance (%)	54.22	79.60	92.28
Spectral parameters			
Wavelength of emission maximum at $\lambda ex = 310$	0.835	-0.289	0.456
Blue shift $\lambda 1$ (nm)	-0.493	0.793	-0.332
SUVA	0.757	-0.462	-0.384
$E_2:E_3$	-0.943	-0.316	0.057
E _{270/400}	-0.934	-0.352	0.018
E _{280/472}	-0.926	-0.367	-0.068
$E_4:E_6$	0.683	-0.247	-0.134
SDR _{267/280}	0.798	-0.236	-0.417
FDR _{240/280}	-0.881	-0.424	-0.203
Eigenvalue	6.01	1.58	0.71
Total variance (%)	66.79	17.55	7.86
Cumulative variance (%)	66.79	84.35	92.21

eigenvalues more than one. PC1 explains 54 % of total variance and shows strong loadings for percentages of C, O and N, and O/C and N/C atomic ratios. PC2 explains 25 % of the total variances and shows strong loading for H content and H/C. For spectroscopic dataset, PCA yielded two PCs with eigenvalues more than one, explaining 84 % of total variance. About 67 % of total variance is explained by PC1 with strong loadings for all the parameters, except E_4/E_6 and the blue shift. PC2 explains 17 % of variance with strong loading for the blue shift.

Figure 5 illustrates possibilities to identify OM-sources, using either solely simplest chemical characteristics (elemental content and atomic ratios, Fig. 5a) or a set of spectroscopic parameters (Fig. 5b). PCA plots show that basing on chemical parameters, there was no distinct separation between PCA scores of HA-like fractions, associated with OM origin (Fig. 5A), whether spectroscopic dataset allowed dividing the samples into three groups, depending in the OM origin: fossils, peat and sapropel, and lignosulphonate. HA-like fractions from fossil-derived HPs form a separate group with positive loadings on PC1. HA-like fractions from non-fossil-derived HPs

fall into one group with negative loadings on PC1 and positive on PC2. The most distinct separation is observed for HA-like fractions from lignosulphonate-derived HP (OW-LhK).

4 Overall views

Absorption spectra of HA-like fractions isolated from HPs varying in OM origin have similar wavelength-dependent character of UV-Vis spectra with HA from corresponding environments (peat or coal). For some HA-like fractions, weak maxima around 230 and 280 nm are observed in the derivative spectra with enhanced spectral resolution, most probably due to the presence of low-molecular-weight phenolic compounds, products of lignin decomposition. UV-Vis absorbance ratios SUVA, $E_{270/400}$, $E_{280/472}$ and derivative indices FDR_{280/240} and SDR_{267/280} showed the highest descriptive ability for identification of OM source of HPs. In contrast, the values for $E_{280/665}$, $E_2:E_3$, $E_4:E_6$, $\Delta \log K$, and Sr have close magnitudes for studied HA-like fractions derived from HPs from fossils,

Fig. 5 Factor score plot for HAlike fractions isolated from coal (*BC*-), leonardite (*Le*-), peat (*Pe*-), sapropel (*Sa*-), and lignosulphonate (*OW*-)-derived humic products in the principal components 1 and 2 obtained using chemical (**a**) and spectroscopic (**b**) parameters



peat, and sapropel (see Table 3) and may not be used for the discrimination of peat from fossil HPs.

The fact, that the "blue shift" was observed for HA-like fractions from most of peat-derived HPs and from lignosulphonate-derived HP, while it was not detected for HA-like fractions from sapropel-derived HP and majority of HA-like fractions from fossil-derived HPs can denote that the fossil-derived HP with higher degree of humification are more homogeneous in respect to their fluorescent behavior. Therefore, we suppose that the position of emission maximum and presence of "blue shift" can also be considered as other diagnostic criteria for distinguishing between organic source materials for HPs. These considerations are supported by PCA, which showed strong loadings for the position of emission maximum, "blue shift", SUVA, $E_2:E_3$, $E_{270/400}$, $E_{280/472}$, FDR_{280/240}, and SDR_{267/280}.

HA-like fractions from lignosulphonate-derived HP showed the most distinct differences in all the parameters studied. This HP is manufactured using oxidation-hydrolytic degradation of lignosulphonate and therefore its genesis is different from other HPs. Nevertheless, many chemical properties of this product are similar to commercial HPs from peat and coal, as well as its effectiveness as plant growth stimulator (Ertani et al. 2011). Although in this study we examined only one sample of this product, high stability of this HP's composition has been reported (Poloskin et al. 2012). Simple chemical data can hardly discriminate lignosulphonate-derived HP from the ones produced from fossils, whether UV-Vis and fluorescence proxies were able to reveal the differences.

Thus, spectroscopic and fluorescent proxies in combination with derivative spectroscopy applied in this study might serve as inexpensive and robust tools to compare HPs from various environments. Although the dataset in this study included only four samples from fossil-derived HPs and four from plantresidue-derived HPs (peat and sapropel) which is not enough for wide generalizations, the approach is promising and worth further development.

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