

Properties of soil and peat humic substances from Latvia

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Abstract. The acidity, elemental, functional and spectral (UV, fluorescence, IR spectra) characteristics of humic substances isolated from soils of different origin and peat in Latvia are described and compared with values common for humic substances of different origin, to evaluate the character of processes during humification. Substantial dependence of properties of humic substances on the humification conditions are found.

Key words: humic substances, soil, peat.

INTRODUCTION

Humic substances (HS) are the most widely found naturally occurring organic substances. Humic substances are a general category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in colour, of high molecular weight and refractory. Humic substances can be divided in three fractions: a) humin is the fraction of humic substances that is not soluble in water at any pH; b) humic acid (HA) is the fraction of humic substances that is not soluble in water under acidic conditions (below pH 2), but becomes soluble at greater pH; c) fulvic acid is the fraction of humic substances that is soluble under all pH conditions (Gerke, 2018). Humic substances form most of the organic component of soil, peat and natural waters, they influence the process of formation of fossil fuels, they play a major role in the global carbon geochemical cycle. About 60–85% of the organic matter in soil consists of humic substances (Thurman, 1985) and their concentrations change depending on the soil origin. Interaction of humic substances with xenobiotics may modify the uptake and toxicity of these compounds, and affect fate of pollutants in environment (Lipczynska-Kochany, 2018). In the same time the soil and peat humic substances may be regarded as a valuable resource for applications in agriculture (Gerke, 2018). Thus humic substances are of great importance not only considering processes in environment, but also as an important resource.

Despite the long history of humus research, comparatively recently reliable and unified methods have been introduced for isolation of humic substances (Mallick, 2017) to adequately determine the HS structure and properties, depending on its sources. The major questions are concerning the changes in properties of humic matter depending on their origin. It has been shown, that the structure and properties of soil humic matter differ between soil humic matter of different origin (Орлов, 1990), in the same time, considering the great variability in soil properties and thus the humification conditions, the actual

relationship is far from being explained. Another open question can be related to the genesis of soils and development of peat soils. Just as soil humic substances also humic matter form peat has been studied in comparatively few studies (Wiszniewska et al., 2016).

The aim of the present paper is to describe the properties of soil humic substances isolated from different soils in Latvia and peat humic substances, to evaluate the impact of the soil and peat composition on the humus structure (humification process, generally) and soil formation.

MATERIALS AND METHODS

Humic substances were isolated from peat of Latvia, representing different peat types and mechanisms of their formation. Soil samples were from different sites in Latvia from upper horizon during summer season 2018. The selected soils (for some soils several samples were taken to get representative samples) from all territory of Latvia represent the principal soil types in Latvia. The soils were classified according to the International Soil classification system (IUSS Working Group WRB, 2015). The analytical characterisation of soil samples were done within 1 month. The taxonomic and chemical properties of the soils and peat are presented in Table 1. Organic carbon content was determined by wet combustion (Walkley-Black method) and soil, and peat pH in a 0.1 M KCl solution.

Table 1. Characteristics of soil and peat samples

Soil group/peat type	Symbol	Organic carbon, %	pH (in 0.1M KCl)
Leptosols	VKz	5.8	7.32
Phaozems	VKt	2.7	7.13
Stagnosol	VKg	6.3	7.24
Luvisol	BRt	22.9	6.51
Retisols	PVv	3.7	6.87
Podzols	Pot	4.6	6.76
Anthrosols	ANt	20.6	6.73
Technisols	ANu	3.7	7.23
Gleysols	GLh	6.3	6.48
Histosols	TZh	31.3	5.64
<i>Peat</i>			
Pine peat	P-1	34.5	6.2
Sedge- <i>hypnum</i> peat	P-2	22.4	6.4
Wood- <i>Sphagnum</i> peat	P-3	28.4	5.3
<i>Fuscum-Sphagnum</i> peat	P-4	16.3	4.7
Cotton-grass- <i>Sphagnum</i> peat	P-5	26.4	4.8

Humic acids were extracted and purified using procedures recommended by the International Substance Society (Klavins, 1998). Briefly, 1 kg of 2 mm (sieved) air dry soil or peat was reacted with 10 L of 0.1 M HCl for 1 h. The slurry was allowed to settle, and the aqueous phase was decanted and discarded. Approximately 1 L of H₂O was added to the soil or peat mass and the resulting slurry was allowed to incubate for 30 min, after which the pH of the slurry was adjusted to 7 with the addition of 1 M NaOH. This was followed by the addition of a sufficient quantity of 0.1 M NaOH (done under N₂ gas), to bring the total volume of the solution phase to 10 L and the resulting slurry was

stirred under N₂ gas. After 24 h the alkaline slurry was filtered through glasswool and the particle free filtrate was acidified to a pH value of 1, with the addition of 6 M HCl. This solution was then allowed to settle then it was centrifuged, and the supernatant was discarded. The sediment (which contained humic acid) was washed with distilled water and repeatedly centrifuged discarding the supernatant. Solid residue after centrifugation was then suspended in a mixture of 0.1 M HCl and 0.3 M HF, to remove mineral particles. This treatment was repeated until the ash content was reduced less than 2%. Afterwards humic acid dispersion in distilled water were dialyzed against water to remove chlorides and resulting humic acids were lyophilized. Clear brown coloured solution containing the fulvic acids (FA) were obtained after acidification with HCl. The obtained FA solutions were transferred to the H⁺ form by passing through a strong cationite KY-23 column and finally freeze dried. To ensure representative samples for analysis two extractions were made for some soil samples.

The obtained humic substances were characterized as follows:

1. Elemental analysis: C, H, N and ash concentrations were determined by a Perkin Elmer 240/A CHN-Analyzer. Oxygen concentrations were calculated by difference;
2. Concentrations of functional groups were determined by standard methods (Klavins, 1998);
3. Molecular weight distribution was determined with gel filtration on Sephadex G-100 (bead diameter 40–120 μm) column (1 × 40 cm). The eluent: 0.01 M Tris-HCl buffer (pH 9.0) was pumped through the column at 1 mL min⁻¹, gathering 1 mL fractions. 25 mg of humic substances were applied on the top of the column as 1% solution in 0.1 M NaOH. A standard set of proteins was used for column calibration. The void volume of the column was determined using Blue Dextran 2000000. Detection was performed at 280 nm;
4. UV-Vis spectra were obtained over a range of 200 to 700 nm using a Specord UV 40 UV-Vis spectrophotometer on 0.05 N NaHCO₃ solutions at a concentration of 50 mg L⁻¹ for FA and 33 mg L⁻¹ for HA and a pH between 8 and 9. For analysis of E4/E6 ratio the humic matter samples (2 mg) were dissolved in 10 mL 0.05 M NaHCO₃ and absorbances at 465 and at 665 nm were measured against a reference of 0.05 M NaHCO₃.
5. Fluorescence spectra were recorded using a Hitachi 850 fluorescence spectrometer, on aqueous solutions of each sample at a concentration of 100 mg L⁻¹, adjusted to pH 8 with 0.0 M NaOH. Emission spectra were recorded (scan speed 60 nm min⁻¹, response 2 s) over the wavelength range 360 to 640 nm at a fixed excitation wavelength of 335 nm.
6. Infrared (IR) spectra were recorded, in the 4,000 to 500 cm⁻¹ wavenumber range using a Perkin Elmer 400 IR spectrophotometer, of KBr pellets obtained by pressing mixtures of 1 mg samples and 400 mg KBr with precautions taken to avoid moisture uptake;
7. The Van Krevelen graphical-statistical method was applied as used for the study of the structure on the basis of elemental analysis data (Орлов, 1990).

RESULTS AND DISCUSSION

The selected soils and peat samples (Table 1) are representative for Latvia. To study the processes influencing their properties soil humic substances have been compared

with aquatic humic substances studied in previous paper (Klavins et al., 1997). The composition of major elements, atomic ratios and ash contents of HA and FA samples examined are presented in Table 2. The elemental composition of humic substances from soils and peat of Latvia is generally similar to that of humic substances presented in literature (Орлов, 1990).

Table 2. Elemental and functional composition of humic substances from soils, peat and related environments of Latvia

Humic substances	C, %	H, %	N, %	O, %	COOH, mmol g ⁻¹	ArOH, mmol g ⁻¹
<i>Humic acids</i>						
HA-VkzA	52.34	4.83	3.08	38.92	2.85	1.94
HA-VKzB	52.05	4.69	3.16	39.23	3.10	2.15
HA-VktA	53.46	4.81	3.61	36.78	2.85	2.10
HA-VKtB	53.96	4.76	3.85	35.95	2.20	2.05
HA-VKtC	52.76	4.95	3.55	37.39	2.50	1.50
HA-VKg	51.32	4.53	4.38	38.60	2.15	2.48
HA-BrA	55.10	5.23	4.35	33.17	1.90	1.85
HA-BRtB	57.03	5.04	4.35	33.17	1.90	1.05
HA-BRtC	56.13	4.89	3.86	34.00	2.10	0.95
HA-PVv	54.05	5.21	3.71	35.89	2.10	1.56
HA-Pot	53.68	5.12	3.56	36.68	2.15	1.15
HA-Ant	57.91	4.03	3.85	32.89	2.85	2.10
HA-Anu	57.25	3.94	2.95	34.71	2.95	2.05
HA-GLh	54.68	4.08	3.84	36.55	1.90	0.75
HA-TZh	53.01	5.03	1.07	38.63	1.95	0.80
HA-Rāznas Lake	48.74	4.06	1.65	44.17	5.56	0.72
HA-Rusonu Lake	50.38	4.03	1.04	37.40	4.28	0.65
HA-Nordic Reference	55.20	4.12	1.04	39.02	4.12	1.21
HA-P-1	48.11	5.60	1.95	43.00	n.d.	n.d.
HA-P-2	52.47	4.18	2.15	39.06	n.d.	n.d.
HA-P-3	50.71	4.43	2.73	40.95	n.d.	n.d.
HA-P-4	53.01	5.03	1.65	38.63	n.d.	n.d.
HA-P-5	51.39	4.08	2.25	41.03	n.d.	n.d.
<i>Fulvic acids</i>						
FA-VkzA	49.31	4.64	2.95	41.93	3.28	2.16
FA-VKzB	48.65	4.54	2.87	42.98	3.80	2.20
FA-VktA	43.68	4.81	3.65	46.18	3.68	1.75
FA-VKtB	44.36	4.31	3.48	46.51	3.75	1.60
FA-VKtC	42.95	4.55	3.71	47.64	3.75	1.70
FA-VKg	48.75	3.85	2.63	43.59	3.30	2.20
FA-BrA	48.23	4.43	3.93	41.54	2.82	0.90
FA-BRtB	49.35	4.56	2.76	42.19	2.85	1.15
FA-BRtC	48.17	4.15	2.83	43.90	2.70	0.85
FA-PVv	46.13	4.09	2.48	45.99	3.10	1.65
FA-Ant	45.53	4.31	2.95	46.06	3.50	1.60
FA-Nordic Reference	52.62	4.09	0.74	41.93	7.53	1.53

Humic acids commonly have much higher carbon concentrations than fulvic acids, but at the same time oxygen, carboxylgroup concentrations are higher in fulvic acids. To

characterise elemental composition, atomic ratios (Van Krevelen graphs) are often used. H/C versus O/C ratios (Fig. 1) reflect the relative percentage of aromaticity in structures of humic substances. Chemical processes influencing the formation of HA's can be evaluated by the van Krevelen diagram. As far as most important processes influencing the structure of HS can be described as demethylation (removal of $-CH_3$ group), dehydration and decarboxylation (removal of $-COOH$ group), the same diagram (Fig. 1) can be also used to study humification process. Demethylation, dehydration and decarboxylation processes result in increased aromaticity and increased humification degree. Fig. 1 shows that demethylation and dehydration processes dominate in the generation of humic acids. Highest is the aromaticity of peat HA and HA from hydromorphic soils. Lowest among humic acids is the humification degree for HA's from *Anthrosol* and *Phaozem* soils.

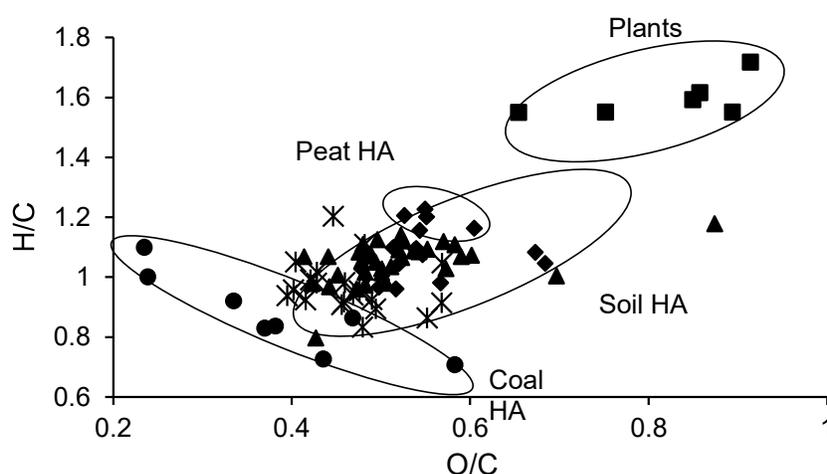


Figure 1. Van Krevelen (H/C vs. O/C atomic ratio) graph of humic matter precursors (Klavina et al., 2015)) (plants) (■); peat humic acids (◆), coal humic acids (Krumins et al., 2017) (●) and soil humic acids (▲★).

In humic substances from *Gleysol*, *Histosol* and *Podzol* soils, concentrations of carboxylgroups and phenolic hydroxylgroups are lower (Table 1), than in humic substances from *Phaozem* soils. The most dominant functional groups in the structure of AHS are carboxyl and phenolic hydroxylgroups. The concentrations of carboxylgroups are higher in fulvic acids ($3.5\text{--}6\text{ mmol g}^{-1}$), while humic acids have more phenolic hydroxylgroups ($1.0\text{--}1.5\text{ mmol g}^{-1}$). In general, the concentrations of carboxylgroups correlate to the O/C ratio, thus indicating that the dominant portion of the oxygen in humic molecules is in form of carboxylgroups. Nitrogen concentrations in humic acids are ~ 1.5 times higher than in fulvic acids.

The UV-Vis spectra of soil and peat HA examined (Fig. 2) are featureless, and they monotonically decrease with increasing wavelength. Only in case of humic substances from *Anthrosol* soils shoulders at 360 nm can be observed. The ultraviolet spectra of both humic and fulvic acids are similar, differing only slightly in optical density.

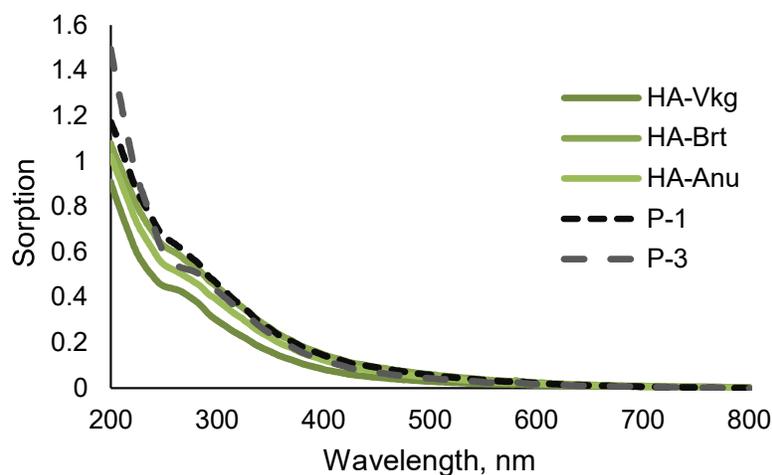


Figure 2. UV-Vis spectra of soil and peat humic substances (*Phaozem* soil VKg *Cambisol* soil BRt *Anthrosol* soil ANu Pine peat P-1 *Wood-Sphagnum* peat P-3).

Table 3. Light absorbance properties and aromaticity of soil humic substances

Sample*	Molar absorption, L (mole C) ⁻¹ cm ⁻¹	E ₄ /E ₆	Aromaticity, %	M _w
FA Missouri River**	247	13.9	20.4	-
FA Ohio River**	274	17.2	24.3	-
FA Minnesota groundwater**	122	3.53	12.6	-
FA Suwannee River**	389	20.7	24.8	880
FA Lake Fryxell**	150	-	13	-
FA Coal Creek**	401	20.7	27.4	-
FA Lake Burtnieku***	214	8.6	17.4	2,200
FA Lake Liepajas***	189	9.4	16.1	1,650
HA-VKg	501	5.4	41.4	8,200
HA-BrA	444	6.5	36.7	4,250
HA-PVv	392	7.8	32.4	3,800
HA-Pot	379	6.7	31.4	5,100
HA-GLh	269	6.3	22.3	4,400
HA-TZh	681	5.6	56.3	9,300

* – for soil symbols see Table 1; ** – Chin et al., 1994; *** – Klavins, 1998.

The slope of the adsorption curves as measured by the ratios of UV absorbance at 465 and 665 nm, have been suggested to be inversely related to the condensation of aromatic groups (aromaticity), and also to particle size and molecular weight. The higher E₄/E₆ ratios measured for the aquatic FA, with respect to those of soil origin, are in general agreement with data in the literature and suggest a lower degree of condensed aromatic systems and smaller particle sizes or molecular weights than for aquatic HS. The E₄/E₆ ratio for fulvic acids is higher than for humic acids, thus this ratio correlates with the changes in molecular mass of humic substances (Table 3). Within the UV spectra, the molar absorptivities of the humic substances were measured at 280 nm. This wavelength was chosen, since $\pi - \pi^*$ electron transition occurs in this region for phenolic substances, aniline derivatives, benzoic acids, polyenes, and polycyclic aromatic

hydrocarbons (Fuentes et al., 2018). Since many of these substances are precursors of components of certain types of humic substances, molar absorptivity (ϵ) may serve as a basis for calculation of aromaticity of humic mater and evaluation of its origin using the following equation (Chin et al., 1994):

$$\text{aromaticity} = 0.05 \epsilon + 6.74$$

(ϵ – molar absorption, L (mole C)⁻¹ cm⁻¹)

Comparison of molar absorptivities and calculated values of aromaticity found in this study, with those from literature (Table 3) allows to evaluate the impact of different sources on the structure and properties of humic substances. Humic substances from *Histosol* soils have a high degree of aromaticity and molar absorptivity.

Fluorescence emission spectra (excitation at 330 nm) of humic and fulvic acids (Fig. 3) are characterised by broad bands centered around $\lambda = 435$ nm and between $\lambda = 445$ nm and $\lambda = 485$ nm, respectively. Some samples of humic substances influenced by human activities show also shoulders at lower wavelengths. Although the exact nature of the fluorescing groups is still far from clarified, fluorescence at the higher wavelengths, typical for humic substances from *Cambisol* and *Histosol* soils, may be attributed to either highly substituted aromatic nuclei, possibly bearing at least one electron-donating group, and/or conjugated unsaturated systems capable of high degrees of resonance. Soil humic and fulvic acids have fluorescence spectra with maximum at lower wavelengths than aquatic HS (Klavins, 1998), indicating low degrees of aromatic polycondensation and lower levels of conjugated chromophores. Also, lignin-derived, coumarin-like structural units and conjugated phenolic aldehydes, which have $\lambda_{em\ max} = 465\text{--}470$ can be suggested as possible contributors to fluorescence of soil humic substances (Shirshova et al., 2017). The presence of lignin-like structures seem to play a secondary role. Substantially different are spectra for humic substances influenced by human activities (*Anthrosol* soils) which have spectral maximums possibly related to conjugation products with contaminants.

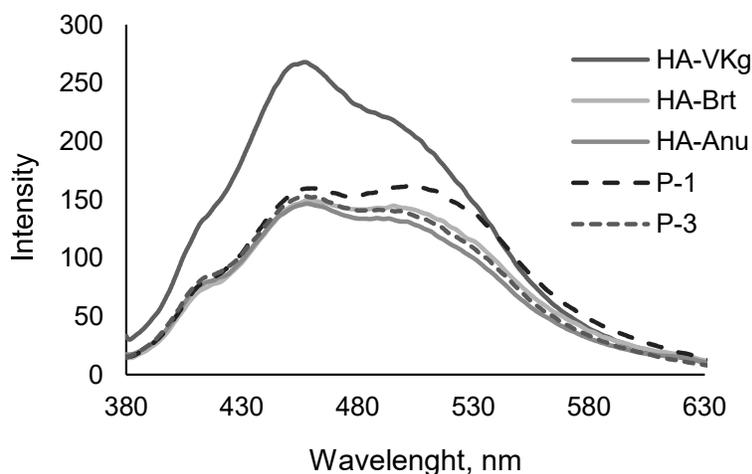


Figure 3. Fluorescence emission spectra of soil and peat humic substances (*Phaozem* soil VKg *Cambisol* soil BRt *Anthrosol* soil ANu Pine peat P-1 Wood-*Sphagnum* peat P-3).

The IR spectra (Fig. 4) of the peat and soil HA examined are in general similar to one another in the main position of adsorption, but differences of various entity are apparent in the relative intensity of some bands, depending of origin and nature of the sample. IR spectra of analysed humic substances can be divided by regions depending on informativity and the presence of important functional groups. Absorption bands in spectral region $3,600\text{--}2,800\text{ cm}^{-1}$ are very broad and are typical not only for humic substances, but also for mineral constituents. Absorbance in this spectral region is determined by the presence of OH groups. Sorption at wavelengths $2,920$ and $2,860\text{--}2,850\text{ cm}^{-1}$ identifies the presence of CH_3 and CH_2 groups. IR spectra data of soil humic substances show that methylene groups $\text{-(CH}_2\text{)}_n\text{-}$ exist in the form of comparatively short alkane chains ($n < 4$). Typical intensive sorption lines are common for the region around $1,700\text{ cm}^{-1}$ ($1,725\text{--}1,700\text{ cm}^{-1}$), which is characteristic for carbonyl groups in aldehydes, ketones and carbonic acids. The actual sorption maximum greatly depends on the conjugation degree, presence of substituents and hydrogen bonding. In spectral region $1,690\text{--}1,500\text{ cm}^{-1}$ it is possible to identify the sorption maximum of amide bonds ($1,650\text{--}1,640\text{ cm}^{-1}$ and $1,550\text{--}1,540\text{ cm}^{-1}$). In region $1,625\text{--}1,610\text{ cm}^{-1}$, the sorption indicates the presence of aromatic $\text{C}=\text{C}$ and carbonyl groups, quinones. At wavelengths $1,470\text{--}1,370\text{ cm}^{-1}$, there are bands typical for C-H and O-H bonding and sorption maximums typical for C-O. For wavelengths below $1,000\text{ cm}^{-1}$ fingerprint patterns are evident. Sorption in this spectral region provide information about possible role of carbohydrate percentage in the structure in humic molecules. Sorption at $1,080\text{ cm}^{-1}$ shows OH deformation or C-O stretch of phenol and alcohol OH groups, and $1,040\text{ cm}^{-1}$ indicates C-O stretch of polysaccharide components.

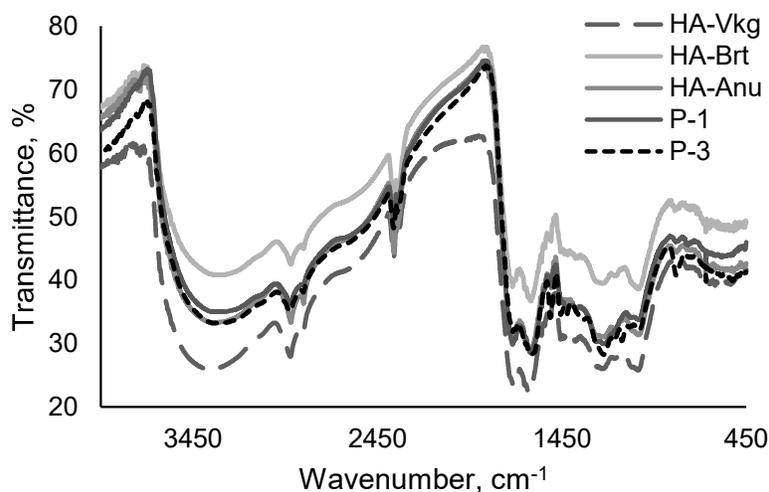


Figure 4. Fourier transform infra-red spectra of studies humic substances Fluorescence emission spectra of soil and peat humic substances (*Phaozem* soil VKg *Cambisol* soil BRt *Anthrosol* soil ANu Pine peat P-1 Wood-*Sphagnum* peat P-3).

The distinctive distribution of functional groups and the major building blocks of humic substances may reflect the way of their production and structure alteration due to microbial degradation and geochemical alteration. Fulvic acid formation may occur through condensation reactions involving compounds derived from precursor organic materials or through production of residual recalcitrant compounds in the microbial

degradation of precursor organic material. For peat humic acids, lignin is not included in the set of precursor materials and carbohydrates and aliphatic structures are relatively abundant. Carbohydrate structures, however, are the most labile and are rapidly degraded by microorganisms. Similarity between humic substances from peat and soil indicate either the dominance of allochthonous peat humus or the deep transformation of autochthonous humic material.

CONCLUSIONS

The elemental, functional, mass-molecular and spectral characterization of soil and peat humic substances stresses the role of their origin on the properties of humic substances. Humic substances from different soil and peat types differ in properties. The similarity in the structure and properties of humic substances originating from *Podzol*, *Phaozems*, *Gleysol* soils on one hand and *Cambisol* and *Histosol* soils on other hand, suggests the importance of intensity of biological transformation of organic matter on their structure. Humic substances from *Cambisol* and *Histosol* soils are similar to peat humic substances and they are highly aromatic. The pattern of differences between Latvian peat and soil humic substances, demonstrates that even within small territory as Latvia it is impossible to isolate one reference sample that would be representative of soil humic substances.

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