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COMPOSITION AND PROPERTIES OF HUMIC ACIDS IN PEAT

Kūdras humīnskābju sastāvs un īpašības

Thesis for Doctor's Degree in Environmental Sciences

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ABBREVIATIONS

TOC – total organic carbon

HS – humic substances

HA – humic acids

FA – fulvic acids

FTIR – Fourier transform infrared (spectroscopy)

ESR – electron spin resonance

NMR – Nuclear magnetic resonance (spectroscopy)

Py-GC/MS – pyrolysis coupled with gas chromatography and mass spectrometry

UV-Vis – Ultraviolet-visible (spectroscopy)

INTRODUCTION

There are enormous peat resources in the world – about 2% of the total land surface (≈3 million km^2) are covered with peatlands, main deposits being located in North America and the northern parts of Asia, Eastern Europe, Scandinavia, Ireland, Scotland, northern Germany, the Netherlands, and Indonesia. The total volume of peat *in situ* is up to 4,000 billion m^3 (Lappalainen, 1996). At present, the principal producers of peat are Ireland, Finland, Belarus, the Russian Federation, Sweden, and Ukraine. Despite the widespread distribution of peat resources, consumption of peat, especially for energy production, outside Europe is comparatively insignificant. The global consumption rate is approximately 17 million tonnes per year, extracted from a very small part of the total area of peatlands (in the EU, only around 0.34% of the total peatlands (≈1,750 km^2) are used for energy production) (Lappalainen, 1996).

Peat has many applications – it is widely used for power generation, in agriculture and horticulture, for production of various fertilizers, mulching, as well as insulating construction materials. Chemical and thermal processing of peat may yield different substances – humic acids, alcohols, activated charcoal, wax, furfural, and feed yeast (Spedding, 1988).

One of the major challenges of environmental sciences is to study the possibilities of use and protection of natural resources, especially focusing on an effective use and production of innovative and high added-value products. Peat processing products, especially humic substances, are environmentally friendly and have low toxicity; besides, humic substances have major anti-virus, anti-inflammatory, hormonal system stimulant, profibrinolytic and heavy metal-binding capacities. Therefore, nowadays they are used as feed and food additives, in dyes and cosmetics.

Peatlands cover 10.4% of the territory of Latvia, which contains substantial volumes (10.8 billion m^3) of peat. 0.4% of the world's peat resources are situated in Latvia, ranking it 8th among the calculated world's peat resources per capita (World Energy Council, 2007). Therefore, peat is one of the most important natural resources of Latvia with a high application potential. In the present decade, peat mining output is 825,000 tonnes per year on average, reaching up to a million tonnes per year during the last few years. It is estimated that at the current peat extraction intensity (taking into account the growth rate of peat), the peat resources can last for the next 800 years (Kalniņa, 2000).

In Latvia this essential natural resource has been thus far used mainly as a source of fuel and in agriculture. Considering the need for a wise use of natural resources, it is important to study the possibilities of developing new peat-based products. Extensive studies of peatlands and peat resources in Latvia have been carried out in the first half of the 20th century, under supervision of Professor *Dr.* Pēteris Nomals at the Peatland and Peat Utilisation Department of the University of Latvia, later the Peatlands Institute (Šnore, 2004). No extensive and detailed scientific studies of peat bogs and peat properties have been conducted in Latvia in the last 20 years. Despite the fact that different peat products and humic substances are currently produced in significant quantities worldwide, the possibilities of the use of peat for production of humic substances, as well as the production process itself, have been investigated relatively little.

As proved by foreign experience, it is necessary to do research on peatlands and the uses of peat resources, creating new high value-added innovative products that do not require large-scale peat extraction, thus contributing to sustainable use of Latvian peat resources, preserving nature and promoting competitiveness.

The Aim of the Thesis

The aim of the work is to explore the properties and composition of humic acids isolated from raised bog peat prospective for industrial use, and the factors that influence their properties and composition.

The Tasks of the Thesis:

1. to collect peat samples from different bogs, study their properties, humification of peat and factors influencing it;
2. to explore the extraction process of humic acids from peat using different high-intensity extraction methods;
3. to characterise the properties of peat humic acids and evaluate the influence of the peat properties on the properties and composition of humic acids.

The Scientific Novelty of the Study

The thesis is a complex study of the prospects of peat for industrial use, as well as the impact of the humification process on the composition of humic substances of peat. The possibilities of using intensive extraction methods for extraction of humic substances were verified, as were the changes of the properties of humic substances depending on the intensity of processing. Humification indexes proposed for characterisation of the humification process were compared, and a new index that could be prospective specifically for determination of the humification degree was developed. Humic substances in preparative quantities were extracted from the peat samples selected for this study, analysis of the elemental composition and functional group content of these substances was performed, using UV-Vis, fluorescence, FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopy methods. The impact of the peat-forming material and its humification process on the properties of peat humic substances was determined.

Major Achievements

The complex multiparameter research of the composition and properties of peat can serve as a useful reference in the process of researching and developing new areas of use of peat. Furthermore, commercial production of peat HA can benefit from the studied peat HA extraction methods. The humification indexes of peat can be used as an effective instrument for exploration and characterisation of peat.

Complex research allows to assess the variation trends of raised bog humic acids in the peat mass prospective for industrial production of humic acids, as well as to evaluate variability of the features of HA and the approaches to standardisation of industrially produced products.

Approbation of the Results

The results of the thesis are published in 8 articles; the author has 12 publications in total. The author has presented the results of the research at 13 international conferences.

Scientific publications (related to the thesis):

1. Kļaviņš M., **Šīre J.**, Eglīte L. (2004) Humic substances and their potential use in agriculture. Proc. Latv. Acad. Sci, B., 58(2), pp. 39 – 49;
2. Kļaviņš M., **Šīre J.** (2007) Character of transformation of organic matter in peat and peat humic substances. *Proc. Latv. Acad. Sci*., B., 61 (1/2), pp. 1 – 14;
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4. **Sire J.**, Klavins M., Kreismanis J., Jansone S. (2009) Impact of the process of isolating humic acids from peat on their properties. Can. J. Civ. Eng, 36, pp. 1 – 11.
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6. **Sire J.**, Klavins M. (2008) Characterisation of peat humic substances from Latvia. In: After wise use – the future of peatlands (Ed. C.Farrell, J.Feehan). Proceedings of the 13th International Peat Congress, Tullamore, vol. 1, Ireland, pp. 341 – 344;
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Patents, Licences

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Contribution of the Author

The author has made a contribution in the form of the following activities: collection and treatment of peat samples, analysis of the metal content, functional group concentration, cation exchange capacity, PO_4^{-3}; introduction, elaboration, and measurement of peat humification indicators; analysis of the conventional extraction methods of humic acids, isolation in preparative quantities of humic acids, preparation of UV spectra, participation in the acquisition of KMR spectra; summarisation, evaluation, and analysis of the research results, assessment of the relationship between different parameters and the properties of peat humic acids; a study and experimental testing of the impact of the isolation methods of humic acids on the properties of humic acids.

1 LITERATURE REVIEW

1.1 Bogs – the Genesis of Peat

Peat is an eluvial sediment that forms from little transformed plant remains in bogged-up places (overgrown water bodies, land depressions, as well as areas where the surface water runoff is hindered or high groundwater level persists). Dry peat consists of over 50% of organic matter (Kivinen and Pakarinen, 1981), it is formed from incompletely decomposed bog plants in a process when due to a high water level and low oxygen content decomposition of the plant material is significantly retarded. As a result of condensation processes and microbial activity, during the humification process dark-coloured heterogeneous organic substances with high molecular weight – humic substances (HS) – are formed (Kuršs and Stinkule, 1997).

The following features pertain to peat.

- Decomposition degree – the ratio of fibrous and undecomposed peat material to the dense, amorphous part which forms during the humification process (Fuchsman, 1980). It depends on the environmental conditions (mainly the oxygen and moisture levels), plant composition, and the age of peat. According to the decomposition degree of peat, it can be subdivided into:
  - peat of a low decomposition degree (< 20%);
  - peat of a medium decomposition degree (20–35%);
  - well-decomposed peat (> 35%).
- There is a close relationship between the decomposition degree of peat, its colour, and plasticity – decomposed peat is darker and more plastic than peat of a low decomposition degree. Therefore, for fieldworks, the von Post scale with 10 divisions was created – the decomposition degree can be determined by squeezing a fresh, wet peat sample in hand (von Post, 1924).
- Peat moisture level – percentage of the amount of water in the whole peat sample. It depends on the peat type and decomposition degree, and on some occasions it can reach 95–98%.
- Peat ash content – percentage of inorganic or mineral particles in a dry peat sample. Peat ash can be divided into constitutional (mineral content in plant

residue) and fetched (by wind and water). This parameter substantially influences the physical and mechanical properties of peat.

- Botanical composition – the types of peat-forming plants which characterise peat-forming conditions. Depending on the percentage of various trees, shrubs, grasses, and mosses, peat types are determined. We can distinguish three types of peat (Fuchsman, 1980):
    - fen type peat – formed from plants which feed on groundwater rich in biogenic elements. For this peat type hypnum moss (*Hypnos*), reed (*Phragmites*), and sedge (*Carex*), as well as trees – alder (*Alnus*), willow (*Salix*), and birch (*Betula*) are characteristic;
    - raised bog type peat – formed from plants which feed mostly from atmospheric precipitation. It is mainly composed of *Sphagnum* moss (*Sphagnum*), cotton grass (*Eriphorum*), and different genera of heather. Not all of the aforesaid plants are always represented, but *Sphagnum* moss occurs the most frequently;
    - transition mire type peat – formed from mixed vegetation;
    - some plants such as rush (*Scheucheria*) and cattail (*Scirpus*) are found in both raised bog peat and fen-type peat. Classification by the botanical composition is used to supplement and confirm the geological conditions, such as the impact of groundwater, since the plants typical of raised bog peat grow on waters poor in mineral elements, whereas for vegetation found in low-type peat, more nutrients are necessary (Fuchsman, 1980).
- Subtypes of peat can also be determined by geobotanical classification: forest peat, forest-mire peat, and mire peat. Peat groups can also be described by vegetation: tree peat, tree-moss peat, grass peat, grass-moss peat, and moss peat.
- Botanical composition is closely related to plant nutrition conditions, properties of the underlying layers of the peat bog, the mineralisation degree of the groundwater; it significantly affects the degree of decomposition, moisture, and physical and mechanical properties of peat (Fuchsman, 1980).

- Peat deposits are formed from one or more types of peat. Three types of deposits are distinguished: low, transitional, and mixed type deposits.
- Naturally peat is characterized by a high moisture content (85–96%) and low density (1,400 to 1,600 kg/m^3). Dry peat has an average density of 300 kg/m^3, porosity from 70 to 80%, a small coefficient of thermal conductivity, high capability of gas absorption, capability of moisture absorption from 500 to 1 500%, and the combustion heat from 18.4 to 23.9 MJ/kg (Danilāns, 1995).

The development of peatlands takes many thousands of years, and for the accumulation of peat as well as prevention of complete decay of biomass produced by the plant growth, excess water is needed. Up to recent time, it was considered that the water level keeps above the surrounding landscape due to capillary action, and the groundwater rises within the peat column (Charman, 2002). However, studies of capillarity in *Sphagnum* peat with a low humification degree showed that the capillary rise of water is insufficient to create a groundwater rise. Today it is generally accented that domed profile probably forms mainly due to low hydraulic conductivity, decay processes, and the different values of conductivity in the catotelm, i.e., the lower, anoxic part of the peat column (Charman, 2002).

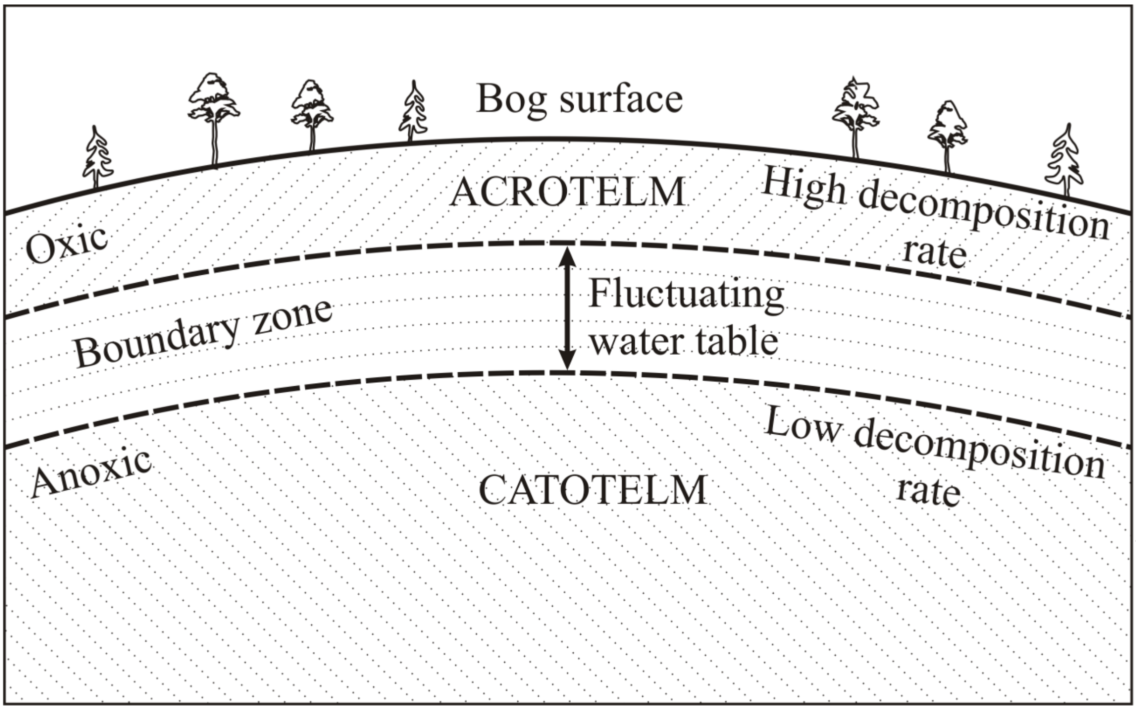

Figure 1. The acrotelm and catotelm – the main functional zones of a raised peat bog (Borgmark, 2005)

Decay of plant material occurs mainly in the acrotelm – the upper, aerated layer of peat (Figure 1) (Ingram, 1978, 1983; Charman, 2002). The decay rate in the acrotelm considerably varies. Water content, temperature, oxygen conditions, plant material, and microbial populations are the main factors controlling the decay rate of the peat (Clymo, 1983; Charman, 2002). In the catotelm (Figure 1) the decay processes are almost nonexistent; the decay rate is only ≈0.1% of that in the acrotelm (Ingram, 1978, Belyea and Clymo, 2001). The rate of decomposition is a complex issue not yet fully understood because it is affected by various factors and interactions. The border between the acrotelm and catotelm is not evident, but approximately matches the lowest level of groundwater level in summer (Clymo, 1984). The acrotelm supplies the catotelm with organic material, and in this transfer process from the aerobic to anaerobic layer about 80–95% of the total organic production is lost (Clymo, 1984, Warner *et al.*, 1993).

Decomposition of peat mainly depends on the thickness of the acrotelm, where the plant decay takes place. The thickness of the acrotelm equals the degree of surface wetness; therefore, decomposition of peat is a function of the degree of surface wetness (Caseldine *et al.,* 2000). In turn, surface wetness mainly depends on precipitation and evapotranspiration, but the exact relationship is unknown (Charman, 2002). There are a number of internal and external factors that affect surface wetness and humidity of bogs on different scales; climate is the most significant of these factors (Charman, 2002).

1.2 The Significance of Humic Substances in the Carbon Biogeochemical Cycle

Among the various organic substances that occur naturally, humic substances (HS) are the most widespread (Stevenson, 1982). Humic substances form most of the organic component of soil, peat, and natural waters (Figure 2).

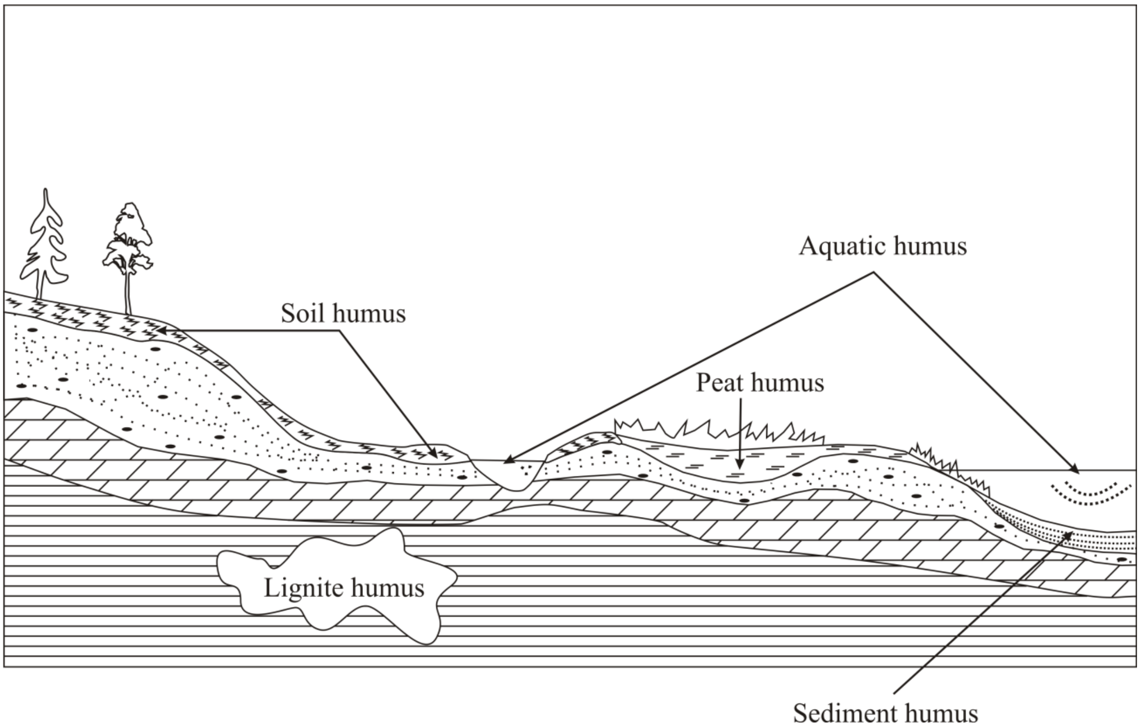

Figure 2. Humic substances in the environment

Up to 70% of the organic carbon in soil and up to 90% in peat may occur in the form of humic substances. Humic substances influence the formation process of fossil fuels and are significant in the global carbon geochemical cycle. The global pool of humic matter is an important component in the formation of atmospheric carbon dioxide. The estimated amount of carbon in the atmosphere is 638–702 × 10^{15} g, which is in the same range as carbon accumulated as soil humus (700–3,000 × 10^{15} g), peat humus (250 × 10^{15} g), and humic substances (850 – 1,700 × 10^{15} g) (Woodwell *et al.*, 1978). It has been recently estimated that the global transport of organic carbon to oceans is about 0.4 to 0.9 × 10^{15} g/yr (Hope *et al.*, 1994).

The significance of humic substances is determined by their formation during the humification (decay) process of living matter. Humic substances form in an intermediate phase of the transformation process of living matter (organic carbon reservoir) which continues in the organic carbon cycle, or are deposited (as fossil materials). Properties of humus depend on its sources. Depending on the character of the humification process, the largest reservoirs of humic matter are either soil or peat.

1.3 Humic Substances and Their Properties

1.3.1 Classification of Humic Substances

The present knowledge indicates that humic substances form one of the most important groups of biomolecules (Stevenson, 1982). Humic substances do not belong to any unique chemical category. They are defined by combining all known aspects of their properties, including the process of their isolation. The most common definition is that “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 (Figure 3) (Stevenson, 1982):

- humin is the fraction of humic substances that is insoluble in water at any pH;
- humic acids (HA) belong to the fraction of humic substances that is insoluble in water under acidic conditions (pH < 2) but becomes soluble at pH > 2;
- fulvic acids (FA) belong to the fraction of humic substances that is soluble under all pH conditions”.

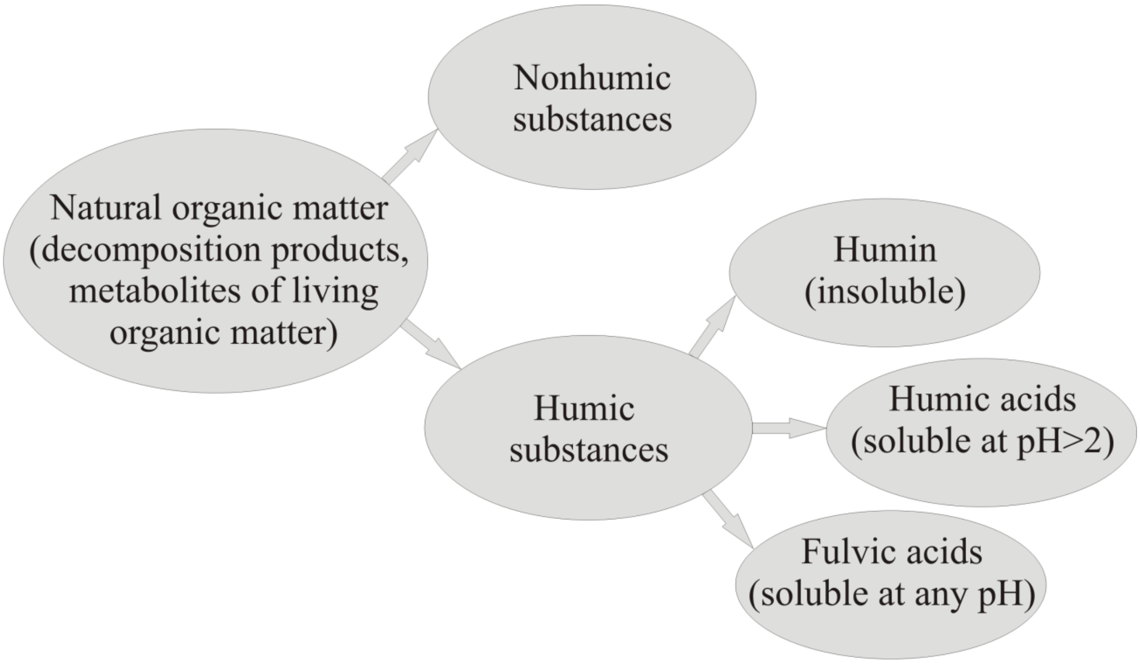

Figure 3. Classification of humic substances

1.3.2 Isolation of Humic Substances

Isolation of humic substances from the natural environment is hindered by complicated environments, where alongside humic substances other macromolecular compounds (amino acids, carbohydrates, and lipids) can be found. Currently there are many methods of isolation and purification of humic substances, but in each case one method may be more useful than others, and the results are difficult to compare. Therefore, standard methods for isolation of humic substances from different sources have been developed to obtain comparable results. Stevenson (1982) has stated that the ideal method of isolation of HS from peat must meet four criteria.

1. The method should not alter the natural characteristics of the isolated material.
2. The isolated HS must be free of inorganic contaminants, such as clay particles and polyvalent cations.
3. Isolation is ideal if the acquired fractions of HS represent the entire molecular mass series.
4. The isolation method is applicable in different environments.

The main problem with isolation of humic substances from peat is that only a small proportion of humus is in a free form. Most of humus is bound to minerals and decaying moss in different forms and, in order to obtain the HS, it is necessary to break these associations (Kononov, 1966; Stevenson, 1982; Orlov, 1990).

For isolation of HS from peat, sodium hydroxide solutions are most frequently used (Kononov, 1966; Schnitzer, 1978). Many researchers disapprove of the use of NaOH solution because the presence of an alkaline solution transforms the structure and properties of obtained humic substances due to hydrolysis and oxidation reactions (Stevenson, 1982). It is recommended to perform isolation of humic substances with NaOH solution in N_2 atmosphere, reducing the structural changes during the isolation process. Already in the 1930s, attempts were made to use other solutions for isolation of HS, such as mineral acid salts (NAF, $Na_2P_4O_7$, and Na_3PO_4), low molecular acids, inorganic and organic solvents, etc.

The use of mineral acid salts has significant benefits, especially the use of sodium pyrophosphate ($Na_2P_4O_7$). The efficiency of this solution is determined by the good solubility of the obtained salts of humic substances. Nevertheless, it is believed that HS are bound in complex forms in peat and mineral fractions, and the isolation efficiency of $Na_2P_4O_7$ or other solutions of such type is lower than that of alkaline solutions. However, in some cases sodium pyrophosphate gives higher HS yields (Drozdova, 1959).

According to standard methods (Schnitzer, 1978; Thurman and Malcolm, 1981), isolation of HS is performed with 0.1 N NaOH, 0.5 N NaOH, 0.1 N NaOH, or 0.1 N $Na_2P_4O_7$.

The following substances are obtained with a NaOH solution:

- non-extractable peat organic matter;
- extractable peat organic matter – water-soluble fractions of humic substances.

Humic acids are obtained by acidification of the alkaline extract with HCl solution to pH of 1.5. Humic acids precipitate as dark brown or black residue, but fulvic acids remain in the extract, usually of yellow colour.

1.3.3 Characterisation of Peat Humic Acids

Contemporary analytical methods have provided a large amount of information on the characteristics of humic substances. Among such basic characteristics are, firstly, the elemental composition of HS. Knowledge of the elemental composition of HS allows to evaluate the role of basic elements in the building of macromolecules of humus and to analyze, in simplified terms, the influence of the origin of humus on its composition and even structure. The great variability of natural waters is related to diverse humification processes and the composition of humus. The elemental compositions of humic substances isolated from different types of peat indicate that elemental composition depends on the origin of humus, seasonal factors, and other variables (Table 1).

The variations of the elemental composition of humic substances in different peat samples is similar to the differences between humic substances of different origin.

The variations of the elemental composition of humus indicate the effect of environmental conditions, especially humification, on the structure and properties of humic substances.

Table 1

Properties of peat humic acids

Source materials	**Ash, %** in dried HA	**Elemental analysis, %**				**Functional groups**		**Reference**
		C	H	N	O	COOH (cmol/kg)	Phenolic OH (cmol/kg)	
***Sphagnum* peat** (Canada)	2.1	46.1	4.9	2.1	35.0	192	86	Yamaguchi *et al.*, 1998
Reed-sedge peat (Belarus)	3.8	48.3	5.2	3.1	28.6	186	71	
Reed peat (Japan)	4.4	36.3	5.9	2.1	33.6	134	68	
Woody peat (Indonesia)	1.7	52.6	4.8	1.5	28.7	324	25	
Reference peat (Italy)	n.d.	44.0	5.0	1.6	49.0	429	224 meq/mg	Francioso *et al.*, 2003
***Sphagnum* peat** (Switzerland)	0.64	48.2	5.97	0.6	45.2	n.d.	n.d.	Zaccone *et al.*, 2007
Sawgrass peat (Florida)	n.d.	57.5	5.6	2.8	36.5	n.d.	n.d.	Hatcher *et al.*, 1986
Ringinglow bog peat (England)	n.d.	49.8	4.5	2.3	42.0	3.00 meq/g	1.65 meq/g	Gao *et al.*, 1999

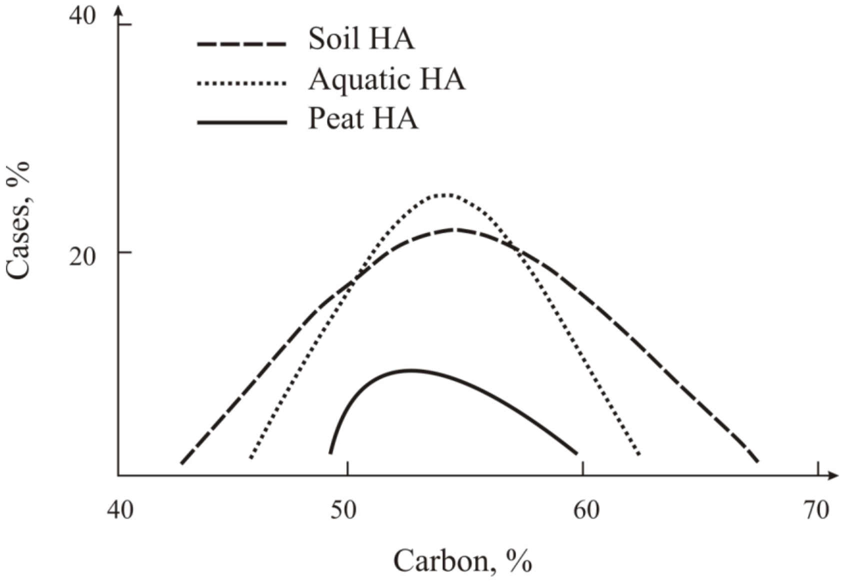

Figure 4. The content of carbon in humic acids isolated from peat, soil, and aquatic samples (by Orlov, 1990)

The data on elemental analysis of 340 humic substances indicates differences in the content of carbon in substances of different origin (Figure 4). The carbon content of peat and soil humic substances is similar; nevertheless, there are clear differences in the composition of different humic substances. Humic and fulvic acids also differ in composition, except when the impact of the source of HS on their composition is more pronounced than the differences between FA and HA.

In general, the carbon content in humic acids is higher than in fulvic acids (Orlov, 1990). The content of other elements can also indicate structural features of humic substances. The content of oxygen indicates the number and the relative content of oxygen-containing moieties in the structure of humic substances: carboxyl, hydroxyl, and carbonyl groups and oxygen-containing heterocyclic structures, as well as carbohydrates. Elimination of oxygen-containing moieties usually takes place in the humification process. Nitrogen content in humus also indicates the character and intensity of the humification processes. The nitrogen content in humic acids is commonly ≈1.5 times higher than in fulvic acids.

Since humic substances also are able to bind elements by formation of complexes in isolated and purified HS samples, microelements may be linked to the structure of humic substances. Therefore, the content of bound microelements reflects both natural conditions and the ability of humic substances to form complexes (Riise and Salbu, 1989). In HS reference samples, elements such as Ca, Mg, Ba, Al, Fe, Na, and K have been found in quantities of 50 to 200 μg/g. The concentrations of Ag, B, Cd, Cr, Hg, Mo, Ni, Pb, Sc, Sn, Sr, Th, Ti, and Zr are lower, ranging from 0.01 to 5 μg/g.

Elemental composition is often used for the calculation of atomic ratios (O/C, H/C, N/C). Plots of these values (van Krevelen graphs) can help to identify the correlation between the elemental composition and origin of humic substances (Visser, 1983; van Krevelen, 1984; Kļaviņš, 1993; Pettersson *et al.,* 1994). Plots of H/C vs O/C, acidity or molecular weight (M_w) allow to assess the degree of the humification process, or the degree to which plant residue has transformed into biochemically more stable, but more condensed aromatic structures. The degree of dehydratation or oxidation of humic matter can also be estimated with van Krevelen graphs.

Based on the C/O and H/C proportions, the percentage (α) of aromatic groups can be estimated (Orlov *et al.,* 1987) as follows:

$$\alpha = [C_{aromatic}]/[C_{aromatic}] + [C_{aliphatic}];$$

$$C_{aliphatic} = [(H/C + 2O/C \times K) / 1.67] - 0.20;$$

where: $K = [A - (B + C + D)/A$; A – number of moles of oxygen in humic sample; B, C, D – number of moles of oxygen in phenol, carboxyl, and carbohydrate structural units, respectively.

Functional groups such as carbonyl, hydroxyl (alcoholic and phenolic hydroxyls), methoxyl groups, aldehyde, amino-, and other functional groups, are an important part of the structure of humus; therefore, methods for estimation of their number have been proposed. It is recommended to perform measurements of hydroxyl group content by acetylating them with acetic anhydride and subsequently determining the numbr of bound acetates (Tan, 2005). The content of alcohol and phenol hydroxyls is mostly calculated as a difference based on the total acidity and content of carboxyl groups and hydroxyl groups. It is recommended to determine the total content of carbonyl groups after oxime formation, titrating the excess of used hydroxylamine with perchloric acid. Concentrations of quinoidal carbonyl groups are determined by reducing Fe^{+2} in an alkaline triethylamine solution (Tan, 2005).

The concentration of hydroxyl groups in HS ranges from 0.3 to 2.5 mg Eq/g (the concentration of hydroxyl groups in humic acids is typically higher than in fulvic acids). The concentration of methoxyl groups in HS has been determined in few studies, and it has been suggested that the high concentration of methoxyl groups indicates humic substances of terrestrial origin and a low degree of humification. Ketone and aldehyde carbonyl groups have been found in very small amounts (0.05–0.3 mMol/g). Chemical methods are time- and work-consuming, and the obtained results can be influenced by the conditions during the preparation of the sample. Contemporary analytical processes are ^{13}C NMR, IR and ESR spectroscopy, often together with corresponding derivatisation methods (Leenheer and Noyes, 1989a). The formation of humic substances, i.e., humification involves transformation of living organic matter, which, depending on its origin, can contain proteins, carbohydrates, fragments of nucleic acids, and other biomolecules. Peat humic substances contain these biomolecules in varying amounts, linked by bonds of different strength – from very loosely ionogenically bound substances that can be partly lost during isolation to stable links into the structure of humus throughout subsequent transformations. More loosely bound substances may be isolated after hydrolysis, but more permanently linked substances can be identified only by analysis of degradation products of humic substances.

Various spectroscopic methods have been widely used to characterise humic substances, from simple but least informative methods (UV-Vis spectroscopy) to more sophisticated methods. The UV-Vis spectra of HS do not give much information due to the absence of individual sorption maximums and the evenly decreasing intensity of sorption. The main structural units that influence the sorption in UV-Vis spectral regions are condensed structures conjugated with oxygen-containing moieties and the polyaromatic core of humic molecules (Larina and Kasatockhin, 1966). Humic acids with higher percentage of aromatic condensed structures have higher sorption at lower wavelengths (220–280 nm) than fulvic acids, which have higher percentage of aliphatic structural units. Sorption in the spectral region around 280 nm is determined by $\pi - \pi^*$ electron transitions typical of phenolic substances, aniline derivatives, benzoic acid derivatives, and polycyclic aromatic hydrocarbons. It is possible to evaluate the percentage of aromatic structural units in the molecules of humic substances based on their sorption in an UV region (Larina and Kasatockhin, 1966; Gauthier *et al.,* 1987; Chin *et al.,* 1994; Traina *et al.,* 1990).

UV-Vis spectra can also be used to evaluate molecular mass of humic substances. The proportion of E_4/E_6 (Kononova, 1966; Chen *et al.,* 1977) can be used for this purpose because of a rather good correlation with the molecular masses determined with gel filtration. The E_4/E_6 ratio also correlates with free radical content, contents of O, C, -COOH; thus, this parameter can be regarded as a simple means of measuring molecular mass and humification degree.

Infrared spectra of humic substances can provide information on their structure, particularly in regard to typical functional groups and their links in the structure of humic substances. A great advantage of this method is that the samples do not require pre-cleaning, but rather concentration with evaporation. However, an unambiguous interpretation of the obtained spectra is not always possible due to interferences by adsorbed water and inorganic substances that are linked to the structure of humic substances. IR spectral analysis is a common method (Dereppe *et al.*, 1980; Senesi *et al.,* 1986; Peschel and Wildt, 1988; Senesi and Sakellariadou, 1994) to describe the properties and the basic characteristics of humic substances; therefore, there is much information on the IR spectra of humic substances.

IR spectra allow to identify the presence of most of the important functional groups, to compare humic substances of different origin, and to estimate the concentration of functional groups, and they are important for the development of structural models.

Wide scan range (5,000 G) ESR spectra of humic substances are dominated by a strong, very narrow symmetrical resonance common to ESR spectra of humic matter of any origin. However, differences are apparent in the relative intensity of the two dominant resonance lines and in the presence of a minor resonance. Line widths (ΔH_{pp}) and spin contents of free radicals (I') are among the ESR spectral parameters that provide the most information (Atherton *et al.*, 1967). The structures most possible to bear organic free radicals are semiquinone structures conjugated with condensed ring systems or substituted with aliphatic or heteroatom-containing substituents. ESR spectra indicate the differences in the molecular structures of humic and fulvic acids. The applicability of ESR spectra in characterisation of humic substances is limited to some extent (Peuravuori, 1992). However, this method is a powerful tool for the study of the complexation process between HS and paramagnetic metal ions since it allows to measure the content of bound metal atoms and the types of their links in the molecules.

The fluorescence spectra of humic substances show one broad band in the excitation spectrum (λ_{max} = 320–330 nm) and one in the emission spectrum (Ewald *et al.,* 1983; Belin *et al.,* 1993). Studies of homogeneity of HS samples provide some evidence for the presence of lignine fragments in the structure of humic substances. Fluorescence spectra have been comparatively widely used to characterise interactions between humic substances and metal ions or organic molecules since these substances induce the quenching of fluorescence of HS.

NMR techniques are a very efficient tool to determine the properties of humus. Among the NMR spectra, ^{1}H – NMR spectra give less information, but they are the most widely used, and provided valuable information on the structure of humic substances (Lundquist *et al.,* 1985). ^{1}H NMR spectra have been used to estimate the location and quantity of hydrogen atoms in HS molecules and the number of specific functional groups.

The ^{13}C NMR technique allows to determine directly the relative number of major structural units in humic substances. Signals in these NMR spectra represent different types of carbon atoms and allow to determine their concentration (the intensities of carbon signals are proportional to the number of carbon atoms in the substances). However, interpretation of ^{13}C NMR results can be misleading since the nuclei can be located in a wide variety of chemical environments that produce different chemical shifts due to the very heterogeneous macromolecular nature of humic substances (Peuravuori and Pihlaja, 1990; Fründ and Lüdemann, 1991; Fründ *et al.,* 1994a; Fründ *et al.,* 1994b).

The dimensions and shapes of humic substances directly influence their solubility, their ability to migrate, and their interactions with suspended particulate matter and living matter. Major problems in the studies of molecular masses of humic substances are their polydispersity and the fact that humic substances are mixtures of structurally similar substances. Average values of variables must be used in characterisation of the molecular properties of humic substances. The molecular masses of humic substances can be characterised by such variables as mean molecular mass, the shape and conformation of molecules, the degree of polydispersity, and distribution of molecular masses. The molecular characteristics of humic substances are closely connected with their environmental functions and geochemical roles. The evaluation of the molecular mass and shape of the molecules is directly related to analysis of the structure of humic substances.

Since humic substances are polydisperse mixtures, they can be characterised by averaged molecular masses: number-averaged (M_n) and weight-averaged (M_w) (Peuravuori, 1992).

The most common methods for analysis of molecular weights of humic substances are gel filtration, high-performance size-exclusion chromatography, vapour pressure osmometry, low angle X-ray scattering, ultracentrifugation, ultrafiltration, field flow fractionation, viscosimetry, electron microscopy, a.o. (Pansu and Gantheyron, 2006). The obtained values of molecular masses of humic substances greatly differ, depending on the methods used and on the origin of HS.

Molecular weights of humic substances vary in a broad range (400 to 1,000,000), thus supporting the idea of two types of organisation of humic molecules: organisation can take place at the molecular or molecular aggregate levels.

1.4 Studies of the Properties of Humic Substances of Peat

A significant part of the total organic carbon (TOC) pool is stored in the form of peat humic substances. From this perspective, peat humic substances are of definite importance in industrial mining and processing of peat. It has been stated that up to 500 Gt of humic acids of industrial importance can be mined worldwide (Markov *et al.*, 1998). Studies of peat humic acids are of importance to understand better the biogeochemical cycling of carbon, the development of peat resources, as well as the genesis of fossil fuels. At the same time, it is surprising that in-depth studies of peat humic substances are rather scarce. Only a few studies of peat humic substances focus on the changes of their properties within the peat profile, also considering the influence of peat-forming plants on the properties of peat and its humic substances.

Anderson and Hepburn (1986) studied HS in two peat profiles with different stratification and botanical composition. Some problems regarding their solubility in aqueous alkali solution, especially in peats with a significant amount of relatively low decomposed plant material were noted. In this study, several products of hydrolysis were studied to determine relevance to molecular structure and changes in the humification process. For example, HS are showed to contain a higher concentration of levulinic acid, which originates from primary polysaccharides, representing undecomposed plant carbohydrates, than can be obtained by conventional methods of carbohydrate hydrolysis. This can be explained by the presence of altered carbohydrates attached to the molecular structure of humic acids.

The humification process affected lignin-derived moieties as well (Anderson and Hepburn, 1986). Characteristic phenolic acids were separated and quantified by the conversion of the ether-soluble fractions of humic acids to volatile trimethysilil hydrolysates.

The conditions hostile to biological activity stop the processes of humification in peat at an early stage. Additionally, analysis of the mixture of extractable organic substances which comprise peat humic substances is more complex than analysis of mineral soils because the partial humification of peat appears to be much faster than the subsequent changes. These alterations of the original plant material determine the properties of humic substances. Oxidation of aromatic or alicyclic compounds can produce a series of very different products which can form more complex condensed structures. This means that humic substances are indeed products derived from peat, but their properties are influenced during the isolation process.

Cellulose, hemicellulose, lignins and tannins are the four main plant ingredients. Organic extracts of peat contain quantitatively important fractions of polysaccharides, but it is difficult to extract polysaccharide preparations covalently bound within humic substances. Fractions of fulvic acids that are rich in polysaccharides vary in their content depending on the source, and contain important peptide components (Anderson and Hepburn, 1986).

In the process of peat formation, lignin appears to undergo gross degradation and condensation, resulting in the formation of fractions with a higher molecular weight.

During humification peat-forming plants, such as *Phragmites, Carex,* and moss, are subjected to demethylation, oxidation, and condensation; however, the losses of lignin are much smaller. Pyrolysis mass spectrometric analyses of HA in well decomposed peat confirm that peat humic acids from deeper layers are of a higher humification degree, decreasing lignin and polysaccharide pyrolysis products.

Tannins are considered to be precursors and components of humic substances; however, although some degradation products of humic acids can be derived from tannins (e.g. resorcinol and phloroglucinol derivatives), more typical tannin fragments (benzylsulphides and ketones) are not reported (Anderson and Hepburn, 1986).

In the peat samples studied by Anderson and Hepburn (1986), the amount of total carbon presented as humic acid C in general increased with the sample depth, except for the topmost samples which were subjected to increased oxidation and therefore had higher concentrations of organic carbon.

Anderson and Hepburn (1986) also report that the use of the E_4/E_6 ratio as a humification degree indicator rather reflects the molecular weight and particle size, not the chemical structure of the sample.

Phenolic acids in peats are most probably produced by the microbial degradation process of plant residue that contains high amounts of phenols. Removal of these structures because of the water transport and washout of fulvic acids would lower the values of decomposition degrees. Therefore, better understanding of the flows of organic components in peat bogs is needed.

In a study by Zaccone *et al.* (2007), HA isolated from a peat core (depth 81 cm) divided into 27 slices of 3 cm each were studied. Humic acids were analyzed using Fourier transform infrared spectroscopy and UV-Vis spectroscopy (E_4/E_6 ratio).

Different conditions along the peat profile were shown in respect to physical and chemical properties. In total 3 distinct zones were distinguished in the bog: the acrotelm – the zone of active decay, seasonally oxygenated; the catotelm – always anoxic with a very slow and partial decomposition of organic matter; the transitional layer where complex interactions between organic and inorganic compounds take place. E_4/E_6 ratio and FT-IR spectra of HA confirmed different stages of humification of peat in the profile. In the deeper layers a greater degree of aromatic condensation was observed than in the surface layer.

Hatcher *et al.* (1986) investigated sawgrass (*Mariscus jamaicensis*) peat from Florida by doing elemental analysis and ^{13}C NMR spectroscopy. It appears that during humification loss of cellulose and other carbohydrates present in living plants takes place, and humic substances are formed from lignin. Since the peat samples have a low degree of humification, humic substances may contain comparatively unchanged compounds found in the living plants. In some situations the amount of humic acids decreased despite an increase in humification. That can be explained by selective oxidation and solubility of fragments of humic acids, as well as by the fact that drainage waters remove humic acids.

Geochemical studies of organic matter testify that plant polysaccharides and proteins are degraded in the upper 20 cm of peat, and with increasing depth, microbial polysaccharides (e.g. polyuronic acids) are more frequent. Therefore, early decomposition removes plant-derived carbohydrates.

To understand humification better, humified fractions of peat and plant polymers that form a significant part of humic substances must be examined. Lignin is relatively unaltered (in comparison with proteins or carbohydrates) in peat humic substances. Humic substances undergo the most significant changes in the upper layers (0–20 cm) of peat, where an active humification process takes place: the amount of humic substances increases, but the amount of polysaccharides sharply decreases as the plants degrade. In lower layers an unexplained decrease of humic substances takes place; the trend is opposite to the increasing humification. This can be explained with the mobilisation or loss of HS.

H/C and O/C ratios allow to characterise the humification process. Near the surface the atomic H/C ratio is higher, but it declines with increasing depth due to hydrolysis and increased aromaticity. O/C ratios also decline with depth. That can be explained by the abundance of carboxyl and carbonyl functional groups as well as oxygen-rich polysaccharides at the surface.

Solid-state ^{13}C NMR allows to study the chemical and structural composition of peat during the humification process. An important structural change is the loss of carbohydrates with increasing depth, while aliphatic and aromatic groups gradually increase with depth. In the deepest layers peat is less saturated with carbohydrates, but it is enriched with lignin molecules. Humic substances have relatively high aromaticity at all depths, which suggest that humic substances in peat form independently from the plant material. That could be related to the fact that humic substances are produced selectively from one component of peat. Considering the high aromaticity of humic substances, they are most likely derived from lignin (Hatcher *et al.*, 1986).

Humic substances are of similar structure at all depths of peat. They are produced by oxidative processes and have more aromatic groups than other peat components due to being derived from lignin.

A study by McDonnell *et al.* (2001) estimated the variation of concentrations and characteristics depending on the source of humic substances to understand better the significance of humic substances in carbon dynamics. Humic substances from 6 peat and peaty mineral soil samples from the Vartry Catchment were isolated, fractionated, quantified, and characterised using the method adapted by the International Humic Substance Society. Using XAD-8 resin columns and dialysis, samples were isolated and analysed for ash, elements (carbon, hydrogen, nitrogen, and oxygen), amino acids, and carbohydrates and by means of FT-IR spectroscopy.

Although details of the chemical processes were not reported, some differences between forested and non-forested sites were identified – the HS from forested sites consisted of more humified organic matter and had undergone different decomposition processes. Two variables important for the formation of HS were identified – land use and altitude – since trends in HS properties related to altitude and land use were observed: for example, increased aromacity indicates greater humification and elevated elemental ratios (both H/C and C/N). Humic acids showed that the humification degree increased with increasing altitude at non-forested sites. The soil chemical environment at forested and non-forested sites differed in amino acid and saccharide concentrations.

One-dimensional NMR analysis of a fulvic and a humic acid obtained from *Eriophorum* peat provided semi-quantitative contents of humic constituents with limited resolution in structural detail (Hertkorn *et al.,* 2002). More accurate arrangement of humic substructures was achieved when information from homo- and hetero-nuclear two-dimensional NMR spectra was used. Detailed analysis showed significant impacts of amino acids, amino- and desoxy- sugars, and highly oxidized aliphatic chains of intermediate length in the chemical shift region normally attributed to carbohydrates. Combined analysis of all NMR spectra showed a very significant effect of the fractionation procedure on the composition and chemical structure of humic substances. Comparison of the structures forming humic and fulvic acids indicates that the biopolymer degradation model of humification applies as the fulvic acids are formed from humic acid constituents.

In a study by Chefetz *et al.* (2002), the structure of peat humic acids was studied in comparison with soil humic acids using bleaching (NaClO oxidation) and acid hydrolysis with ^{13}C NMR and pyrolysis-gas chromatography/mass spectrometry. The suggested approach (mild oxidation and acid hydrolysis) allows to remove known structural fragments and to study the building blocks and components of HS macromolecules. Bleaching and hydrolysis allows to remove lignin structures and carbohydrates. However, this study does not provide more specific conclusions that would shed some light on the specific structural features of peat humic substances.

Chemical methods and spectroscopy techniques, including UV-Vis, FT-IR, and ^{13}C NMR used for characterisation of peat humic and fulvic acids isolated from a surface horizon (0–60 cm) and deeper horizon (> 60 cm) of an ombrotrophic peat bog located in NW Spain showed that in the deeper horizon peat humic substances have a greater degree of aromatic condensation than in the surface horizon, judging from the E_4/E_6 ratios. The alkyl-C content of humic substances remained almost unchanged, whereas the O-alkyl-C content of humic acids decreased with depth. Ionisation constants of the carboxylic groups or the phenolic groups showed no variation with depth. Ratios of carboxylic/phenolic content were similar to other peat bogs, with a higher content of acid groups in the humic substances isolated from the lower horizon, mainly attributable to the carboxylic groups (Gondar *et al.,* 2005).

Use of flash pyrolysis gas chromatography-mass spectrometry, solid-state ^{13}C NMR, and FT-IR for analysis of different fractions of humic substances from different depths of the peat bogs in Spain showed significant differences of molecular weight, composition, and structural characteristics (alkyl-, O-alkyl and aromatic structures) in different humic materials (Gonzalez *et al.,* 2003). Humic acids contained lignin structures, while fulvic acids consisted largely from carbohydrates and peptides. As shown by infrared spectra, lignin originates from vascular plants, but humic substances do not characterise the changes of biodiversity during the peat formation process since homogenisation and transformation of organic materials happens.

The disaggregation kinetics of peat humic acids at several pH values can be studied using fluorescence correlation spectroscopy (Avena and Wilkinson, 2002). It measures diffusion coefficients of fluorescent molecules and aggregates, allowing to determine the disaggregation rates with a resolution of seconds to minutes. Disaggregation is initiated by dilution of a peat concentrate containing large aggregates and free monomers. At different pH values the size of aggregates decreases and the proportion of free monomers in the solution increases until complete disaggregation occurs. The mechanism involves release of monomers from the surface of the aggregates, with pH significantly affecting the disaggregation rate. These results may be of importance for environmental systems, implying that at low pH HS could temporarily trap, protect, and transport organic molecules, pesticides, metal ions, and other substances in large aggregates, releasing them at higher pH values by disaggregation. Therefore, this mechanism is prospective for transport and release of hydrophobic pollutants from areas of high HS concentration, such as soils, to water bodies with lower concentrations, including rivers and lakes.

Investigating humic acids and humin from acidic peat with solid state ^{13}C NMR and pyrolysis with tetramethylammonium hydroxide (TMAH) and tetraethylammonium acetate (TEAAc) derivatisation, and comparing the results obtained with solvent extraction, it was evident that more complex structures (lignins, saturated and unsaturated hydrocarbons, methyl esters) retained from insoluble material were dominating the humic fractions whereas lipids originating mostly from plants were extracted with solvent (Guignard *et al.*, 2005). The use of these methods allows to identify a wide range of constituents of humic substances, indicating that hydrogen bonds play a key role in their structure. The combination of the said methods allows to determine certain fatty acids and their esters in the structure of peat humic substances.

Quantitative estimation of peat, brown coal, and lignite humic acids using chemical parameters, ^{1}H-NMR spectroscopy, and differential thermal analysis showed a rather high heterogeneity of peat HAs. Only some variables out of 14 obtained (C concentration, aliphatic and aromatic components and the heat of reaction of the second exothermic peak) were selected for analysis due to low data correlation. The multivariate discriminant analysis performed on these variables clearly distinguished different HA extracted from distinctive sources and therefore is suggested as a method for investigation of the humification process during coalification (Francioso *et al.*, 2003).

Characterisation of organic matter from peat, leonardites, and lignites can be done by combining humification parameters and electrofocusing. Humification index can be used for distinguishing peat from leonardite and lignite, since their values overlap, whereas humification rate allows to identify only leonardite. The electrofocusing method reveals different band patterns in all studied materials: HA from peat samples do not show bands in the region with isoelectric point while leonardite HA show very intense bands, but lignite HA samples have very different band patterns, and bands are poorly resolved (Cavani *et al.*, 2003).

In the following studies, humic substances in bog profiles have been analyzed.

Dudarchik *et al.* (1997) analysed humic substances from fen-type sedge peat using ^1H NMR spectroscopy and stressed that NMR can provide important information on the origination and structural features of HA. However, the authors failed to differentiate major structural units of peat humic acids.

A distinctive aspect of studies of peat humic substances is that their properties have been analyzed from samples obtained in different places of the world (Garnier-Sillam *et al.,* 1999, Fong and Mohamed, 2006), with comparatively simple methods (analysis of elemental composition, functional groups, E_4/E_6 ratio, UV-Vis, and IR spectroscopy).

A study of HS isolated from peats in Sumatra (Indonesia) confirms that the studied humic acids are generally aliphatic and significantly differ from humic substances extracted from soil (Garnier-Sillam *et al.,* 1999).

A study of humic substances in peat of Sarawak (Malaysia) shows high concentrations of carboxylic and phenolic groups, rich in carbohydrates and oxygen. The studied HA have comparatively high molecular weight and, as indicated by the E_4/E_6 ratio, are dominantly aliphatic. A study of metal complexing capacity demonstrates a high detoxifying capacity of humic substances (Fong and Mohamed, 2006).

In-depth characterisation of peat fulvic acids has been done by Hänninen *et al.* (1993). Fractions of peat fulvic acids have been analyzed with IR, NMR spectroscopy, and CuO oxidation. CuO oxidation demonstrated that the main products of all fulvic acid fractions were 4-hydroxybenzaldehyde and 4-hydroxyacetophenone, both indicators of 4-hydroxyphenylpropane building blocks. The authors of this study emphasise that the number of aromatic structures in peat fulvic acids is low and it is determined by presence of olephinic double bonds. However, aromaticity signals found with ^{13}C NMR can only in part be explained by aromaticity. Thus, the structure of peat humic substances could much differ depending on their source.

The impact of anthropogenic factors on the structure of humic substances has been assessed, comparing humic acids from peat in polluted and unpolluted remote regions of the United Kingdom using the method of ion-exchange equilibrium between humic acids and metal ions. The analyses showed that polluting metal ions (Fe, Cu, Pb) can be found in rather high concentrations closer to the urban and industrial regions, but the concentration of commonly occurring metal ions (Na, Mg, Ca) decreased with proximity to industrial areas. This indicates that pollution of peat with heavy and transition metals may occur by displacement of light metals in an ion-exchange process as well as by precipitation and migration from soil (Gao *et al.*, 1999).

As described by Helal *et al.* (2006), peat humin can serve as a good sorbent for removal of radionuclides and it demonstrates reasonable sorption capacity and can retain up to 1.04 mmol/g of Ag^+, 1.63 mmol/g of Zn^{2+} and 1.73 mmol/g of Co^{2+}. The sorption process is time-dependent and the percentage of uptake increases with pH (maximum pH $\approx$7.5), which corresponds to the pK_a value of humin.

Studies of the binding of Cu^{2+} to humic and fulvic acids extracted from two horizons of an ombrotrophic peat bog with the help of metal titration experiments at different pH values and at fixed ionic strength showed that the amount of Cu^{2+} bound to the humic fractions was greater than the amount bound to the fulvic fractions and only at the highest concentrations of metal ions, the amount of Cu^{2+} sorbed by both fractions became equal. The proton-to-metal ion exchange ratios are similar for all humic substances, with values ranging from 1.0 to 2.0, and decreasing with increased pH. The amount of bound Cu^{2+} is basically independent of the horizon from which the sample was extracted. The results indicate that humic substances show similar cation-binding behaviour despite the differences in chemical composition. The copper-binding data are quantitatively described with the NICA-Donnan model which allows to characterise only the binding sites of carboxylic type. The values of the binding constants are higher for humic acids, not fulvic acids (Gondar *et al.*, 2006a).

Investigation of sorption of humic and fulvic acids (International Humic Substance Society (IHSS), reference samples) on hematite and kaolinite showed that humic substances dissolved in groundwater can serve as sorbents for hydrophobic organic compounds (HOC) as they adsorb to certain mineral surfaces, rendering hydrophilic surfaces hydrophobic. The amount of sorption depends on the concentration of hydroxylated surface sites on the mineral and the properties of the humic substances, but sorption of humic substances to two solids is proportional to their aromatic carbon content and inversely proportional to the O/C ratio. Increased quantities of sorbed humic substances (f_{oc} 0.01% to 0.5%) increased the sorption of carbazole, dibenzothiophene, and anthracene; besides, the most aromatic peat humic acid showed the greatest sorption enhancement of HOC when sorbed to hematite. Sorption capacity can be affected by the surface of the mineral and the ionic strength of the electrolyte, determining the interfacial configuration of the sorbed humic substance, altering the size or accessibly of hydrophobic domains on the humic molecule to HOC (Murphy *et al.*, 1992).

Studies of the interaction between pesticides (chlordimeform and lindane), herbicides (paraquat, 2.4-dichlorophenoxyacetic acid, and atrazine) and humic substances (humic and fulvic acids) showed that humic substances, especially fulvic acids, can participate in the transport of pesticides (Hesketh *et al.*, 1996).

Gondar *et al.* (2006b) studied the binding of Pb(II), Cd(II), and Cu(II) to humic and fulvic acids isolated from an ombrotrophic peat bog with metal titration experiments at different pH values (pH 5.5 and 6.0) and fixed ionic strength (0.1 M KNO_3), measuring free metal ion concentrations in solution using ion-selective electrodes. Maximum adsorption capacity was observed for Cu, with almost the same saturation for Pb, but in the case of Cd, at the lowest concentrations of metal, the binding isotherm was an intermediate between Cu and Pb. The proton-to-metal exchange ratios followed the order $Cu^{2+} > Pb^{2+} > Cd^{2+}$. The binding data allowed to characterise only the binding sites of carboxylic type using the NICA-Donnan model. The values of the binding constants were higher for humic acids than for fulvic acids and higher for Pb than for Cd.

It has been proposed that peat derived from *Sphagnum* moss can serve as a media for preservation of different biodegradable material, such as human bodies, carcasses of domestic materials, dried fruits, kegs of butter, etc, for up to 2000 years. This can be explained by α-keto-carboxylate groups that comprise ≈60% of the holocellulose in the hyaline cell walls of the moss. The condensation of highly reactive carbonyl compounds with primary amino-groups or ammonia in the Maillard reaction inhibits microbial growth. It has been suggested that these properties of *Sphagnum* moss peat could find broader or entirely new applications in preservation of food and other products in the future (Painter, 1998).

A brief review of studies done on the properties of peat humic substances clearly reveals the major contradictions in previous studies and their conclusions, especially those relevant for studies of peat HS from raised bogs in Latvia:

1) the differences in the composition of peat-forming plants, impacts of climatic and hydrological factors and humification conditions are not considered in the reviewed studies of peat humic substances;
2) a major contradiction exists regarding the properties of peat HA: if some studies stress their aromaticity, others indicate that aromaticity and impact of lignin residue is low;
3) a general statement could be made: classic studies of the properties of peat HA are necessary, especially if peat HA are prospective for industrial mining.

2 MATERIALS AND METHODS

2.1 Peat Sampling

28 peat samples from profiles of five raised bogs of industrial importance located in Latvia were collected (Figure 2.1). A sampling strategy was developed to study the properties of peat mass of industrial importance in sites where industrial peat extraction is going on. Considering the volume of peat needed for the experiments, the peat monoliths (width, length, and depth were approximately 0.5 m × 0.5 m × 1.5 m) with the total dry peat weight of about 15 kg per monolith were collected. The peat samples were obtained by cutting the monolith into layers in the height of 20 to 30 cm. Profiles were obtained after removal of top layers, which consisted of bog plants, undecomposed peat (the acrotelm) in depths varying from 0 cm to 145 cm (the acrotelm and catotelm). During the peat mining process in the selected sampling sites, the upper (living) moss and plant material had been previously removed and the necessary melioration had been done to reduce the groundwater. The age (by ^{14}C dating) of the "zero" horizon representing peat horizons of industrial importance was between 400 and 1 400 years. Peat samples then were placed into plastic bags for transportation and dried afterwards at the laboratory, at first air-dried, then dried at a temperature of 105 °C. Then the samples were homogenized and sieved through a 1 mm sieve.

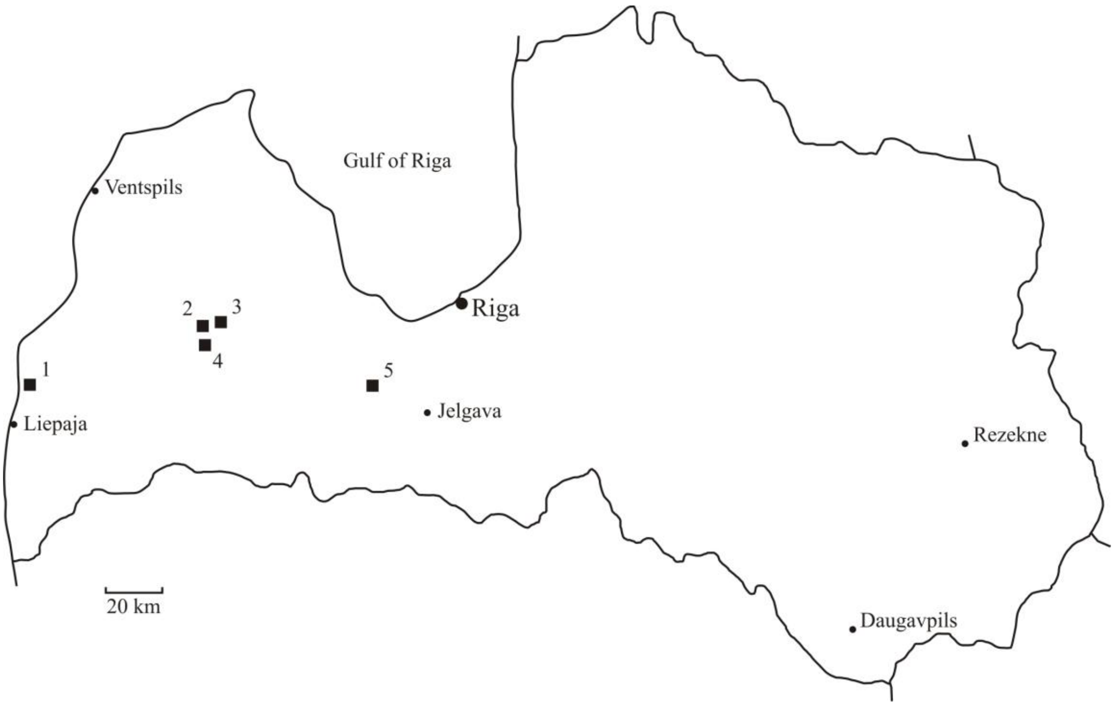

Figure 5. Peat sampling sites: 1. Ploce Bog; 2. Dižais Veiķenieks Bog; 3. Mazais Veiķenieks Bog; 4. Gāgas Bog; 5. Kaigas Bog

2.2 Peat Characterisation

The analysis of botanical composition was performed using a Carl-Zeiss binocular microscope. The decomposition degree (von Post, 1924; Lishtvan and Korol, 1975) was determined using the usual well-known methods. ^{14}C dating was conducted at the Institute of Geology of Tallinn Technical University (Estonia). Elemental analysis (C, H, N, S, and O) was carried out using an Elemental Analyzer Model EA – 1108 (Carlo Erba Instruments). The ash content was measured after heating 50 mg of peat sample at 750 °C for 8 h. Metals (Na, Mg, K, Ca, Mn, Fe, Ni, Cu, Zn, Cd, Pb) were determined after wet digestion with HNO_3 + $HClO_4$ (Tan, 2005) with flame atomic absorption (Perkin-Elmer AAnalyst 200). FTIR spectra were collected using Nicolet AVATAR 330 (Thermoelectron Co) spectrophotometer in KBr pellets. Transmittance ratios $T_{3400}/_{2900}$ and $T_{3400}/_{1600}$ were used to characterise major functional groups of humic substances (transmittance at 3400 cm^{-1} characteristic of O-H stretching to transmittance at 2900 cm^{-1} characteristic of aliphatic C-H stretching and transmittance at 3400 cm^{-1} to transmittance at 1600 cm^{-1}

characteristic of aromatic C=C and strongly H-bonded C=O of conjugated ketones). The organic carbon concentration in peat extracts was determined using a Shimadzu TOC-VCSN. For carbohydrate analysis, humic samples (10 mg) were suspended in 10 ml of 6 M HCl and heated at 100 °C for 3 hours. The concentration of carbohydrates in hydrolysates was determined using a phenol-sulphuric acid assay following the method described by Chaplin and Kennedy (1994). Cation exchange capacity (the ammonium acetate method) and the concentration of phosphates were determined using methods suggested for soil analysis (Tan, 2005). pH was determined in demineralised water extracts using a pH meter Hanna pH 213 (Tan, 2005). For scanning electron microscopy (SEM) analysis, after extraction residue of peat was washed with distilled water and dried. Then dry residue was placed in a SEM sample holder and covered with gold. The prepared samples were investigated using a JEOL ISM T – 200 scanning electron microscope.

2.3 Technologies for Extraction of Humic Substances from Peat

The efficiency of five technologies for extraction of humic substances from peat has been studied and compared.

1. Conventional extraction technique: low temperature treatment. Humic acids were extracted and purified using procedures recommended by the International Humic Substances Society (IHSS) (Tan, 2005). Briefly, 20 g of air-dried and finely ground peat samples were treated in N_2 with 1 l of extractant (P1 and P2 in Table 2.1.) for 24 h, stirring. The suspension was filtered and the filtrate was acidified with concentrated HCl to $pH < 2$ to precipitate humic acids. The obtained humic acids were further purified by repeatedly dissolving and precipitating as well as dialyzing against Millipore water with final drying. To study the kinetics of the extraction process, 10 ml of the reaction mixture were removed in definite time intervals, and the concentrations of humic substances were determined using transmittance at 410 nm (calibration using IHSS reference humic acids).
2. Conventional extraction technique: extraction under heating (samples P3, P4 and P5 in Table 2) is based on thermal treatment of the peat fibres with the same

extractants as above (heating temperatures 80 °C and 100 ºC). In this method, high hydroxide concentrations are used.

3. Intensive treatment extraction: extraction using cavitation is based on generation, subsequent growth, and collapse of cavitation resulting in very high energy densities of the order of 1 to 10^{18} kW/m^3. Cavitation can occur at millions of microlocations in a reactor simultaneously, and generate microconditions of very high temperatures and pressure locally (a few thousand atmospheres of pressure and a few thousands of Kelvins), when the overall environment is one of ambient conditions. Moreover, free radicals are generated in the process due to dissociation of vapors trapped in the cavitation bubbles. Cavitation also results in generation of local turbulence and liquid microcirculation in the reactor, enhancing the rates of transport processes (Lin and Yen, 1993; Gogate *et al.*, 2006). Extraction of humic acids from peat using a hydrodynamic cavitation reactor (P6 and P7 in Table 2.1) designed as described previously (Keil and Swany, 1999; Gogate *et al.*, 2006) by Professor of Riga Technical University Semyon Tsyfansky (Tsyfansky, 2007) allows to treat peat in flow mode under intensive hydrodynamic and local heat impact arising during generation and burst of cavitation bubbles. The extraction was performed using 200 grams of peat and 3 l of extractants for 2 hours; the extractants varied (1% NaOH or 1% KOH) (Table 2 and 11). The extraction experiments were performed at Riga Technical University.
4. Intensive treatment extraction: extraction using electrical discharge is based on extraction of humic substances from peat in flow mode by processing peat-extractant (KOH or NaOH) media with electrical discharge (18 kV). During discharge, a plasma field with high temperatures and shock wave is formed between the electrodes, which induces intensive mixing of the extraction media as well as degradation of peat fibres (Kurets *et al.*, 1999; Gogate *et al.*, 2006). Extraction of humic acids from peat using an electrical discharge reactor (P10 in Table 2 and 11) designed as described in Boiko and Mizinenko (1970); Keil and Swany (1999); Kurets *et al.* (1999) also allows to treat peat in flow mode and to disperse peat in solution of extractant under strong electrical discharge (18 kV) conditions (Kurets *et al.*, 1999). Extraction was performed using 5 kg of peat and 20 l of 1 % NaOH as an extractant for 15 minutes (Table 2) on a reactor provided

by Mainland Tech. The HS obtained in certain periods were filtered, and the filtrate was acidified with concentrated HCl at pH < 2 to precipitate humic acids. The obtained humic acids were further purified by repeatedly dissolving and precipitating as well as dialyzing against Millipore water with final drying.

5. Intensive treatment extraction: extraction using ultrasound has been suggested to facilitate extraction from plant material (Proestos and Komaitis, 2006). In this case pressure variations in the liquid phase are effected using sound waves (0.07 and 0.27 kW power). The ultrasound method involves sonification of extractant-peat mixture with ultrasound in commercial ultrasound baths (Mason *et al.*, 2004; Yasuai *et al.*, 2005; Gogate *et al.*, 2006). Extraction of humic acids from peat by treating it with ultrasound was done using differing ultrasound power (0.07 and 0.27 kW) (P8 and P9 in Table 2 and 11) provided by industrially produced ultrasound baths. The extraction was performed using 200 g of peat and 3 l of 1 % NaOH as an extractant either for 2 hours (Table 2) or changing the extraction period and the extractant.

Table 2

Conditions of extraction of humic acids (HA) from peat samples

Number of technique	**Extraction procedure**	**Extractant**	**°C**	**Time, h**
P1	Stirring	5% K_2CO_3	20	24
P2	Stirring	1% NaOH	20	24
P3	Stirring with heating	1% NaOH	100	24
P4	Stirring with heating	1% NaOH	80	24
P5	Stirring with heating	1% $K_2P_4O_7$	100	24
P6	Extraction in cavitation reactor	1% NaOH	60	1
P7	Extraction in cavitation reactor	1% KOH	60	1
P8	Treatment with ultrasound 0.07 kW	1% NaOH	30	6
P9	Treatment with ultrasound 0.27 kW	1% NaOH	40	6
P10	Extraction in electrical discharge reactor	1% NaOH	70	0.25

Reference humic acid samples from industrial sources (Tehum (P11) and Aldrich (P12)) as well as the International Humic Substances Society (IHSS) reference sample from Pahokee peat (P13) were used. Information on the extraction conditions was unavailable, except for the IHSS sample, for which 1M NaOH was used.

2.4 Estimation of Humification Indicators

1) Humification index (HI) (Cavani *et al.,* 2003). 2.0 g of peat sample in a 250 ml flask was shaken with 100 ml of 0.1 M NaOH plus 0.1 M $Na_4P_2O_7$ for 48 h at 65 °C in a thermostated shaker (Biosan, Latvia) in N2 atmosphere. After extraction the samples were centrifuged at 5,000 rpm for 15 min, and the supernatant was filtered through a 1 μm filter. This filtered solution constituted the total extract (TE) and was fractionated into humified (humic acids – HA, fulvic acids – FA) and non-humified (NH) fractions. In short, 25 ml of TE was put into a 50 ml centrifuge tube and acidified at pH < 2 by adding a small volume (0.3–0.5 ml) of 9 M H_2SO_4, then centrifuged at 5,000 g for 20 minutes. The precipitated fraction (HA) was collected and stored, and the supernatant solution fed onto a small column packed with about 5 cm^3 of insoluble polyvinylpyrrolidone, previously equilibrated in 0.005 M H_2SO_4. The eluate (NH) was collected in a 50 ml volumetric flask, then diluted to volume with 0.005 M H_2SO_4 and stored. The retained fraction (FA) was eluted with 0.5 M NaOH solution and collected in the centrifuge tube containing the HA precipitate, which was redissolved. Total organic carbon (TOC) and humified carbon (in HA, FA) were determined using a TOC analyser as described elsewhere. The humification index (HI) was calculated as follows:

   $$HI = \frac{C_{NH}}{C_{HA} + C_{FA}}.$$

2) Pyrophosphate index (PyI) (Schnitzer and Levesque, 1979). 0.50 g of peat sample was shaken with 50 ml of 0.025 M $Na_4P_2O_7$ for 18 hrs at room temperature. Peat residue was separated by filtration, washed with

distilled water, and the filtrates with washings were diluted to 250 ml with distilled water. The optical density of this solution was measured at 550 nm, and absorbance multiplied by 100 was referred to as the pyrophosphate index PyI.

$$PyI = D_{550} \times 100$$

3) K value of humus quality (Hargitai, 1994). 1.00 g of peat sample was shaken with 50 ml of 1% NaF for 6 hrs, filtered, and in the filtrate adsorption was determined at 410 nm (DHSNaF). Another 1.00 g portion of the same peat sample was shaken with 50 ml of 0.5 % NaOH for 6 hrs, filtered, and in the filtrate adsorption was determined at 410 nm (DHSNaOH). The value K of humus quality was calculated as follows:

$$K = \frac{D_{HSNaF}}{D_{HSNaOH}} \times C ,$$

where C is the percentage of carbon in the sample.

4) Peat humification index (PHI). 1.00 g of peat sample was shaken with 50 ml of deionised water for 24 hrs, filtered, and in the filtrate the total organic carbon of the aquatic extract (Cw) was determined. Another 1.00 g portion of the same peat sample was shaken with 50 ml of 0.1 M NaOH for 24 h, filtered, and in the filtrate the total organic carbon of the alkaline extract (CNaOH) was determined. The peat humification index was calculated as follows:

$$PHI = \frac{C_w}{C_{NaOH}} \times 100 .$$

5) Humification degree (HD). 1.00 g of peat sample was shaken with 50 ml of 1.0 M NaOH for 24 hrs, filtered, and in the filtrate the total organic carbon of the alkaline extract, containing humic substances (CHS), was determined (mg C/g). The peat humification degree was calculated as follows:

$$HD = \frac{C_{HS}}{C_{peat}} \times 100 ,$$

where C is carbon in the sample (mg/g).

2.5 Isolation of Humic Acids

Humic acids were isolated and purified using techniques recommended by the International Humic Substances Society (IHSS) (Tan, 2005). Briefly, 20 g of air-dried and finely ground peat samples were isolated in N_2 with 1 l of 1 M NaOH and stirred for 24 h. The suspension was filtered and the solution was acidified with concentrated HCl at $pH < 2$ to precipitate humic acids, leaving fulvic acids in the solution. The obtained humic acids were further purified by repeatedly dissolving and precipitating as well as dialyzing against Millipore water with final drying. Industrially produced humic acids (Aldrich) and IHSS reference humic acids isolated from Pahokee peat (the USA) were used for comparison.

2.6 Characterisation of Humic Acids

Elemental analysis (C, H, N, and S) was carried out using an Elemental Analyzer Model EA – 1108 (Carlo Erba Instruments). The ash content was measured by heating 50 mg of each HA at 750 °C for 8 h. Amounts of oxygen in the samples were calculated as the difference between the total mass of the sample and C, H, N, S and ash content. FTIR spectra were collected using a Nicolet AVATAR 330 (Thermoelectron Co) spectrophotometer in KBr pellets. Transmittance ratios $T_{3400/2900}$ and $T_{3400/1600}$ (transmittance at 3400 cm^{-1} characteristic of O-H stretching to transmittance at 2900 cm^{-1} characteristic of aliphatic C-H stretching and transmittance at 3400 cm^{-1} to transmittance at 1600 cm^{-1} characteristic of aromatic C=C and strongly H-bonded C=O of conjugated ketones) were used to characterize major functional groups of humic substances. UV/Vis spectra were recorded on a Thermospectronic Helios γ UV (Thermoelectron Co) spectrophotometer in a 1 cm quartz cuvette. The E_2/E_6, E_2/E_3, E_3/E_4, E_4/E_6 ratios (Chen *et al.,* 1977): ratio of absorbance at 280, 340, 465, and 665 nm were determined for the solution of 5 mg of a humic or fulvic acid in 10 ml of 0.05 M $NaHCO_3$. Organic carbon concentration in HS was determined using a Shimadzu TOC-VCSN. An automatic titrator TitroLine Easy (Schott-Geräte GmbH) was used to measure the total acidity of each HA. To estimate that (Tan, 2005), known amount of humic acid, about 20 mg, was dispersed in 10 ml 0.1 M $Ba(OH)_2$ solution, which was then shaken overnight in N_2, filtered and washed with water. The filtrates together with the washing solution were potentiometrically titrated with 0.1 M HCl down to pH 8.4 under N_2 flow.

For nuclear magnetic resonance (NMR) spectra, solutions were prepared by dissolving 50 mg of each sample in 1 ml of 1.0 N NaOD (in D_2O). ^1H and ^{13}C NMR spectra were recorded at 300 and 75.47 MHz, respectively, with a Bruker DRX300 NMR. Inverse-gated decoupling was used under the conditions of 0.2 s acquisition time and 2.0 s interpulse delay to minimise distortions.

Fluorescence spectra were recorded using a Perkin Elmer LS 55 fluorescence spectrometer on aqueous solutions of each sample at a concentration of 25 mg/l, adjusted to pH 7 with 0.5 M HCl. Emission spectra were recorded over the wavelength range of 380 to 650 nm at a fixed excitation wavelength of 350 nm (scan speed 500 nm/min, with a slit of 10.0 nm).

Electron spin resonance (ESR) measurements were carried out with a RE – 1306 spectrometer that operated at X-band frequencies with 100 kHz magnetic field modulation.

High pressure size exclusion chromatography (HPSEC) was used to determine weight-averaged molecular weight (M_w) of humic substances and it was calculated as follows:

$$M_w = \frac{\sum_{i=1}^{N} h_i M_i}{\sum_{i=1}^{N} h_i},$$

where h_i and M_i are the respective peak height and molecular weight at retention time i.

HPSEC was performed using a Waters 510 solvent pump, a Waters 486 variable wavelength detector, and a Waters Protein-Pak 125 modified silica column. Phosphate buffer (0.028 M, pH 6.8) was used as a mobile phase at a flow rate of 1 ml/min. The SEC column was calibrated using polydextrans (kDa: 0.83, 4.4, 9.9, 21.4, 43.5) and sodium polystyrene sulfonates (kDa: 1.37, 3.8, 6.71, 8.0, 8.6, 13.4, 16.9). The standard kits were purchased from the Polymer Standard Service (Mainz, Germany).

The calibration curve was expanded into the low MW region by means of mono- and oligosaccharides (180, 342, 504 Da) and glycerol. Blue dextran (2 000 kDa) served as a void volume probe, methanol – as a permeation volume probe.

2.7 Data Analysis

Principal component analysis (PCA) (rotation method: Varimax with Kaiser Normalisation (rotation converged in 8 iterations)) was conducted using *SPSS* 12.0 for *Windows*. Figures were prepared and data relevance and correlation analysis was performed with *Origin* 6.1 program, using the correlation function.

2.8 Py-GC/MS Analysis

The chemical nature and structural composition of humic acids were analyzed with Py-GC/MS. Humic acid samples before pyrolysis were treated with a tetramethylammonium hydroxide (TMAH) solution. 1.36 mg of humic acid sample were heated in 100 °C for 30 min together with 30 μl of 12.5% TMAH. A Double-Shot Pyrolyzer PY-2020 (Frontier Lab) combined with a Shimadzu GC/MS 2010 (Japan) was used to pyrolyse and analyse humic acid samples. The temperature of pyrolysis was 500 °C in helium (99.555% purity) atmosphere. GC capillary column RTX-1701 and 60 m × 0.25 mm × 0.25 μm film were used. The GC injector temperature was 250 °C and the temperature of ion source was 250 °C with EI of 70 eV. The oven program was 1 min isothermal at 60 °C, then 6 °C min^{-1} to 270 °C, and, finally, 10 min at 270 °C. The MS scan range was m/z 15–350. The carrier gas was helium at a flow rate of 1 ml min^{-1}, and the split ratio was 1 : 20.

The identification of the degradation products was based on NIST library search and manual interpretation. The relative proportions of the degradation products were directly estimated using the peak area of TIC chromatograms.

3 RESULTS AND DISCUSSION

3.1 Properties of the Studied Peat and Approaches to the Estimation of Humification Indicators of Peat

In this study peat from five raised bogs of industrial importance located in Latvia was used. Samples were obtained at different depths, representing bogs that reflect bog development conditions in Northern Europe well, at the same time using peat mass that is of industrial importance. The botanical composition of the deepest layers of peat was mostly raised cotton grass-*Sphagnum* peat and *fuscum* peat, while the upper layers consisted of raised bog *fuscum* peat, and the botanical composition was considered to be comparatively homogeneous. The age of the samples varied from 400 to 2,260 years, and their decomposition degree (the von Post scale) varied from H2 to H6 (Table 3).

The elemental composition of all obtained peat samples was determined (Table 4). The age of peat gradually increased with increasing depth, but the decomposition degree evidently varied within the peat column – these changes can be explained by the changes in climate, the hydrological and oxygen regime of the bog during its development, as well as other factors that commonly affect bog development. Possibly these factors have determined the fact that peat layers with higher decomposition are covered by peat layers with lower decomposition. Moreover, preparation of the bogs for peat mining can be considered to be a major factor affecting the stratigraphy and properties of peat.

However, the basic parameters of peat do not display random changes of elemental composition. The C concentration in the the studied peat samples varies from 41% to 54%, N concentration – from 0.41% to up to 2.38% , H concentration – from 4.96% to 6.15%, S concentration – from 0.5% to 1.3% (Table 4). The carbon and nitrogen content in peat samples from Mazais Veiķenieks Bog increases with increasing age (depth and decomposition degree), but the content of sulphur decreases with increasing age. However, in the other studied bogs, the variability of elemental composition is not so random and most probably depends on the botanical composition and specific peat formation conditions (the hydrological regime in the bog).

Table 3

Basic characteristics of the peat samples

Sample	Depth, cm	Peat type	Peat age, ^{14}C years	Decomposition, H	Decomposition, %
Ploce Bog	0−30	Raised bog *fuscum* peat	1460	H5	24
	31−60		n.d.*	H2	8
	61−85		n.d.		14
	86−110		n.d.		14
	111−130	Raised bog cotton grass-*Sphagnum* peat	1 670		11
Gāgas Bog	0−20	Raised bog cotton grass-*Sphagnum* peat	n.d.	H6	29
	21−40		n.d.		32
	41−60		n.d.		31
	61−80	Raised bog *fuscum* peat	n.d.	H4	20
	81−100		n.d.	H2	13
	101−120		n.d.		13
	121−140		1 640		11
Kaigas Bog	0−25	Raised bog cotton grass-*Sphagnum* peat	810	H6	33
	26−45	Raised bog *fuscum* peat	n.d.	H2	10
	46−70		1 590		11
	71−95	Raised bog narrow-leaf *Sphagnum* peat	n.d.		13
	96−125		n.d.		7
	126−145	Raised bog *fuscum* peat	2 260		9
Dižais Veiķenieks Bog	0−25	Raised bog *fuscum* peat	520	H2	12
	26−52		n.d.		12
	53−75		n.d.		6
	76−98		n.d.		9
	99−118		1 350		6
Mazais Veiķenieks Bog	0−30	Raised bog *fuscum* peat	400	H2	8
	31−60	Raised bog Magellan’s *Sphagnum* peat	n.d.	H3	14
	61−90	Transition type tree grass peat	n.d.	H5	27
	91−125	fen-type tree grass peat	1 180	H6	31

* n.d. – not determined

Alongside a basic characterisation of peat (elemental composition), a study of the changes of properties of peat was performed, which reflected the character of the humification process (Table 5).

The impact of peat development conditions on peat properties is also evident from the carbohydrate concentration, cation exchange capacity, and the metal concentration in the analysed peat samples (Table 5). The results of the extensive characterisation of peat have been used to study the impact of the peat formation conditions on the major peat properties and the character of humification of peat organic matter. The ash content in the studied peat samples can be considered to be low – that influences peat application possibilities. The KCl extract of the peat samples demonstrates a rather acidic environment, but the concentrations of phosphorus and carbohydrates are rather low. Major and trace element concentrations in the samples significally differ. In peat from Ploce Bog and Mazais Veiķenieks Bog rather high concentrations of Ca were found, as were in the upper layers of Gāgas Bog, Kaigas Bog, and Dižais Veiķenieks Bog. Trace element concentrations can be considered to be low, and characterise background impact.

Table 4

Elemental composition of the studied peat samples

Sample	Depth, cm	C, %	H, %	N, %	S, %	O, %
Ploce Bog	0−30	50.78	5.18	1.38	0.69	37.78
	31−60	54.26	5.73	1.08	0.44	36.19
	61−85	48.46	5.34	0.65	0.79	43.72
	86−110	52.41	5.31	1.05	0.67	39.87
	111−130	52.18	5.16	0.79	0.63	40.70
Gāgas Bog	0−20	48.54	5.54	1.15	0.84	40.79
	21−40	49.09	5.67	0.93	1.34	43.06
	41−60	48.82	5.60	0.75	0.58	43.86
	61−80	48.41	5.62	0.68	0.52	44.62
	81−100	49.45	5.52	0.83	0.92	43.28
	101−120	49.68	5.49	0.73	0.67	43.23
	121−140	50.78	5.60	0.85	1.01	42.09
Kaigas Bog	0−25	48.26	5.76	1.15	0.69	41.57
	26−45	49.31	5.76	0.85	1.01	43.39
	46−70	49.56	5.71	0.80	0.77	43.16
	71−95	48.53	5.83	0.86	0.81	43.97
	96−125	48.54	5.59	0.78	0.72	44.44
	126−145	41.75	4.96	0.70	0.67	51.73
Dižais Veiķenieks Bog	0−25	46.85	5.39	0.59	0.89	46.23
	26−52	48.19	5.53	0.66	0.58	44.88
	53−75	47.53	5.52	0.45	0.53	45.82
	76−98	46.96	5.40	0.41	0.58	46.52
	99−118	46.72	5.47	0.43	0.81	46.92
Mazais Veiķenieks Bog	0−30	45.67	5.65	0.73	0.69	46.87
	31−60	46.49	5.80	0.53	0.54	45.87
	61−90	52.46	6.15	1.88	1.08	37.19
	91−125	53.01	6.09	2.38	0.94	35.37

Although knowledge of the transformation process of living organic matter (humification) is of importance in understanding the carbon biogeochemical cycle, the number of studies dedicated to this topic is rather small (Jerzykiewicz *et al.*, 1999; Domeizel *et al.*, 2004; Zsolnay *et al.,* 1999; Cavani *et al.*, 2003; Ikeya and Watanabe, 2003; Rosa *et al.*, 2005; Corvasce *et al.*, 2006). Peat can be considered an especially appropriate subject of studies of humification because it is possible to establish links between the properties of peat and the decomposition degree of original organic matter. In addition, the studies of peat humification are of definite applied and theoretical interest. To study the humification process, principal component analysis was used to determine the main factors that affect peat composition (Table 6, 7). Six principal components were extracted, explaining 87% of the total variance of data (Table 6, 7). The six components define the main peat property factors: component 1 is characterised by the depth of the peat layer, ash content, as well as the concentration of phosphate ions, carbohydrates, and potassium (Table 7). Concentration of metals (mostly of anthropogenic origin, for example, Cd, Pb) in peat can be explained by this very component. Component 2 is associated with the peat decomposition degree, ash content, carbon content, and also metals of, most likely, natural origin (Ca, Mg, Fe); it can also be suggested that this factor describes peat genesis. Component 3 is associated with biogenic processes – presence of carbohydrates, abundant in living plants, but rapidly consumed during the decay process); presence of phosphate ions and nitrogen and sodium. Component 4 is characterised by the depth and age of the peat layer, component 5 – by magnesium and calcium concentration, and component 6 – by the sulphur content in peat and cation exchange capacity.

Table 5

Composition of the studied peat samples

Sample	**Depth, cm**	**pH**	**Ash, %**	**CEC, cmol/kg**	**PO_4^{3-}, mg/kg**	**Carbo-hydrates, mg/g**	**Na, mg/kg**	**Mg, mg/kg**	**K, mg/kg**	**Ca, mg/kg**	**Mn, mg/kg**	**Fe, mg/kg**	**Ni, mg/kg**	**Cu, mg/kg**	**Zn, mg/kg**	**Cd, mg/kg**	**Pb, mg/kg**
Ploce Bog	0−30	2.95	5.18	13.8	0.69	37.78	66	584	109	7483	6.51	659	1.16	2.42	8.94	0.07	4.76
	31−60	2.46	5.73	10.8	0.44	36.19	63	639	66	2590	2.74	534	0.97	2.12	6.67	0.30	5.86
	61−85	2.51	5.34	6.5	0.79	43.72	69	632	54	2628	2.92	279	0.55	1.17	11.38	0.07	5.61
	86−110	2.40	5.31	10.5	0.67	39.87	72	886	27	1434	0.91	146	0.31	0.60	2.02	0.01	0.69
	111−130	2.50	5.16	7.9	0.63	40.70	61	961	51	1984	5.06	168	0.32	2.00	2.42	0.04	0.79
Gāgas Bog	0−20	2.50	5.54	11.5	0.84	40.79	60	502	165	1726	15.15	756	1.23	3.37	9.38	0.58	7.74
	21−40	2.47	5.67	9.3	1.34	43.06	81	389	67	813	6.44	185	0.33	0.64	8.51	0.06	3.88
	41−60	2.41	5.60	7.5	0.58	43.86	74	372	65	523	2.00	147	0.33	0.76	4.56	0.05	6.31
	61−80	2.36	5.62	6.8	0.52	44.62	82	437	68	522	2.55	141	0.27	0.77	3.94	0.03	0.70
	81−100	2.46	5.52	8.3	0.92	43.28	83	365	86	453	2.01	130	0.25	0.69	3.30	0.03	0.96
	101−120	2.42	5.49	7.3	0.67	43.23	77	363	79	425	0.73	106	0.23	0.78	2.19	0.02	0.59
	121−140	2.45	5.60	8.5	1.01	42.09	80	373	72	506	1.72	113	0.33	1.24	2.20	0.03	1.18
Kaigas Bog	0−25	3.02	5.76	11.5	0.69	41.57	64	558	202	1786	17.31	566	1.08	2.00	3.39	0.18	1.78
	26−45	3.07	5.76	8.5	1.01	43.39	51	250	80	443	0.86	113	0.23	0.49	2.09	0.03	0.42
	46−70	3.11	5.71	8.0	0.77	43.16	54	333	74	503	1.73	128	0.28	0.90	3.71	0.03	0.98
	71−95	3.15	5.83	8.6	0.81	43.97	62	313	83	476	1.22	109	0.29	0.63	3.25	0.27	0.84
	96−125	3.26	5.59	7.8	0.72	44.44	47	359	63	424	1.03	83	0.20	0.55	2.46	0.01	0.40
	126−145	3.31	4.96	7.0	0.67	51.73	34	665	54	782	2.70	127	0.19	0.71	2.26	0.01	0.33
Dižais Veiķenieks Bog	0−25	2.94	5.39	5.9	0.89	46.23	81	507	94	704	6.98	115	0.35	0.59	5.84	0.05	5.53
	26−52	2.90	5.53	6.6	0.58	44.88	80	312	90	329	1.05	95	0.18	0.44	2.67	0.02	3.04
	53−75	2.28	5.52	4.5	0.53	45.82	115	336	99	414	0.77	120	0.08	0.53	2.48	0.01	0.55
	76−98	2.29	5.40	4.1	0.58	46.52	102	333	81	537	0.98	157	0.12	0.31	2.57	0.02	0.23
	99−118	2.35	5.47	4.3	0.81	46.92	90	465	69	973	2.36	352	0.12	0.47	3.22	0.03	0.36
Mazais Veiķenieks Bog	0−30	3.01	5.65	7.3	0.69	46.87	51	456	54	1058	1.55	398	0.17	1.14	3.45	0.02	2.43
	31−60	3.14	5.80	5.3	0.54	45.87	30	445	43	2191	2.00	665	0.51	2.11	2.68	0.01	2.63
	61−90	3.66	6.15	18.8	1.08	37.19	28	352	73	3051	5.58	1503	1.24	1.50	4.34	0.03	4.60
	91−125	4.17	6.09	23.8	0.94	35.37	26	387	51	4473	6.48	2464	2.88	2.02	2.84	0.02	1.02

Table 6

Summary statistics of PCA of the data on selected peat properties (VARIMAX rotation and Kaiser's normalisation were used on the initial component structure)

Component	Variance	% of explained variance	Cumulative % of variance
1	6.064	24.254	24.254
2	5.020	20.081	44.335
3	4.841	19.365	63.699
4	2.248	8.991	72.690
5	2.138	8.552	81.242
6	1.362	5.449	86.691

Table 7

Loading structure of the first six components extracted by PCA analysis of the data on selected peat properties

Variable/acronym		Component					
		1	2	3	4	5	6
Type	PV1	0.006	0.393	0.761	−0.042	−0.255	0.084
Depth, cm	PV2	−0.453	0.008	0.129	−0.719	0.061	0.001
C, %	PV3	0.022	0.912	−0.132	−0.229	−0.107	0.082
H, %	PV4	0.057	0.406	0.394	0.197	−0.733	0.116
N, %	PV5	0.172	0.763	0.498	0.051	0.017	0.256
S, %	PV6	0.019	0.143	0.142	−0.010	−0.231	0.826
O, %	PV7	−0.233	−0.948	−0.051	0.057	0.011	−0.095
Ash content, %	PV8	0.609	0.575	0.183	0.271	0.363	−0.004
pH	PV9	−0.073	0.174	0.913	0.162	−0.047	0.146
CEC, mEq/g	PV10	0.234	−0.079	−0.556	0.274	−0.109	−0.465
PO_4^{3-}, mg/kg	PV11	0.557	0.229	−0.675	0.125	0.311	0.026
Carbohydrates, mg/g	PV12	0.557	0.229	−0.675	0.125	0.311	0.026
Decomposition degree	PV13	0.587	0.401	0.307	0.344	0.106	0.294
Age, years	PV14	−0.055	−0.035	0.001	−0.860	0.228	0.068
Na, mg/kg	PV15	−0.112	−0.167	−0.890	0.133	−0.115	−0.042
Mg, mg/kg	PV16	0.128	0.150	−0.080	−0.298	0.792	−0.134
K, mg/kg	PV17	0.708	−0.086	−0.144	0.410	−0.039	0.240
Ca, mg/kg	PV18	0.136	0.668	0.261	0.258	0.502	−0.105
Mn, mg/kg	PV19	0.768	0.153	0.133	0.320	0.228	0.324
Fe, mg/kg	PV20	0.132	0.659	0.608	0.233	−0.051	−0.022
Ni, mg/kg	PV21	0.321	0.707	0.508	0.182	0.053	0.039
Cu, mg/kg	PV22	0.683	0.468	0.248	0.093	0.302	−0.143
Zn, mg/kg	PV23	0.943	0.153	−0.125	0.065	0.058	−0.059
Cd, mg/kg	PV24	0.936	0.085	−0.050	−0.130	−0.139	−0.115
Pb, mg/kg	PV25	0.938	0.158	−0.090	0.088	−0.010	−0.124

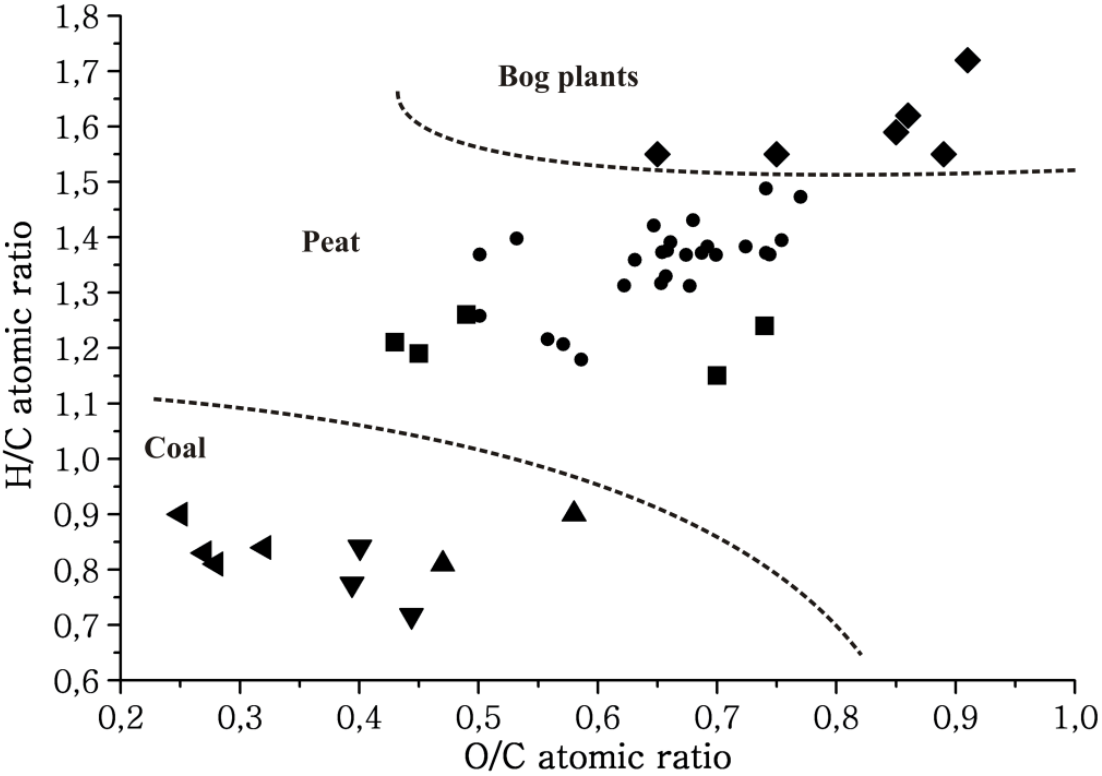

Figure 6. **Van Krevelen (H/C vs O/C atomic ratio) graphs of bog plants (◆), peat samples from studied bogs in Latvia (●), reference peat samples (IHSS) and peat samples from common peat bogs (■), brown coal (▲), coal (◀), lignite (▼) (Орлов, 1990)**

The peat humification process was examined using van Krevelen graphs frequently applied for analysis of the carbon biogeochemical cycle and genesis of fossil fuel (Van Krevelen, 1950). Table 8 shows the elemental ratios of the studied peat samples. According to Steelink (1985), the atomic ratios O/C, H/C, and N/C are useful in the identification of structural changes and the degree of maturity of peat in different depositional environments.

The H/C ratios in the studied bogs range from 1.17 to 1.44, but mostly these values are around 1.35. The N/C ratios typically are around 1.15 for the samples analyzed, only for the deeper layers of Mazais Veiķenieks Bog these values are up to 0.38. The O/C relationship varies between different peat bogs – in Ploce Bog these values are in range between 0.501 and 0.677, but in Dižais Veiķenieks Bog they oscillate between 0.699 and 0.77

Table 8

Elemental ratios and humification coefficients of peat

Sample	**Depth, cm**	**H/C**	**N/C**	**O/C**	**E_2/E_6**	**E_4/E_6**	**E_2/E_3**	**E_3/E_4**	**PHI**	**HI**	**K**	**PyI**	**HD**
Ploce Bog	0−30	1.216	0.023	0.558	24.100	2.700	2.060	4.333	18.139	0.113	0.534	31.6	1.014
	31−60	1.258	0.017	0.501	22.000	2.600	2.056	4.115	27.507	0.155	0.515	18.2	0.942
	61−85	1.312	0.011	0.677	15.875	2.000	2.082	3.813	35.362	0.140	0.636	16.6	1.055
	86−110	1.207	0.017	0.571	17.833	2.000	2.058	4.333	31.742	0.134	0.290	13.8	0.994
	111−130	1.179	0.013	0.586	18.600	2.760	1.938	3.478	39.033	0.139	0.307	15.3	1.102
Gāgas Bog	0−20	1.359	0.020	0.631	10.438	1.813	1.942	2.966	27.007	0.142	0.971	16.2	1.029
	21−40	1.376	0.016	0.658	15.778	2.167	1.986	3.667	52.431	0.150	0.424	16.8	1.107
	41−60	1.368	0.013	0.674	12.313	1.875	1.931	3.400	66.310	0.171	0.400	39.1	1.124
	61−80	1.383	0.012	0.692	12.071	1.929	1.920	3.259	63.467	0.195	0.609	39.0	1.214
	81−100	1.330	0.014	0.657	13.222	2.056	1.935	3.324	48.207	0.171	0.474	21.7	1.168
	101−120	1.317	0.013	0.653	15.955	2.273	2.006	3.500	39.179	0.191	0.305	19.7	1.048
	121−140	1.313	0.014	0.622	19.095	2.476	1.985	3.885	33.268	0.125	0.307	15.8	1.059
Kaigas Bog	0−25	1.421	0.020	0.647	11.444	1.889	1.943	3.118	7.042	0.070	0.946	21.9	1.182
	26−45	1.391	0.015	0.661	10.526	1.842	1.923	2.971	12.334	0.122	0.271	12.1	1.106
	46−70	1.373	0.014	0.654	16.250	2.125	1.970	3.882	7.992	0.083	0.188	14.7	1.113
	71−95	1.431	0.015	0.680	24.091	2.727	2.008	4.400	7.228	0.068	0.191	11.5	1.187
	96−125	1.372	0.014	0.687	16.571	2.000	2.035	4.071	6.442	0.072	0.209	9.1	1.187
	126−145	1.417	0.014	0.930	29.500	2.700	1.916	5.704	1.670	0.067	0.323	10.8	1.348
Dižais Veiķenieks Bog	0−25	1.372	0.011	0.741	24.300	2.650	1.876	4.887	27.909	0.314	0.804	45.5	1.170
	26−52	1.368	0.012	0.699	11.947	1.737	2.009	3.424	69.336	0.404	0.721	24.1	1.123
	53−75	1.383	0.008	0.724	13.308	2.000	2.012	3.308	89.385	0.298	0.843	24.7	1.144
	76−98	1.369	0.008	0.744	12.083	1.833	1.986	3.318	199.601	0.406	1.277	27.7	1.157
	99−118	1.395	0.008	0.754	17.750	2.375	2.014	3.711	126.397	0.349	1.177	22.7	1.206
Mazais Veiķenieks Bog	0−30	1.473	0.014	0.770	16.167	1.667	2.021	4.800	23.807	0.087	0.814	19.2	1.184
	31−60	1.488	0.010	0.741	12.667	1.500	2.000	4.222	10.220	0.077	0.610	17.6	1.237
	61−90	1.398	0.031	0.532	26.333	2.667	2.079	4.750	2.984	0.069	0.326	39.1	1.055
	91−125	1.369	0.038	0.501	23.182	2.727	2.056	4.133	2.485	0.071	0.545	36.6	1.035

The spectroscopic parameters such as E_2/E_6 and E_3/E_4 ratios are fluctuating in relatively wide regions between 10.526 and 29.500, and 2.966 and 4.887, respectively. Fluctuations of E_4/E_6 and E_2/E_3 ratios are less pronounced and stay in the range between 1.667 and 2.760, and 1.876 and 2.082, respectively.

The graph of H/C atomic ratio versus O/C atomic ratio reveals changes in the properties associated with coalification reactions. Figure 6 shows the relationship between H/C atomic ratio and O/C atomic ratio of organic material of a differing decomposition degree – beginning with bog plants up to brown coal, lignite, and coal. Figure 6 could be considered a graphical statistical representation of the humification process, indicating the degree of maturity and intensity of degradation processes such as dehydrogenation (reduction of H/C ratio), decarboxylation (reduction of O/C ratio), demethylation occurring during the decay of peat-forming plants, and peat maturation into coal. These changes are especially evident when atomic ratios of peat-forming plants are compared to the atomic ratio of organic matter of a high decomposition degree (low moor peat, coal). From the point of view of chemistry, humification can be considered to be a process in which more labile structures (carbohydrates, amino acids, etc) are destroyed, but thermodynamically more stable aromatic and polyaromatic structures emerge. It follows that the atomic ratios are associated with the processes transpiring during peat humification. As shown in Figure 6, H/C ratio decreases with increasing decomposition degree of the original living matter, starting with peat-forming plants, cellulose, and proteins, and up to bituminous coal. Comparatively, the studied peats are at the start of the transformation process of living organic matter.

The van Krevelen graphs (Figure 6) also indicate a decrease in O/C ratio, illustrating the decrease in the number of oxygen-containing functional groups, such as methoxyl, carboxylic, and carbonyl functional groups, in the fossil material with increasing humification degree. In order to provide reliable and quantity information about the early diagenesis, we carried out further studies on the dependence of the elemental composition of the peat samples on their age, depth and decomposition degree (Figure 7).

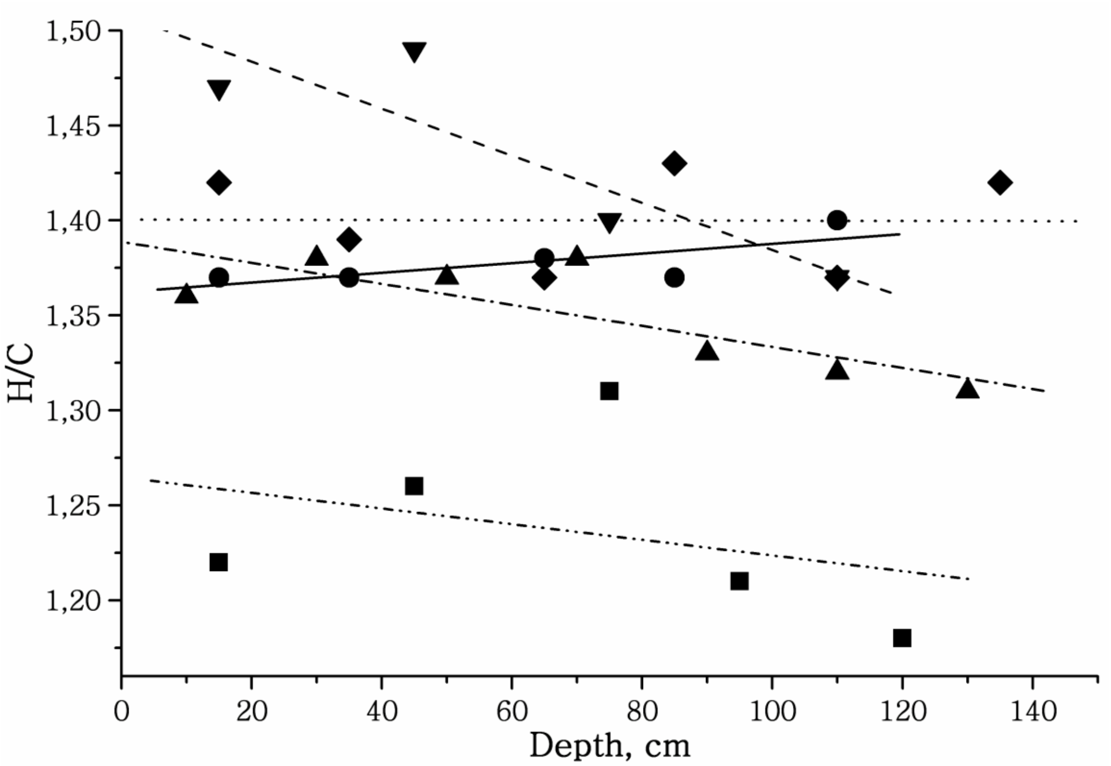

***Figure 7.* Dependence of H/C value on peat depth in different peat bogs**
● **Dižais Veiķenieks Bog ($Y = 1.362 + 2.543^{-4} \cdot X$; $R = 0.742$) $P > 0.05$**
▲ **Gāgas Bog ($Y = 1.389 - 5.536^{-4} \cdot X$; $R = -0.195$) $P > 0.02$**
◆ **Kaigas Bog ($Y = 1.400 - 4.892^{-6} \cdot X$; $R = -0.812$) $P > 0.05$**
■ **Ploce Bog ($Y = 1.265 - 4.118^{-4} \cdot X$; $R = -0.338$) $P > 0.05$**
▼ **Mazais Veiķenieks Bog ($Y = 1.509 - 0.001 \cdot X$; $R = -0.889$) $P > 0.05$**

H/C value showed a negative relation with peat depth; Kaigas Bog and Mazais Veiķenieks Bog had the highest negative correlations. However, Dižais Veiķenieks Bog showed a strong positive relation. This demonstrates that the atomic ratio cannot be used for precise studies of the humification process due to the significant impact of the original plant composition and peat formation conditions on this parameter.

Changes of carbohydrate concentration in peat correlate more strongly with peat depth and, evidently, peat age (Figure 8). The carbohydrate concentration in peat decreased with depth of the studied peat layers (Figure 8), clearly indicating that carbohydrate degradation is amongst the major processes describing peat formation and humification.

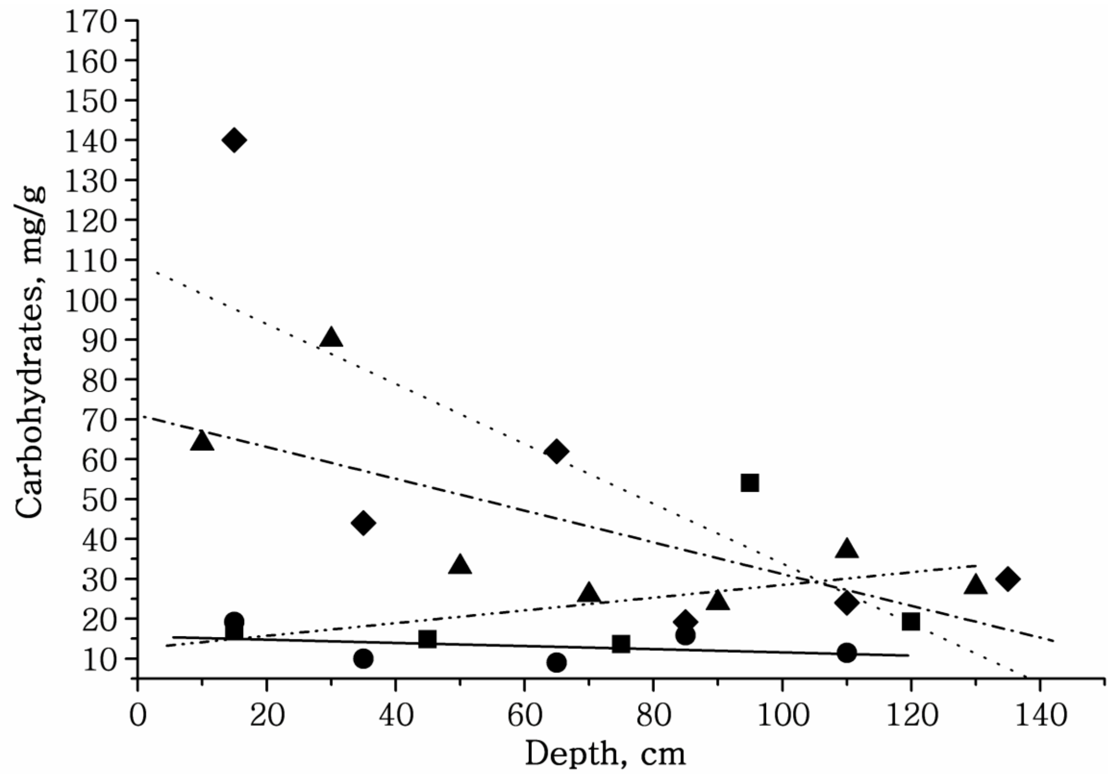

Figure 8. **Relationship between carbohydrate concentration and peat depth in different peat bogs**

● Dižais Veiķenieks Bog (——— $Y = 15.599 - 0.041 \cdot X; R = -0.356$) $P > 0.05$
▲ Gāgas Bog (– · – · $Y = 71.018 - 0.398 \cdot X; R = -0.697$) $P > 0.05$
◆ Kaigas Bog (········· $Y = 108.944 - 0.752 \cdot X; R = -0.751$) $P > 0.05$
■ Ploce Bog (— · · $Y = 12.521 + 0.159 \cdot X; R = -0.338$) $P > 0.05$

Variability of the 18 parameters that describe peat properties, their decomposition degree, age, and peat humification process were examined using principal component analysis. The first five components extracted explained 84% of the total variance (Table 9). The first component involved parameters describing the development of peat – its elemental composition (C, N, and O) and correlated well with the suggested humification degree HD. The second component characterized the correlation between peat type, hydrogen, and nitrogen; these parameters correlated with peat humification index PHI. The third component characterized the humification processes, as it was associated with most of the humification coefficients used in our study. Association of peat age and type with this component was also suggested. It is supposed that all the used humification coefficients can be applied in analysis of the peat humification process since each of them describes different aspects of the humification process. The fourth component was characterized by a close relation between the extinction ratios of humic extracts; it rather described the properties of peat humic substances than the transformation degree of peat organic matter. However, the very simple parameter – extinction ratio E_4/E_6 – is well correlated with the depth of the peat samples and peat age; it can also be efficiently used to analyse the humification process. The fifth component can be designated as the "age component" since it is correlated with peat depth, age, and humification indicators such as E_4/E_6, H/C, K, PyI, and HD. Therefore, these humification indicators can be recommended for characterisation of the peat humification process (Figure 9, 10).

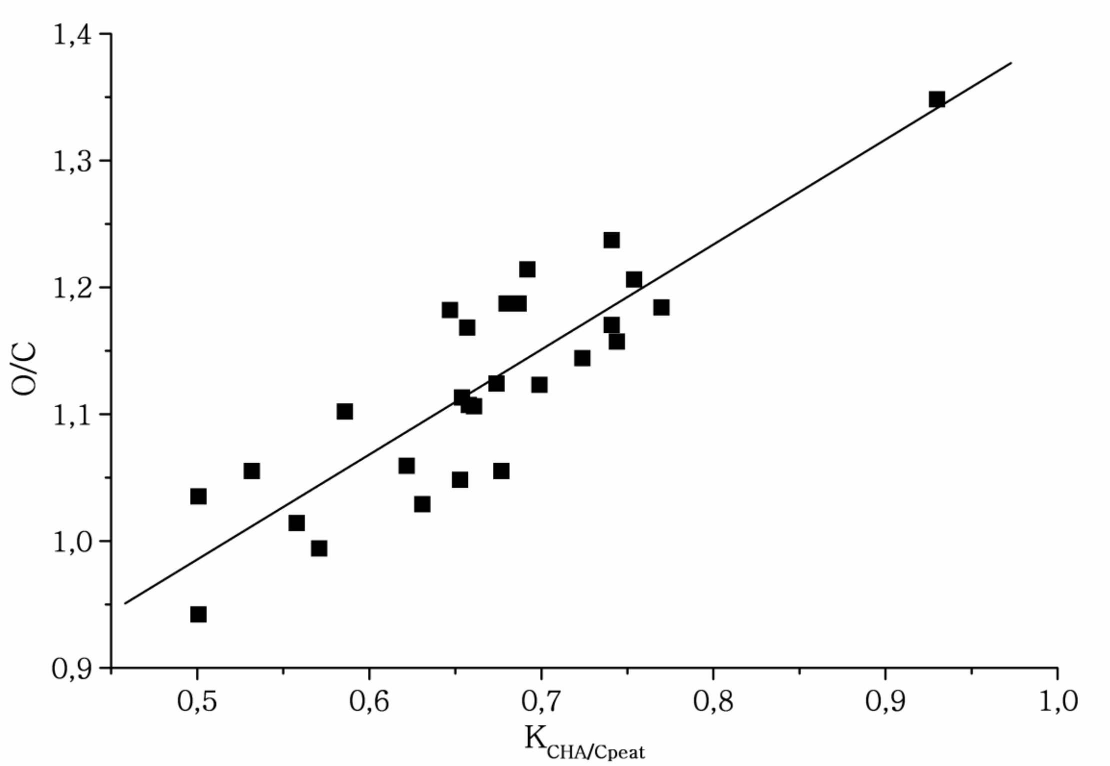

***Figure 9.* Dependence of O/C values on $K_{CHA/Cpeat}$ ($Y = 0.572 + 0.828 \cdot X$; $R = 0.863$) $P < 0.0001$**

Table 9

Summary statistics of PCA analysis of the data on selected peat properties and humification coefficients

Component	Variance	% of explained variance	Cumulative % of variance
1	6.582	34.641	34.641
2	3.201	16.847	51.489
3	3.137	16.509	67.998
4	1.945	10.239	78.237
5	1.091	5.742	83.979

Table 10

Loading structure of the first five components extracted by PCA analysis of the data on selected peat properties and humification coefficients

Variable/acronym		Component				
		1	2	3	4	5
Type	HV1	−0.095	0.836	−0.268	0.093	0.031
Depth, cm	HV2	0.069	0.095	0.077	0.159	0.913
C, %	HV3	−0.939	0.236	−0.063	0.010	0.105
H, %	HV4	−0.158	0.866	−0.028	−0.136	−0.255
N, %	HV5	−0.545	0.600	−0.217	0.433	−0.115
S, %	HV6	−0.061	0.516	−0.108	0.123	0.111
O, %	HV7	0.906	−0.322	0.143	−0.151	0.101
H/C	HV8	0.770	0.487	0.020	−0.125	−0.313
N/C	HV9	−0.482	0.600	−0.249	0.452	−0.138
O/C	HV10	0.937	−0.296	0.094	−0.043	0.041
Age, years	HV11	−0.069	−0.227	−0.325	0.139	0.816
E_2/E_6	HV12	−0.044	0.050	−0.214	0.853	0.271
E_4/E_6	HV13	−0.276	0.028	−0.067	0.803	0.394
HI	HV14	0.085	−0.232	0.889	−0.276	0.146
PHI	HV15	0.093	−0.343	0.860	−0.082	−0.078
K	HV16	0.197	−0.112	0.700	−0.086	−0.436
PyI	HV17	−0.143	0.152	0.530	0.462	−0.456
HD	HV18	−0.471	−0.180	0.079	−0.627	0.247

The peat age, depth, and the suggested humification indicators are located closely in the 3D space of the first three components obtained using PCA analysis (Figure 11). Other parameters, such as H/C ratio and E_4/E_6 ratio (Figure 11), also group according to peat age. These variables reflect the formation conditions of peat.

Humification has been mostly studied with the aim to analyse composting and soil formation processes. However, the humification process in peat very much differs from that in composts and soils in a quite rapid decomposition of organic matter in early humification stages. In bogs, under the impact of the anaerobic and acidic environment, the humification process of the saturated peat layers is very much retarded. Nevertheless, in peat it is possible to follow the humification process for very long periods (several thousand years are not the limit). The obtained results demonstrate that it is possible to study the stage of humification of organic matter in peat according to its age and apply the suggested methods of analysis to the estimation of the humification degree of peat (peat humification indexes).

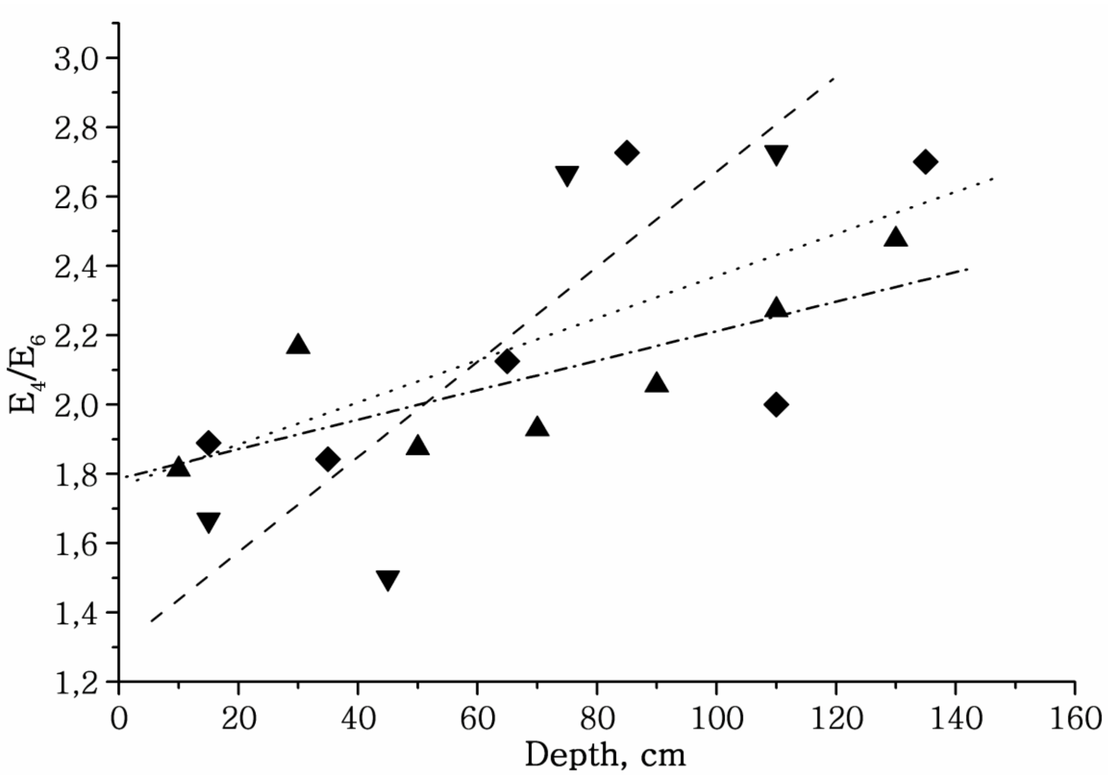

***Figure 10.* Dependence of E_4/E_6 values on peat depth in different peat bogs**
▲ Gāgas Bog (− · − · Y = 1.786 + 0.004·X; R = 0.774) *P* < 0.05
◆ Kaigas Bog (·········· Y = 1.763 + 0.006 X; R = 0.688) *P* > 0.05
▼ Mazais Veiķenieks Bog (− − − Y = 1.30 + 0.014 X; R = 0.863) *P* > 0.05

The first issue to be resolved is the definition of the concepts “decomposition degree” and “humification degree”. The decomposition degree describes the extent to which original (living) organic matter is transformed. The decomposition process thus includes: a) transformation processes of living organisms and their tissues; b) degradation of molecules forming a living organism; c) mineralisation (transformation of organic carbon compounds containing organic nitrogen, phosphorus, and sulphur compounds into their inorganic species); and d) formation of refractory organic substances – humic substances. Decomposition can also be described as the breakdown of plant material by microorganisms that use decaying organic matter as a source of energy and building material.

The estimation of the humification degree is often based on the monitoring of humic substances (HS), humic acids (HA), and fulvic acids (FA) isolated by extraction in an alkaline solution (Stevenson, 1994). Among the proposed indexes of maturity, those most representative of the evolution of the maturity of the compost were presented, particularly indexes measuring the evolution of humic acids (HA) compared to fulvic fraction (FF) or fulvic acids (FA). Humification is a narrower concept, describing the development of humus (humic substances). To calculate the humification degree, the ratio of the total amount of organic matter to the amount of formed humic substances can be suggested for use:

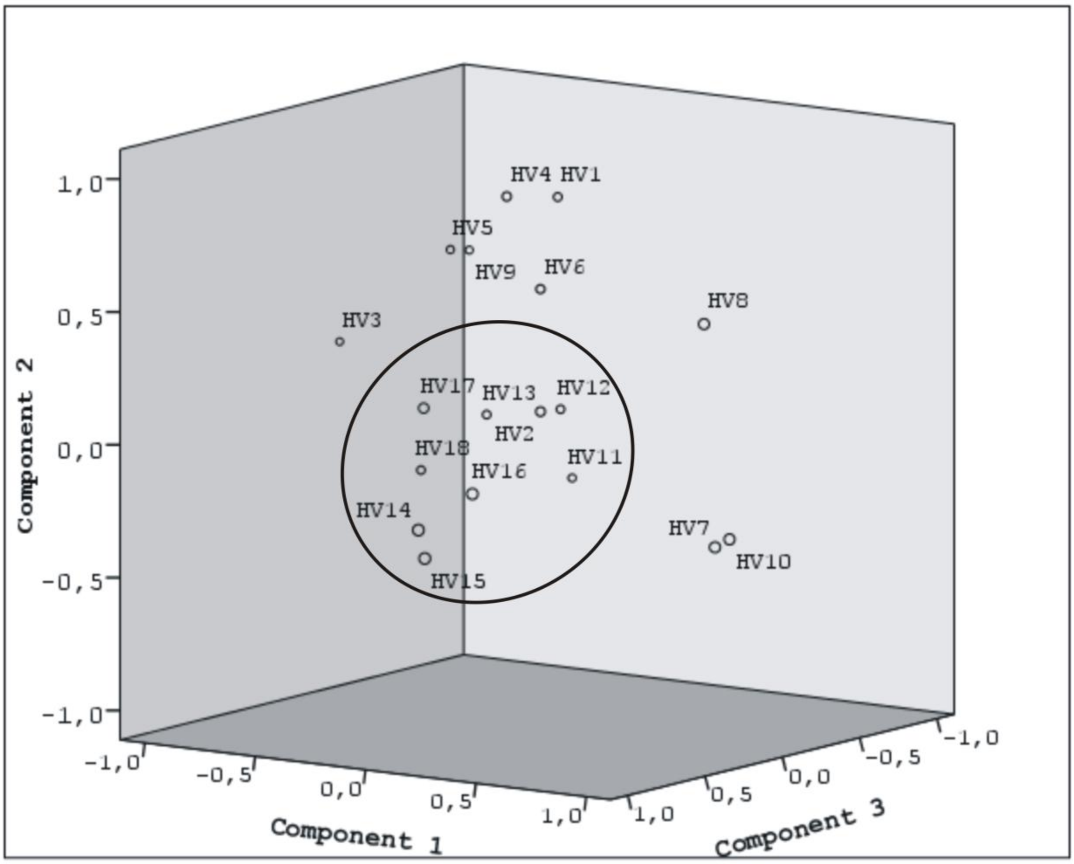

***Figure 11.* Ordination of variables representing selected peat properties and humification coefficients on the first three PCA axes**

$$HD = \frac{C_{HS}}{C_{peat}} .$$

As the results of this study show, this parameter corresponds well to the peat depth and age; thus, it can also be used to study the transformation processes of organic matter.

The peat properties in the bog profiles vary significantly, most probably due to peat formation conditions (oxygen and hydrological regimes) and the botanical composition of peat. Analysis of the changes of peat properties indicate decreasing carbohydrate concentrations and cation exchange capacity as well as variable metal, major and trace element concentrations.

To study the humification process, principal component analysis was used to determine the main factors that affect peat composition, as were van Krevelen graphs to identify structural changes and the degree of maturity of peat. The main factors that impact the peat humification process are: hydrological/oxygen regime, composition of peat-forming plants, geological processes, and climate.

The obtained results were used for the development of new approaches to the estimation of the peat humification degree (peat humification indexes). The ratio of the total amount of organic matter to the amount of formed humic substances is suggested for description of the humification degree, as this parameter corresponds well to the peat properties and parameters describing peat formation.

3.2 Impact of the Process of Isolation of Humic Acids from Peat on Their Properties

It has been estimated that only peat can be a source of up to 500 Gt C of humic acids of industrial production value (Markov *et al.,* 1988). Peat can be considered to be a significant source of humic substances (first and foremost, humic acids), but technologically relevant methods for extraction of HA have not been studied much, and most producers do not use peat as the raw material. To an extent, efficiency of extraction of humic acids depends on the extractant and the intensity of the treatment; thus, from a wide array of different extractants recommended in several studies (Martin-Neto *et al.,* 2001; Olk *et al.,* 1995; Kaschl *et al.,* 2002; Mahieu *et al.,* 2002), hydroxides (NaOH, KOH) and metal salts (Na_2CO_3, K_2CO_3, $K_4P_2O_7$) have been used. However, recently suggested intensive treatment technologies (Gogate *et al.,* 2006; Tsyfansky, 2007) have not been used for extraction of humic substances. Depending on the intensity of treatment, five methods of extraction of humic substances from peat have been studied in this research.

1) Low temperature peat treatment with NaOH or KOH under mixing. Although this method does not require special machinery or energy inputs, each extraction cycle takes rather long time and high hydroxide concentrations; besides, extraction cannot be performed in flow mode. This method is well suited for laboratory experiments, where small amounts of humic substances are required.
2) Extraction under heating is based on thermal treatment of peat fibres with the same extractants under heating. As in traditional methods, this method requires high hydroxide concentrations, but the extraction time can be radically decreased (up to 4 hours less).
3) Extraction using cavitation is based on the generation, subsequent growth, and collapse of cavitation resulting in very high energy densities, generation of local turbulence and increasing circulation in the reactor, thus enhancing the rates of transport processes. High doses of kinetical and thermal energy released in a very small area can cause complete destruction of peat fibres, as well as mixing of the extraction

environment. Cavitation increases velocity of chemical reactions and can be performed in flow mode.

4) The electrical discharge method is based on extraction of humic substances from peat in flow mode by treating the peat-extractant media with electrical discharge; during discharge, a plasma field with a high temperature and shock wave is generated between the electrodes. The shock wave induces intensive mixing of the extraction media and degradation of peat fibres.
5) The ultrasound method involves sonification of extractant-peat mixture with ultrasound in commercial ultrasound baths. Ultrasound does not only shred the peat fibres, but also provides intensive mixing of the extraction environment.

Changes in isolation efficiency of humic substances (their concentration in the extraction mixture) over time (Figure 12) show that the differences in extraction efficiency depend on the intensity of treatment.

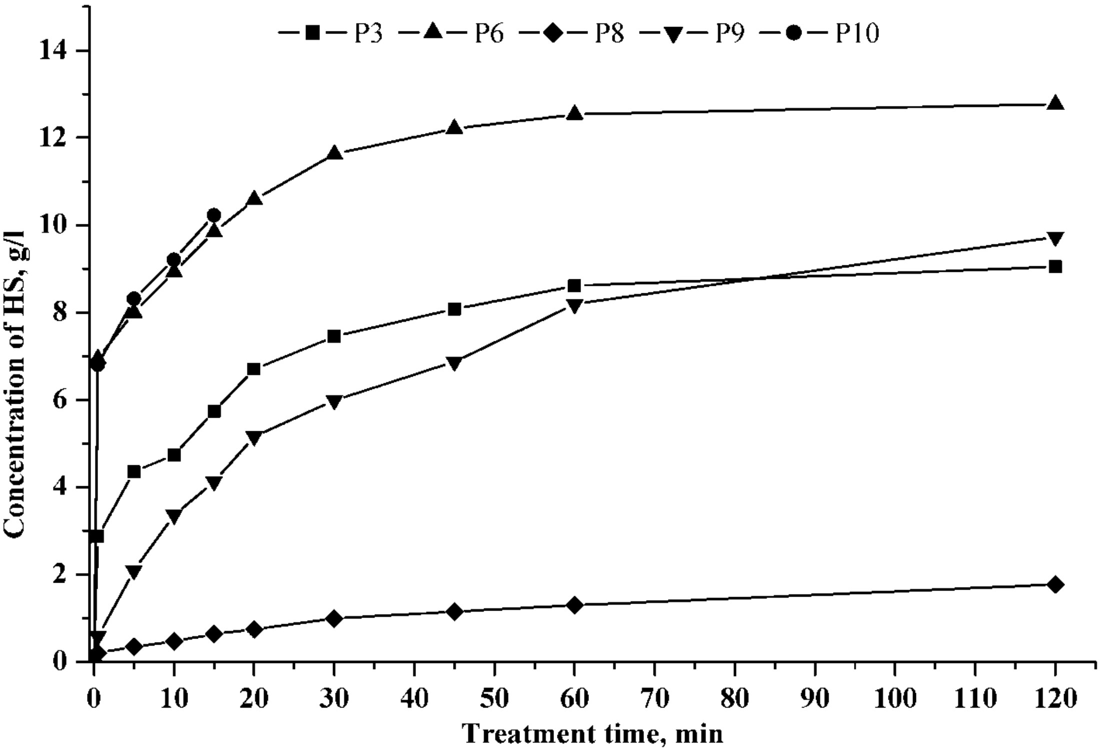

Figure 12. **Changes of the concentration of humic substances in the extraction mixture over time depending on the character of the extraction process (extractant 1% NaOH) (P3 – stirring with heating; P6 – extraction in a cavitation reactor; P8 – treatment with ultrasound of 0.07 kW; P9 – treatment with ultrasound of 0.27 kW; P10 – extraction in an electrical discharge reactor)**

The use of electrical discharge and cavitation methods allows to obtain comparatively high concentrations (11 and 13 g/l, respectively) of solution of humic substances much faster and more effectively than the use of other methods (besides, these two methods are very effective – concentrations of about 7 g/l can be obtained during the first few minutes of extraction). The effectiveness of ultrasound is proportional to its power, but, in general, this process is much slower and less effective than the two former methods. Using ultrasound with a power of 0.27 kW, concentrations similar to those obtained with electrical discharge can be obtained only in 120 minutes of treatment.

The yields of humic substances depend on the properties of the extractant and the intensity of treatment (Figure 13, 14) – NaOH appears to be the most effective from the tested extractants, and the effectiveness of ultrasound is closely related to the used power – ultrasound treatment with a power of 0.27 kW is four times more effective than treatment with a power of 0.07 kW, using NaOH (Figure 14). Comparing different extractants, it is evident that sodium hydroxide is about 20% more effective than potassium hydroxide, but application of potassium pyrophosphate is ineffective because the concentrations obtained are at least five times lower than using NaOH or KOH.

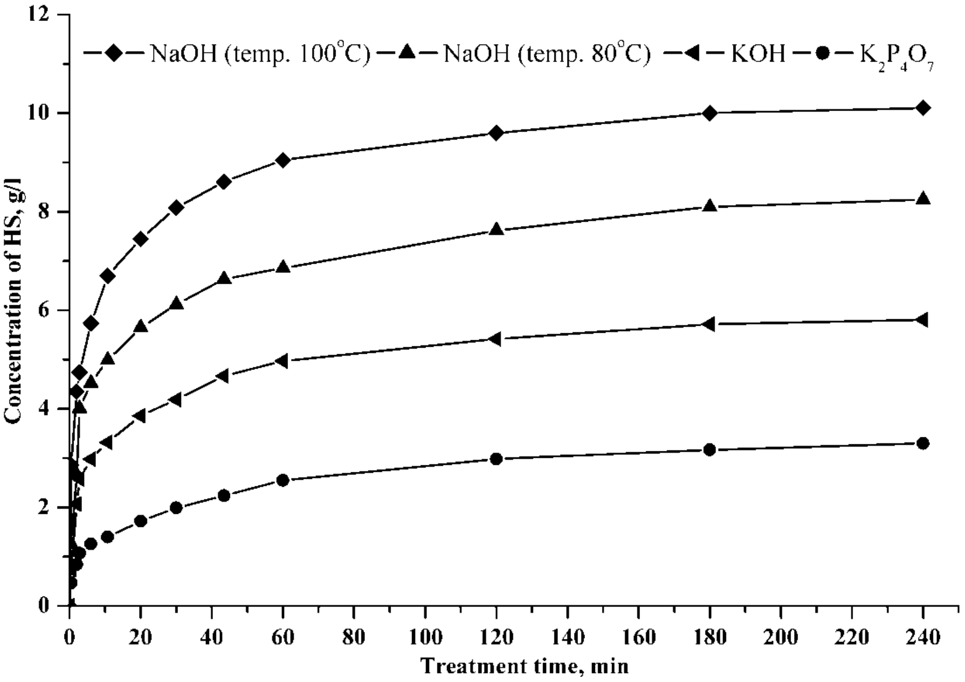

Figure 13. **Changes of the concentrations of humic substances in the extraction mixture depending on the poperties of extractants; extraction under heating**

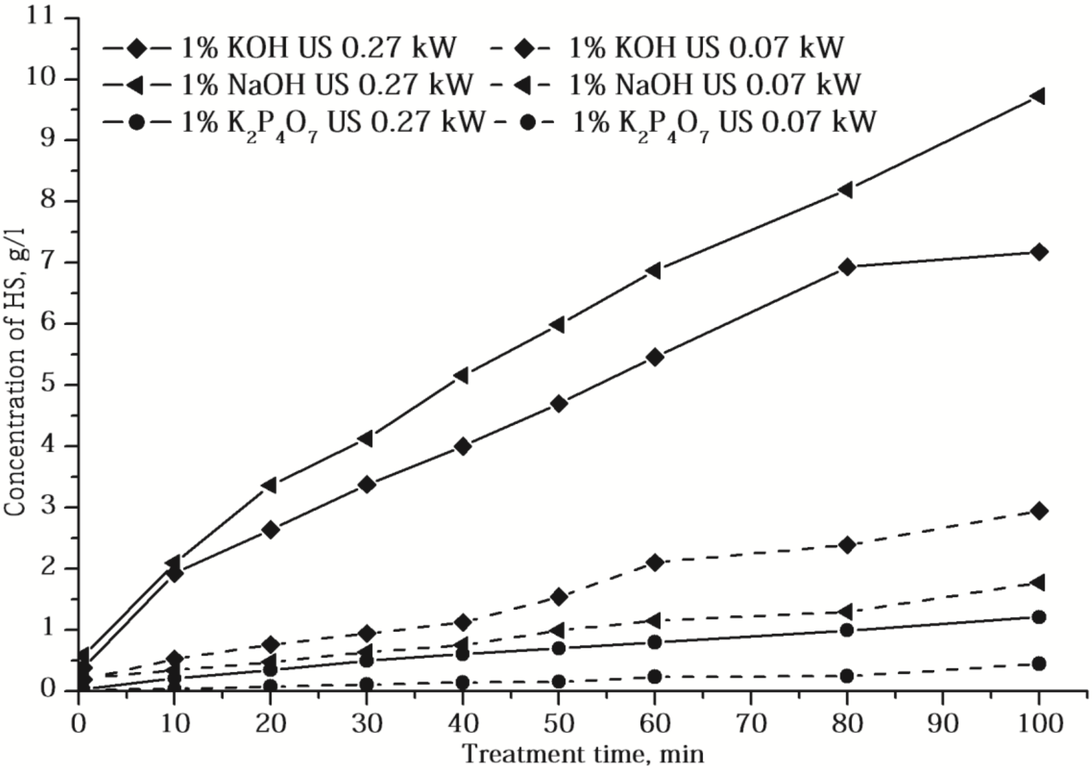

Figure 14. **Changes of the concentration of humic substances in the extraction mixture over time depending on the properties of the extractant and the intensity of ultrasound treatment**

To evaluate the impact of different extraction methods on peat fibres, scanning electron microscopy was used (Figure 15–18). Figure 15 shows peat fibres before treatment. The surface and stomatas of a *Sphagnum* moss leaf without any damage are clearly visible. Treatment with 1% NaOH under elevated temperatures breaks down the structure of peat particles. Figure 16 shows slightly changed forms and shapes of stomatas, and the surface layer of peat fibres has been broken and partly ripped off. When peat is treated with ultrasound, degradation of its structural elements is promoted since treatment with ultrasound destroys the plant and moss remains in peat, increasing the yield of humic substances. After yet more intensive treatment with the cavitation method (Figure 18), a completely eroded surface layer and damaged internal structures can be seen. Thus, we conclude that intensive treatment technologies allow to obtain significantly higher yields of humic substances than traditional extraction methods, and the efficiency of these technologies is explained by destruction of peat fibres during treatment.

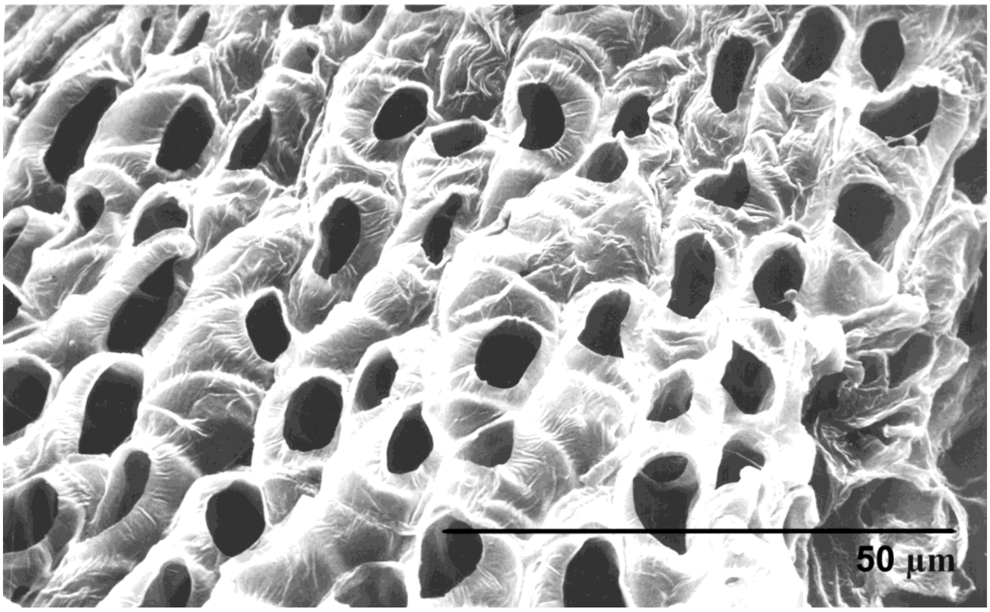

Figure 15. **Peat fibres (raised bog *fuscum* peat, decoposition degree 10%) before treatment**

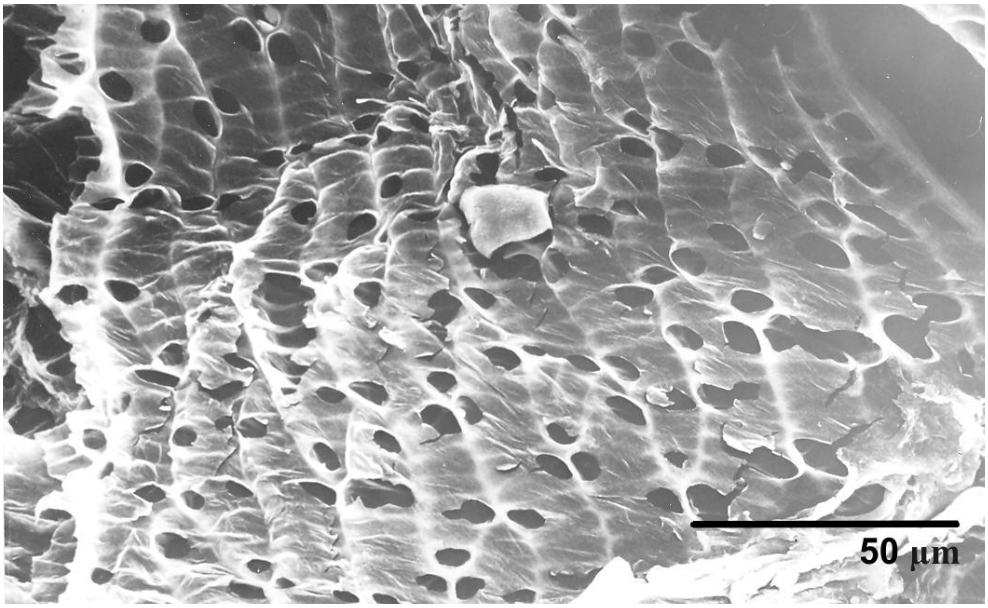

Figure 16. **Peat fibres after 4 h of treatment with 1% NaOH under elevated temperatures**

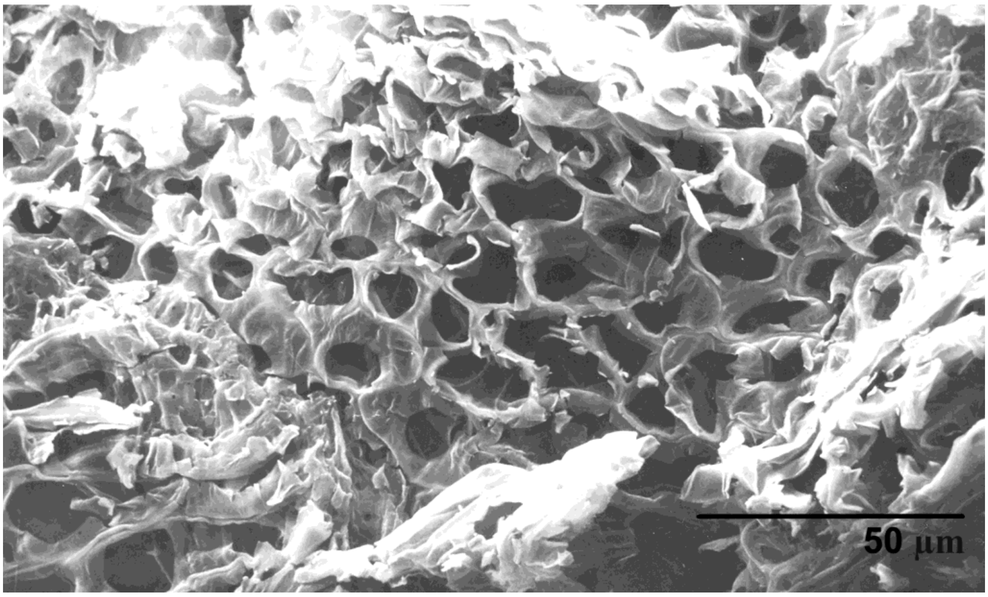

Figure 17. **Peat fibres after 100 m of treatment with ultrasound (0.27 kW) using 1% NaOH as an extractant**

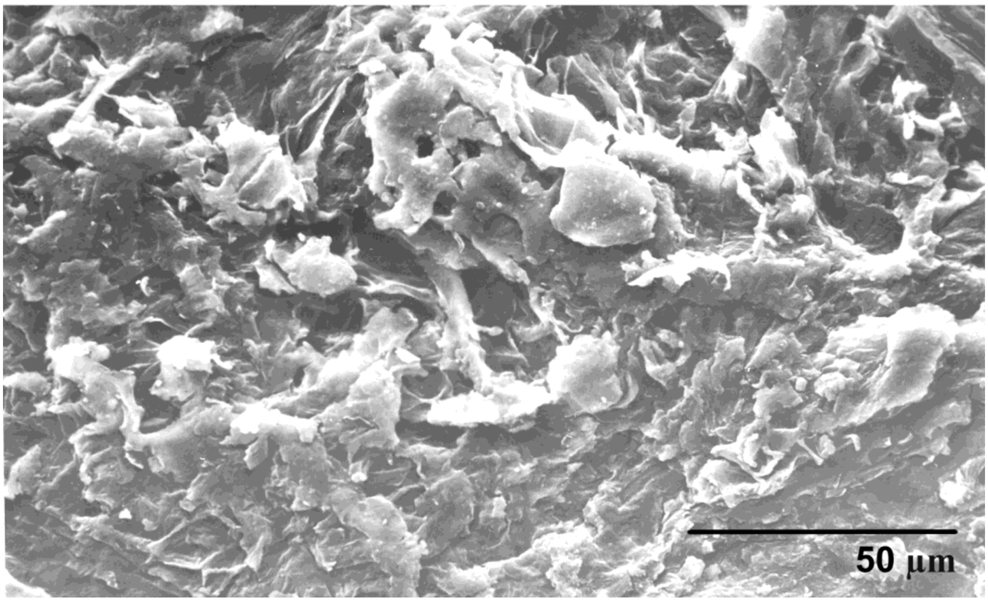

Figure 18. **Peat fibres after 20 m of treatment with the cavitation method using 1% NaOH as an extractant**

The impact of the isolation process on the properties of humic acids from peat was analyzed using elemental, functional, molecular mass and spectral (UV, IR, ^{1}H NMR) analysis. The character of the isolation process does have a major impact on the properties of the isolated humic acids, as the elemental analysis evidently suggests (Table 11). Depending on the intensity of treatment, the amount of carbon in humic substances and their molecular mass are reduced, as is the E_4/E_6 ratio. Van Krevelen graphs, which are usually widely applied for analysis of basic structures of humic acids (Barancikova *et al.*, 1997) were also used for analysis. The van Krevelen graph in Figure 19 shows that variability of humic acids isolated from different sources (from peat of different bogs) is lower than variability of the elemental composition of humic acids isolated using different extraction techniques (treatment).

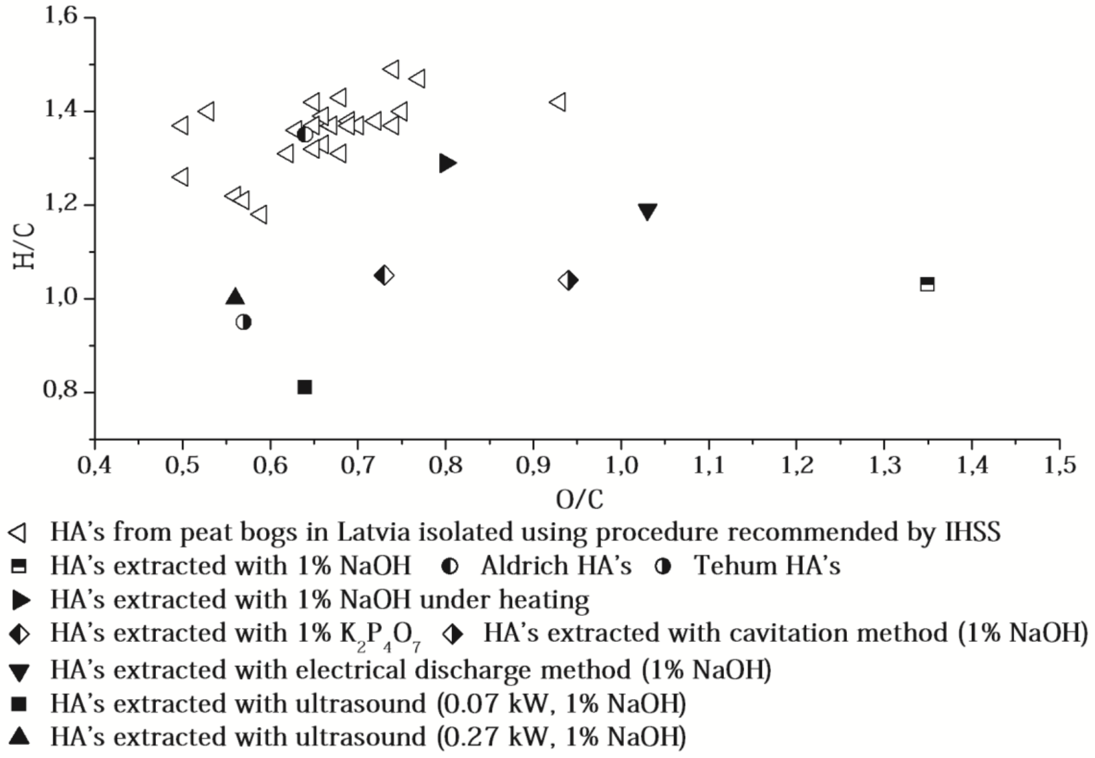

***Figure 19.* Relationships between H/C and O/C elemental ratios of humic acids extracted with various extractants and/or techniques from a peat sample and of HA from several peat bogs in Latvia extracted with the technique recommended by the International Humic Substances Society (IHSS)**

Position in van Krevelen graphs allows to identify major processes that affect the structure and properties of organic matter – dehydration, dehydratation (reduction of the H/C ratio), decarboxylation (reduction of the O/C ratio), and demethylation (Barancikova *et al.*, 1997). The graphs show that intensive extraction techniques result at first in dehydratation and decarboxylation of humic substances. The same trend is evident analyzing the functional characteristics and molecular mass changes of isolated humic acids – intensive treatment methods (ultrasound, electrical discharge, cavitation, as well as treatment with increased concentrations of hydroxides) yield humic material (Table 11) with a reduced number of carboxylgroups.

Table 11

Properties of humic substances extracted using different technologies*

Number of technique	Elemental composition				Elemental ratio			E_4/E_6	-COOH	M_w, dal
	C, %	H, %	N, %	O, %	N/C	H/C	O/C		mEq/g	
P1	53.25	4.27	1.76	40.72	0.03	0.95	0.57	8.00	4.95	4 200
P2	50.74	4.24	1.59	43.44	0.03	1.00	0.64	6.43	5.68	3 400
P3	53.96	4.52	1.23	40.29	0.02	1.00	0.56	4.93	6.79	1 900
P4	52.89	4.32	1.25	41.54	0.02	0.97	0.59	4.93	7.00	1 900
P5	51.31	3.50	1.65	43.54	0.03	0.81	0.64	5.89	5.68	2 200
P6	39.98	4.00	1.43	54.59	0.03	1.19	1.03	5.82	4.96	1 800
P7	42.13	3.68	1.45	52.75	0.03	1.04	0.94	6.09	4.84	3 200
P8	47.93	4.21	1.34	46.52	0.02	1.05	0.73	6.00	6.42	3 000
P9	45.36	4.90	1.55	48.20	0.03	1.29	0.80	4.76	5.93	1 600
P10	43.60	3.65	1.55	51.20	0.03	1.00	0.88	4.71	4.91	1 500
P11	60.70	3.70	1.50	34.10	0.02	1.13	0.64	4.51	NA	NA
P12	49.89	4.76	2.30	43.05	0.04	1.03	1.35	4.00	2.15	4 100
P13	56.37	3.82	3.69	37.34	0.06	0.81	0.50	5.80	3.59	8 500

* Detailed information on the extraction conditions can be found in Table 2.

The molecular mass of humic substances isolated using intensive treatment methods is significantly lower than of substances isolated using the conventional extraction approach. UV-Vis spectra of HA isolated by different methods differ significantly (Figure 20); furthermore, the E_4/E_6 ratio (Table 11) used to estimate molecular weight confirms the character of structural changes of HA depends on the intensity of peat treatment.

IR spectra (Figures 21, 22) reveal the character of changes in the structure of humic acids depending on the isolation process. Comparison of IR spectra shows changes in the relative number of -OH groups (absorption maximum at 3,400 cm^{-1}), in respect to $-CH_2$ groups (maximum at 2,950 cm^{-1}). Impacts of the isolation process on presence of aromatic C=C and conjugated carbonyl C=O groups (absorption maximum at 1,620 cm^{-1}), carboxyl and ketone carbonyl stretching (1,720 cm^{-1}) and presence of carbohydrates (stretching of carbohydrates or alkoholic -OH groups at 1,040 cm^{-1}) are similarly significant. For example, isolation of HA using ultrasound results in significantly reduced intensity of signals characteristic of carboxyl groups and carbohydrate hydroxyl groups. IR spectra also indicate significant reduction in the number of hydroxylgroups characteristic of carbohydrates during extraction using electrical discharge and cavitation.

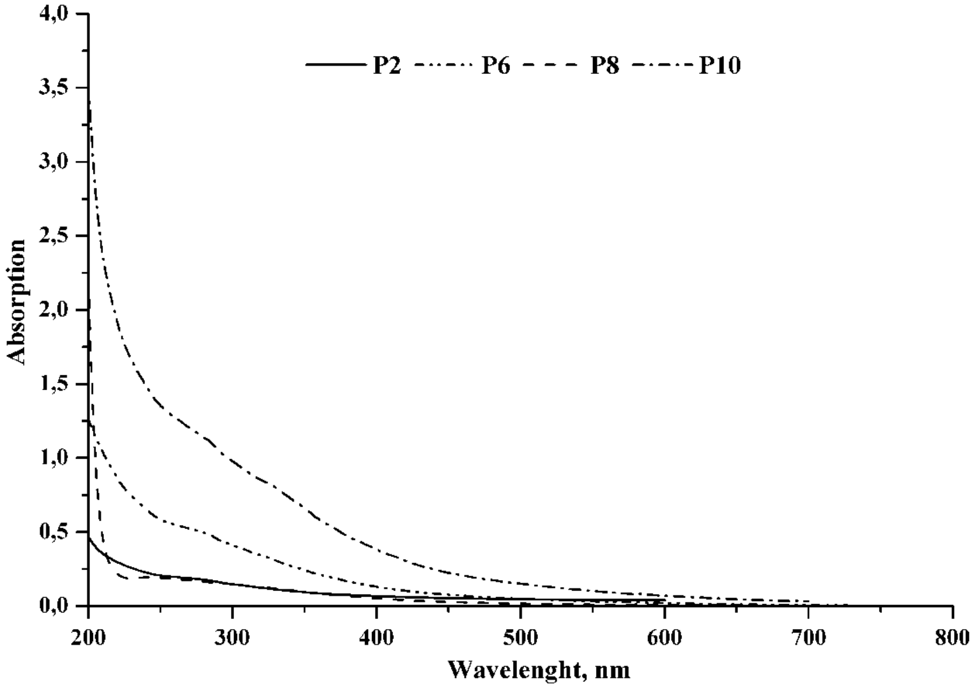

Figure 20. **UV-Vis spectra of humic substances isolated using different extraction methods (1% NaOH, concentration of HS = 0.1 g/l) (P2 – stirring; P6 – extraction in a cavitation reactor; P8 – treatment with ultrasound of 0.07 kW; P10 – extraction in an electrical discharge reactor)**

The ^{1}H NMR spectra of humic acids in peat (Figure 23) showed the following resonances.

The first region between 0.8 and 3.0 ppm is commonly attributed to aliphatic protons (peat assignments as in Barancikova *et al.*, 1997). In particular, the resonances at 0.9 and 1.3 ppm can be attributed to protons of methyl groups of highly branched aliphatic structures and terminal methyl groups of methylene chains, respectively. The signals at 1.3 ppm have also been attributed to hydrogens attached to benzene rings. The peaks in the 1.8 ± 2.5 ppm interval appear due to protons in C atoms adjacent to carbonyl, carboxyl, aromatic rings or unsaturated groups. The second region between 3.0–4.5 ppm is attributed to protons in C atoms attached to the oxygen atom, and C atoms are attributed to sugar-like components, polyether or methoxyl groups. Finally, the third region between ≈6.0–8.5 ppm can be attributed to the presence of both heterocyclic and highly substituted

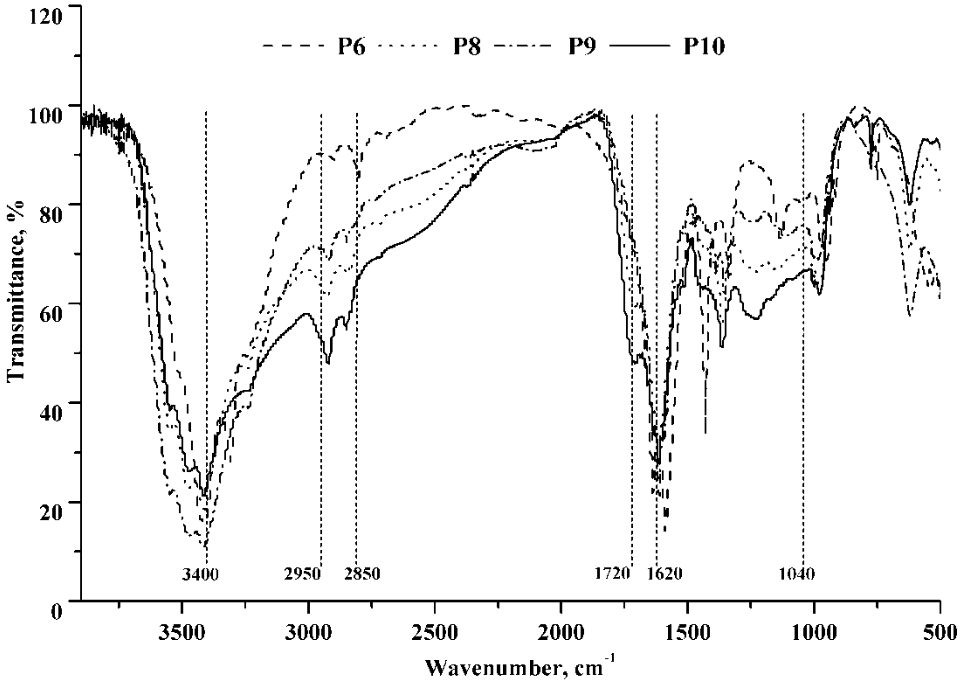

Figure 21. **FTIR spectra of humic substances isolated using different extraction methods (P6 – extraction in a cavitation reactor; P8 – treatment with ultrasound of 0.07 kW; P9 – treatment with ultrasound of 0.27 kW; P10 – extraction in an electrical discharge reactor.**

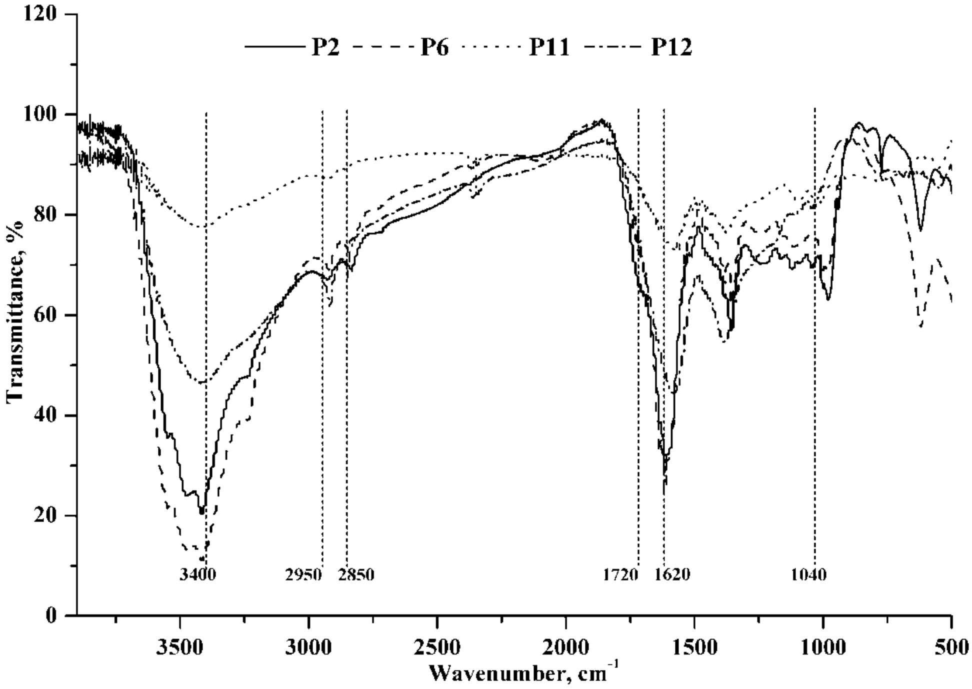

Figure 22. **FTIR spectra of industrially produced humic substances isolated using different extraction methods (P2 – stirring; P6 – extraction in a cavitation reactor; P11 – TEHUM HA; P12 – Aldrich HA).**

aromatic ring hydrogens. The intensity of signals in all studied humic acids is weak. The changes observed in the spectra of HA shown in Figure 23 can be attributed to the chemical changes in peat during the extraction process. During intensive extraction, resonances assigned to sugar-like components can be observed.

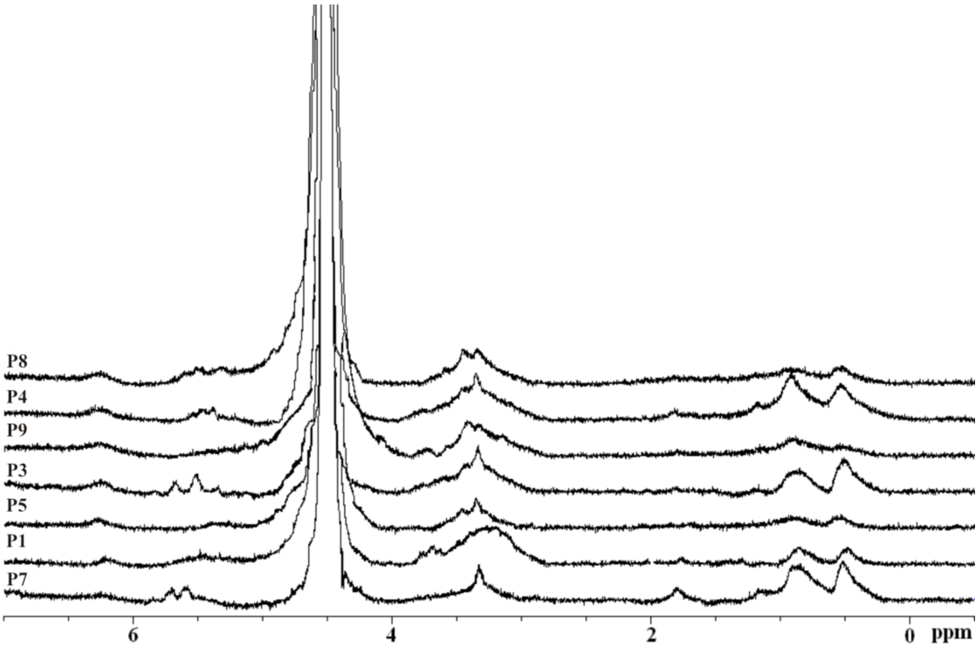

Figure 23. **^{1}H NMR spectra of humic substances isolated from peat using different extraction methods (P1 – stirring; P3 – stirring with heating; P4 – stirring with heating; P5 – stirring with heating; P7 – extraction in a cavitation reactor; P8 – treatment with ultrasound of 0.07 kW; P9 – treatment with ultrasound of 0.27 kW)**

Effectiveness of the methods for extraction of humic substances directly depends on the capacity of the method to degrade raw materials, as well as on the extractant. Since for industrial production of humic substances, acquisition of the highest possible amount of humic material using the smallest possible quantity of raw materials, time and energy is very important, the most effective extraction methods are cavitation and electrical discharge, while the most effective extractant is NaOH, which allows to obtain higher concentrations of humic substances than KOH and shows significantly better results than other extractants, e.g., $K_4P_2O_6$. The temperature of the extraction environment also affects the outcome.

The character of the isolation process has a major impact on the properties of isolated humic acids – depending on the intensity of treatment, the amount of carbon in humic substances, their molecular mass, and E_4/E_6 ratio decline. IR spectra indicate a significant decline in hydroxylgroups characteristic of carbohydrates as well as carboxyl groups. ^{1}H NMR spectra show a decline of resonances attributed to sugar-like components during intensive extraction procedures.

3.3 Variations in Properties of Humic Acids within Peat Profiles

Peat HS can be considered especially appropriate for studies of the carbon biogeochemical cycle because HS properties and the decomposition degree of the original organic matter are connected.

In this study humic acids were isolated from different depths of the peat column in five raised bogs in Latvia, representing bogs of industrial importance that reflected the conditions of bog development in northern Europe (Table 3.10).

Table 12

The elemental composition of peat humic acids extracted from peat bogs in Latvia

Sample	Depth, cm	C, %	H, %	N, %	S, %	O, %	Ash content, %	Total acidity, meq/g	M_w, Da
Ploce Bog	0−30	51.51	4.80	2.12	1.03	40.30	0.24	8.26	22 000
	31−60	51.13	4.81	2.06	0.78	40.97	0.25	8.36	n.d.
	61−85	51.12	4.87	2.05	0.92	40.78	0.26	7.68	18 000
	86−110	52.11	4.61	1.60	0.73	40.70	0.25	7.51	n.d.
	111−130	57.50	4.94	1.92	0.78	34.61	0.26	7.43	22 500
Gāgas Bog	0−20	49.94	4.68	2.11	0.34	41.49	1.45	n.d.	n.d.
	21−40	54.32	5.03	2.36	0.89	36.78	0.62	n.d.	n.d.
	41−60	54.87	4.93	2.10	0.55	36.93	0.62	n.d.	n.d.
	61−80	58.76	5.20	2.15	0.74	32.53	0.62	n.d.	n.d.
	81−100	57.75	4.50	1.98	0.39	35.26	0.12	n.d.	n.d.
	101−120	52.09	4.94	1.66	1.27	39.42	0.62	n.d.	n.d.
	121−140	53.80	5.34	1.96	0.78	37.83	0.29	n.d.	n.d.
Kaigas Bog	0−25	57.05	5.06	2.62	0.57	34.45	0.25	7.61	30 600
	26−45	54.52	5.42	2.52	0.85	36.39	0.30	7.64	32 600
	46−70	55.14	5.07	1.96	0.87	36.85	0.11	7.25	n.d.
	71−95	57.61	5.35	2.22	0.59	33.93	0.30	7.15	n.d.
	96−125	57.60	5.19	1.82	1.59	33.50	0.30	7.71	18 500
	126−145	56.26	5.43	2.37	0.09	35.33	0.52	7.51	21 500
Dižais Veiķenieks Bog	0−25	54.80	5.09	2.19	0.83	36.48	0.61	7.75	n.d.
	26−52	54.11	5.07	2.10	0.95	37.30	0.47	8.25	33 000
	53−75	54.38	5.72	2.46	0.90	35.92	0.62	6.98	n.d.
	76−98	54.31	5.44	2.59	0.82	36.37	0.47	7.17	32 500
	99−118	56.36	5.59	2.23	0.66	34.99	0.18	6.67	26 000
Mazais Veiķenieks Bog	0−30	54.10	5.33	2.24	1.55	36.68	0.10	7.67	37 000
	31−60	57.51	5.35	2.73	0.81	32.94	0.66	7.00	n.d.
	61−90	55.36	5.29	2.56	0.80	35.33	0.66	6.34	n.d.
	91−125	54.89	5.23	2.76	0.79	35.11	1.22	6.72	n.d.

Table 13

The elemental ratios and humification indicators of peat humic acids

Sample	Depth, cm	E_4/E_6	H/C	N/C	O/C	Humification degree (HD)
Ploce Bog	0−30	3.70	1.11	0.04	0.59	1.206
	31−60	3.60	1.12	0.03	0.60	1.184
	61−85	3.00	1.14	0.03	0.60	1.237
	86−110	3.00	1.05	0.03	0.59	1.055
	111−130	3.76	1.02	0.03	0.45	1.035
Gāgas Bog	0−20	2.81	1.12	0.04	0.62	1.029
	21−40	3.17	1.10	0.04	0.51	1.107
	41−60	2.88	1.07	0.03	0.51	1.124
	61−80	2.93	1.05	0.03	0.42	1.214
	81−100	3.06	0.93	0.03	0.46	1.168
	101−120	3.27	1.13	0.03	0.57	1.048
	121−140	3.48	1.18	0.03	0.53	1.059
Kaigas Bog	0−25	2.89	1.06	0.04	0.45	1.182
	26−45	2.84	1.18	0.04	0.50	1.106
	46−70	3.13	1.10	0.03	0.50	1.113
	71−95	2.73	1.11	0.03	0.44	1.187
	96−125	3.00	1.07	0.03	0.44	1.187
	126−145	3.70	1.15	0.04	0.47	1.348
Dižais Veiķenieks Bog	0−25	3.65	1.11	0.03	0.50	1.014
	26−52	3.74	1.12	0.03	0.52	0.942
	53−75	3.00	1.25	0.04	0.50	1.055
	76−98	2.83	1.19	0.04	0.50	0.994
	99−118	3.38	1.18	0.03	0.47	1.102
Mazais Veiķenieks Bog	0−30	2.67	1.17	0.04	0.51	1.170
	31−60	2.50	1.11	0.04	0.43	1.123
	61−90	3.67	1.14	0.04	0.48	1.144
	91−125	3.73	1.14	0.04	0.48	1.157

3.4 Elemental Composition and Functional Characteristics

Depending on the bog and the intervals of changes, the elemental compositions of the studied peat HA (Table 12) were highly variable: C was in the range of 49–57%; H – 4.6–5.7%, N – 1.6–2.8%, S – 0.5–1.5%, and ash – 0.1–1.2%. The O content was in the range of 32–42% and was determined using mass balance. In general, C and H concentrations increased with depth. N concentrations decreased with depth, but the S concentration was very variable down the profile. The elemental composition of HA from peat in Latvia is within a similar range to the peat HAs from other regions of the world described by Anderson and Hepburn, 1986; Qiamg *et al.*, 1993; Yamaguchi *et al.*, 1998; Garnier-Sillam *et al.*, 1999; Zaccone *et al.*, 2007.

The main atomic ratios (H/C, N/C, and O/C) are reported in Table 13.

The most dominant functional groups in the structure of HA were carboxyl and phenolic hydroxyl groups, constituting the total acidity of HA (Table 13). The range of the total acidity was 6.67–8.36 meq/g; acidity was usually higher in HA from upper peat layers.

The molecular weight (Table 12) of peat HA was characterized using M_W. The determined molecular masses of HA were all rather high, up to 37,000 Da.

3.4.1 UV Spectroscopy

Although widely used for characterisation of different HS (Artinger *et al.*, 2000), the UV-Vis spectra of the examined HS were featureless and their intensity monotonically decreased with increased wavelength (Figure 24 and 25). Spectra of HA slightly differed in optical density, especially if HA were from deeper layers, but these changes were insignificant. For qualitative description, the curvature of the absorption continuum of HA can be characterized by either the specific absorbance at one wavelength (A_{280}) or the absorption ratio E_4/E_6 (Table 13) at two wavelengths (Artinger *et al.*, 2000).

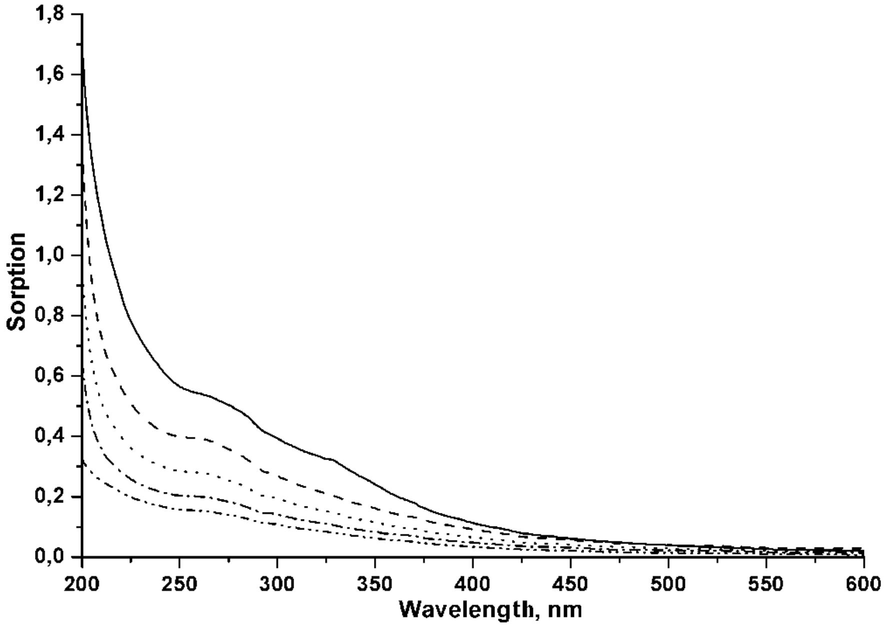

Figure 24. **UV-Vis spectra of peat HA from Dižais Veiķenieks Bog, at a range of depths:**
—— 0–25 cm ‐ ‐ ‐ ‐ 26–52 cm ·········· 53–75 cm ‐ · ‐ · ‐ 76–98 cm
— · · — 99–118 cm

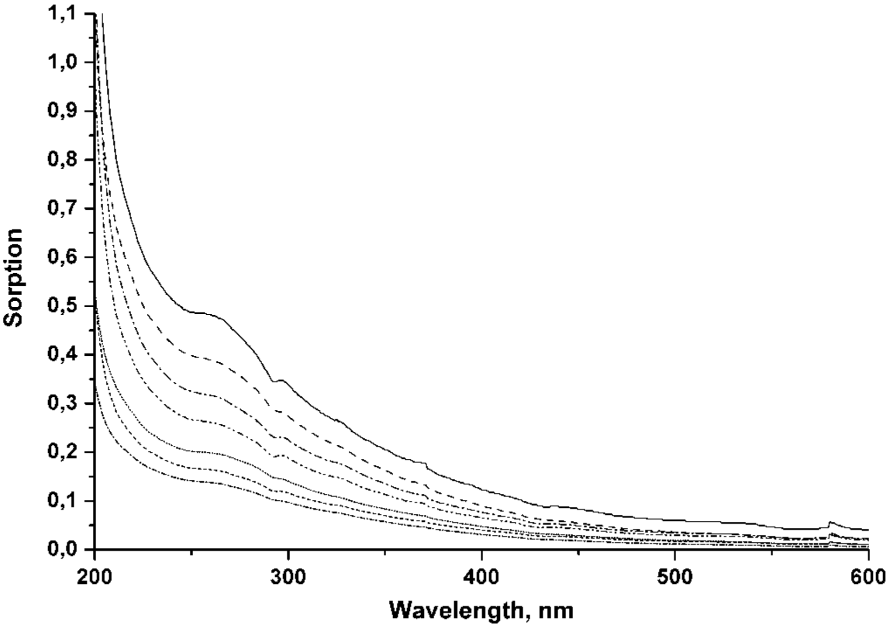

Figure 25. **UV-Vis spectra of peat HA from Gāgas Bog, at a range of depths:**
—— 0–20 cm ---- 21–40 cm ·········· 41–60 cm —·—· 61–80 cm
—··— 81–100 cm -·-·- 101–120 cm —··· 121–140 cm

3.4.2 Fluorescence Spectroscopy

Fluorescence spectra in emission scanning mode were used to obtain more detailed information on the condensed aromatic structures in HA. All HA fluorescence spectra were of similar character, showing maximum peaks at around 450 nm and weak shoulder curves at about 480 nm (Figure 26), but the intensity of fluorescence gradually decreased with depth. These results correspond to the data reported by Alberts and Takacs (2004), who characterized fluorescence emission spectra (excitation at 330 nm) of HA with maximums at the same wavelengths. The exact properties of the fluorescing groups are still uncertain although many scientists agree that fluorescence at higher wavelengths is typical of more humified HA and may be attributed to either highly substituted aromatic nuclei, possibly bearing at least one electron-donating group, or/and conjugated unsaturated systems capable of high degrees of resonance (Alberts and Takacs, 2004). In addition, lignin-derived, coumarin-like structural units and conjugated phenolic aldehydes which have λ_{em} max = 465–470 nm can be suggested as possible contributors to fluorescence of soil humic substances (Senesi and Sakellariadou, 1994). The presence of lignin-like structures seemed to be of secondary importance because the composition of *Sphagnum* moss lignin was a minor contribution.

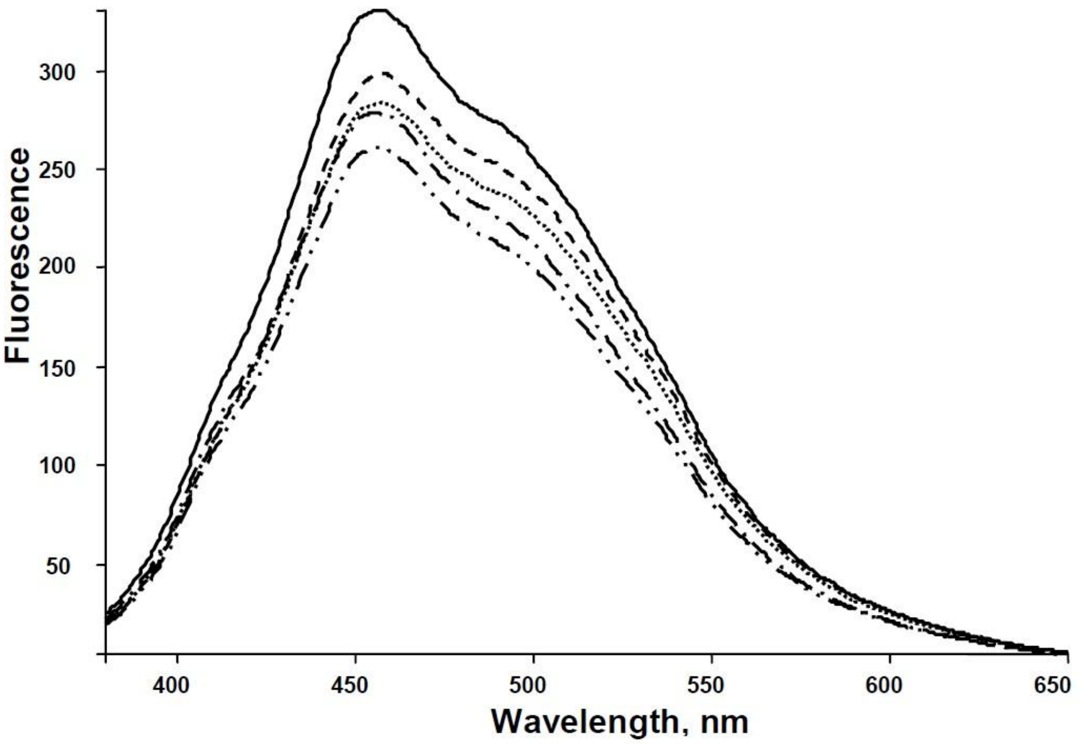

Figure 26. **Fluorescence spectra of peat HA from Dižais Veiķenieks Bog, at a range of depths:**
—— 0–25 cm - - - - 26–52 cm ·········· 53–75cm -·-·- 76–98 cm
—··— 99–118 cm

3.4.3 FT-IR Spectroscopy

Fourier transform infrared spectra (Figure 27 and 28) of peat HA were similar to one another in the main position of adsorption, but differences of various entities were apparent in the relative intensity of some bands, depending on the origin (depth and age) of the sample. IR spectra of analyzed HA can be divided into regions depending on informativity and the presence of important functional groups. Absorption bands in the 3,600–2,800 cm^{-1} spectral region were very broad; absorbance in this region was determined by the presence of -OH groups. Sorption at wavelengths of 2,950 and 2,850 cm^{-1} identified the presence of CH_3- and CH_2- groups, respectively. Typical intensive sorption lines were common for the region around 1,700 cm^{-1} (1,725–1,700 cm^{-1}), which is characteristic of carbonyl groups in aldehydes, ketones and carbonic acids. The actual sorption maximum mainly depends on the conjugation degree, presence of substituents, and hydrogen bonding. In the spectral region of 1,690–1,500 cm^{-1} it was possible to identify the sorption maximum of amide bonds (1,640–1,620 cm^{-1} and 1,550–1,540 cm^{-1}). In the spectral region of 1,625–1,610 cm^{-1}, sorption indicated the presence of aromatic C=C and carbonyl groups and quinones. At wavelengths of 1,470–1,370 cm^{-1}, there were bands typical of C-H and O-H bonding and sorption maximums typical for C-O. In wavelengths < 1,000 cm^{-1} fingerprint patterns were evident. Sorption in this spectral region provides information about the possible significance of carbohydrate percentage in the structure of humic molecules. Sorption at 1,080 cm^{-1} shows OH deformation or C-O stretch of phenol and alcohol OH groups, and at 1,040 cm^{-1} indicates C- O stretch of polysaccharide components (Table 14).

Table 14

Typical IR sorption lines of humic substances

Wavenumber (cm^{-1})	Functional groups
3,400–3,300	O-H, N-H
2,490–2,900	C-H (aliphatic)
1,725–1,720	C=O (carbonic acid, ketone)
1,660–1,630	C=O (amide, quinone, ketone)
1,630	C=C (aliphatic)
1,620–1,600	C=C (aromatic, conjugated ketone)
1,590	C=C (aromatic)
1,590–1,517, 1,400–1,390	COO^{-}, NH (amide)
1,517	C=C (aromatic)
1,400–1,390	OH, C-O, C-H (aliphatic)
1,280–1,200	C-O, O-H, C-O, C-N
1,170–950	C-O (carbohydrate)
750	C-C, C-O (carbohydrate)

Changes in the relative intensities of sorption maxims in FT-IR spectra (Figure 27–31) indicate the character of changes during the development of the structure of peat HA during humification, especially if compared with IR spectra of peat-forming plants (Figure 32). There were remarkable differences in sorption between peat and bog plant HA in the wide line around 3,400 cm^{-1}. The absorption band centred at 1,720 cm^{-1} (indicating acidic carboxylic groups) was clearly stronger for HA from peat with a higher degree of decomposition, especially in comparison with the intensity of the sorption line in bog plants. The absorption band centred at 1,040 cm^{-1}

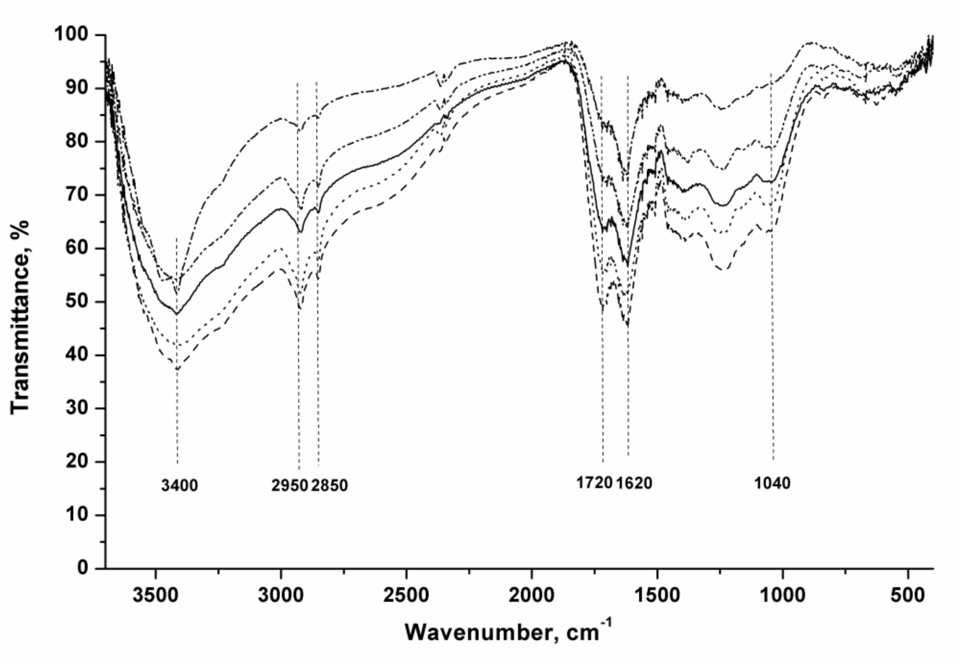

Figure 27. **FT-IR spectra of peat HA from Dižais Veiķenieks Bog, at a range of depths:**

—— 0–25 cm ---- 26–52 cm ··········· 53–75 cm -·-·-76–98 cm

—··— 99–118 cm

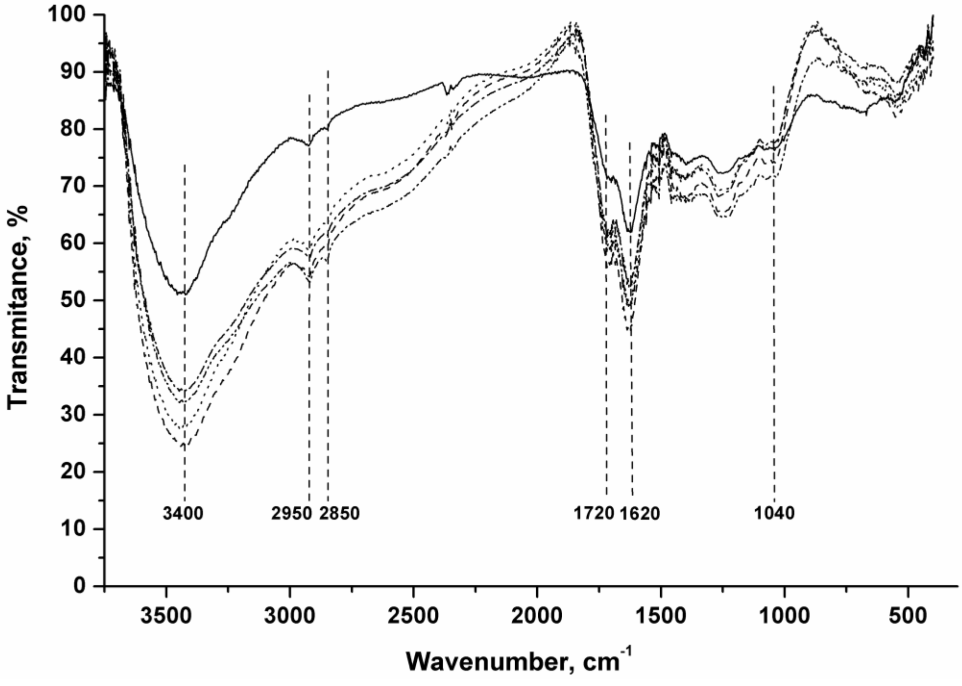

Figure 28. **FT-IR spectra of peat HA from Ploce Bog, at a range of depths:**

—— 0–30 cm ——— 31–60 cm ··········· 61–85 cm —·—· 85–110 cm

—··— 111–130 cm

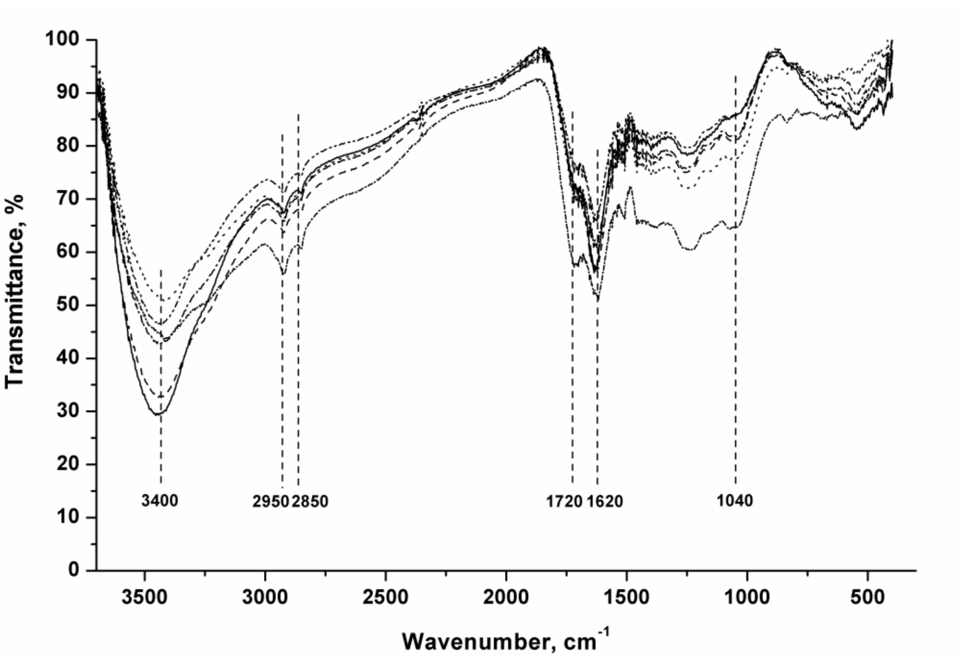

Figure 29. **FT-IR spectra of peat HA from Kaigas Bog, at a range of depths:**
—— 0–25 cm – – – 26–45 cm ·········· 46–70 cm –·–· 71–95 cm
–··– 96–125 cm -·-·- 126–145

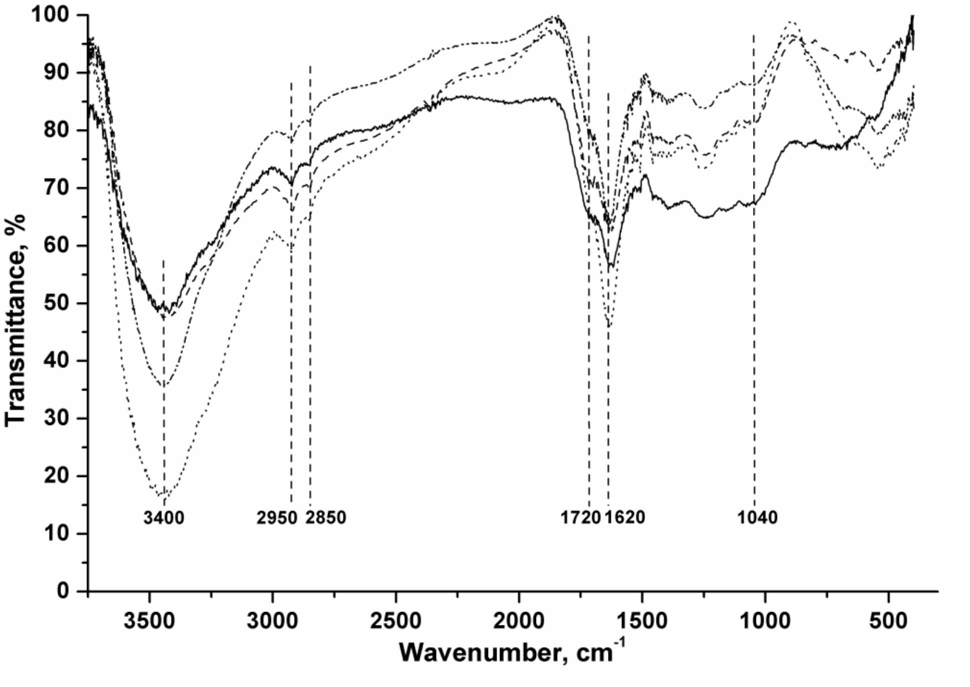

Figure 30. **FT-IR spectra of peat HA from Mazais Veiķenieks Bog, at a range of depths:**
—— 0–30 cm – – – 31–60 cm ·········· 61–90 cm –·–· 91–125 cm

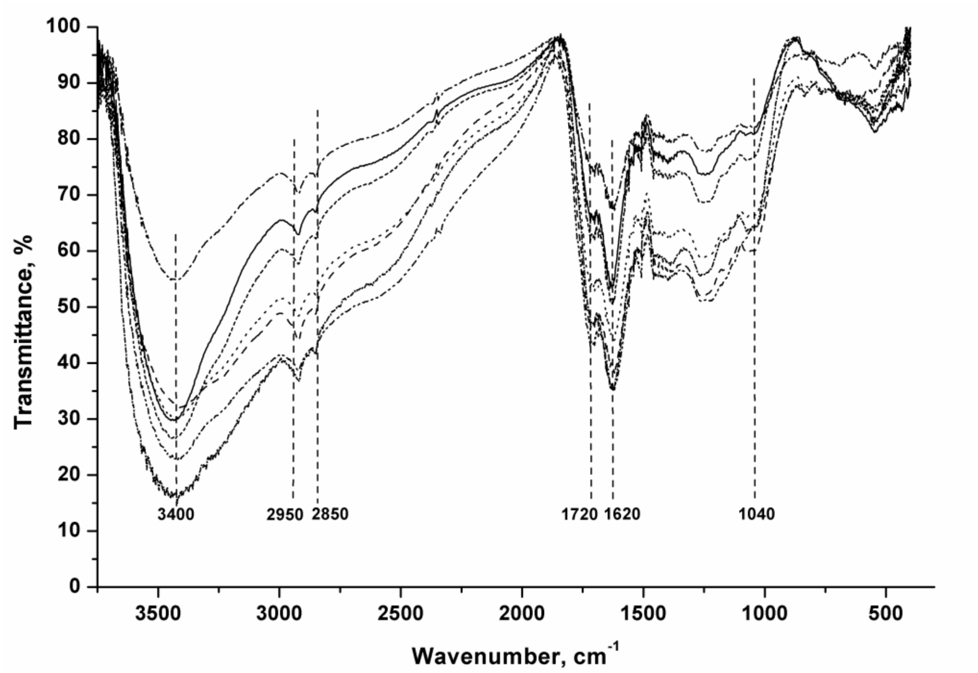

Figure 31. **FT-IR spectra of peat HA from Gāgas Bog, at a range of depths:**
—— 0–20 cm — — — 21–40 cm ······· 41–60 cm —·—· 61–80 cm
—··— 81–100 cm -·-·- 101–120cm ············ 121–140 cm

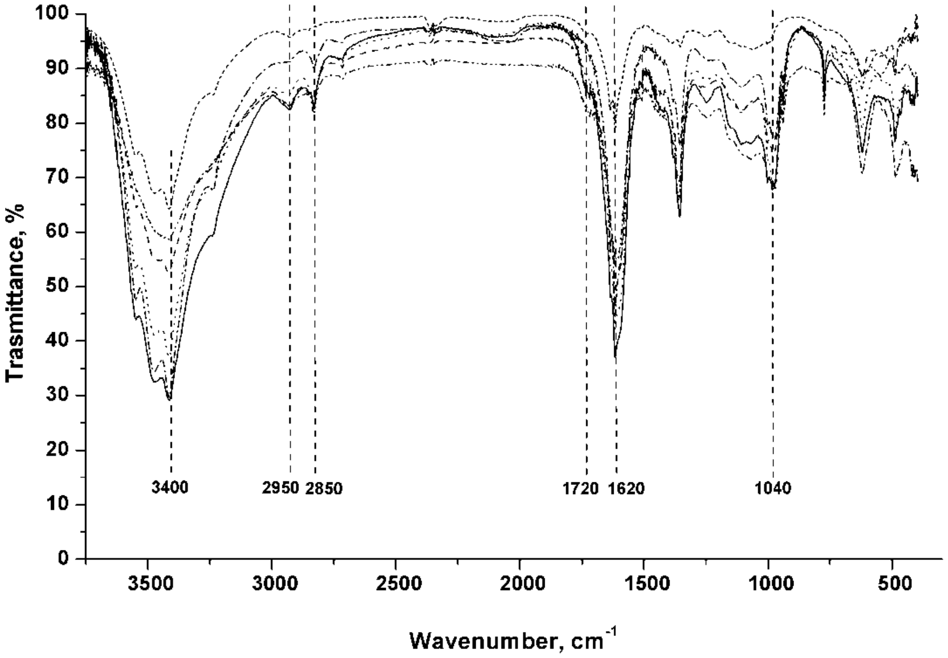

Figure 32. **FT-IR spectra of peat-forming plants**
—— ***Pleurozium schreberi*** **— — — Heather ······· Sedge**
—·—· ***Sphagnum magellanicum*** **—··—** ***Sphagnum angustifolium***
- - - - ***Hylocomium splendens***

(C-O stretching, e.g., in polysaccharides) was equally weak for all studied HA, even if their decomposition degree was low, indicating that decomposition of carbohydrates takes place at very early stages of humification. However, there was still a trend of decline of polysaccharides and polysaccharide-like substances with depth. Such pattern of changes illustrates that the development of the structure of peat HA was similar in all samples.

3.4.4 NMR Spectrometry

^{1}H NMR spectra can be divided into five regions (Corvasce *et al.*, 2006). The region at 0.2–1.49 ppm indicates presence of methyl and methylene protons of carbons directly bonded to other carbons. The region at 1.48–2.92 ppm is attributed to methylene and methine protons located in the α position to aromatic rings, or carboxyl and carbonyl groups. The region at 0.2–2.92 ppm represents aliphatic protons. The region at 2.92–4.21 ppm is determined by protons attached to C atoms bound to oxygen (methoxyl, carbohydrate, peptide, and amino acid protons). The region at 5.94–8.14 ppm is attributed to protons attached to olephinic and aromatic carbons; in this region, aromatic protons dominate. The ^{1}H NMR spectra of peat HA from the peat column are similar (Figures 33 and 34), they do not reflect major changes within peat columns and exhibit resonances in the carbohydrate region. ^{1}H NMR spectra show that in peat HA, the relative concentrations of aliphatic, carbohydrate and methoxyl protons are usually larger than those of aromatic protons.

The structure of humic matter was also studied using ^{13}C NMR spectroscopy and the cross polarisation/magic angle spinning (CP/MAS) technique. Comparison of ^{13}C CP/MAS NMR spectra allows to determine directly the major HS structural units (Figure 35). The signals in NMR spectra represent different types of C atoms and allow to determine their concentrations. ^{13}C NMR spectra can be divided into eight chemical shift ranges (Figure 35). Region A (0–50 ppm) consists primarily of aliphatic C resonances (methyl, methylene and methine carbons). Region B (50–60 ppm) consists of methoxyl carbon ($-OCH_3$) resonance signals. Region C (60–90 ppm) is primarily attributed to C-O since the N content of HS is commonly low. In this region, carbohydrate-type compounds and C in ether-type bonds can resonate. Region D (90–110 ppm) is determined by resonance signals of dioxygenated carbons (present in polysaccharides).

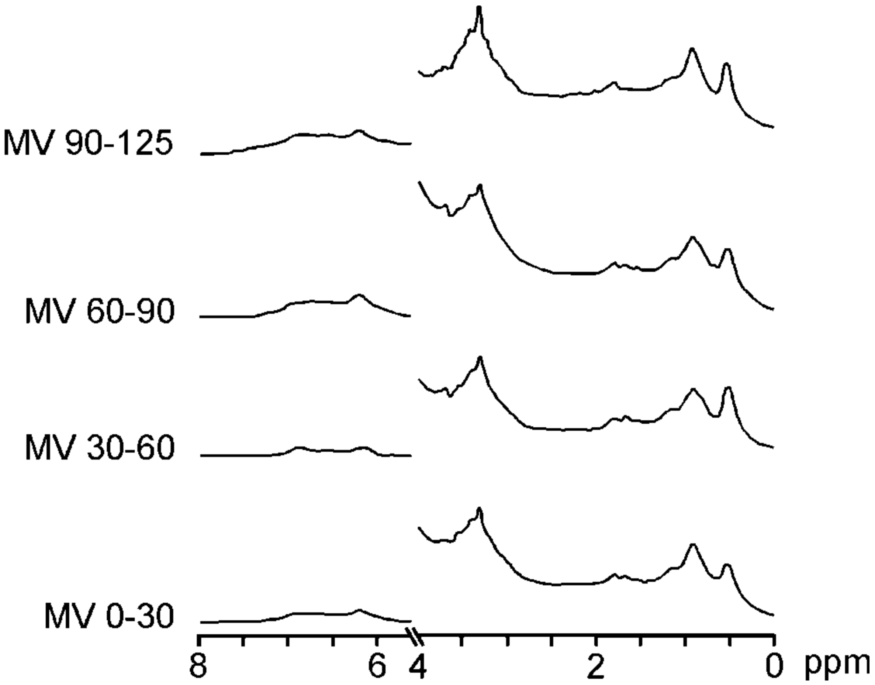

Figure 33. **^{1}H NMR spectra of peat HA from Mazais Veiķenieks Bog**

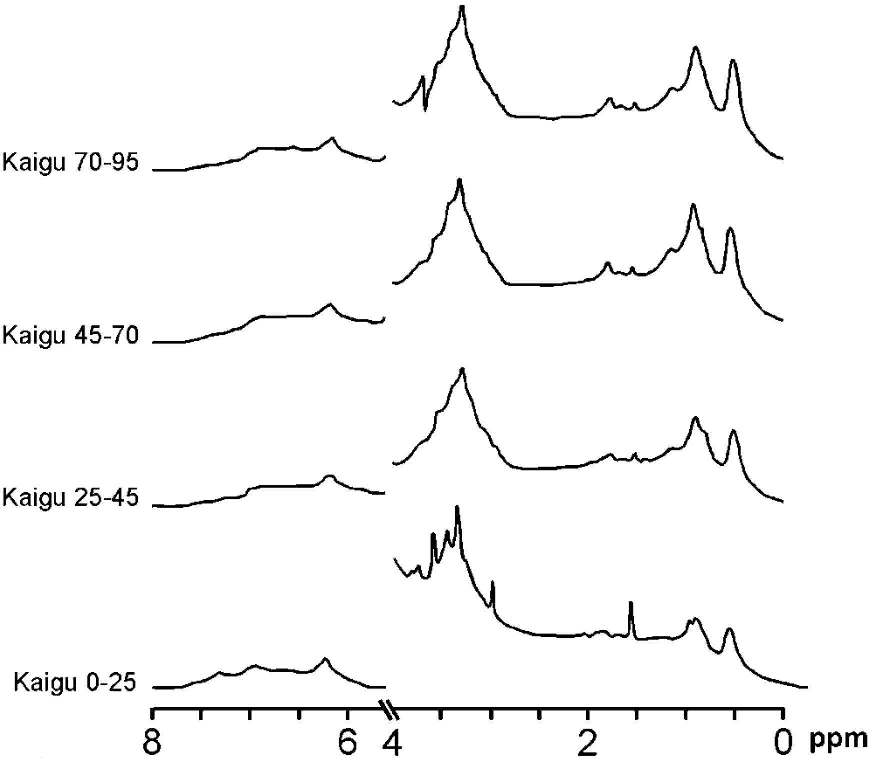

Figure 34. **^{1}H NMR spectra of peat HA from Kaigas Bog**

Signals at 50–110 ppm correspond to carbons bonded to electronegative atoms (O, N, and Cl), but this range can be dominated by resonance signals from carbohydrate structures. Region E at 110–145 ppm is attributed to resonance of unsubstituted and alkyl-substituted aromatic carbons (aromatic C *ortho* to O-substituted aromatic C).

Region F at 145–160 ppm is attributed to resonance of aromatic carbons substituted by O and N (e.g. phenols, aromatic ethers, or amines). Region G at 160–190 ppm represents resonances mainly caused by carboxyl carbons, and Region H at 190–220 ppm represents carbonyl carbons (aldehydes and ketones). ^{13}C NMR spectra show that the studied peat HA have marked differences in their structure, namely higher aromaticity (intensity of signal 110–160 ppm) of HA from deeper layers in comparison with HA from upper peat layers, but especially in comparison with peat-forming moss – *Sphagnum* moss (Figure 35).

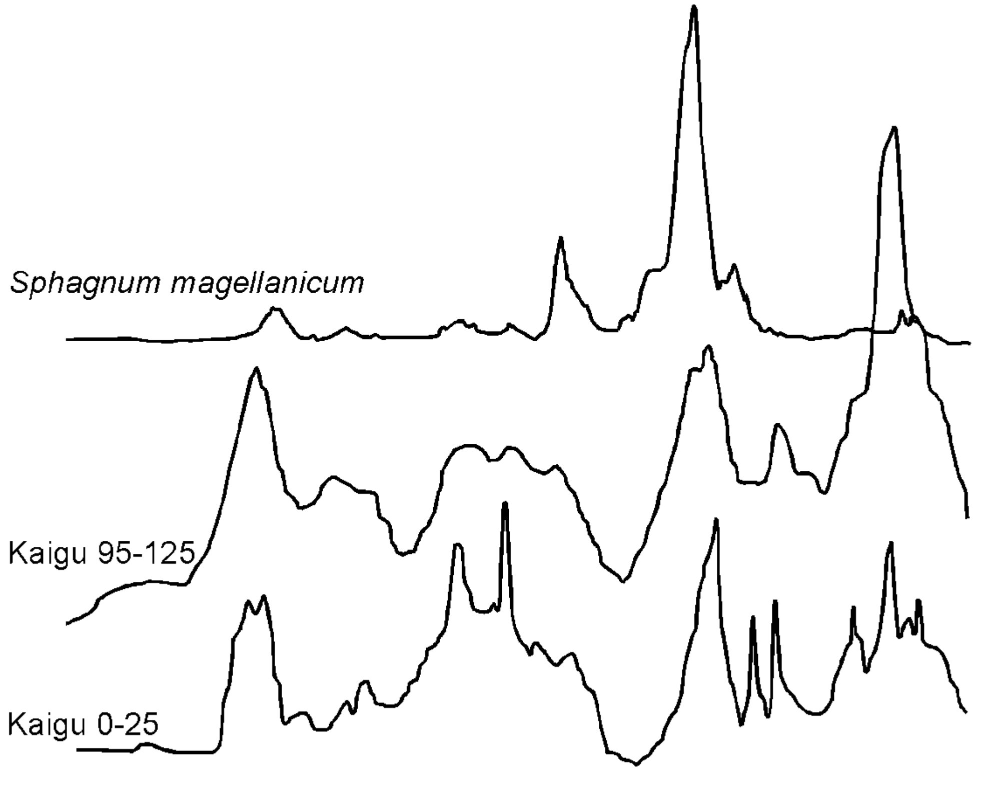

Figure 35. **^{13}C NMR spectra of the upper and lower layers of peat HA from Kaigas Bog and *Sphagnum magellanicum* moss**

The number of aromatic structures in the studied peat samples was rather low, especially compared to soil and aquatic HS (Thorn *et al.,* 1989; Hertkorn *et al.,* 2002; Schumacher *et al.,* 2006; Conte *et al.,* 2007), but it was similar to the aromaticity of peat HA (Hertkorn *et al.,* 2002). We could assume that resonance signals in this region consist not only of resonance of "truly" aromatic C, but also of unsaturated (olephinic) C atoms. Aromaticity of the studied peat HS did not change much with the depth of the peat column (Table 15). ^{13}C NMR spectra demonstrated that the relative percentage of carboxyl groups (signals at 160–190 ppm) did not change much, but increased with depth. The dominant structural units in peat HA probably are aliphatic structures – alkyl groups (0–50 ppm) and residue of carbohydrate structures (O-alkyl, 50–112 ppm). Even in the deepest layers the percentage of aliphatic structures was high, especially compared with HA from coal, soil, and aquatic sources (Table 15). In general, ^{13}C NMR spectra confirmed the information provided by elemental, functional, and other spectroscopic studies of HS and the proportions of aliphatic structures calculated from the spectral properties of HS.

Table 15

Distribution of C (peak areas) among the main structural groups of peat HA isolated from a peat column and from other sources determined with ^{13}C NMR spectra

Sample	**Depth, cm**	**Range of δ ^{13}C and assignment (ppm)**					
		220–190, ketonic	190–167, carboxyl	167–145, phenolic	145–110, aromatic, olephinic	110–50, O-alkyl	50–0, alkyl
Ploce Bog	0−30	2.8	14.3	8.3	23.5	27.5	23.6
Gāgas Bog	0−20	3.2	15.5	8.7	23.8	26.5	22.3
Dižais Veiķenieks Bog	0−25	3.8	13.6	7.5	22.5	25.2	27.4
Mazais Veiķenieks Bog	0−30	3.4	15.5	8.5	26.3	26.5	19.8
	31−60	3.6	15.5	9.3	26.8	28.8	19.0
	61−90	4.0	16.4	9.4	27.4	17.2	25.6
	91−125	4.0	18.5	10.1	29.5	9.6	28.3
Peat HA[i]		3.8	10.8	10.3	28.7	22.2	16.0
Lignite HA[ii]		22		36		10	28
Aquatic HA[iii]		6	12	5	11	42	24
Peat HA (Waskish)[iv]		8	18	42		14	18
Soil HA[iv]		6	18	50		10	16

[i] Hertkorn *et al.*, 2002
[ii] Conte *et al.*, 2007
[iii] Schumacher *et al.*, 2006
[iv] Thorn *et al.*, 1989

3.4.5 ESR Spectroscopy

ESR spectroscopy was used to estimate the free radical content in the studied HA. Organic radicals of semiquinonic nature conjugated with extended aromatic systems or paramagnetic metal ions (Fe, Mn, and V) can be the source of free radicals (Chen *et al.*, 2002). ESR spectra of investigated peat HA (Figure 36) were of relatively simple shape and similar to one another. All spectra were dominated by strong, very narrow symmetrical lorentzian resonance signals. The g-values (g-electron free-spin g factor) and line widths (Table 16) were similar in all samples, and consistent with organic free radicals of semiquinone nature conjugated with an aromatic network (Kļaviņš, 1995). Another feature common to all spectra was an asymmetrical broad line centered at g = 2.038 attributed to Fe^{+3} ions. The ESR spectra of all investigated samples were characterized by weak resonance lines at g = 4.26 and g = 9.4, also consistent with Fe^{+3} ions at sites of rhombic symmetry.

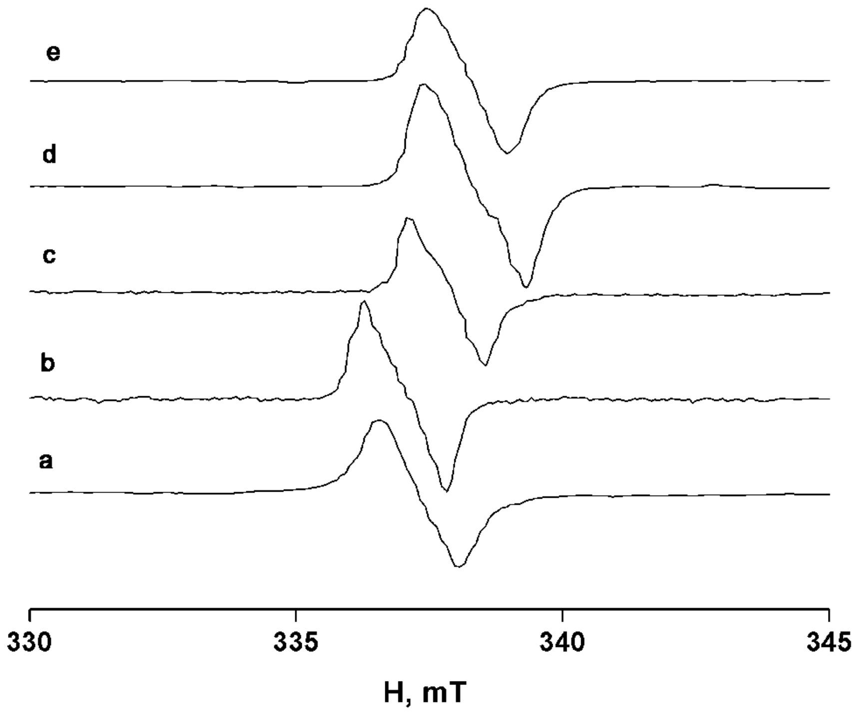

Figure 36. **Electron spin resonance spectra (typical HA semiquinone-type free radical spectrum) of HA extracted from peat: (a) Dižais Veiķenieks Bog 0–25 cm; (b) Gāgas Bog 0–20 cm; (c) Kaigas Bog 0–25 cm; (d) Mazais Veiķenieks Bog 0–30 cm; and (e) Ploce Bog 0–30 cm**

Table 16

ESR parameters of peat HA spectra

Sample	Depth, cm	Spin concentration × 10^{-19}/g	g-value[a]	Line width, Mt
Ploce Bog	0–30	0.110713	2.0056	1.5
	111–130	0.032206	2.004	1.4
Gāgas Bog	0–20	0.020888	2.006	1.5
	121–140	0.008430	2.006	1.5
Kaigas Bog	0–25	0.009795	2.006	1.6
	26–45	0.028002	2.006	1.5
	46–70	0.000589	2.005	1.4
	71–95	0.001753	2.0065	1.4
	96–125	0.017086	2.005	1.5
	126–145	0.011009	2.006	1.5

[a] g-electron free-spin g factor

In general, the spectral characteristics (g-values and line widths) of ESR spectra were the same in all samples. However, there were differences in the relative intensity of the principal resonance line and minor resonances at first, indicating differing concentrations of paramagnetic metal ions.

3.4.6 Chemical Properties of Peat Humic Acids and Factors that Influence them

For industrial mining of peat, the homogeneous, “bulk” peat mass (the catotelm) is of major importance. For peat extraction, living and undecomposed plant material is of minor value and is usually removed. This study investigated the variability of properties of peat HA only from the catotelm.

There was a significant variation in the elemental composition of HA within the peat profiles. It is assumed that periodic drying and wetting the topmost peat layers are subjected to during the peat extraction process would increase aerobic decomposition and oxidation (Anderson and Hepburn, 1986). As a result, the changes of the elemental composition of peat HA do not regularly coincide with the depth of the peat column.

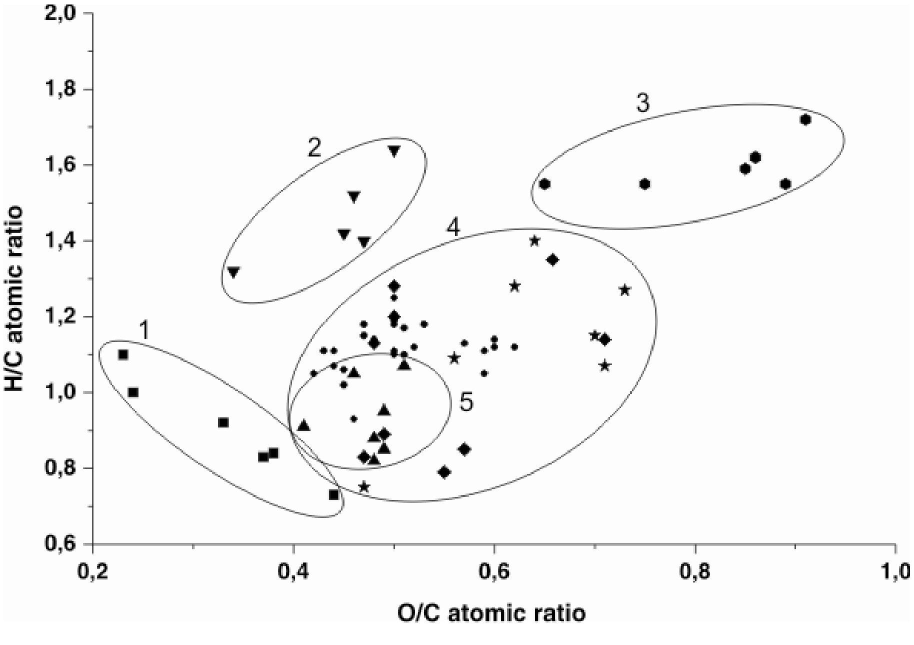

Figure 37. **Van Krevelen (H/C vs O/C atomic ratio) graphs of bog plants (⬢); HA isolated from peat samples from bogs in Latvia (•); reference peat HA (IHSS, 2008) and peat HA (★) (Беляева, 2004); soil HA (◆); HA from different coals and lignite (■) (Кречетова, 1994; Dick *et al.*, 2002; Беляева, 2004); sedimentary HA (▼) (Senesi and Sakellariadou, 1994); and aquatic HA (▲) (Reckhow *et al.*, 1990; Pettersson *et al.*, 1994; Artinger *et al.*, 2000)**

Peat HA were analyzed using van Krevelen graphs that are frequently applied in studies of HS and the carbon biogeochemical cycle (van Krevelen, 1950). Table 13 shows the elemental ratios of the studied peat HA samples. According to Orlov (1990), the index of O/C, H/C, and N/C atomic ratios is useful for identification of structural changes and the degree of maturity of HS obtained from different environments. The relations between the H/C atomic ratio and O/C atomic ratio of HA of different decomposition degree – beginning with bog plants up to brown coal, lignite, and coal – reveal changes in the associated elemental composition (Figure 37). To provide reliable quantity information on the diagenesis of HA, we carried out further studies on the dependence of the elemental composition of the peat samples on their age (depth and decomposition degree) (Figure 38). Dependence between H/C values and the depth of the peat samples was mostly negatively correlated, demonstrating that dehydrogenation is among the dominant processes during ageing of peat HA.

O/C vs N/C atomic ratio indicates that the decarboxylation processes were in line with the reduction of N concentration relative to C content in HS (Figure 39).

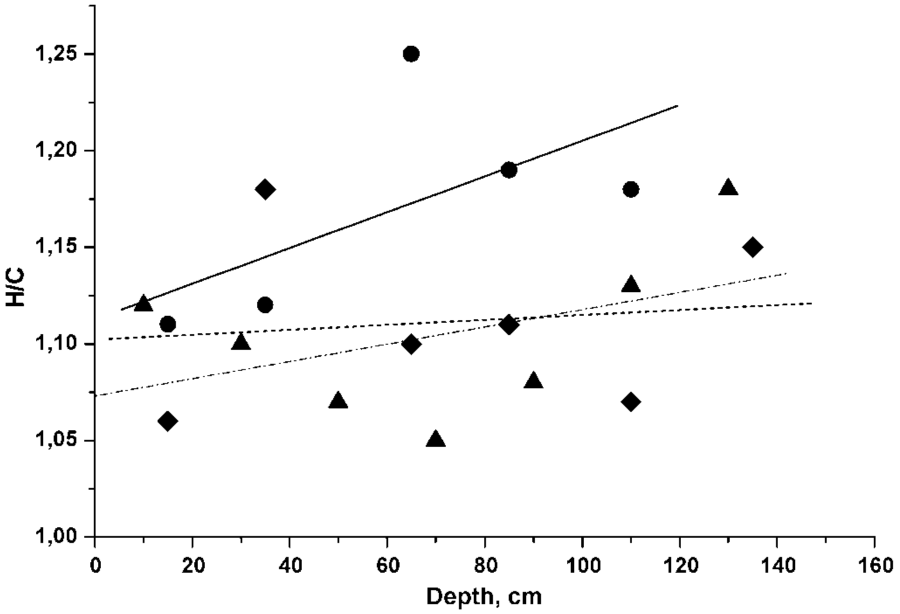

Figure 38. **Dependence of H/C values of peat HAs on the depth of the peat column in different peat bogs**
● Dižais Veiķenieks Bog (——— Y = 1.11261 + 9.25606^{-4} · X; R = 0.61719; P = 0.26739)
▲ Gāgas Bog (– · – · Y = 1.07304 + 4.46429^{-4} · X; R = 0.44305; P = 0.31945)
◆ Kaigas Bog (········· Y = 1.10217 + 1.28007^{-4} · X; R = 0.12521; P = 0.81317)

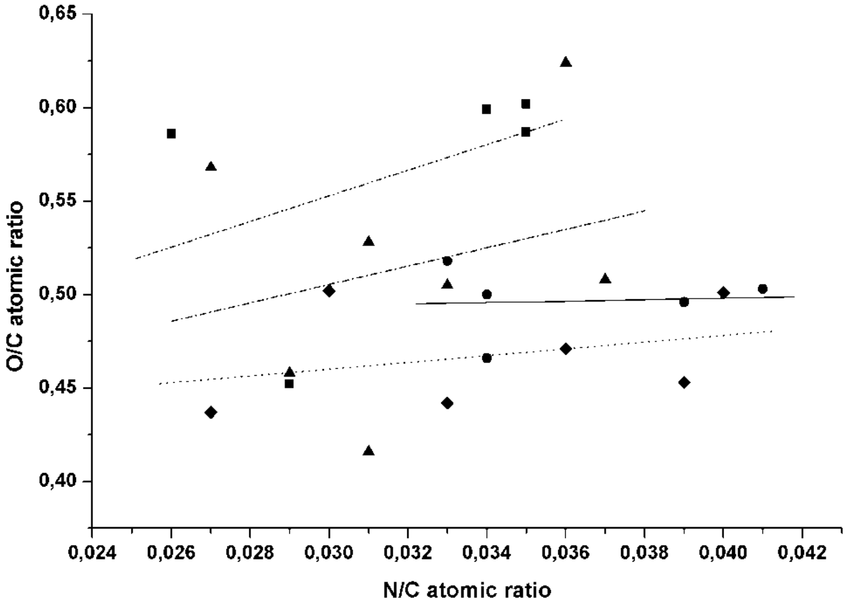

Figure 39. **Van Krevelen graphs of O/C and N/C atomic ratios in different peat bogs**
● Dižais Veiķenieks Bog (———Y = 0.48206 + 0.40157 · X; R = 0.07524; P = 0.9043)
▲ Gāgas Bog (– · – · Y = 0.35816 + 4.91026· X; R = 0.2589; P = 0.57506)
◆ Kaigas Bog (········· Y = 0.40595 + 1.80637 · X; R = 0.32208; P = 0.53359)
■ Ploce Bog (— · · Y = 0.34707 + 6.85928 · X; R = 0.44021; P = 0.45818)

Studies of the elemental composition of peat HA extracted from a peat column can provide information on humification reactions ongoing during peat development. The decomposition degree is related to humification indicator HD, confirming that during the decomposition process, development of humic substances occurs since peat with a higher decomposition degree yields more humified humic acids (Figure 40)

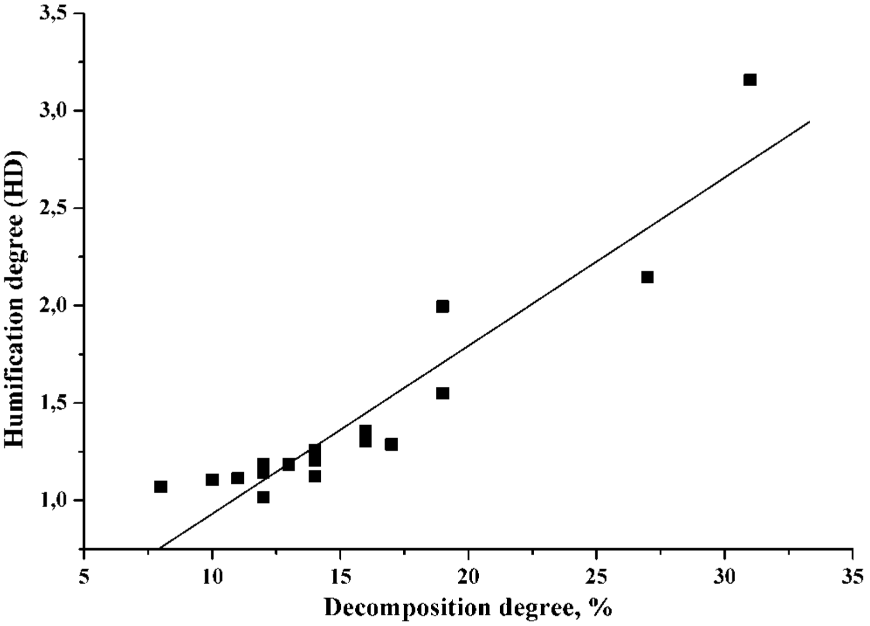

Figure 40. **Correlation between the humification degree of humic acids and peat decomposition degree**

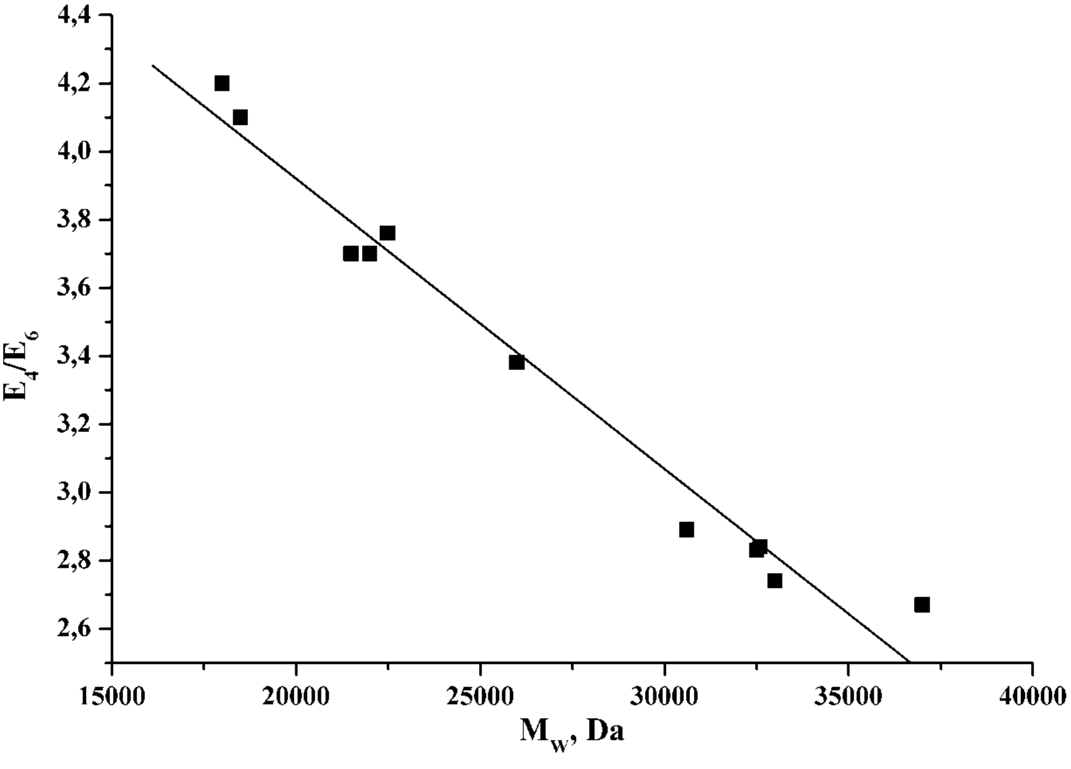

Figure 41. **Effect of the molecular mass of peat HA on E_4/E_6 ratio**

UV-Vis absorption ratios were measured to obtain information on the humification degree of peat samples. The E_4/E_6 ratio is often used to describe the extent of condensation of aromatic C-containing structures; low ratios reflect high degrees of condensation of these structures while high ratios mean presence of large quantities of aliphatic structures and low quantities of condensed aromatic structures (Chin *et al.,* 1997). This ratio also is inversely related to the degree of aromaticity, particle size, molecular weight, and acidity (Uyguner *et al.,* 2004), which is confirmed in this study (Figure 41).

This study proved that the variability of E_4/E_6 ratios in peat profiles was generally low (Table 8). Correlations between different chemical parameters were investigated and the plot of the total acidity values and E_4/E_6 ratios showed strong negative correlation in peat profiles (Figure 42). Increase of acidic groups in HA samples resulted in a reduced E_4/E_6 ratio. Ratios of previously mentioned spectral wavelengths were also connected with the humification indicators suggested in our study; when humification index increased, the E_4/E_6 ratio decreased or stayed relatively constant. However, these changes were insignificant and can be explained by the overall low decomposition degree of the peat samples or the effect of dissimilar botanical composition on these values. In some cases the humification index and E_4/E_6 ratio can be used for description of the transformation processes of organic matter within peat profiles.

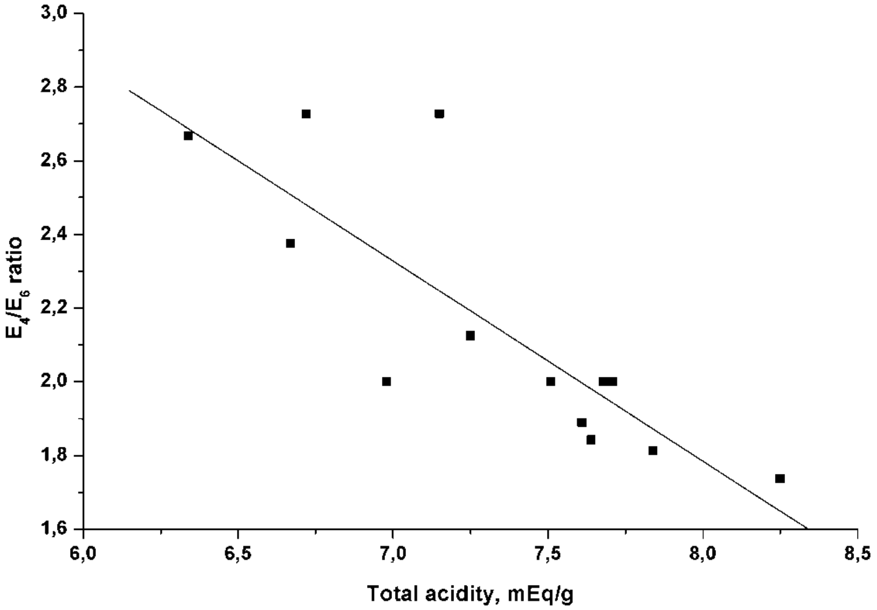

Figure 42. **The correlation between E_4/E_6 ratios and the total acidity in Dižais Veiķenieks Bog, Gāgas Bog, and Kaigas Bog ($Y = 6.13374 - 0.54365 \cdot X$; $R = -0.82782$; $P = 0.002$)**

The elemental composition of humic acids substantially varies due to inconsistent moisture and oxygen levels, as well as the botanical composition of peat. Spectroscopic indicators such as E_4/E_6 ratio clearly demonstrate that the humification degree increases with increasing depth of the sample of humic acids. It was confirmed by such parameters as molecular mass and total acidity.

The different spectroscopic methods (UV-Vis, Fluorescence IR, ^{1}H NMR, ESR) do not provide any valuable information on the structure and properties of peat humic acids, except for ^{13}KMR spectra, which give detailed information on the distribution of carbon among the main structural groups of peat HA isolated from a peat column. The data show increasing amounts of aromatic structures and thus confirm the transformation of humic acids during the humification process.

The correspondence of the data of analysis of the composition of humic acids to peat properties allow to use the humification indexes of peat developed by us to describe peat transformation processes during the humification process.

Conclusions

1. Detailed analysis of peat is a very important prerequisite for studies of peat humic acids, since it allows to link the peat formation conditions with the properties of newly formed humic material (humic acids).
2. The degree of decomposition indicates the intensity of transformation of organic matter and characterises the degree of humification of the living organic matter. Analysis of humification parameters in several bogs in Latvia describes the transformation of organic matter to humus (humic substances); therefore, the degree of humification and the humification indexes developed during this research can be used to study the process of transformation of organic matter.
3. Intensive mixing technologies (electrical discharge, cavitation, and ultrasound) promote significantly higher yields and can operate in flow mode (electrical discharge plasma, cavitation). Therefore, these technologies can be applied to develop highly efficient production processes. The effectiveness of the extraction of HS directly depends on the capacity to degrade the raw material, as well as on the extractant. Cavitation and electrical discharge technologies are best suited for large-scale production because they allow to achieve the highest yields possible using the least amount of raw materials, time, and energy. However, depending on the intensity of the treatment process, significant changes in the structure of HS may occur, e.g. degradation of the carbohydrates associated with HS.
4. The elemental, functional, mass-molecular, and spectral characterisation of peat HA demonstrate the impact of the peat properties on the properties of HA. The genesis of peat HA takes place in the upper bog layer (the acrotelm); within the bulk peat mass (the catotelm), changes in the structure of peat HS are insignificant. In anaerobic and acidic conditions, the humification process of the saturated peat layers is very much retarded. The source of changes in peat HA properties extracted from industrially important bogs could be both natural variability and structural changes induced by preparation of bogs for industrial extraction.

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