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HPLC Fingerprints of Porewater Organic Compounds as Markers for Environmental Conditions

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1. Introduction

Lake sediments are considered invaluable natural archives that provide long-term records of past changes in climate and environment related to catchment processes as well as in-lake changes in biological communities. Moreover, lake sediments also register anthropogenic activities and man-made environmental problems. Lake sediments are known to accumulate different compounds during their formation and adsorption processes, and thus sediment investigations can be used as an important tool to assess the contamination of aquatic ecosystems. The organic matter in sediments is distributed between the particulate and dissolved phases, and usually the aquatic phase is named porewater (*pw*).

The dissolved organic matter (DOM) is an important component of aquatic ecosystems and of the global carbon cycle. It is known that changes in DOM quality and quantity have effects on the whole ecosystem. Quantitative and qualitative changes in DOM are related to precipitation, runoff, and seasons. DOM consists of a mixture of macromolecular compounds with a wide range of chemical properties and diverse origins. The DOM in lakes can serve as a molecular proxy for identification of previous inputs of organic matter. Moreover, detailed knowledge about DOM is greatly needed in order to reconstruct palaeoclimate or land-use. The biogeochemical transformation of DOM helps to elucidate past and present environmental conditions. For all those reasons, detailed DOM characterization at the molecular level is of utmost importance.

Since DOM is naturally a very complex mixture of molecules, the determination of its exact chemical composition is a complicated task. Only detailed chemical characterization using various analytical methods could be carried out. A part of DOM is optically active, enabling spectroscopic methods based on UV absorption to be used for the characterization. Another possible method of DOM analysis is chromatographic size fractionation using high-performance liquid chromatography (HPLC) with a size exclusion option (high-performance size exclusion chromatography – HPSEC). HPSEC has been widely used in studies of DOM together with spectroscopic methods. The reliability and sensitivity of this method have been reported and discussed previously (Chin et al., 1994; Hoque et al., 2003; Minor et al., 2002; Nissinen et al., 2001; Pelekani et al., 1999; Perminova et al., 1998, 2003; Specht & Frimmel, 2000; Zhou et al., 2000). Although HPSEC characterization of lake sediment *pw*DOM has demonstrated great potential for

palaeolimnological research (Leeben et al., 2008a; Lepane et al., 2004, 2010a; Makarõtševa et al., 2010) it is not widely used for evaluating the long-term changes in aquatic ecosystems. At present, no comparative investigations of *pw*DOM from lake sediments are available. Coupling of HPSEC as a separation method with diode-array detection (DAD) allows DOM fingerprints and spectra of DOM molecular fractions to be obtained for qualitative and semi-quantitative analysis. The non-destructive analysis, small sample volume, and minimal sample pretreatment are great advantages of the HPSEC-DAD approach, making the method suitable for environmental studies. HPSEC-DAD has been adapted and optimized for analysis of *pw* samples under various conditions (Lepane et al., 2004; 2010a; O'Loughlin & Chin, 2004). The advantage of the usage of this chromatographic system is a better understanding of the qualitative and quantitative *pw*DOM properties by detecting aromatic fractions (chromophoric compounds). This method has recently been applied for monitoring and detection of organic matter from surface waters after oxidation treatment (Liu et al., 2010).

This study aims: (1) to investigate temporal changes in *pw*DOM components' qualitative and quantitative characteristics by exploring different sediment core records; (2) to find the similarities and differences in HPSEC-DAD fingerprints of *pw*DOM after applying the statistical data treatment methods; (3) to explore the potential impact of environmental change on *pw*DOM records in investigated sediment cores.

2. Materials and methods

2.1 Study area and sampling

The case studies were conducted at two sediment cores from Estonia: Lake Peipsi and Lake Rõuge Tõugjärv.

Lake (L.) Peipsi is the largest transboundary lake in Europe shared between Estonia and Russia. It is the fourth biggest lake in Europe. Its surface area is 3,500 km^2 with an average depth of 7 metres. The maximum depth is only 15 metres. The catchment area covers more than 47,000 km$^{2.}$. The catchments area has been used for agricultural purposes for several millennia. On the northern side of the lake, extensive mining areas and several electric power plants operating on oil shale exist. As a consequence, enhanced delivery of nutrients to L. Peipsi has induced an increase in primary productivity within the lake and anthropogenic eutrophication during the last few decades. Today the lake is classified as eutrophic.

A 43-cm long sediment core was collected from L. Peipsi in March 2007 from location 58°47′13″N and 27°19′18″E. The sampling point was located in the middle of the lake. The water depth at the sampling site was 9.8 m. Sediment samples were taken by a Willner corer. The core was cut into 1-cm thick subsamples, packed into plastic bags, and transported to the laboratory. The chronology of the core was established via correlation of its loss-on-ignition (LOI) curve with that of the 2002 year core, which was previously dated by the ^{210}Pb radiometric method using gamma spectrometry (Appleby et al., 1986). For calculations of the ^{210}Pb dates the Constant Rate of Supply model (Appleby & Oldfield, 1978) was applied and the results were compared to two other independent dating approaches – the sediment distribution of artificial radionuclide ^{137}Cs and spheroidal fly-ash particles (Alliksaar et al., 1998). The methodology and results of the dating methods used and the reliability of the chronology are explained in detail in Heinsalu et al. (2007).

The second lake investigated is situated in South Estonia, where the anthropogenic pressure is not too high and is expressed mainly through the agricultural activity. L. Rõuge Tõugjärv (57°44′30″N; 26°54′20″E) is a small-size stratified hard-water mesotrophic lake with a surface area of 4.2 ha and a maximum depth of 17 m. The main source of pollutants in L. Tõugjärv sediments is the catchments area. The studied sediment core was visibly laminated, reflecting the annual changes in the lake. Annual laminations, or varves, typically consist of two visible layers (a clastic inorganic layer and a darker organic humic layer), and each varve can be considered as representing one year's deposition.

The topmost 13 cm of the sediment was loose unconsolidated dark gyttja (dated until 1986 AD), while the rest of the sediment sample was laminated gyttja with well-developed varves (dated until the year 1852 AD). The L. Rõuge Tõugjärv sediment core was taken in May 2006 with a Willner-type sampler. The core was transported in a tightly closed Plexiglas tube to the laboratory, immediately sliced into 1-cm thick sub-samples, and packed into plastic bags to maximally avoid oxygen exposure.

The age-scale for the sediment sequence was obtained by correlating marker varve horizons and LOI values with another sediment core sampled in 2001, which had been carefully dated by several parallel dating methods (varve counting, ^{210}Pb, ^{137}Cs, ^{241}Am, and spheroid fly-ash particles) (Alliksaar et al., 2005; Veski et al., 2005; Poska et al., 2008). According to this correlation the obtained sediment core covered about 150 years (1850–2005).

Pw samples for analysis were obtained by extraction of unfrozen sediments by centrifugation at 3,500 rpm for 30 minutes and filtration through 0.45 µm filters (Millex, Millipore). Samples were stored at 4 ^{o}C in the dark.

2.2 Chemical analyses

Absorbance spectra of the *pw* samples were collected using a Jasco V-530 UV/VIS Spectrophotometer (Japan), with 1-cm-pathlength fused silica cells and ultrapure water as the blank. Spectra were measured over the range of 200–500 nm with a 2.0-nm bandwidth. The dissolved organic carbon (DOC) concentration in *pw* samples was calculated from absorption spectra using the equation given by Højerslev (1988). The absorbance ratio at 250 and 360 nm (A250/A360), which reflects the aromaticity of dissolved molecules (Peuravuori & Pihlaja, 1997), was calculated from the spectra.

2.3 HPLC analyses

The molecular characteristics of DOM in sediments were determined using an HPLC system. The HPLC system comprised a Dionex P680 HPLC Pump, Agilent 1200 Series (Agilent Technologies, UK) diode array absorbance detector (DAD), and a Rheodyne injector valve with a 20-µL sample loop. A BioSep-SEC-S 2000 PEEK size exclusion analytical column (length 300 mm, diameter 7.50 mm, Phenomenex, USA) preceded by a suitable guard column (length 75 mm, diameter 7.50 mm, Phenomenex, USA) was used for separation. The applied flow rates were 0.5 mL min^{-1} (L. Peipsi samples) and 1.0 mL min^{-1} (L. Rõuge Tõugjärv samples). The column packing material was silica bonded with a hydrophilic diol coating, with a particle size of 5 µm and a pore size of 145 Å. The mobile phase consisted of 0.10 M $NH_4H_2PO_4$ - $(NH_4)_2HPO_4$ buffer at pH 6.8. The HPLC system was calibrated using five different molecular mass protein standards (Aqueous SEC 1 Std, Phenomenex, USA) (see Fig. 1). All solutions for HPLC measurements were prepared using ultrapure water passed through a MilliQ water system, filtered with 0.45-µm pore size filters

(Millipore), and degassed. Samples were analysed in triplicate. In general, the relative standard deviations for the replicated measurements did not exceed 5% (obtained by comparison of total peak areas). For quality control the aqueous protein standard was analysed each day. The chromatograms were recorded and processed by Agilent ChemStation software. Full details of the method used are described previously (Lepane et al., 2004).

Weight-average and number-average molecular masses of DOM (M_w and M_n, respectively) were determined using the formulae $M_w = \Sigma(h_i M_i)/\Sigma h_i$ and $M_n = \Sigma h_i / \Sigma(h_i/M_i)$, where h_i is the detector output and M_i is the molecular mass, both at the i-th retention time (Mori & Barth, 1999).

As a semi-quantitative DOM characteristic, the total chromatogram peak areas, representing the total UV-absorbing fraction of the specific molecular size fraction of DOM in each sample, were used in the data analysis. The total chromatogram peak areas obtained with DAD actually represent the variations in optical intensities of DOM fractions at the chosen wavelength of 280 nm. The detector response (the height of the chromatogram at the i-th elution volume) refers to the amount of DOM in a specific molecular size fraction. The sum of all peak heights represents the total amount of DOM capable of UV adsorption in the sample (Matilainen et al., 2006; Peuravuori & Pihlaja, 1997; Vartiainen et al., 1997). Peak areas were used as a semi-quantitative characteristic to present age-related variations in the DOM fractions. To obtain qualitative DOM characteristics the chromatograms were divided into two molecular size fractions: 1) high molecular mass (HMW), and 2) humic substances (HS) (Lepane et al., 2010a, 2010b). The polydispersity M_w/M_n, describing the homogeneity or heterogeneity of organic matter, was calculated from the data obtained.

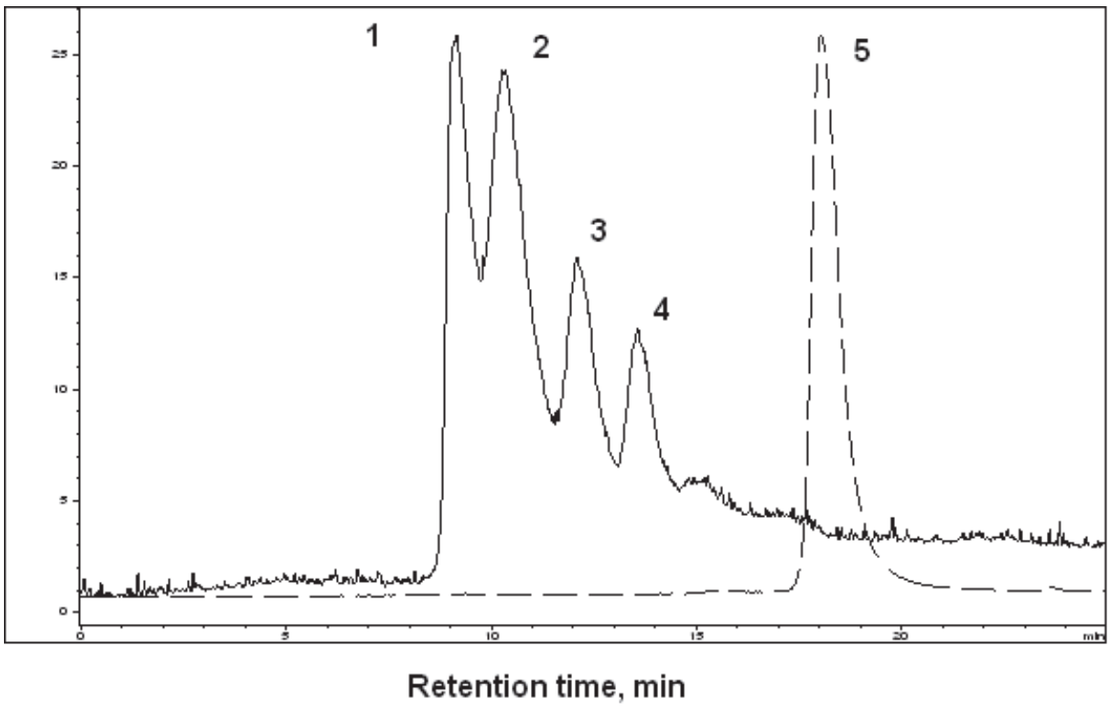


Fig. 1. Separation of calibration standards by HPLC. Protein molecular masses (1) 670 kDa, (2) 150 kDa, (3) 44 kDa, (4) 17 kDa, (5) 244 Da; detection wavelength 280 nm.

2.4 Statistical analyses

Cluster analysis using the Ward method was applied to reveal age-related periods in the analysed samples (Brereton, 2003). The analysis was performed on the chromatographic data. As descriptors of the DOM, all of the separated peak areas and total chromatogram areas, molecular masses, and their ratios, DOC and A250/A360, for all samples were included in the analysis. The Euclidean distance was used as a measure of the similarity–dissimilarity of the samples. The statistical analyses were carried out using WinSTAT for Excel software (R. Fitch Software, Germany).

3. Results and discussion

3.1 Main characteristics of the sediment profiles

The descriptions and dating values for analysed sediment cores are reported in Table 1. Both sediment cores covered roughly 150 years of sediment deposition. The characteristics of L. Peipsi sediment core solid-phase have been reported previously (Lepane, 2010b). Briefly, the organic matter content was 23 to 25% for the period 1860–1950 and increased up to 27% after the 1950s. This increase was followed by an increase in DOC values from the 1960s.

In the second case investigated, L. Rõuge Tõugjärv, the organic component of the particulate sediment matrix had low values (11-13%) between 1850 and the 1880s, followed by a distinct peak (18%) around the 1900s and a progressive rise to 27% thereafter (Alliksaar et al., 2005). The rest of the sediment consisted mainly of terrigenous mineral matter eroded into the lake from the catchment. In L. Rõuge Tõugjärv, the low organic matter values in sediment probably indicated land derived input from human-induced topsoil erosion and dilution of organic matter by addition of clastic mineral particles, while the increase in organic matter presumably reflected a lower contribution of terrestrial mineral material with a reduction in the overall rural activities. The DOC values corresponded to an increase in organic matter from 1850 to the 1890s; thereafter the decrease has been significant up to the present. The low DOC values may be due to a high amount of aliphatic organic compounds resulting from the microbial activity. Since the determination of DOC was based on the spectroscopic method, the aliphatic organic matter fraction was not determined in present study.

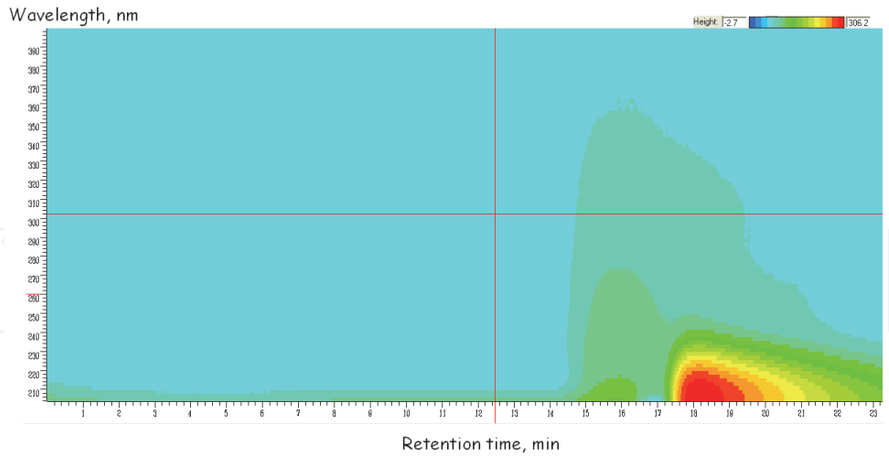
3.2 Multi-wavelength HPLC analyses

The sediment DOM was characterized by *pw* sample analysis using HPLC (HPSEC) with DAD. Absorbance spectroscopy with a single detection wavelength has been verified as a suitable detection method after HPLC separation (Lepane, 2010a). The UV absorbance at 250–280 nm has been widely used to provide an estimation of aromatic compounds (Filella, 2010). In the present study, multi-wavelength HPLC analysis has been carried out to detect changes in *pw*DOM composition and molecular mass profiles down the cores. The absorbance spectra were examined from 200 to 400 nm. Figures 2 and 3 display the HPLC chromatograms of *pw* from studied sediment cores and from different layers, respectively. The UV detector response range from 205 to 400 nm has been plotted against the retention times to obtain the multi-wavelength contour plots. Plot colours provide the visual representation of the relative absorbance intensity. The multi-wavelength plots allowed the most suitable detection wavelength for separated DOM components to be selected. Simultaneously, the chromatograms were registered at 280 nm. The chromatogram patterns suggested that the *pw*DOM molecules included two fractions in both lakes. The first peak, with shorter retention times of 6–7 min and of 8–9 min, corresponded to the

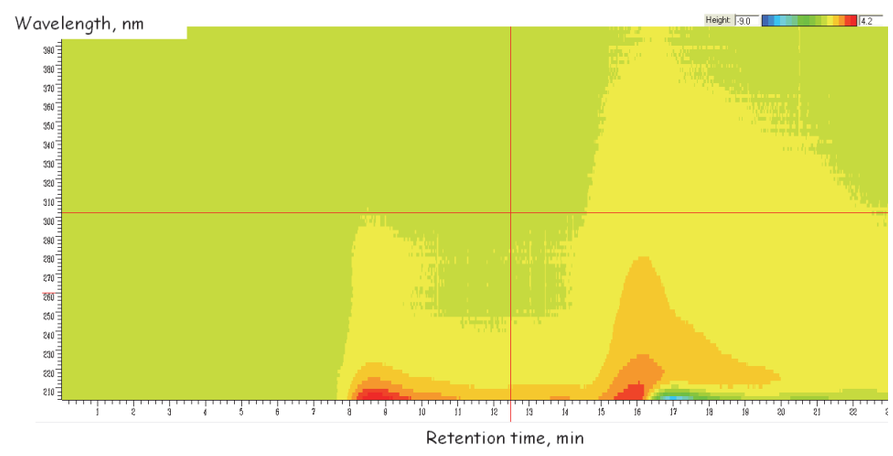
fraction with larger molecules and was operationally named the high molecular mass (HMW) fraction. The second peak, whose maximum was at retention times of 11–12 min and of 15–16 min, was assigned to smaller DOM molecules and named the humic substances (HS) fraction.

Lake		**Peipsi**			**Rõuge Tõugjärv**		
Period	Sample no	Depth, cm	Age dating, y	Period	Sample no	Depth, cm	Age dating,y
I	1	0/-1	2006	**I**	1	0/-1	2006
	2	-1/-2	2005		2	-1/-2	2005
	3	-2/-3	2004		3	-2/-3	2005
	4	-3/-4	2003		4	-3/-4	2004
	5	-4/-5	2002		5	-4/-5	2002
	6	-5/-6	2000		6	-5/-6	2000
	7	-6/-7	1997		7	-6/-7	1998
	8	-7/-8	1994	**II**	8	-7/-8	1996
	9	-8/-9	1990		9	-8/-9	1994
	10	-9/-10	1986		10	-9/-10	1991
	11	-10/-11	1982		11	-10/-11	1989
	12	-11/-12	1978		12	-11/-12	1986
	13	-12/-13	1974		13	-12/-13	1984
II	14	-13/-14	1970		14	-13/-14	1981
	15	-14/-15	1966		15	-14/-15	1979
	16	-15/-16	1962		16	-15/-16	1976
	17	-16/-17	1958		17	-16/-17	1974
	18	-17/-18	1954	**III**	18	-17/-18	1971
	19	-18/-19	1950		19	-18/-19	1968
	20	-19/-20	1945		20	-19/-20	1964
	21	-20/-21	1940		21	-20/-21	1961
	22	-21/-22	1934		22	-21/-22	1958
	23	-22/-23	1928		23	-22/-23	1954
	24	-23/-24	1922		24	-23/-24	1951
	25	-24/-25	1916		25	-24/-25	1948
	26	-25/-26	1911		26	-25/-26	1944
	27	-26/-27	1907		27	-26/-27	1941
	28	-27/-28	1904		28	-27/-28	1938
	29	-28/-29	1901		29	-28/-29	1934
	30	-29/-30	1897		30	-29/-30	1931
	31	-30/-31	1892		31	-30/-31	1928
	32	-31/-32	1887		32	-31/-32	1924
	33	-32/-33	1882		33	-32/-33	1921
III	34	-33/-34	1880		34	-33/-34	1917
	35	-34/-35	1877		35	-34/-35	1914
	36	-35/-36	1875		36	-35/-36	1911
	37	-36/-37	1872	**IV**	37	-36/-37	1905
	38	-37/-38	1870		38	-37/-38	1899
	39	-38/-39	1867		39	-38/-39	1893
	40	-39/-40	1865		40	-39/-40	1887
	41	-40/-41	1862		41	-40/-41	1881
	42	-41/-42	1857		42	-41/-42	1875
	43	-42/-43	1852		43	-42/-43	1869
					44	-43/-44	1864
					45	-44/-45	1858

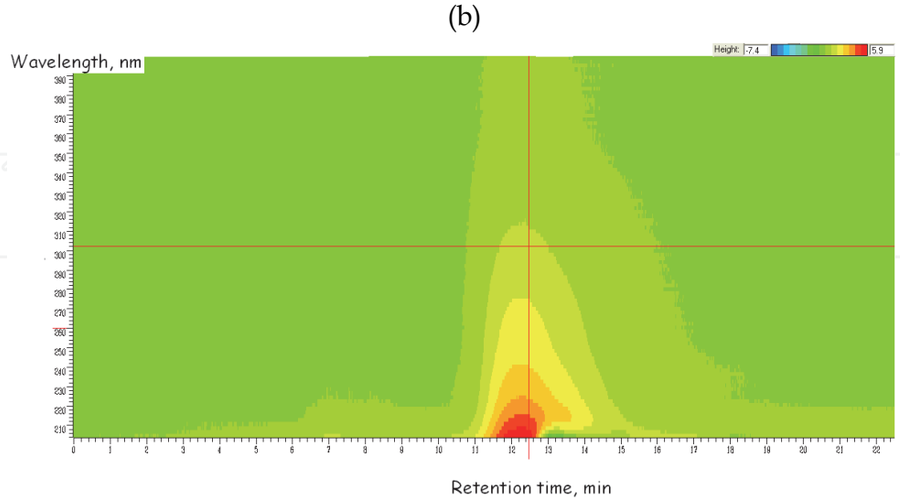
Table 1. Sediment samples numbering and depths down the profiles with dating values for Lake Peipsi and Lake Rõuge Tõugjärv.



(a)



(b)



(c)

Fig. 2. Multi-wavelength HPLC chromatograms of porewater samples from Lake Peipsi sediment core at different depths: (a) 4 cm, (b) 37 cm, (c) 41 cm.

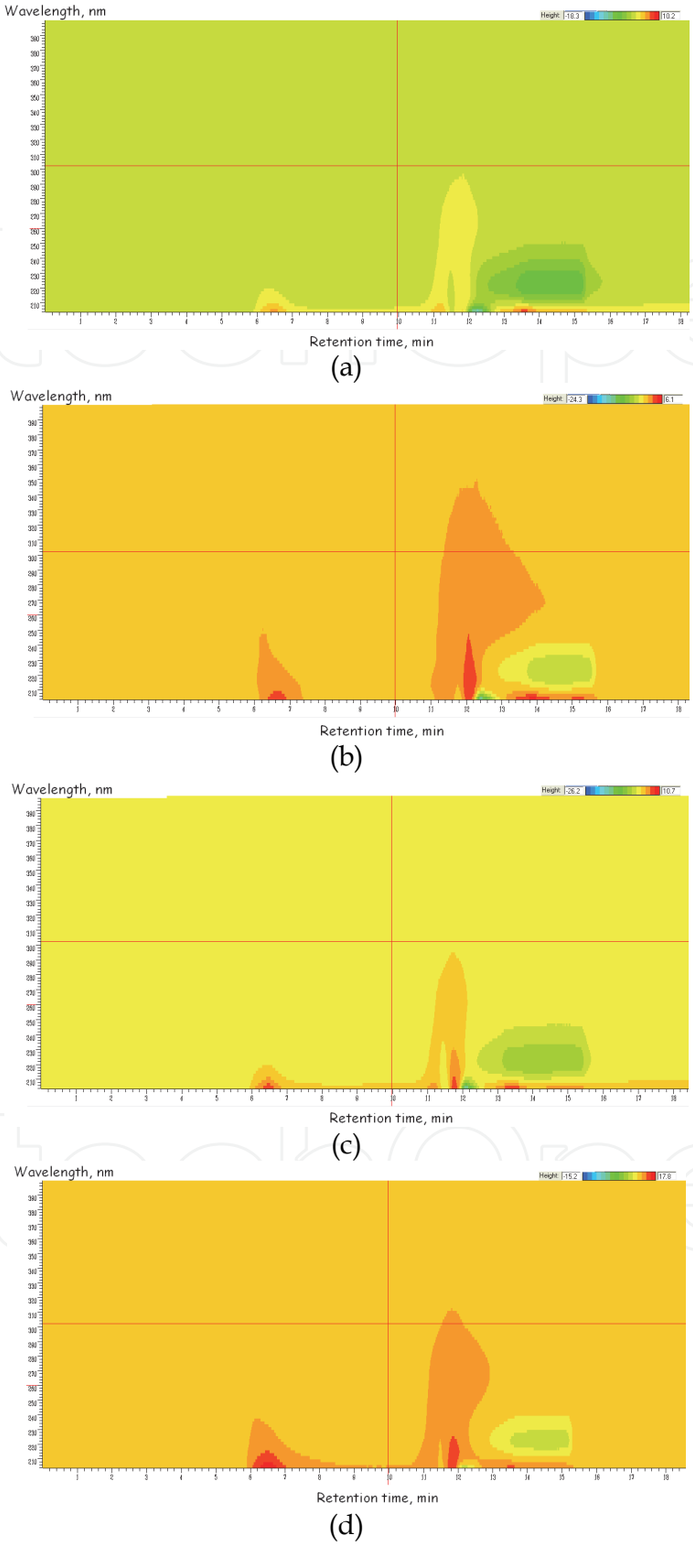


Fig. 3. Multi-wavelength HPLC chromatograms of porewater samples from Lake Rõuge Tõugjärv sediment core at different depths: (a) 6 cm, (b) 13 cm, (c) 27 cm, (d) 42 cm.

All chromatograms of L. Peipsi sediment *pw*s were very similar, consisting of two main peaks representing HMW and HS fractions. The intensities and positions of the peaks (*i.e.* fractions) changed in different sediment layers reflecting age-related changes in the concentrations and transformation of organic constituents. The area of the HMW fraction was always smaller (~3% of the total area) than the second HS fraction (~97% of the total area). The calculated molecular masses for the HMW fraction varied between 200 and 270 kDa. The HMW fraction was absent from samples dating from the 1990s and from older samples from the nineteenth century. The HS fraction, with molecular masses between 700 and 3,700 Da, was dominant. The calculated average M_w was 1,500 Da, which is characteristic for aquatic humic and fulvic acids. The profiles of the determined chemical characteristics and HPLC variables are presented in Fig. 4.

The L. Rõuge Tõugjärv *pw*DOM was also separated into two peaks. The components of the second peak eluted as a broad distribution and sometimes with a partially resolved sub-shoulder. Possibly, the composition of the *pw*DOM from those sediment layers where the sub-shoulder appeared (some layers from the 1980s and 1960s) might have been somehow different from the major DOM composition. According to DAD spectra, components eluted with the first peak contained proteinaceous material, while the second peak spectra were characteristic of HS. The retention times of both peaks remained stable down the core. The calculated molecular masses for the HMW fraction varied between 800 and 1,000 kDa (M_w). The HMW fraction varied between 6 to 13% of the total peak area and was thus present in a significantly higher amount than in L. Peipsi sediment *pw*s. Possibly, HMW material might have been formed from some proteins encapsulated into HS aggregates or micelles. The ability of HS to aggregate into large supramolecules has been reported previously (Havel & Fetsch, 2007; Piccolo, 2001). Generally, average M_w values of the analysed L. Rõuge Tõugjärv sediment *pw* HSs slightly exceeded 1,000 Da, and M_n was close to 400 Da. Molecular mass values of HS were in good agreement with molecular mass distributions reported for aquatic fulvic acids (Klavinš, 1997; Lepane et al., 2004).

The depth profiles for both lake cores (Fig. 4) indicated corresponding changes in M_w and M_n values. The molecular mass values for HS from L. Peipsi were slightly higher: 1,500 Da *vs.* 1,000 Da. The high fluctuations in HS molecular masses during 1870–1930s were not detected for L. Rõuge Tõugjärv. The down-core profiles of the chromatogram total peak areas and HS fraction areas were similar and exactly followed the changes in DOC.

The M_w/M_n ratio, or polydispersity, which is a measure of the homogeneity of organic matter, was mostly stable down the core, varying from 2.3 to 3.5 for L. Rõuge Tõugjärv and from 1.9 to 3.0 for L. Peipsi. This indicated the relatively homogeneous HS fraction in both lakes studied. The results showed that the molecular mass distribution and the polydispersity of DOM from L. Peipsi and L. Rõuge Tõugjärv were quite similar to those of sediment *pw*DOM from other lakes from Estonia and other regions studied by HPSEC (Fu et al., 2006; Leeben et al., 2008a; Lepane et al., 2004, 2010a; Makarõtševa et al., 2010; O'Loughlin & Chin, 2004).

The absorbance ratio of DOM at wavelengths of 250 and 360 nm (A250/A360) indicates the source of organic matter in the sediments (Peuravuori and Pihlaja, 1997). A higher ratio is related to autochthonous organic matter, which is produced within the lake, and the substances of smaller size and lower aromaticity are present in DOM molecules. Lower values of absorbance ratio reflect a higher aromaticity with an extent of allochthonous organic matter that originates outside the lake and is carried into the lake by inflows (McKnight et al., 2001). The ratio for L. Peipsi core samples was constant until the 1960s, with an average value close to 4.0. Thereafter, up to the present, it increased to 6.5, meaning that the origin of the organic matter changed to autochthonous. Constant values were also obtained for the L. Rõuge

Tõugjärv core until the 1940s, indicating higher degree of allochthonous organic matter in the lake. In the mid-twentieth century the ratio slightly increased, which coincided with the period when the lake sediments received decreased proportions of allochthonous organic compounds due to the decline in rural land-use practices and decreased sub-soil erosion. However, since 1980s there was a sharp increase in the absorbance ratio of the DOM up to 8, which also indicated the dominance of a more aliphatic autochthonous organic matter.

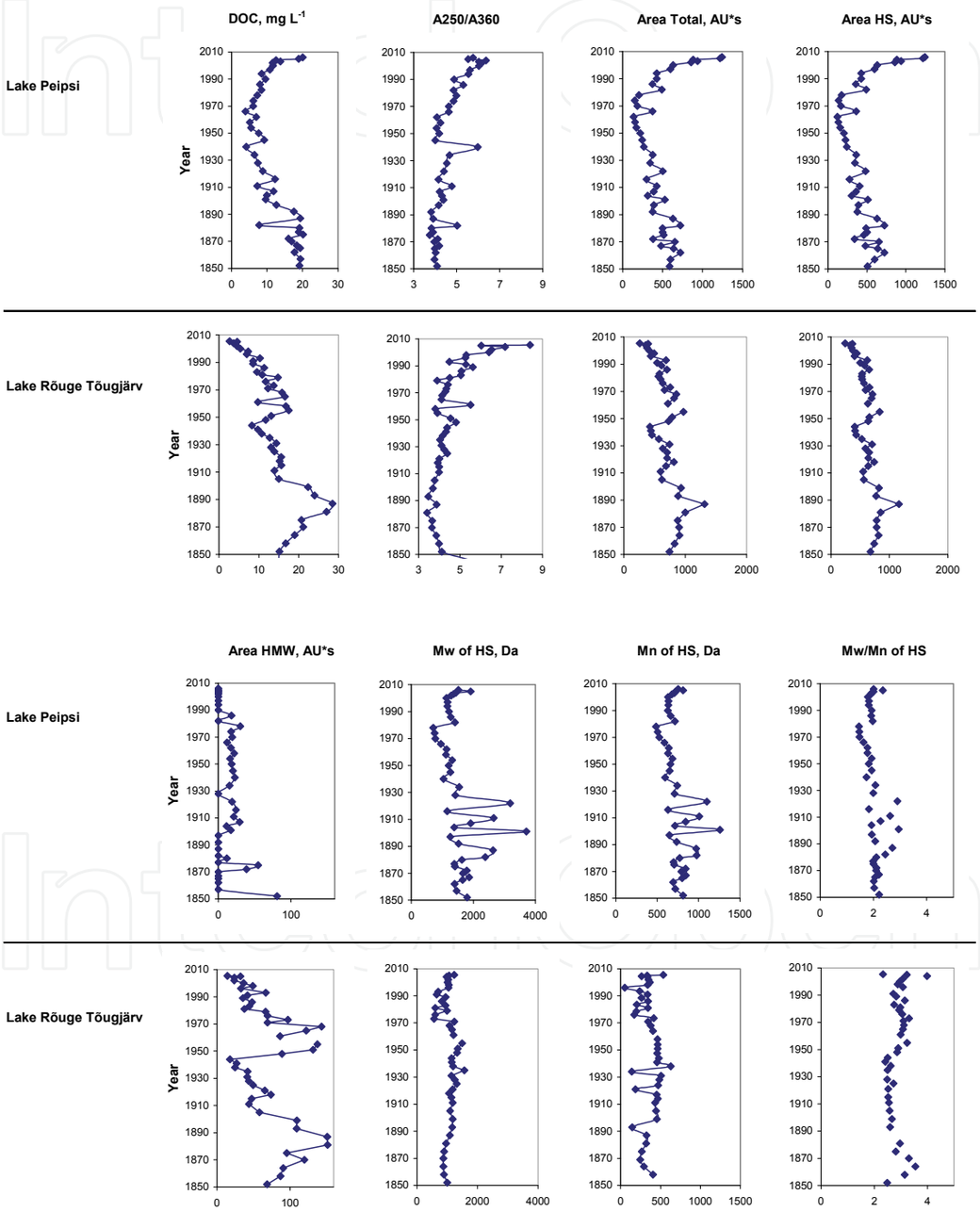


Fig. 4. Profiles of general chemical characteristics and variables by HPLC of Lake Peipsi and Lake Rõuge Tõugjärv sediment porewaters. The year denotes the year of sediment deposition.

3.3 Age-related changes in DOM characteristics of sediment cores

The statistical analysis of data was performed to reveal periods in the characteristics of separated DOM fractions. Based on DOC and absorbance ratio data, the L. Peipsi sediment core was divided into three age/depth periods: (I) 0–13 cm of sediment core depth, dated to 2006–1974; (II) 14–33 cm core depth, dated to 1970–1882; and (III) 34–43 cm core depth, dated to 1880–1852. L. Rõuge Tõugjärv sediment core was operationally separated into four age/depth periods: (I) 0–7 cm, dated to 2006–1998; (II) 8–17 cm, dated to 1996–1974; (III) 18–36 cm, dated to 1971–1911; and (IV) 37–45 cm, dated to 1905–1858 (Table 1). The mean values of the analysed variables divided into three or four periods with 95% confidence limits are shown in Figs. 5 and 6.

3.3.1 Lake Peipsi

The HMW fraction data (peak area, molecular masses, and polydispersity) were statistically similar down the core, as was the HS fraction polydispersity, and therefore did not allow the differentiation of sediment layers (Fig. 5). The DOC, total chromatogram peak area, and HS peak area changed similarly, thus proving the suitability of peak areas as semi-quantitative characteristics of DOM. The 1880–1852 dated samples had elevated DOC values. The upper 0–13 cm sediment DOM had statistically relevant differences in comparison to period III as revealed by DOC and HS molecular masses. The recent DOM accumulating into sediments has lower molecular masses and the highest absorbance ratio in comparison with preceding sediment layers. The obtained results indicate that recent *pw*DOM in L. Peipsi is more aliphatic and contains lower average molecular mass organic compounds which are likely of autochthonous origin. This might be the result of the microbial degradation of labile organic matter constituents such as carbohydrates (Zaccone et al., 2009). The absorbance ratio in L. Peipsi *pw*s shows significant differences throughout the sediment profile and can thus serve as an excellent variable for revealing the changes in sediment core.

3.3.2 Lake Rõuge Tõugjärv

As in the first lake sediment core studied, the polydispersity of HMW and HS fractions did not show any particular trend along the L. Rõuge Tõugjärv core profile (Fig. 6). The DOC, total chromatogram peak area, and HMW and HS fraction peak areas changed similarly. The obtained results indicated a general increase in all those variables with depth. However, it was not possible to differentiate between periods II and III (*i.e.* corresponding to years 1996–1911) by using DOC and semi-quantitative chromatographic data. Also, in the case of this lake the highest *pw*DOC was registered in the deepest layer 37–45 cm. The molecular masses of both HMW and HS of this undisturbed sediment core show different trends down the profile in comparison with L. Peipsi core. Similarities were found between the most recent and the oldest layers (dated to 2006–1998 and 1905–1858, respectively) and differences were found between the intermediate ones (periods II and III, dated to 1996–1974 and 1971–1911, respectively). Thus, the upper sediment layer (0–7 cm) variables indicate decreased DOM input with the characteristic high molecular mass compounds. The molecular mass data variations may reflect the influence of the watershed but also the seasonal climatic factors, like in-lake primary production. The observed distinct increase in absorbance ratio that was synchronous with a decrease in the DOC content possibly indicates the enhanced algal productivity and eutrophication of the lake, but also the lower contribution of allochthonous organic matter into the lake.

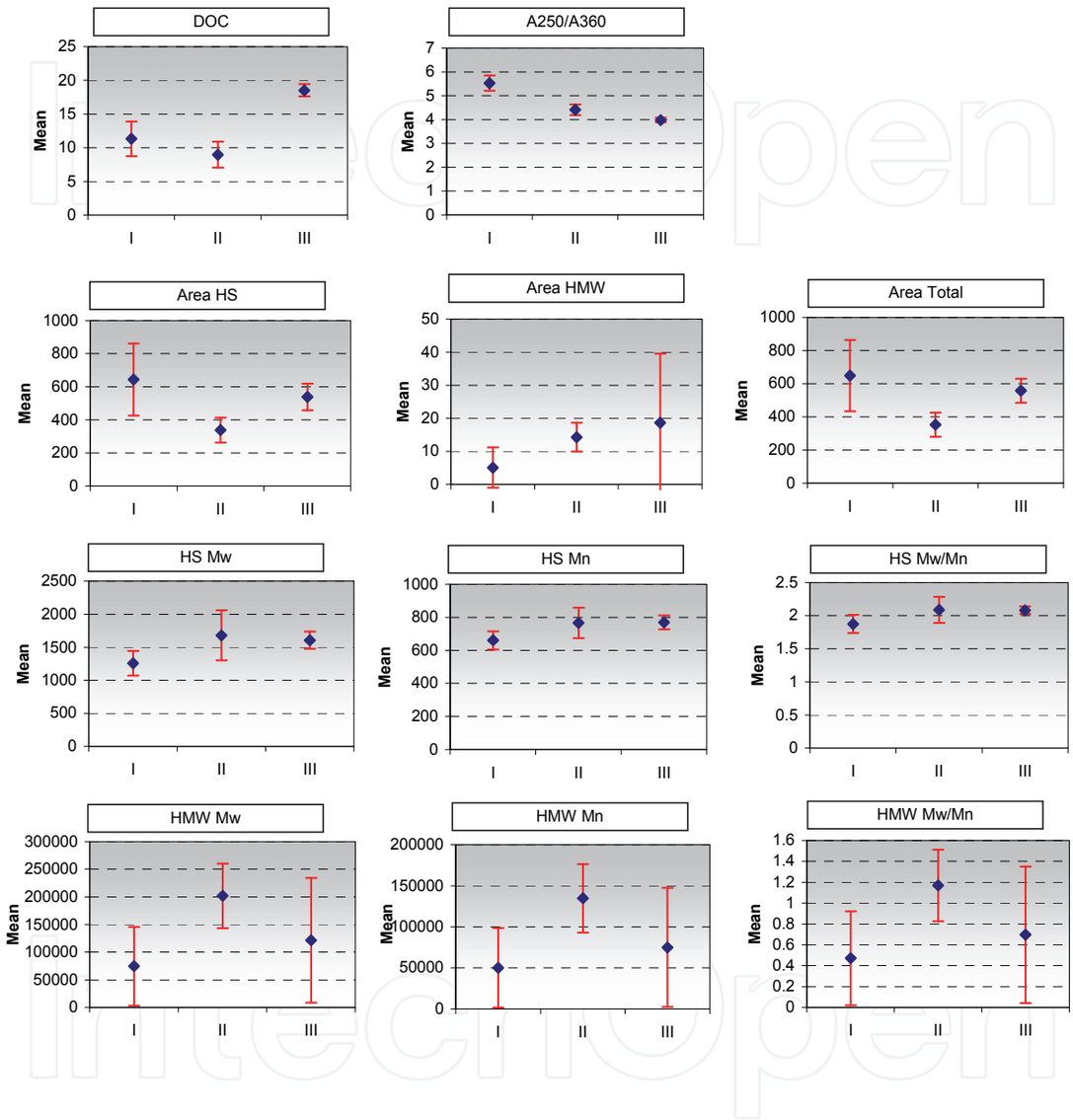


Fig. 5. Plots describing mean values of Lake Peipsi DOM semi-quantitative (areas), molecular, and spectroscopic characteristics arranged into three age/depth periods (see text). Red bars indicate confidence limits at the 95% level. DOC, mg L^{-1}; A250/A360: absorbance ratio at respective wavelengths; Mw/Mn: polydispersity; Mw and Mn: weight – and number-average molecular masses, respectively, Da; Area Total: total chromatogram peak area; Area HMW and Area HS: HMW and HS fraction peak areas, respectively, mAU*s.

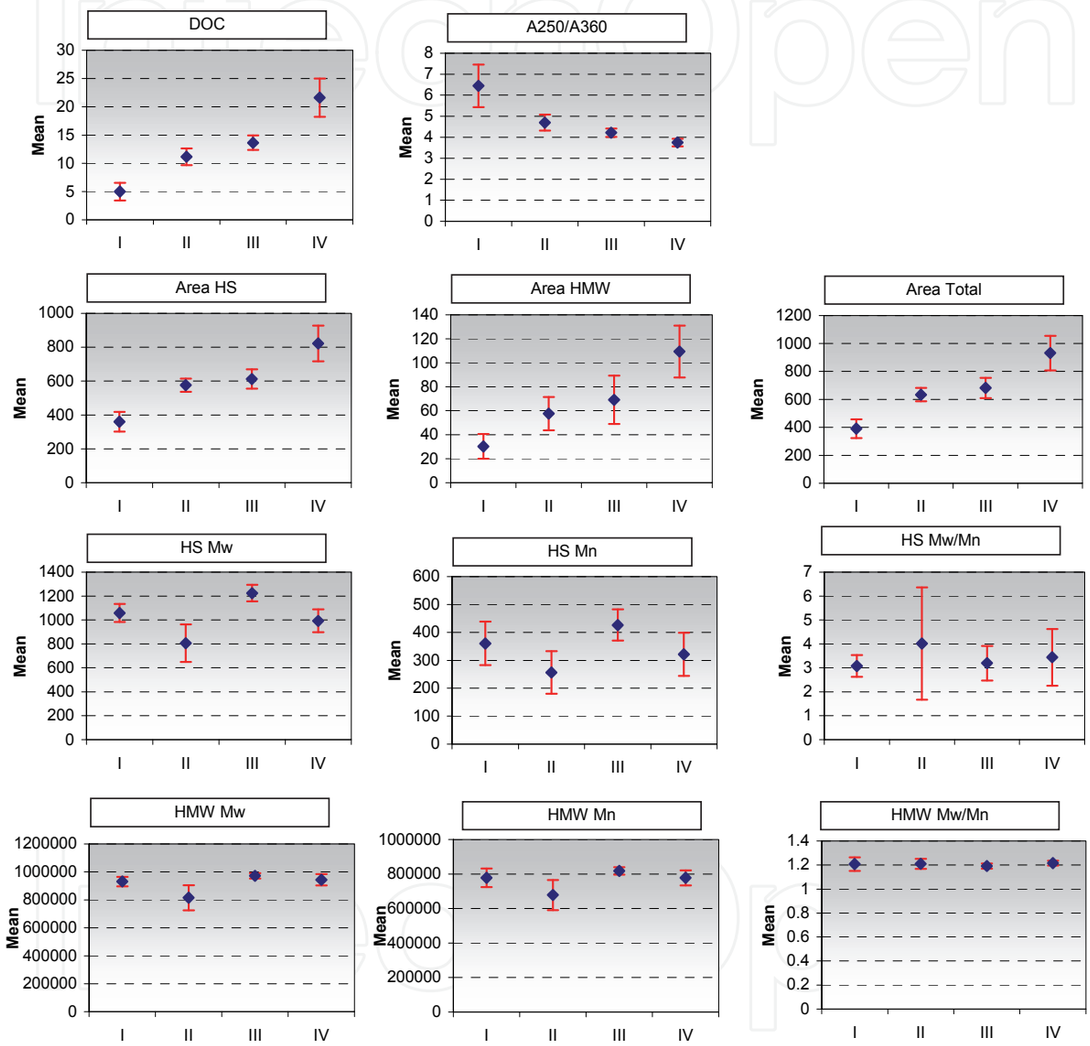


Fig. 6. Plots describing mean values of Lake Rõuge Tõugjärv DOM semi-quantitative (areas), molecular, and spectroscopic characteristics arranged into four age/depth periods (see text). For abbreviations see Fig. 5 legend.

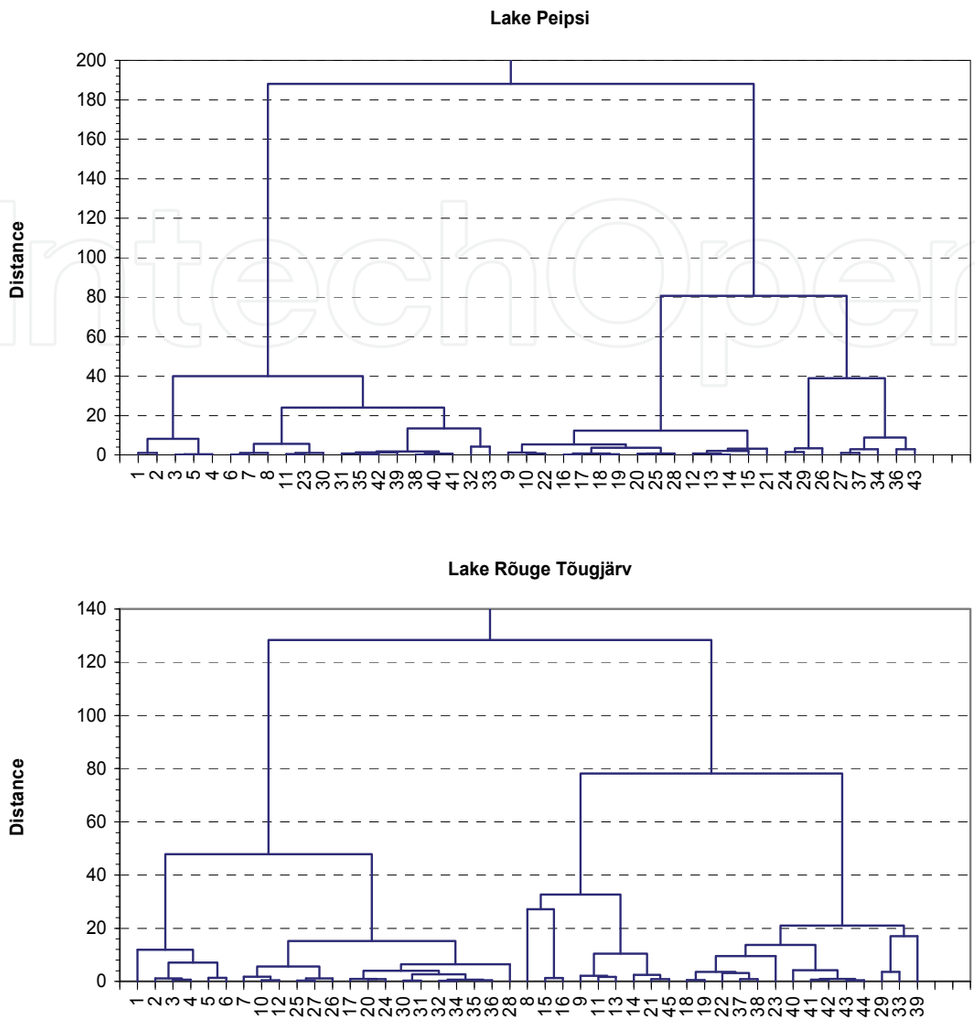


Fig. 7. Cluster analysis of Lake Peipsi and Lake Rõuge Tõugjärv sediment core samples from different depths (numbers indicate sediment depth in centimetres).

3.4 Tracking environmental change in organic compounds records

During the second half of the nineteenth and early twentieth centuries, L. Peipsi had a stable ecosystem similar to natural reference conditions as indicated by low autochthonous productivity. During the second half of the twentieth century, the ecological conditions of L. Peipsi worsened constantly. In the 1960s the lake was classified as mesotrophic. Eutrophication is the major environmental issue in the L. Peipsi basin due to the nutrient load to the lake. The main source of nutrient pollution of L. Peipsi is agriculture and municipal wastewaters. The decline in agriculture during the 1990s caused pollution to decrease and the quality of waters to improve. The lake area has been in a period of transition for more than decade.

Cluster analysis of *pw*DOM data was performed to reveal periods with similar characteristics in the studied L. Peipsi and L. Rõuge Tõugjärv sediment cores. The

dendrograms are shown in Fig. 7. The aim was to identify the subgroups within the HPLC dataset and relate them to environmental changes. The L. Peipsi data allowed the layers to be grouped into two major groups. According to the results, the DOM from 2006–1994 formed a homogeneous subgroup that was in the same cluster as samples from 1982, 1928, and 1897–1857. The second major group was also divided into two subgroups. The first included sediment layers from years 1880–1872, 1911–1901, 1922 and 1940. The second subgroup covered mainly the sediment layers from 1990–1934, excluding the 1982 layer. Palaeolimnological studies state that anthropogenic impact on the lake has increased since the 1950s. Until that time, the lake was considered mesotrophic (Leeben et al., 2008b). Biomanipulation of the lake was carried out in 1993–1994 and was reported to have improved the lake ecosystem. This event can be seen in HPLC data considering the grouping results of organic compounds. Sedimentary pigment analysis indicated and thus confirmed the eutrophication of the lake since the 1980s (Leeben et al., 2008a).

L. Rõuge Tõugjärv experienced anthropogenic catchment disturbances up to the beginning of twentieth century, as indicated by extensive farming and increased drainage. During the first part of the twentieth century the development of efficient agricultural practices and reforestation improved the water quality. During the second part of the twentieth century the cultivated area declined and reforestation continued but the widespread use of mineral fertilizers caused an increase in primary production. After old agricultural practices stopped in the 1990s the lake was recovered and is reported to be mesotrophic today. The anthropogenic activities can be tracked by sediment investigations. L. Rõuge Tõugjärv sediments were annually laminated and thus possessed records with calendar year chronology. Thus, changes in this lake ecosystem and climate could be resolved seasonally. The L. Rõuge Tõugjärv HPLC organic matter data enabled the sediment layers to be classed into two major homologous groups. The recent sediment layers (2006–1998) formed a separate subgroup and were included in the same cluster as samples from 1951–1911 and some separate layers from 1991, 1986, 1974, and 1964. The second major group was also divided into two subgroups: the first one was similar to period II (1996–1976) and the second was similar to period IV (1905–1864), together with some separate layers from years 1971–1968, 1958–1954, 1934, 1921. The organic matter characteristics from period IV samples may reflect long-term agricultural impact because the lake has been mediated by human activity over hundreds of years (Heinsalu & Alliksaar, 2009). The massive utilization of fertilizers led to increased primary production in the 1960s–1980s (Alliksaar et al., 2005). Thus, one of the major clusters might reflect the eutrophication of L. Rõuge Tõugjärv. Since the 1990s the lake has been classified as mesotrophic with a decrease in diatoms and very good water quality. Historically, the same is reported for the time period 1920–1940. The above-described periods correlate well with the major cluster that included the most recent organic matter data together with data from the first part of the twentieth century.

The obtained results for both lakes show quite good agreement with some common eutrophication indicators (diatoms, fossil pigments) and thus confirm the suitability of organic compounds data for the assessment of the ecological state of the water bodies.

4. Conclusion

The results presented in the present study allowed the changes in the sediment porewater organic compounds to be assessed and related to the environmental conditions of the studied lakes. The applied HPLC method with multi-wavelength detection did not alter the

nature of DOM. It was useful to reveal changes in *pw*DOM and molecular mass profiles, enabling the separation of organic high molecular mass and humic substances fractions in sediment cores of both lakes. Additionally, the qualitative analysis of DOM components based on UV-spectra can provide insights into their sources. The statistical analyses confirmed that porewater organic component variables obtained by HPLC could be used to differentiate between sediment layers and to track environmental changes.

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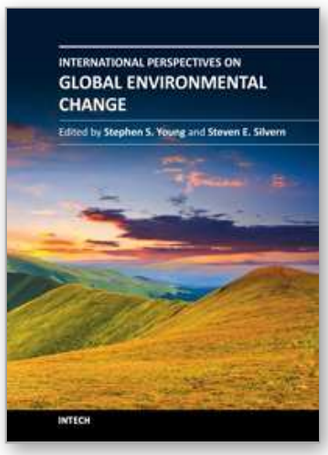

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