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Analysis of Organic Species in Sediments and Soil by High Performance Separation Methods

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Declaration: Hereby I declare that this doctoral thesis is my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology, has not been submitted for any degree.

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NATALJA MAKARÕTŠEVA

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LIST OF PUBLICATIONS

This thesis is based on the following publications which are referred to by Roman numerals within the text:

I. A. Leeben, I. Tõnno, R. Freiberg, V. Lepane, N. Bonningues, N. Makarõtševa, A. Heinsalu, T. Alliksaar, History of anthropogenically mediated eutrophication of Lake Peipsi as revealed by the stratigraphy of fossil pigments and molecular size fractions of pore-water dissolved organic matter. *Hydrobiologia*, 599, 2008, 49-58.

II. N. Makarõtševa, V. Lepane, T. Alliksaar, A. Heinsalu, A 10,000 year record of sediment pore-water dissolved organic matter characteristics from Lake Peipsi as revealed by HPSEC. *Chem. Ecol*., 26, 4, 2010, 13-24.

III. N. Makarõtševa, A. Seiman, M. Vaher, M. Kaljurand, Analysis of the degradation products of chemical warfare agents using a portable capillary electrophoresis instrument with various sample injection devices. *Procedia Chem*., 2, 2010, 20-25.

IV. A. Seiman, N. Makarõtševa, M. Vaher, M. Kaljurand, The detection of nerve agent degradation products in different soil fractions using capillary electrophoresis with contactless conductivity detection. *Chem. Ecol*., 26, 2010, 145-155.

THE AUTHOR'S CONTRIBUTION TO THE PUBLICATIONS

I. The author prepared the samples, optimized and carried out experiments concerning HPSEC analysis. She discussed the results and participated in the preparation of the manuscript in collaboration with other co-authors.

II. The author planned, optimized and carried out all experiments. She interpreted the resuts and wrote the manuscript.

III. The author participated in method development, prepared the samples and carried out CE measurements. She interpreted the results and wrote the manuscript.

IV. The author participated in the preparation and CE analysis of samples. She discussed the results and participated in writing the manuscript.

ABBREVIATIONS

AEDHP	2-aminoethyl dihydrogenphosphate
BGE	background electrolyte
BPA	1-butylphosphonic acid
CCD	contactless conductivity detector
CE-CCD	contactless-conductivity detection capillary electrophoresis
CE	capillary electrophoresis
CWA	chemical warfare agent
DAD	diode-array detector
DOC	dissolved organic carbon
DOM	dissolved organic matter
EMPA	ethyl methylphosphonic acid
EPA	ethylphosphonic acid
GC	gas chromatography
HPSEC	high-performance size exclusion chromatography
HS	humic substances
IMPA	isopropyl methylphosphonic acid
LC	liquid chromatography
LOD	limit of detection
LOI	loss on ignition
M_n	number-average molecular weight
MPA	methylphosphonic acid
MS	mass spectrometry
MW	molecular weight
M_w	weight-average molecular weight
M_w/M_n	polydispersity
NOM	natural organic matter
OM	organic matter
PA	phosphonic acid
PMPA	pinacolyl methylphosphonic acid
PPA	propylphosphonic acid
RSD	relative standard deviation
SEC	size exclusion chromatography
SPE	solid-phase extraction

INTRODUCTION

Concerns about the global environment has induced intensive research into ways of its functioning. One of the most important areas of research deals with biogeochemical processes and the fate of various organic substances in the environment. This is a matter of great interest in the investigation of processes involving environmental pollution. Without this knowledge it is impossible to comprehend and predict the distribution, behavior, and the relationships of organic species in different environments (water, soil, sediments, air). Analysis of organic materials in environmental samples is a problematic task due to the complex nature of matrices and their possible interactions with analytes. Matrix properties play an important role in the fate of organic molecules, including pollutants. A fundamental parameter of all natural substances is their size as the latter defines a substance's physical and chemical properties. The physical and chemical properties of natural molecules or particles define and predict their behavior and interactions under various conditions. For example, in the case of natural organic matter (NOM), its bioreactivity and adsorption ability, as well as interactions with metals and environmental pollutants, are strongly related to size. Therefore, the determination of the molecular weight (MW) of NOM has aroused special interest among environmental researchers. Moreover, NOM is an integral and predominant part of almost all environmental matrices.

To obtain adequate information about MWs of NOM, it is very important to have the state of analytes and analysis conditions both being close to environmental conditions. In view of this, minimal sample pretreatment and non-destructive analysis are appreciated. Enormous research has been done on the properties, behavior and fate of different organic species in complex environments. However, knowledge of environmental processes themselves is yet insufficient and vast areas of pertinent investigations have remained uncovered.

The properties of sediments and soils play an important role in the fate of different pollutants. When investigating the behavior of hazardous species in different matrices, properties of the latter should be taken into account. Therefore, these, as well as interactions of matrices with pollutants need to be studied.

The present study aims at filling in gaps in our understanding of the distribution and fate of organic species in complex sediment and soil environments by applying modern analytical methods which require very small sample volumes and provide a wide range of information.

Analysis of dissolved organic matter in lacustrine sediments

Lacustrine sediments are usually rich in organic matter (OM). The origin, quantity and structure of OM depend on the development of a lake's ecosystem,

changes in climatic conditions, and human impact. Pore water dissolved organic matter (DOM), which is in equilibrium with the particulate phase, participates actively in biogeochemical processes, plays an important role in the carbon cycle, and affects the availability of nutrients and contaminants. Sediment investigations are widely carried out in the framework of (paleo)limnological research, while pore water studies are fewer. Pore water DOM could be used as a geochemical determinant to reveal changes in the aquatic system. DOM is tightly linked to the lake catchment site of and vegetation development in a lake. The size (and thus, MW) of DOM reflects its origin and its quantity provides information about climatic conditions and other factors. In the long term, pore water DOM might provide valuable data about temporal changes in a lake ecosystem, its development, and eutrophication. Moreover, DOM plays a significantl role in the environment due to its ability to interact with different compounds (metals, minerals, environmental pollutants, etc.). This ability also depends on the MW of DOM. However, studies of lacustrine pore water DOM are infrequent, especially that from deeper and older sediment layers. Most investigations in this field have dealt with the pore water DOM taken from upper sediment layers, usually at a depth of several dozens of centimeters. To understand the biogeochemistry of lacustrine DOM, as well as the various impacts affecting the accumulation of sediments, samples should also be taken from much deeper layers (at a depth of several meters, for instance).

DOM studies are often focused on the characterization of its properties and determination of MW. Various analytical approaches have been applied to the characterization of pore water DOM. However, high-performance size exclusion chromatography (HPSEC) is the one most widely used. The HPSEC analysis may provide information on the MW distribution and compositional changes of lacustrine DOM downward through the sediment profile. Thus, it may be useful for tracking changes related to environmental conditions or eutrophication of a lake. The direct HPSEC analysis of pore water DOM is easy and requires very low sample volumes (μL). The separation of pore water from the particulate phase is not as time-consuming as OM extraction from sediments. Moreover, sample pretreatment prior to analysis is non-destructive and disturbance of analytes minimal.

Analysis of phosphonic acids adsorption onto soil

The fate of different contaminants and other hazardous substances in soil is a subject of continuous research. Among the most toxic compounds ever synthesized are organophosphorus nerve agents. Nerve agents quickly hydrolyze to produce less toxic phosphonic acids (PAs). Analysis of PAs in complex soil matrices is complicated due to the interactions of the acids with matrix components. For the analysis of PAs in soil, the analytes must be extracted from the matrix. However, PAs recoveries from soil by water extraction have been very low. This has been attributed to the adsorption of PAs onto soil particles.

These mechanisms are yet unclear. No data on the adsorption mechanism of PAs onto soil has been published in the literature.

Capillary electrophoresis (CE) with contactless conductivity detection (CCD) is a suitable method for the separation and analysis of PAs. CE is a well-established and widely-used technique for the analysis of small charged molecules. The advantages of CE include instrumentation simplicity, ability to separate high number of analytes in a short time, minimal sample volumes (μL) and simple sample preparation such as dilution and filtration. The latter feature is very important because PAs may be analysed directly without any derivatization like in the case of gas chromatographic analysis, which is also widely used for PAs determination. The CCD detection of PAs is preferrable as those acids do not contain chromophores and they can not be detected with a UV detector. The CE-CCD instrumentation can be easily miniaturized. Miniaturized portable instruments can be taken outside the laboratory and the determination of contaminants can be quickly and easily performed in the field.

AIMS OF THE STUDY

The main goal of the present study was to analyse organic species present in complex environmental matrices by using HPSEC and CE-CCD. Properties of matrices play an important role in environmental processes, including pollution. Therefore, investigation of matrix properties arouses environmental researchers' interest. In the present work, special emphasis was placed on the study of the characteristics and properties of sediments and soils which are determined by the size of natural matter constituents. Specific aims of the research were:

- to characterize the molecular composition and size of dissolved organic matter in sedimentary pore water by HPSEC
    - to chromatographically and spectroscopically characterize the pore water DOM from two well-dated sediment cores from one of the largest lakes in Europe, Lake Peipsi, Estonia;
    - to study the effect of storage conditions of sediments on the characteristics and molecular weight (size) of DOM. For this purpose the first sediment core was frozen after sampling, while the second sediment core was stored fresh at low temperature;
    - to construct age-resolved profiles of DOM characteristics as a function of sediment depth covering 120 and 10,000 years of sediment accumulation in Lake Peipsi. For Estonia, this lake is an important economic resource which has been extensively exposed to human activity for a long time. Therefore, the first sediment core (120 years of accumulation) should reflect the period of anthropogenic impacts on the lake ecosystem. The second sediment core (10,000 years of accumulation) should reflect the period of natural baseline conditions affected by the post-glacial lake system development and climatic conditions. This is the first investigation of the pore water from Lake Peipsi and the first study ever of pore waters obtained from sediments covering an accumulation period of over 10,000 years;
    - to evaluate the applicability of the HPSEC method combined with spectroscopic approach to limnological and palaeolimnological research. For this purpose profiles of the pore water DOM characteristics were studied to determine possible environmental and conditional changes in the lake ecosystem due to human impact, eutrophication, or climatic conditions. The results were compared with the data obtained by traditional palaeolimnological methods published in the literature.

- to study interactions of contaminants with different soil types. A particular aim was to determine the phosphonic acids concentration in soil samples by using a portable CE-CCD:

- to work out a universal and simple procedure for an on-site extraction and analysis of PAs in soil samples and demonstrate the possibility of using the portable CE-CCD instrument for the rapid determination of PAs in the field;
- to investigate the adsorption of PAs onto sand and loamy soil in respect to the size of matrix particles.

1 LITERATURE OVERVIEW

1.1 HPSEC analysis of dissolved organic matter

1.1.1 Dissolved organic matter from lacustrine sediments

Lacustrine sediments accumulate organic matter (OM) which originates from the detritus of plants, as well as from the tissues of dead organisms formerly having lived in the lake and its watershed. Sediments of mesotrophic to eutrophic temperate lakes generally contain more than 20% OM called gyttja[1]. The OM buried on the bottom of the lake consists of an extremely heterogeneous and complex mixture of humic substances (HS), proteins, carbohydrates, lipids, amino acids, and other biomolecules. According to origin there are two types of organic material. Autochthonous OM is formed within the aquatic system from organisms and plants which have lived in the lake. Allochthonous OM is mostly land-derived and originates outside the lake. It may come to the lake from its catchment via rivers, streams, and land runoff. Allochthonous OM contains mostly humic, high-molecular weight components, whereas autochthonous OM usually consists of low-molecular-weight molecules, such as amino acids or carbohydrates.

OM is a dynamic biogeochemical fraction of sediments. During deposition and accumulation in the lake it undergoes processes which lead to alterations in its original structure and character. On sinking from their origin to the lake bottom organic materials undergo degradation via oxidation in the water column. Reaching the bottom, OM is still subject to oxidation and destruction. Resuspension of sediments and bioturbation prolong exposure to oxidative conditions. When organic material is below the zone of bioturbation, it undergoes alterations caused by anaerobic bacteria. Generally, autochthonous OM is more sensitive to bacterial degradation than allochthonous organic material. Less reactive components, such as HS and lipids, become more dominant in the sedimentary OM while more reactive forms (amino acids, carbohydrates) are processed by the lake biota. During reprocessing, components utilized and synthesized by microorganisms are added to the OM mixture. As a result of multiple processes, the composition of OM in sediments may significantly differ from that of original organic residues produced in the lake and its catchment[2].

OM is distributed between the particulate and dissolved phases. This distribution is assumed to be in equilibrium. The dissolved organic matter (DOM) is a unique fraction of sedimentary pore water. The term “dissolved organic matter” is operational and its definition is mostly connected with the physical size of molecules. According to Zsolnay[3], DOM consists of molecules which pass through a 0.45 μm filter. This term defines DOM as molecules entirely dissolved in water and colloidal OM[4,5].

Analysis of pore water DOM has to be faced with several problems. First of all, oxygen exposure to sediments during sampling and storage should be maximally avoided in order to prevent oxidation. Secondly, sediment storage conditions may affect DOM properties. Freeze-preservation is a common procedure for sediment storage. However, freezing and subsequent thawing may disintegrate OM in the particulate phase and alter the structure of OM molecules in pore water. Otero *et al.* found that freezing of sediments caused increase in dissolved organic carbon (DOC) concentration, absorbance and fluorescence once the samples were defrozen[6]. Thirdly, DOM must be separated from the particulate phase. The most common way to isolate pore water from sediment is by centrifugation followed by filtration[7]. The speed and time of centrifugation may vary between different studies (e.g. from 2,500×g to 20,000×g for centrifugation speed and from 20 to 30 min for centrafigation time[7,8]). Other pore water sample preparation or isolation procedures include peeper, suction, and pressurization[9]. However, different isolation techniques may also change the properties of original DOM as there is a danger of alteration of DOM structure during sample preparaton before analysis, e.g., while concentrating via ultrafiltration or reverse osmosis[3]. The knowledge of these possible changes is still limited. Moreover, the use of different handling procedures and conditions complicate the comparison of the results. The fewer sample preparation procedures, the better, then there is a higher chance of undisturbing DOM structure.

Humic substances are reported to be the main constituents of lacustrine DOM[10,11,12]. These substances result from the accumulation and natural chemical reactions during biodegradation of OM. The chemical nature and structure of HS are still issues of debate. According to the latest concepts, HS are assumed to be supramolecular conformations of chemically diverse, relatively low molecular weight components. Humic molecules form dynamic self-associations linked by hydrogen bonds and hydrophobic interactions (van der Waals, π-π, CH-π)[13,14,15]. HS are amphiphilic molecules formed mainly by the enzymatic depolymerization and oxidation of aromatic and lipid plant components. The nonpolar part of these molecules consists of relatively unaltered segments of plant polymers, while the polar part is composed of carboxylic acid groups[16]. It was also postulated that HS exhibited micellar properties forming micellar-like aggregates in aqueous solutions and membrane-like aggregates on mineral surfaces groups[16]. Structurally, in addition to carboxylic groups, humic molecules have aromatic and aliphatic chains with hydroxylic, phenolic, enolic, quinine, ether and carbonyl functional groups.

HS can be classified according to solubility in water into humic (non-soluble below pH 2, but soluble at higher pH) and fulvic acids (soluble under all pH conditions), and humin (non-soluble at any pH)[17].

Due to the ability to form conformations of differents shapes and sizes, HS display a high reactivity with other materials under environmental conditions[14]. Due to the presence of carboxylic groups, they are powerful chelating metal

agents reducing the toxicity of certain heavy metals (e.g. Cu^{2+}, Cd^{2+}, Pb^{2+})[18]. Moreover, HS bind xenobiotics and other classes of ecotoxicants (e.g. petroleum, pesticides, chlorinated hydrocarbons, nitroaromatic explosives, azo dyes, radionuclides, and actinides)[19].

HS as a major part of DOM are involved in biogeochemical processes in aquatic systems, such as flux of sediment organic carbon[20], preservation and remineralization of OM[21,22] and in the mobility[23], distribution[24,25] and bioavailability[26] of contaminants. Therefore, a systematic and detailed research of DOM properties is needed to understand environmental phenomena processes.

1.1.2 Molecular weight of DOM

The molecular weight (MW) distribution of DOM (and, consequently, HS) is a key factor in its chemical reactivity. MW affects such HS properties as metal complexation/binding[27,28], interactions with organic pollutants[19], bioreactivity and water treatment[29]. According to Cabaniss *et al*.[30] molecules with lower MW have smaller radii, they are able to enter nanopores and have a higher diffusion coefficient resulting in faster adsorption kinetics. Low MW compounds are more hydrophilic and water-soluble and, thus, more bioavailable to aquatic organisms and are faster transported with the flowing water. In its turn, higher MW molecules are more aromatic and hydrophobic; they have enhanced metal-binding capacity due to greater electrostatic potential and more available ligands. Also, high MW material has greater adsorption affinities which results in decreased water solubility and slower mobility through the water column.

Considering the importance of MW of DOM and HS molecules in a wide range of processes and to understand the physical and chemical characteristics of DOM, an accurate determination of MW distribution is crucial. A number of analytical techniques have been applied for this purpose: ultracentrifugation, ultrafiltration, field-flow fractionation, vapor-pressure osmometry, small-angle X-ray scattering, mass spectrometry (MS) and high-performance size exclusion chromatography (HPSEC)[17,31]. However, the results vary greatly between studies. Earlier data in the literature reported MWs from 500 for aquatic HS to more than 10^6 for soil HS[32]. The reason for those huge differences is in the use of different analytical methods and analysis conditions as well as various sample isolation and pretreatment procedures. The high MWs reported earlier in the literature were observed for HS supramolecular associations but not for separate molecules[15]. With development of modern analytical techniques a new insight into MWs of HS was obtained. In recent years, MS and HPSEC have become the most powerful and widely used analytical approaches for determination of the MWs of humic material from different origins. MS coupled with various ionization techniques has been applied to the analysis of HS from aquatic sources[33,34,35,36,37,38,39] as well as from other origins[40,41,42,43,44]. MS results showed

that the MWs of HS from different origins were not higher than 1,000. For example, DOM from sea water, mangrove pore water and pore water from continental shelf sediments had general mass distributions of compounds with a mass-to charge ratio in the range of 300–700 with a typical mass spacing pattern of 14, which corresponded to an increasing number of $–CH_2–$ groups in the analytes[36,38]. MS results for humic and fulvic acids standards as well as natural OM from different origins (derived from soil, water, or peat environments) seemed to be very similar suggesting that during humification processes molecules containing similar chemical structures were formed.

HPSEC is another approach commonly used for the MW distribution determinations of HS from different environments, including DOM (e.g. [10,11,12,45]). When determining MW distribution of DOM (mostly HS) by the SEC method, one should keep in mind that separation is conventionally based solely on size-exclusion effects. In practice, the size of HS molecules is not only related to their mass but is strongly affected by other factors. Polydispersity and charge effects may cause stretching (uncoiling) or aggregation of the HS molecule under different analysis condition[31,46]. For example, depending on pH, functional groups of the humic molecule are protonated/dissociated. The dissociated functional groups are charged negatively. This causes an electrostatic repulsion between neighbouring negatively charged sites which leads to the stretching of the molecule. Electrostatic forces are also influenced by ionic strength and the presence of cationic species or ion-pairing agents. Thus, the same humic molecule with a certain MW may have different sizes under different media and analysis conditions[31]. Therefore, MWs determined by various analytical techniques and under different conditions may be difficult to compare[17,31].

HPSEC is traditionally used with UV detection[17,29]. However, detecting DOM by UV-absorbance may provide a bias in favor of molecules containing chromophores and absorbing light at a chosen wavelength as different DOM components may have different molar absorptivity (ε). It was found that higher MW fractions had a greater ε[47]. Wavelengths from 230 to 280 nm have been recommended for DOM detection[29,48]. This detection range is very suitable for the humic components of DOM, while other possible DOM compounds are more sensitively detected at other UV wavelengths (*e.g.* 210 nm for proteins and amino acids). Hence, at 230–280 nm higher MW fractions may appear to be more abundant than they actually are and possible lower MW components might not be represented properly. Using the SEC system with additional detectors such as online DOC or fluorescence detectors for a precise quantification of DOC was recommended[49,50,51]. Lankes *et al.* proposed that though DOM components detected with UV and DOC detectors were different, UV provides the basis for the more quantitative assessment of SEC data of DOC[52].

To estimate MWs adequately, calibration standards of similar structure and character should be chosen. DOM is a heterogeneous mixture of molecules. Therefore, it is not easy to choose appropriate calibration standards. As HS

constitute the main fraction of DOM, it is widely accepted that the standards should be of the same structure. However, no good calibration standards for humics have been proposed yet. It has been recommended to use random coil standards such as polystyrene sulphonates[12,53]. Some authors used polysaccharides[45,54] and globular proteins[55,56] of known MWs for calibration.

Despite that, HPSEC with UV detection, having obvious advantages over other separation methods, is still one of the most well-established techniques for the analysis of aquatic DOM. Minimal sample pretreatment, which does not affect the structure of DOM (filtration), and small sample volumes (μL) are very important in aquatic DOM analysis[29]. Relatively fast separation achieved under isocratic conditions and the easy sample pretreatment procedure favor convenience. Another advantage is the SEC's column excellent recovery to mass and biological activity because the stationary phase is designed to eliminate interactions with the sample[57].

1.1.3 The basic principle and theory of HPSEC

HPSEC is a separation method which is based on analyte size. This technique is primarily used to determine the distributions of the molar mass or MW of polymers, as for those of the same chemistry and architecture, size correlates with MW. However, HPSEC can also be applied to the analysis of long-chain branching and compositional distributions in samples, as well as to detecting polymer additives, and fractionate samples for further analysis[58,59].

Size separation takes place when the sample under analysis is pumped through a porous medium in the column (stationary phase). During separation the analytes are distributed between the solvent in the pores of the column stationary phase and the solvent outside the pores (mobile phase). In contrast to other chromatographic methods, SEC (under ideal conditions) involves no interaction between the analytes and the stationary phase.

The total volume of the packed column V_T is represented by the sum of the total volume of all pores V_p, the volume of the particle matrix V_m, and the interstitial volume outside the particles V_0:

$$V_T = V_m + V_0 + V_p \quad (1)$$

Largest molecule analytes are not able to penetrate into the pores and can move with the mobile phase only in the interstitial volume. Their retention (elution) volume is equal to V_0. Smallest molecule analytes can penetrate into all available pores and their retention volume is equal to (V_0+V_p). Analyte molecules of intermediate size can penetrate into pores according to the ratio of their size to the pore size. Their retention volume is within V_0 and (V_0+V_p). Thus, the retention volume V_R of an analyte of uniform size is:

$$V_R = V_0 + K_{SEC}V \tag{2}$$

where K_{SEC} is a formal distribution coefficient. K_{SEC} can be written in a standard thermodynamic form:

$$K_{SEC} = \frac{c_p}{c_0} \tag{3}$$

where c_p and c_0 refer to the analyte concentrations in the pores and the interstitial volume, respectively.

For the analyte molecules which are excluded from pores, K_{SEC}=0. K_{SEC}=1 for totally penetrating molecules. For the analyte molecules that can penetrate into the pores and when no other interactions occur besides size exclusion, $0<K_{SEC}<1$[46]. In case $K_{SEC}>1$, the separation is controlled by enthalpic interactions which depend on the analyte chemical composition and not necessarily on the molecular weight[60]. From the SEC column high MW molecules elute first, followed by those of low MW.

Although SEC is used to estimate MW, it should be pointed out that retention is actually determined by the hydrodynamic diameter of the analyte, which is only indirectly related to molecular weight. The hydrodynamic diameter of a molecule is related to its radius of gyration or the Stokes radius, and this may vary with analyte hydration and molecular shape[46,60].

1.1.4 MW averages of polymers

While molecules of a pure substance are characterized by a single MW value, those of polymers have a wide range of values. In this case, MW is described by distribution. The MW distribution of polymers can be expressed statistically, commonly as MW averages. Commonly reported MW averages are number-average and weight-average MW (M_n and M_w, respectively):

$$M_n = \frac{\sum N_i M_i}{\sum N_i} = \frac{\sum h_i}{\sum h_i / M_i} \tag{4}$$

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} = \frac{\sum h_i M_i}{\sum h_i} \tag{5}$$

where N_i is the number of i molecules with the molecular weight M_i and h_i is the height of the chromatogram in the retention volume of point i[59].

M_n is a simple arithmetic mean representing the total weight of molecules in the solution divided by the total number of molecules. M_w puts the emphasis on the contribution of molecules according to their sizes.

The width of distribution is often described by the ratio of the weight- to number-average molecular weight, M_w/M_n, which is called polydispersity[59]. If this ratio is equal to 1, it indicates that the solution is homogenous. The high ratio indicates that the range of MWs in the mixture is wide.

1.1.5 Characterization of DOM as a function of depth

Lake sediments represent historical archives reflecting past environmental changes in a lake ecosystem, such as climatic changes and human impact as well as the development of the lake basin. Once OM is buried on the bottom, it contributes to palaeolimnological record preserved in accumulated sediments. Variations in OM content and composition in the sediment core correspond to different types of lake biota and different origins of organic material. Thus, it is possible to use these as proxies to reconstruct paleoenvironmental conditions[2,61,62].

There is a genuine interest among researchers in the characterization of OM from lacustrine[10,63] and marine[64,65,66] sediments, as well as the study of its distribution as a function of sediment depth. These investigations provide information about the evolution, composition, and reactivity of OM and offer insight into the influence of OM on the biogeochemical cycling of different components (heavy metals, organic pollutants) in the lake system. The majority of the studies have been focused on the characterization of OM from the particulate phase of sediments (e.g., [67,68]). However, there is only a handful of papers dealing with the characterization of freshwater pore water DOM [10,11,55,63,69]. These publications report HPSEC characteristics of pore water DOM distributed downward through lacustrine sediments (Table 1).

Apparently, MWs of DOM from frozen sediments were significantly higher than those of DOM from fresh sediments. MWs around 1,000–1,500 are typical for aquatic fulvic acids[10,70,71] and freshwater sediment pore waters[11]. Polydispersities of aquatic DOM were relatively small showing that DOM in freshwater sediments existed in a narrow range of MWs[10]. These MW values for aquatic DOM are in good agreement with those obtained by MS (e.g., [33,34,37,38,39]).

Pore water DOM retains the characteristics of DOM form the particulate phase which reflect environmental conditions in a lacustrine system. Spectroscopic techniques have already been applied for revealing temporal changes in lakes (e.g.[69,72,73]). Apparently, the HPSEC method can also be applied to palaeolimnological research to track changes related to climatic conditions, erosion, eutrophication, and anthropogenic factors[74].

Table 1. HPSEC characteristics of sedimentary pore water DOM from different origins

	Lake	M_w	M_n	M_w/M_n
Fresh (unfrozen) sediments	Green Bay, Lake Michigan, USA[10]	1,500–2,000	600–900	2.0–2.7
	Old Woman Creek, Lake Erie, USA[11]	1,100–1,300	600–700	1.6–2.0
	Lake Erhai, Southwest China[63]	1,500–2,000	600–900	2.0–3.0
Frozen sediments	Lake Harku, Estonia[69]	5,000–70,000	2,000–36,000	2.1–3.7
	Lake Karujärv, Estonia[55]	2,000–5,000	500–3,000	1.5–3.0

1.2 CE analysis of organophosphates in soil matrices

1.2.1 Capillary electrophoresis

Capillary electrophoresis (CE) is a quick and convenient separation method where the migration behavior of molecules depends on their charge-to-size ratio. CE can be described as a high-efficiency technique of separation of sample ions in a narrow bore (25–100 μm) capillary tube filled with an electrolyte solution. The components of the CE system are a high-voltage power supply, anode and cathode compartments containing reservoirs of a buffer solution, and a capillary that passes through a detector connected to a data acquisition device[75,76]. The capillary is first filled with the buffer solution. The sample is then introduced at the end of the capillary away from the detector (usually the anode). The capillary ends are then dipped into the reservoirs with high-voltage electrodes and the buffer solution. Application of the voltage (usually 10–30 kV) across the capillary causes movements of analyzed species[75].

1.2.2 The basic principle and theory of CE separation

Electrophoresis is the movement of sample ions under the influence of an applied voltage[76,77]. Separation by electrophoresis is based on differences in ions velocity in the electric field[78].The velocity v_i aquired by the solute under the influence of the applied voltage H is the product of the solute electrophoretic mobility μ_e and the applied field E ($E=H/L$, where L is the length of the field)[76]:

$$v_i = \mu_e E \tag{6}$$

μ_e is a property of a particle, and is proportional to its charge and inversely proportional to the frictional forces acting upon it in solution. The electrical force can be given by

$$F_{el} = qE \tag{7}$$

and the frictional force for a spherical ion is

$$F_{fr} = -6\pi\eta r v_i \tag{8}$$

where q is the ion charge, η is the buffer solution viscosity, r is the ion radius.

During electrophoresis a steady state is attained resulting in the balance of these two forces. At this point the forces are equal but opposite

$$qE = 6\pi\eta r v_i \tag{9}$$

Thus, rearranging the equations, the mobility μ_e is decribed in terms of physical parameters

$$\mu_e = \frac{q}{6\pi\eta r} \tag{10}$$

The electrophoretic mobility of the analyte is a property of both the charge and size. The mobility is the highest for more highly charged and smaller size solutes, while a large, minimally charged species will have a low mobility[77]. Since q is positive for cations and negative for anions, these species migrate in opposite directions. Neutral species have q equal to 0, thus, their μ_e is 0[76].

Application of voltage across the capillary filled with the electrolyte causes the flow of the solution along the capillary. This introduces a fundamental constituent of CE operation that is electroosmotic flow (EOF). EOF is a consequence of the surface charge on the interior capillary wall[75,78]. The walls of the capillary are electrically charged. The surface of the silica capillary contains a large number of silanol groups (Si–OH). At pH greater than approximately 2 or 3, the silanol groups ionize and form negatively charged silanoate ions ($Si–O^-$). Cations from the buffer are attracted to the silanoate ions and bind to them tightly forming an inner, or fixed, layer. Other cations are more loosely bound and form an outer, or a mobile, layer. Cations in the outer layer migrate toward the cathode. Because these cations are solvated, the solution is also pulled along producing the electroosmotic flow[76].

Electroosmotic flow velocity, v_{EOF}, is a function of the magnitude of applied electric field and the electroosmotic mobility of the electrolyte in the capillary (buffer), μ_{EOF}

$$v_{EOF} = \mu_{EOF} E \tag{11}$$

Electroosmotic mobility is defined as[76]

$$\mu_{EOF} = \frac{\varepsilon \zeta}{4\pi\eta} \tag{12}$$

where ε is the buffer solution's dielectric constant, ζ is the zeta potential.

The level of EOF is highly dependent on the pH of the buffer solution since the zeta potential ζ is largely governed by the ionization of silanol groups. Below pH 4, the ionization is weak, and EOF is not significant. Above pH 9, the silanols are fully ionized and the EOF rate is high. Increasing the ionic strength of the buffer solution results in a higher concentration of cations, decreasing the ζ potential[75,76].

Thus, the overall mobility μ_{tot} of a sample analyte is related to both its electrophoretic mobility and the mobility of EOF[76]:

$$\mu_{tot} = \mu_e + \mu_{EOF} \tag{13}$$

In CE, cations elute first in an order corresponding to their electrophoretic mobilities: small highly charged species elute before larger cations of lower charge. Neutral molecules elute as a single band with the elution rate corresponding to the EOF velocity. Anions are the last components to elute with the smallest highly charged species having the longest elution time[76].

1.2.3 Phosphonic acids

Phosphonic acids (PAs) are degradation products of some highly toxic chemical warfare agents (CWAs), such as VX, sarin and soman. CWAs contain alkyl methylphosphonate moiety in their structure and produce characteristic compounds, alkyl methylphosphonic acids, while decomposing. As a result of hydrolysis, the agents produce intermediate degradation products: VX hydrolyzes to ethyl methylphosphonic acid (EMPA), sarin produces isopropyl methylphosphonic acid (IMPA), and soman is decomposed to pinacolyl methylphosphonic acid (PMPA). The final degradation product of all of them is methylphosphonic acid (MPA)[79]. PAs are good markers for identifying parental

CWAs. They are stable and significantly less toxic compounds than original nerve agents.

The chemical structure of the five PAs investigated in this work is presented in Figure 1. The hydrolysis of CWAs is depicted in Appendix I.

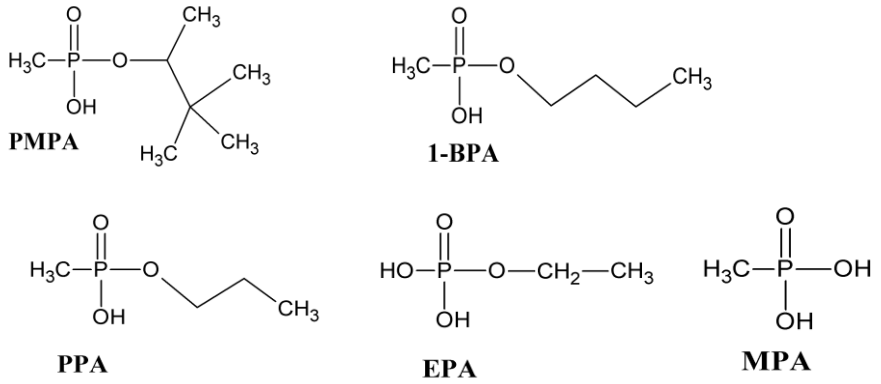

Figure 1. The chemical structure of PAs. PMPA – pinacolyl methylphosphonic acid, 1-BPA – 1-butylphosphonic acid, PPA – propylphosphonic acid, EPA – ethylphosphonc acid, MPA – methylphosphonic acid.

1.2.4 Adsorption

Adsorption is the process of accumulation of particles at a surface. Generally, adsorption is based on two processes: physical adsorption and chemisorption. Physical adsorption is based on physical attractive forces between the adsorbate and the adsorbent, e.g. van der Waals interactions, while in chemisorption particles stick to the surface by forming chemical bonds (usually covalent)[80,81].

Adsorption is described by adsorption isotherms, i.e. the relationship between the adsorbate and the adsorbent. The ratio between the amounts of the adsorbed and remaining adsorbates on the adsorbent at a constant temperature at equilibrium can be described by various adsorption isotherms[80].

1.2.4.1 Langmuir isotherm

The Langmuir isotherm is the most widely-known adsorption isotherm. It describes adsorption in one monolayer based on the assumption that every site is equivalent and the ability of a particle to bind to it is independent of whether or not nearby sites are occupied[80]. Adsorption takes place following the scheme A+S↔AS, where A is the adsorbate molecule and S is the adsorption site. The

Langmuir isotherm can be expressed as the relationship between the adsorbed amount and the final equilibrium concentration of the adsorbate:

$$q_e = \frac{q_{max} K_a C_e}{1 + K_a C_e} \tag{14}$$

where q_e is the amount of the adsorbate adsorbed at equilibrium, C_e is the final equilibrium concentration of the adsorbate, q_{max} is the maximum adsorption at the monolayer coverage, and K_a is the adsorption equilibrium constant related to the energy of adsorption.

There are four possibilities to fit equation (14) to a linear form: plot C_e/q_e vs. C_e, $1/q_e$ vs. $1/C_e$, q_e vs. q_e/C_e, or q_e/C_e vs. q_e[82].

However, the Langmuir isotherm equation does not take into account the heterogeneity of an adsorbent surface (a surface on which different areas have different affinities to the adsorbate), and, therefore, it does not satisfactorily represent physical adsorption data[83].

1.2.4.2 Freundlich isotherm

Freundlich isotherm is sometimes more successful than the Langmuir isotherm. It may be derived assuming a heterogeneous surface with adsorption on each class of sites obeying the Langmuir equation. However, the Freundlich isotherm equation is unsatisfactory for high concentrations as the adsorbed amount increases indefinitely with increasing concentration[83]. The Freundlich isotherm is described by the following equation:

$$q_e = K_F C_e^{\frac{1}{n}} \tag{15}$$

where K_F is the Freundlich constant expressing the adsorption capacity and n is a constant representing the adsorption intensity. The isotherm can be transformed into linear form by taking logarithm on both sides[82]:

$$\ln q_e = \frac{1}{n} \ln C_e + \ln K_F \tag{16}$$

1.2.4.3 Redlich-Peterson isotherm

The Redlich-Peterson isotherm is more sophisticated than the Langmuir and Freundlich isotherms as this model is described by three parameters instead of two:

$$q_e = \frac{K_R C_e}{1 + a_R C_e^{b_R}} \tag{17}$$

where K_R is the Redlich-Peterson constant describing the adsorption capacity, a_R is the Redlich-Peterson isotherm constant, and b_R is the Redlich-Peterson isotherm exponent which lies between 0 and 1[82]. Transforming the Redlich-Peterson isotherm to linear form can be done plotting $\ln(A^*(C_e/q_e)-1)$ vs. $\ln(C_e)$. If b_R is 1, the Redlich-Peterson isotherm transforms to the Langmuir isotherm.

1.2.4.4 BET isotherm

The isotherms described above ignore the possibility that molecules can form multilayers during adsorption as adsorbed molecules can act as a substrate for further adsorption. The BET* theory assumes that the surface possesses uniform, localized sites and that adsorption on one site does not affect that at neighbouring sites. According to this theory the adsorption mechanism is described as follows:

$A+S \leftrightarrow AS$

$A+AS \leftrightarrow A_2S$

$A+ A_2S \leftrightarrow A_3S$ and etc.

For the liquid phase adsorption the BET isotherm is[84]:

$$q_e = \frac{q_{max} K_S C_e}{(1 - K_L C_e)(1 - K_L C_e + K_S C_e)} \tag{18}$$

where K_S is the equilibrium constant of adsorption for the first layer and K_L is the equilibrium constant of adsorption for the upper layer. If K_L is equal to 0, i.e. there is no adsorption on the upper layers, the BET isotherm transforms into the Langmuir isotherm.

* The name has been given after the developers of the theory, Stephen Brunauer, Paul Emmett, and Edward Teller.

1.2.5 CE analysis of PAs in soil matrices

The development of reliable and fast analytical methods for the analysis of degradation products of CWAs has been a subject of interest for almost two decades. The presence of a specific PA in environmental matrices is the evidence of the use of its parental nerve agent. The development of a method for the detection and quantification of PAs in complex environmental matrices is a complicated task. The detectability of PAs in soils and natural waters is low due to interactions of the acids with organic matter. Humic material has a negative effect on the extraction recovery of phosphonic acids[85,86]. It was reported that alkyl methylphosphonic acids, especially MPA, were bound onto spodosol soil[87]. Recoveries of MPA and several other studied PAs decreased with increasing HS concentrations[85]. The low level of an aqueous extraction recovery of PA has been attributed to the adsorption of phosphonates onto the soils[86]. Since PAs are structurally and physicochemically similar to phosphates, they might be adsorbed onto the same adsorption sites. The adsorption of PAs may be considered as the result of both physical adsorption onto inorganic soil particles and chemisorption with OM. PAs are charged negatively, therefore they would be adsorbed onto positively charged sites. Though soil particles are predominantly charged negatively, they may have positive charges as well. Oxide surfaces (notably Fe- and Al-oxides/hydroxides) and the edges of clay minerals, or alumosilicates (e.g., kaolinite, illite, chlorite) contain positively charged sites. Clay minerals and Fe- and Al-oxides/hydroxides form chemical bonds with phosphate anions[88]. Thus, in soil matrices positively charged Fe- and Al-oxides/hydroxides cations, which are adsorbed on HS and clays, and edges of clay minerals would be the main sites of PAs adsorption[86]. However, no detailed research on the adsorption of PAs on different soil matrices has been reported so far.

To improve the recovery of PAs from soil matrices different extraction procedures were developed: alkali extraction followed by neutralization and solid-phase extraction (SPE)[86], a three step superfluidic extraction[89], extraction with carbonate[90], SPE with molecularly imprinted polymers[91], ion-pair SPE[92], pressure-assisted solvent extraction by water[93], electro membrane isolation[85]. Compounds isolated by different techniques were subjected to further analysis by various analytical approaches, such as LC[90,94] or GC[86,92] coupled with MS. CE with indirect UV[95] or contactless conductivity detection (CCD)[85,96] has also been applied to PAs determination.

For a rapid determination and quantification of PAs on-site a reliable and easy to perform method should be developed. For an on-site determination of PAs miniaturized and simple to use instruments and procedures should be developed. CE instruments with CCD are very suitable for miniaturization and measurements on-site. Also, the procedure for PAs extraction should be as easy and quick as possible.

2 EXPERIMENTAL

2.1 Chemicals

Salts for HPSEC buffer: ammoniumdihydrogen phosphate was purchased from Riedel-de Haën (Germany), di-ammonium hydrogen phosphate was purchased from Lach-Ner, s.r.o. (Czech Republic), potassium hydrogenphosphate and potassium dihydrogenphosphate were purchased from Реахим, Russia
Standards for HPSEC calibration: immunoglobulin, ovalbumin, lactalbumin, D,L-tyrosine were from *Реахим*, Russia. The standard mixture of bovine thyroglobulin, human gamma globulin, ovalbumin, myoglobin, and uridine (Aqueous SEC 1 Std.) was from Phenomenex (USA).
Chemicals for CE procedures: L-histidine (His) and 2-(N-morpholino)ethanesulphonic acid hydrate (MES hydrate) for BGE were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium hydroxide was purchased from Chemapol (Prague, Czech Republic).
Standards for CE: methylphosphonic acid (MPA), ethylphosphonic acid (EPA), and 1-butylphosphonic acid (1-BPA) were purchased from Alfa Aesar, Lancaster Synthesis (Windham, NH, USA). Propylphosphonic acids (PPA), pinacolyl metylphosphonic acid (PMPA), and 2-aminoethyl dihydrogenphosphate (AEDHP) were purchased from Sigma-Aldrich (Steinheim, Germany).
All solutions for HPSEC and CE analyses were prepared with high-purity deionized water from the Milli-Q water system (Millipore, France).

2.2 Methods and materials

2.2.1 Analysis of pore water DOM

2.2.1.1 Study site

Lake Peipsi is an unstratified, eutrophied and shallow lake with a mean depth of 7.1 m and maximum depth of 15.3 m. It is the fourth largest lake in Europe by area (3,555 km^2, maximum length ~150 km, width – 42 km). The lake is shared between the Republic of Estonia and the Russian Federation. The lake system consists of two basins (Lake Peipsi *sensu stricto* and Lake Pihkva) joined by a narrow strait (Lake Lämmijärv). Lake Peipsi has an outflow into the Gulf of Finland via the Narva River[97].

The bottom topography of the lake is monotonous and the sediment composition is homogenous. According to Niinemets *et al*.[98] the sediments of Lake Peipsi consist of dark brown and greenish gyttja, homogeneous light bluish-grey lake marl, silt, sand, and brownish glaciolacustrine homogeneous or varved clay. The shallow areas consist mainly of sands, silts and late-gracial

clays, in the areas with >9 m of water depth post-glacial OM rich gyttja sediments are present[99].

In Lake Peipsi *sensu stricto* the amount of total phosphorus is 40 mg P m^{-3} and that of dissolved inorganic phosphorus is 7 mg P m^{-3}. The content of total nitrogen is 660 mg N m^{-3} and of dissolved inorganic nitrogen 121 mg N m^{-3}. The Secchi depth is 1.7 m[100]. The water is brown due to HS (0.05–2.00 mg L^{-1})[101].

Lake Peipsi is an important economic resource for Estonia. It has been exposed to extensive human activity for over several centuries. The lake itself and the rivers in its catchment area have been extensively used for fishing and water transport, whereas the catchment area has been used for agricultural purposes. There are several mining areas and two oil shale-fuelled electric power plants on the northern side of the lake.

Investigations of Lake Peipsi have been carried out for one and a half centuries, complex studies have been conducted since the 1930s[102].

2.2.1.2 HPSEC instrumentation

All HPSEC measurements were carried out on a BioSep-SEC-S 2000 PEEK size exclusion column filled with glycerol covered silica-based gel (300×7.50 mm, Phenomenex, USA). The particle size was 5 μm and the pore size was 145 Å. For the analysis of pore water from the short sediment core, the analytical column preceded a guard column (75×7.50 mm, Phenomenex, USA) and a single wavelength UV detector and a pump from Knauer (Germany) were used. For DOM from the long core, the SEC column preceded a SecurityGuard™ cartridge (10 × 10 mm, Phenomenex, USA) and a diode-array detector (DAD) from Agilent Technologies and Dionex (USA) pump were used. HPSEC data were analysed using the Agilent ChemStation software. The HPSEC system was equipped with a Rheodyne injector (USA).

HPSEC was calibrated using protein standards. For the pore water samples extracted from the short sediment core immunoglobulin (MW 160,000), ovalbumin (MW 45,000), lactalbumin (MW 18,000), and D,L-tyrosine (MW 182) were used for calibration. The proteins dissolved in the buffer were run individually. For the long sediment core pore water analysis HPSEC was calibrated using a mixture of the following proteins dissolved in the buffer: bovine thyroglobulin (MW 670,000), human gamma globulin (MW 150,000), ovalbumin (MW 44,000), and myoglobin (MW 17,000). Uridine (MW 244) was run separately for this calibration. To construct a calibration curve, the elution times of the peak maxima of protein standards were plotted against the logarithm of their MWs. The mobile phase was the phosphate buffer with pH 6.8.

From HPSEC chromatograms total peak areas were computed. The total peak areas were used for the evaluation of DOM contents in pore water samples. M_n and M_w were calculated by equations (4) and (5), respectively.

2.2.1.3 UV spectroscopy

Absorbance spectra were measured with a Jasco V-530 UV/VIS spectrophotometer (Japan) using a quartz cuvette with a 1 cm path length. Milli-Q water was used as a blank. The spectra were collected over the range of 200–500 nm with a bandwidth of 2.0 nm. From the absorbance spectra dissolved organic carbon (DOC) concentrations were calculated using Højerslev equations as follows[103]:

$$DOC_{320} = 472 A_{320} * e^{0.014(320-450)} \quad (19)$$

$$DOC_{340} = 472 A_{340} * e^{0.014(340-450)} \quad (20)$$

$$DOC_{360} = 472 A_{360} * e^{0.014(360-450)} \quad (21)$$

$$DOC = \frac{DOC_{320} + DOC_{340} + DOC_{360}}{3}, \quad (22)$$

where DOC_{320}, DOC_{340}, and DOC_{360} are DOC concentrations calculated from the absorbance spectra at 320 (A_{320}), 340 (A_{340}), and 360 (A_{360}) nm, respectively. The numbers given in the brackets are wavelengths of 320, 340, 360, and 450 nm.

The ratio of absorbances at 254 (or 250) and 360 nm (A_{254}/A_{360}), which reflects the aromaticity of dissolved molecules[104], was calculated.

2.2.1.4 Procedures

Two sediment cores were taken on ice from the center of the broadest part of Lake Peipsi by a group of scientists from the Institute of Geology, Tallinn University of Technology. During collection and sample preparation sediment cores were handled following precautions to maximally avoid oxygen exposure. All cores were sliced into consecutive 1-cm sub-samples.

The first shorter core (40 cm) was obtained at the point 58º47´14´´N; 27º19´20´´E with a freeze corer in winter 2002. After slicing to sub-samples, the sediments were frozen. The sediment samples were dated using ^{210}Pb, ^{226}Ra and ^{137}Cs by gamma spectroscopy using a well-type coaxial low background intrinsic germanium detector[105]. The core covered approximately the last 120 years. The frozen sediments were thawed at +4 ºC prior to extraction.

The second core, 4 m long, was taken from 58º47´213´´N; 27º19´299´´E in March 2007 using a Russian-type peat corer. The sliced 1 cm sub-samples were stored in small plastic bags in the dark at +4 ºC. The chronology of sediments was established using nine ^{14}C dates on the bulk sediment. The radiocarbon dates were converted to calendar years before the present (BP; 0 = AD 1950). The age of the upper part of the sediment record was correlated with that of the

shorter core taken in winter 2002. The obtained sediments covered 10,000 years of accumulation.

Table 2. Pore water extraction procedures and HPSEC analysis conditions for pore water DOM from Lake Peipsi

Samples	Pore water extraction conditions			Phosphate buffer concentration, mM	Flow rate, mL/min	Sample volume, μL	Detection wavelength, nm
	Filter*, μm	Centrifugation speed, rpm	Time, min				
Short core	0.20	10,000	15	20	1.0	100	254
Long core	0.45	3,500	30	100	0.5	20	280

* Millex, Millipore

Pore water extraction procedures and analysis conditions are presented in Table 2. All pore water samples were extracted before analysis. While not being analysed, the samples were stored in tightly-closed test tubes in the dark at +4 ºC.

2.2.2 Analysis of PAs in soil matrices

2.2.2.1 CE instrumentation

On-site CE analysis of phosphonic acids in soil extracts: for an on-site analysis of PAs from soil aqueous extracts an in-house made portable CE instrument with a CCD detector described in Seiman *et al*.[106] was used. A fused silica capillary with (i.d. 75 μm, o.d. 360 μm; Agilent Technologies, USA) with a total length of 44 cm (effective length 36 cm) was used. The separation voltage was 16 kV. For the injection 0.2 mL of the sample was injected into the cross-sampler inlet channel. As BGE 15 mM Mes/His buffer was used for all CE experiments.

A new capillary was flushed with 1 M NaOH for 10 min, with water for 10 min, and with BGE for 10 min. For every day conditioning, before starting the experiments, the capillary was flushed with 0.1 M NaOH for 3 min, with water for 10 min and with BGE for 10 min. Between the runs the capillary was rinsed with BGE.

CE experiments of phosphonic acids adsorption: all adsorption experiments with soil extracts were carried out on a commercial Agilent Technologies CE instrument (Germany) with DAD. However, an in-house made CCD was used for detection. For experiments, an uncoated fused-silica capillary (i.d. 75 μm, o.d. 360 μm; Agilent Technologies, USA) with a total length of 55 cm was used.

The capillary length to the CCD cell was 45 cm and to the DAD cell 49 cm. The CE instrument was controlled using the Chemstation software (Agilent Technologies, Germany). Data acquisition in CCD was done using the in-house written software. Data processing was performed in MatLab (The Math Works, Inc., Natick, MA, USA) using the in-house written software.

For the new capillary flushing and every day conditioning the same procedures as described in the previous section were used. Between the runs the capillary was rinsed with water for 2 min and with BGE for 3 min. For all experiments 15 mM Mes/His buffer as BGE was used. The samples were injected hydrodynamically (50 mbar) for 10 s, the applied separation voltage was 20 kV. The capillary temperature was constantly kept at 25 ºC.

2.2.2.2 Procedures

Standard stock solutions of PAs were prepared by dissolving an exact amount of an analyte in MilliQ water to obtain a concentration of 10 mM. The procedure was followed by the mixing of five PAs into the standard solution of a final concentration of 2 mM.

On-site aqueous extraction of phosphonic acids from soil

5 mL of the PAs stock solution with a concentration of 2 mM was sprayed on a small ground area. After exposing to PAs (1.5 h) 2 g of soil from the upper top layer was taken. The analytes were extracted with 10 mL of water, sonicated for 30 min and filtered through a medium fast paper filter (Whatman, Maidstone, UK) and a 0.45 μm Millipore filter. For an on-site analysis, sonication was replaced by shaking and the sample was filtered through a 0.45 μm Millipore filter and directly injected to CE instrument.

Soil samples for adsorption experiments

Two types of soil samples were collected. The sand sample was collected in a park in Tallinn (59º23´42.13´´N; 24º40´37.02´´E). The loam sample was collected in a forest in Kõpu rural municipality, Viljandi county (58º19´34.72´´N; 25º17´45.19´´E). The samples were collected from the soil surface layers at a maximum depth of 5 cm.

The OM content in soil fractions was measured by heating them at 550 ºC for 4 h to gain constant weight. First, empty crucibles were heated at 550 ºC for 4 h. After cooling for 30 min, the crucibles were weighed and ~1 g of soil was added to the crucibles. Heating at 550 ºC for 4 h was repeated followed by a 30 min cooling and weighing. The OM content was calculated as a difference between the two masses (Table 3).

Table 3. Organic matter in analysed soil samples

Size fraction, μm	Organic matter, %*	
	Sand	Loamy soil
< 100	1.24	5.64
100–200	0.55	7.03
200–400	0.50	6.05

* Based on three parallel analyses

Sample preparation of soil extracts for adsorption experiments

Soil samples were dried at room temperature until constant weight was obtained. After drying the samples were fractionated according to particle size using sieves with different hole sizes. The size fractions were <100, 100–200, and 200–400 μm. Basically, the fractions represented very fine, fine and medium-grained sand; silt and clay were found only in the finest fraction.

To prepare the samples, 0.5 g of soil material from each fraction was weighed into 2 mL plastic vials. The samples were spiked with 12.5, 25, 37.5, 50, 75 and 100 μL of a 2 mM solution of five PAs. The samples were exposed to PAs for 50 min and then water was added to obtain a volume of 1 mL. Thus, the concentration of PAs in the soil samples was 25, 50, 75, 100, 150, and 200 μM, respectively. The samples were shaken for 10 min and centrifuged at 6000 rpm for 10 min. 500 μL of an unfiltered supernatant was placed into a 0.5 mL plastic vial and an exact amount of AEDHP was added as an internal standard to achive a final concentration of the latter of 500 μM. The prepared soil extracts were analysed unfiltered.

Isotherms for phosphonic acids adsorption on soil

For evaluation of the adsorption of PAs the Langmuir, Freundlich, Redlich-Peterson, and BET isotherms were used. The equations for the isotherms were (14), (15), (17), and (18), respectively. Instead of the final equilibrium concentration of the adsorbate C_e, the initial concentration C_{init} of PA applied to the soil was used in the equations. All calculations for fitting the adsorption isotherms to the experimental data were done using in-house written programs in MatLab software (The Math Works, Inc., Natick, MA, USA). For the Redlich-Peterson isotherm the solver add-in of Microsoft Excel was applied to determine the isotherm parameters by using an optimization routine to maximize the determination coefficient between the experimental data and calculated isotherms.

3 RESULTS AND DISCUSSION

3.1 Analysis of pore water DOM

3.1.1 Methodological aspects

When analysing DOM by HPSEC, there is a danger of interactions between HS molecules with the stationary phase of the SEC column which may distort analysis results and should therefore be avoided by choosing appropriate analysis conditions. All pore water samples were analysed using a BioSep column (Phenomenex, USA) which has been reported to be suitable for HS characterization[45,56]. The stationary phase of BioSep columns has diol-functionality and residual silanol groups on the surface. Thus, HS with negatively charged functional groups can not enter the pores of the stationary phase because of electrostatic repulsive forces. To minimize this effect and supress the dissociation of silanols, the ionic strength of the mobile phase should be increased or pH should be lower than 4. In the present study the pH of the mobile phase was neutral (6.8) as this value was characteristic of natural waters. Thus, only the ionic strength could be optimized. For the present method ionic strengths of 0.02 to 0.1 M were chosen in order to minimize undesirable effects[56].

Another important factor in HS analysis by HPSEC is the choice of a proper detection wavelength. The present study employed UV detection at the wavelengths of 254 and 280 nm, these being most suitable for HS detection[48]. However, other possible DOM components which do not absorb UV at that wavelength (e.g. amino acids, carbohydrates) may not be represented properly or even may remain undetected. Therefore, the DOM content and MW distributions obtained by this method will be discussed only in terms of UV-absorbing, mostly humic, matter.

The choice of calibration standards for HS analysis is a crucial factor in MWs determination. In earlier works protein standards have been reported to overestimate MWs of HS[12,107]. However, as shown below, the results of the present study did not confirm this overestimation and protein standards were found to be suitable for the calibration of the BioSep column for HS separation.

High and low MW cutoffs may affect MW values significantly, therefore the proper choice of these parameters is important. To obtain adequate results, MW distributions were calculated only for the compounds which eluted within the calibrated range. For the short sediment core pore water the MW range of 160,000–182 was used and for the long sediment core pore water the MW range was within 670,000–244. The conditions of extraction and analysis of pore water from two cores were different, therefore, the results are directly not comparable. The conditions were different because those found to be optimal for the analysis of pore waters from frozen and fresh sediments differed.

Total SEC peaks areas were used as rough estimations of the UV-absorbing DOM content to construct its changes downward through the cores. The DOC concentrations calculated empirically showed good correlation with total SEC peak areas (R^2=0.86 for the long core DOM).

HPSEC performance data is presented in Table 4. It can be seen that the relative standard deviations (RSD) for DOM from the short core are higher than those of the long core DOM. The experiments were performed with pumps and detectors of different qualities. The DOM from the long core was analysed on the newest instrument, therefore the results were preciser and the RSD was less than 2% for all parameters. The calibration curves demonstrated good linearity (R^2=0.97 for the short core DOM analysis and R^2=0.99 for the long core DOM analysis).

Table 4. HPSEC performance data for DOM*

	Retention time	Peak area	M_w	M_n	M_w/M_n
RSD % (short core), n=120	4.93	7.50	9.60	10.80	5.22
RSD % (long core), n=120	1.06	1.69	0.97	0.67	0.02

* Calculated as an average of all experiments

The aromaticity index A_{250}/A_{360} of the pore water sample indicates the origin of OM[104]. Lower A_{250}/A_{360} values reflect the high content of molecules with aromatic structures which are characterstic to OM from land-derived sources (allochthonous OM), whereas the higher A_{250}/A_{360} index is related to the domination of molecules with aliphatic structures of authochthonous DOM[108]. Therefore, this parameter is useful to predict the origin of OM when reconstructing changes of DOM as a function of depth.

3.1.2 DOM elution profiles by HPSEC

Generally, DOM from all analysed pore waters from both sediment cores eluted from the HPSEC column with quite a similar profile (Figure 2). A typical profile of DOM elution consisted of two peaks: a small peak of high MW components and a broad distribution, sometimes with a partially resolved sub-shoulder. The position of the maxima of both peaks was quite stable for all samples, though the shape of the peaks varied significantly with depth. DAD revealed the presence of proteinaceous material in the first peak, while the second peak exhibited a UV spectrum characteristic to HS. The low MW subfraction is usually represented by small organic acids. However, the retention time of the first peak was above the calibration range, thus, it was not possible to estimate the subfraction's MW adequately. Apparently, high MW peak represented typical supramolecular associations of HS (or aggregates). The presence of biomolecules bonded to humic material has been previously pointed out, though usually it was excluded

from traditional definitions of HS[13]. Usually, biomolecules are degraded by microbes. However, binding to humic molecules results in the encapsulation of biomolecules making them unavailable to microbial degradation. Piccolo and Conte[15] proposed that the high MW fraction eluting at the void volume of the HPSEC column (the first peak in our case) consisted of mainly apolar components which were self-associated into hydrophobic domains. The lower-sized fractions (in the present study, the second peak with sub-shoulders) was composed of polar compounds in hydrophilic associations. Thus, the first peak in our HPSEC chromatograms may have been composed of apolar associations of proteins with humic molecules held together by hydrophobic interactions.

These elution profiles were similar to the HPSEC profiles published in some papers concerning analysis of aquatic DOM (e.g. [10,48,55,74]). However, the high MW fraction with UV absorbance characteristic to proteins was not always detected. It was detected only in pore waters from Lake Võrtsjärv[74]. This may mean that DOM from these two lakes was similar.

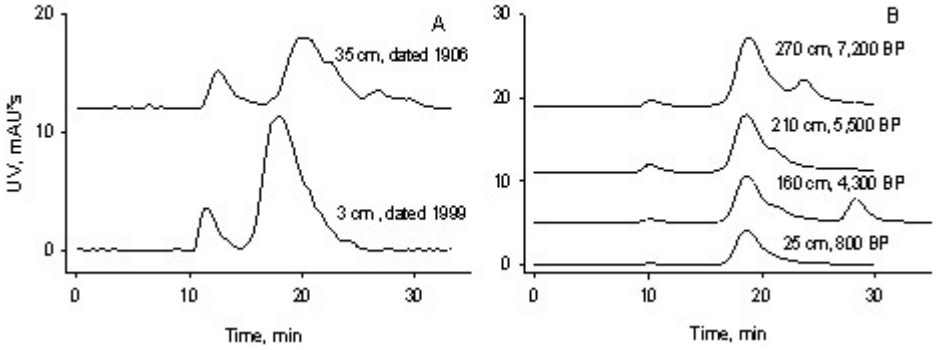

Figure 2. HPSEC chromatograms of pore water DOM from Lake Peipsi: (A) short sediment core, detection wavelength 254 nm, (B) long sediment core, detection wavelength 280 nm. Dated sediments collected at different depths.

3.1.3 HPSEC analysis of DOM from Lake Peipsi as a function of depth

The pore water from the short sediment core was extracted from previously frozen and thawn sediments, while the pore water from the long core was extracted from fresh sediments. Moreover, HPSEC column calibration for the short core pore waters was performed with globular proteins run individually and for the long core waters with a mixture of proteins. Therefore, one should keep in mind that the MW distributions, as well as spectroscopic properties of pore water DOM from two cores are not the same. The two cores covered very different time periods, thus, the comparison of age-related changes of DOM in the lake is not possible. Though the sediment storage and pore water extraction

conditions were different, DOM parameters still may reflect age-related changes in the lake ecosystem. Therefore, the applicability of the HPSEC method to palaeolimnological research was studied.

3.1.3.1 Analysis of pore water DOM from the short core (Publication I)

The elution profiles of DOM of the thawn sediment pore water varied downward through the core (Figure 2A). The DOM from upper layers (first 10 cm of the core; dated 2001–1989) eluted in two fractions (a small peak of the high MW fraction and a high peak of HS), whereas in deeper layers the third peak of the low MW fraction appeared (10–40 cm, dated 1989–1882). This may reflect the decomposition of OM and formation of low MW compounds. The proportion of the low MW fraction was the highest in intermediate layers (11–25 cm; dated 1988–1949), the proportion of high MW aggregates increased in older layers (28–39 cm; dated 1939–1887). The increase in the formation of aggregated matter may be due to condensation processes in the sediments.

DOM content and MW characteristics varied downward through the core (Figure 3). In the upper layers (1–19 cm) MW averages varied more significantly than in the layers deeper than 20 cm showing dynamic and unstable changes in the composition and characteristics of DOM. Sediment upper layers represent the most active part where DOM undergoes significant transformations via biodegradation supported by bioturbation and resuspension[2]. This might have caused great variations in the characteristics of DOM from the sediment surface layers.

In the upper layers (0–10 cm, dated 2001–1989) the DOM content was the highest continuously decreasing downward through the core (Figure 3A). In the deeper layers (32–38 cm; dated 1922–1892) the DOM content temporarily increased. According to the total peak areas and the proportions of different MW fractions in the dissolved phase, the most obvious change was observed in the layers dated to the late 1980s–1950s (Figure 3B). Simultaneously with the decrease in overall DOM content, the proportion of the low MW fraction increased significantly and the proportion of the high MW fraction decreased (Figure 3B). Judging by the higher values of the aromaticity index (Publication I, Fig.1o; data not presented here as the index was measured by another author) in the late 1980s–1950s autochthonous OM dominated in the lake. Autochthonous DOM molecules are produced by aquatic algae[108] and they have usually smaller size than allochthonous DOM. Thus, increase in the proportion of the low MW fraction and decrease in the content of the high MW fraction in the pore waters was attributed to significant changes in the lake column conditions. These changes indicated eutrophic conditions of the lake caused by elevated in-lake bioproduction and anthropogenic impacts. Indeed, agricultural and other anthropogenic activities in the lake catchment would lead to a release of nutrients into the lake causing elevated in-lake bioproduction. The

eutrophication of Lake Peipsi in the 1950s–1980s was confirmed by sediment diatom assemblages studies[73]. Also, analysis of selected fossil pigments revealed simultaneous changes in the sediments implying eutrophication of the lake at the same time period (see Publication I, analysis of fossil pigments).

In the 1990s the overall DOM content in the lake increased, while the proportion of low MW molecules significantly decreased. Simultaneously, the aromaticity index showed lower values indicating the dominance of allochthonous land-derived DOM with aromatic structures. These changes implied a decreased contribution of OM produced within the lake due to the deceleration of eutrophication. A slight recovery of the lake was also confirmed by diatom studies[73].

In general, M_w was less than 8,000, varying mostly between 3,500 and 5,000; M_n varied in the range of 1,000–4,000. Polydispersities varied from 2 to 4, indicating that general changes in MW distribution were small. The MW distributions obtained were similar to those published by Lepane *et al*. for other pore waters from frozen sediments in Estonian lakes[55].

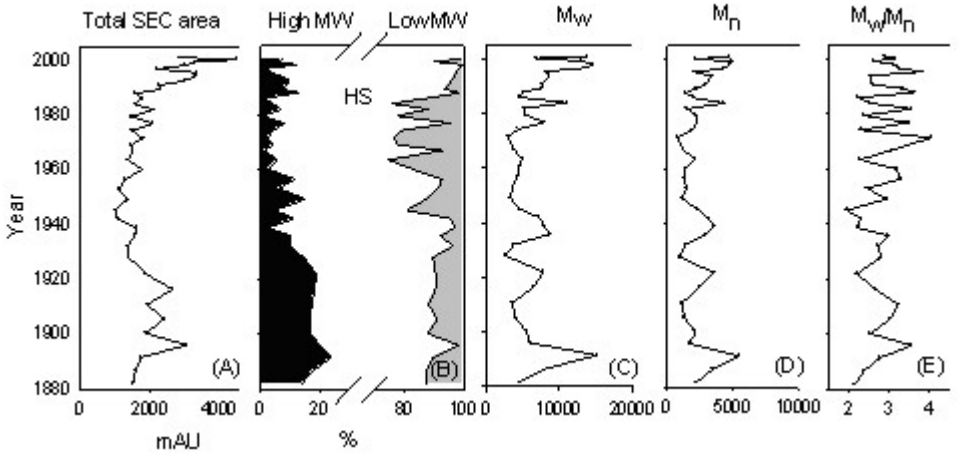

Figure 3. Age-resolved profiles of the characteristics of DOM from the short core pore waters of Lake Peipsi: (A) total HPSEC peak area, (B) proportions of high MW, HS and low MW fractions in the sediments, (C) weight-average molecular weight M_w, (D) number-average molecular weight M_n, (E) polydispersity M_w/M_n.

The conditions of the lake ecosystem preceding the eutrophication in the middle of the 20th century were reported to be close to natural baseline conditions when the influence of human activity was negligible[73]. The pore water DOM from the sediments dated to the 1890s–1940s had quite stable characteristics: minimal variations in content and composition, and stable aromaticity indices implied that no drastic changes appeared in the lake during that period. The proportion of the high MW fraction was larger and that of the

low MW fraction smaller than those in newer sediments, implying that allochthonous DOM constituted the largest part of lacustrine OM.

3.1.3.2 Analysis of pore water DOM from the long core (Publication II)

The high MW aggregates eluted with the first peak were very unstable in the pore waters from the deepest layers (samples dated 10,300–6,600 cal BP[†]) as the peaks varied in shape and height greatly; this peak did not vary for the DOM from pore waters younger than 6,200 cal BP (Figure 2B). However, the peak of the high MW aggregates was negligible in comparison with that of the second fraction (~5–6% of the total area of all detected DOM).

For HS from deeper layers the peak shape varied showing a sub-shoulder (time period 10,300–3,600 cal BP). This indicated possible differences in the structure or composition of DOM and it was possible to resolve different groups of components chromatographically. The peak of HS from samples younger than 3,600 cal BP did not vary revealing a smooth distribution of molecules with similar MWs.

Figure 4A,B shows changes in DOM content downward through the sediment core. There was a significant decrease in the DOM content in pore waters: older pore waters (10,300–2,400 cal BP) contained DOM twice as much as those accumulated after 2,400 cal BP (an average DOC concentration 16.4 mg L^{-1} and 7.7 mg L^{-1}, respectively). Also, the DOM content in older pore waters varied greatly, whereas that in more recent sediments had a smooth decreasing trend with minimal variations. The reason might be that DOM in recent sediments consisted of molecules with aliphatic structures which did not absorb the UV-light. Aliphatic molecules are typical of autochthonous OM which is usually rich in lipids and polysaccharides. Thus, there is a possibility that UV-detection failed to detect this part of DOM. Additional loss-on-ignition (LOI) experiments showed that the OM content in the particulate phase increased after 2,400 cal BP (Publication II, Figure 1; data not presented here as this content was measured by another author). This may indicated the dominance of autochthonous OM with aliphatic structures in recent sediments. However, in the case of long sediment core pore waters the aromaticity index did not allow distinguishing OM sources as the index was generally stable downward through the core showing minimal variations (Figure 4F). The proportions of high and low MW fractions were distributed similarly downward through the sediment core (Figure 4C). In the oldest layers the proportion of the low MW fraction was slightly higher than in newer ones.

[†] cal BP = calibrated years before the present; ^{14}C dates calibrated to calendar years before the present, the time scale used with radiocarbon dating in archeology, geology, etc. As a zero point of the age scale, 1 January 1950 is used; e.g. 1500 BP means 1500 years before 1950, that is 450 AD.

The M_w of HS varied from 1,400 to 1,900 and M_n was about 1,000 (Figure 4D). The polydispersity of all the pore water samples was quite low (1.6–2.0) showing that DOM was relatively homogeneous (Figure 4E). There were no drastic changes in MW averages and polydispersity depending on the age of the sediments. However, in the oldest sediment layers (dated 10,300–7,200 cal BP) the MW averages were slightly higher than in other layers, being approximately 1,900 for M_w and 1,000 for M_n. After 7,200 cal BP the MW values decreased reaching minimum after 2,400 cal BP (M_w 1,500 and M_n 900). The values of MW distribution were in good agreement with the MWs reported for aquatic fulvic acids[10,70,71], lacustrine[63] and freshwater sediment pore waters[12]. Lake Peipsi is a large lake where sediment mixing due to turbulence and bioturbation significantly affect the deposition of original organic matter. Moreover, diagenetic processes in sediments should also be taken into account when interpreting the results. Resuspension of sediments in such large lakes is more intensive in comparison with smaller lakes with stagnant water, thus, changes in the original stucture of buried OM are greater. OM is subject to aerobic degradation in the upper sediment layers and undergoes anaerobic degradation in the deeper layers. Autochthonous organic material is usually more sensitive to biological degradation than terrestrially-derived OM[2]. Once components from aquatic sources are utilized by microbial activity, more resistant forms of OM (HS and lipids) remain. Thus, humic-like material dominates in the deeper sediments. Changes of the DOM content in Lake Peipsi pore waters may, in addition to the depositional history, reflect also diagenetic processes.

However, the agreement between environmental and conditional changes and the DOM characteristics was observed.

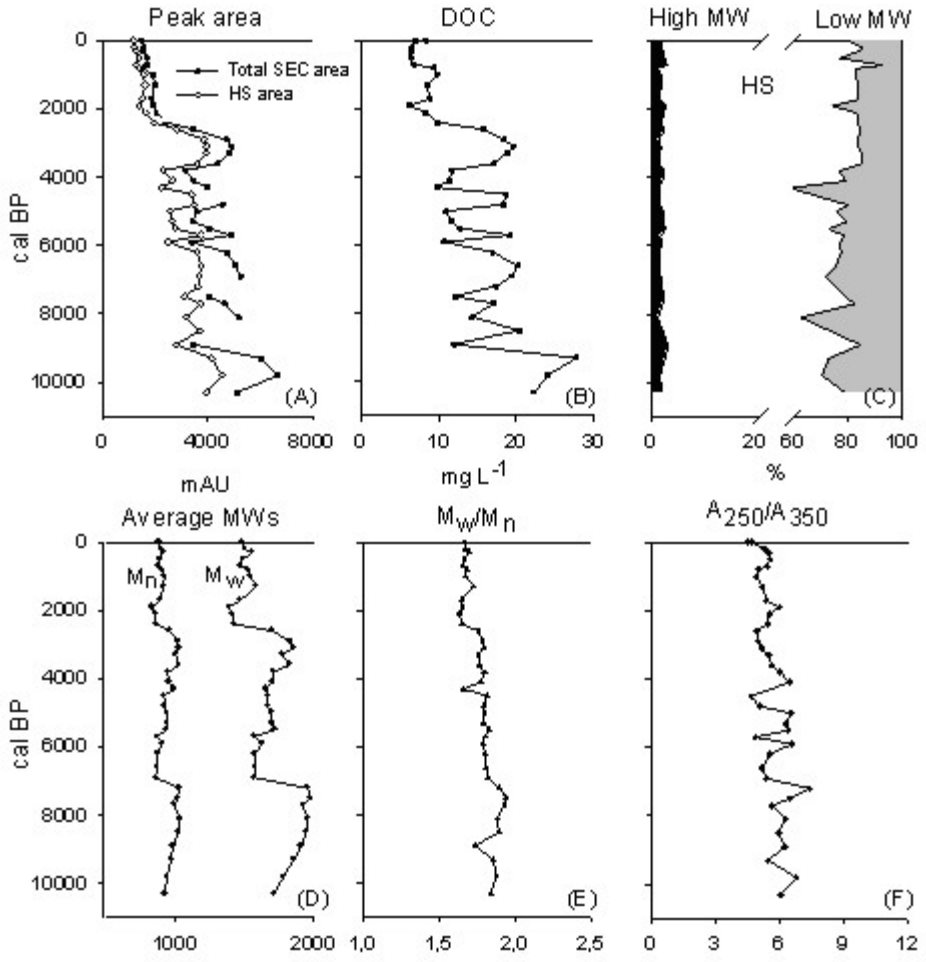

Figure 4. Age-resolved profiles of the characteristics of DOM from the long sediment core pore water of Lake Peipsi: (A) total HPSEC area, (B) DOC concentration, (C) proportions of high MW, HS and low MW fractions in the sediments, (D) number- and weight-average molecular weights, (E) polydispersity M_w/M_n, (F) aromaticity index A_{250}/A_{350}.

3.1.3.3 Temporal changes in Lake Peipsi during the Holocene as revealed by DOM variables

A 4-m sediment core represented ~10,400 years of OM accumulation. Changes in the content of UV-absorbing DOM, as well as of DOC, may be roughly devided into several periods (Figure 4A,B). The DOM content in the older sediment layers was twice higher than in the youngest sediment layers. LOI data for the particulate phase (Publication II, Figure 1; data not presented here as the LOI was measured by another author) was used for comparison of the variables of the dissolved phase.

According to earlier studies of Lake Peipsi, the beginning of the Holocene was associated with a low water level stand in the lake when it was 8 m lower than today[98,109,110,111]. Therefore, the highest pore water DOM contents in the oldest sediment layers (the Early Holocene, 10,400–9,000 cal BP) were attributed to a progressively deepening water body. During the water level rise OM-rich gyttja accumulated onto the bottom. The elution profiles of the pore water DOM from the samples of the oldest sediments varied greatly resulting in a very unstable high MW aggregate and partially resolved HS components with different degrees of resolution (Figure 2B). The polydispersity and aromaticity index A_{250}/A_{360} varied as well (Figure 4E,F), whereas M_w and M_n values were constant (Figure 4C). These strong variations may reflect quickly changing conditions in the lake during the water level rise and, hence, a continuous input of different types of OM. As the lake water level changed rapidly, forcing the coastline to retreat, large areas of coastal soils, terrestrial plants and marginal peatlands were eventually flooded, abraded and transported to the basin. Thus, land-derived OM would provide a source of sediment OM. On the other hand, the increased release of nutrients from the intensively abraded and drowned shoreline, combined with an increased run-off to the lake, possibly brought abundant dissolved nutrients to the lake and stimulated higher rates of primary in-lake production. Average MW values of those samples were slightly higher than those in the other pore water samples (M_w ~1,900 and M_n ~1,000). This indicated the probable dominance of terrigeneous OM. However, it was not possible to distinguish the source of OM by the aromaticity index A_{250}/A_{360} as the latter was mostly the same. Thus, in the early stages of lake formation both the land-derived influx and in-lake bioproductivity were responsible for OM accumulation.

LOI data showed a continuous increasing trend in OM percentage during 10,400–7,400 cal BP supporting the hypothesis about a rapid water level rise (Publication II, Figure 1; data not presented here as the LOI was measured by another author). After 7,400 cal BP the content of the particulate OM was mainly constant, negligible variations were mostly due to minor gradual oscillations, additionally indicating relatively uniform environmental conditions. This probably implies that approximately at 7,000 cal BP Lake Peipsi attained the size and morphology comparable with those of today.

However, the structure and content of DOM were not constant yet. The HPSEC elution profiles of pore waters varied greatly until 3,800 cal BP, especially in the MW region lower than 800. As the lake system had stabilized by that time, the changes in DOM content and structure were most probably related to the lake's inner processes like changing bioproduction. Also, climatic conditions warmer than today could have significantly influenced in-lake production. During the period between 9,000 and 5,000 cal BP, called the Holocene thermal maximum, mean annual temperature in Estonia was 2.5 ºC higher than today[112]. Warmer and more humid climatic conditions could result in an elevated bioproduction in the lake. Leeben *et al*. found that the OM accumulated in Lake Peipsi between 7,200 and 3,000 cal BP consisted mainly of land-derived material which was transported to the lake as a result of erosion[72]. The A_{250}/A_{360} values varied significantly until ~4,000 cal BP as shown in our study, so, we suggest that the accumulation of both autochthonous and allochthonous matter was dependent on the particular period and climatic conditions, though allochthonous OM dominated in all the periods. Great variations in the HPSEC elution profiles of DOM and, hence, in composition is most probably related to the continually changing type of OM in the sediments.

The HPSEC elution profiles of DOM from younger sediments (accumulated after 3,600 cal BP) were all stable and similar. Hence, the composition of OM stabilized, or at least was represented by a more uniform organic material. However, the content of UV-absorbing DOM decreased sharply after 2,400 cal BP. The pore waters from older sediment layers contained DOC twice as much as those from younger sediments. Leeben *et al.* also observed a decreasing trend in the fluorescence intensity of pore waters of Lake Peipsi at 3,000 cal BP[72]. The proportion of OM in the particulate phase increased in the same period. These changes coincided temporally with the beginning of human activity around Lake Peipsi where the earliest traces of cereal farming date back to the Late Bronze Age at 3,000–2,500 cal BP and the overall formation of the agrarian landscape in Estonia took place during the Iron Age from 2,500 cal BP[113]. Extensive agricultural land use led to a gradual deforestation of the area. The opening of the catchment resulted in increased intensities of erosion and produced an outbreak of soil-derived nutrients to the basin subsequently stimulating an increase in lake bioproductivity. Later, manuring of fields obviously added nutrients to the lake catchment stimulating in-lake production even more. Fluorescence indicators clearly showed the predomination of biomass in the lake at approximately 2,500 cal BP[72]. Despite that the aromaticity index did not show any tendency towards elevation which would indicate the domination of autochthonous DOM, a decrease in the UV-absorbing OM content in the dissolved phase and a simultaneous increase in the OM content of the particulate phase is mostly attributed to the high concentration of the non-UV-absorbing material. Due to the presence of large amounts of polysaccharides and lipids with aliphatic character in the microbially derived autochthonous OM, the DOM content in the younger sedimentary pore water was probably understimated by

this method. Analysis of fossil pigments from the short sediment core also revealed the recent eutrophication of the lake which led to an elevated microbially-derived OM input (Publication I, Analysis of fossil pigments).

HPSEC and spectroscopic characteristics of the reconstructed changes of pore water DOM were reasonably compatible with those obtained by traditional palaeolimnological methods (sediment diatom and plant macrofossil assemblages, ostracod assemblages), indicating different conditions in the lake (e.g., [73] for the short sediment core and for the long sediment core[98,110,112,113]). Also, fluorescence spectroscopic analysis of the sedimentary pore water of Lake Peipsi revealed that DOM underwent similar changes during the Holocene[72]. This confirmed the fact that the pore water DOM retained the evidence of conditional changes in the lake. Therefore, HPSEC and spectroscopic analyses of pore water DOM may supplement palaeolimnological research and give valuable information on the eutrophication history and other environmental changes in the lake ecosystem.

3.1.4 MW distribution of lacustrine DOM (Publications I and II)

A significant difference in MW distribution between the pore waters extracted from frozen and fresh sediments was observed. The MWs of the pore waters from frozen sediments varied greatly and were several times higher than those from unfrozen sediments. The MW distributions in DOM from frozen sediments covered the range of from 3,000 to 15,000 for M_w and from 1,000 to 6,000 for M_n, whereas for the fresh sedimentary DOM it was only between 1,400–1,900 for M_w and between 900–1,000 for M_n (Figures 3C,D and 4D). The MW values of the frozen sedimentary DOM varied greatly and even chaotically (especially in the upper sediment layers at 1–19 cm of; the whole sediment core depth was 40 cm) in comparison with those of the fresh sedimentary DOM, which were quite stable downward through the core of 4 m. Apparently, the freezing and subsequent thawing of OM in aqueous solution disintegrated the particulate OM and disrupted dissolved organic molecules. It was previously reported that the freeze-preservation of freshwater sediments altered greatly the properties of OM (an increment in DOC concentration, absorbance and fluorescence once the samples were defrozen) and was therefore not applicable to the analysis of pore water DOC[6]. Therefore, the MW distributions of pore water DOM from the short core may not reflect the "real" MWs of aquatic HS. MWs up to 1,000 for freshwater DOM were reported by many authors (e.g. [10,47,48,63,71,104]). We expect to have MWs in this range and MW values of over 3,000 would be most likely overestimated. The MWs of DOM from fresh sediments were in good agreement with those published in the literature. Despite the disturbance of DOM properties during freezing and thawing, trends in DOM content and spectroscopic properties seemed to be still reflected by the properties of OM originally preserved in the sediments. This was confirmed by a synchronous matching of DOM characteristics with the data from sediment diatom assemblages[73]. It may

be possible if the properties of DOM changed in all samples simultaneously and proportionally. However, our findings also confirmed that freeze-preservation affected the properties of DOM, such as increment in MWs.

The MW of UV-absorbing DOM from the long sediment core did not vary downward through the core. Thus, MWs and properties of this DOM fraction were not dependent on the sediment depth and age of OM. Critical factors which may distort an adequate determination of MWs of aquatic DOM are the choice of calibration standards and a bias provided by UV detection techniques. The MWs of pore water from fresh sediments were in agreement with those published earlier for aquatic DOM. The present study revealed that calibration of the HPSEC column with protein standards did not lead to the overestimation of MWs of the DOM extracted from fresh sediments. The wavelengths chosen for the detection in this study (254 and 280 nm) were suitable for DOM detection[48]. However, detection at these wavelengths provided a bias in favor of components which had higher molar absorptivity (ε). These components are usually humic and fulvic acids whose MW is higher than that of other possible DOM constituents of usually low MW (e.g. amino acids)[49]. Thus, the MWs calculated from 254 and 280 nm wavelengths were attributable to only those molecules which had a higher ε at the chosen wavelengths.

The MW distributions of the pore water DOM extracted from fresh sediments (a long core) were also in good agreement with the reported molecular masses of aquatic DOM and fulvic acids measured by various MS modes [33,36,37,38,39]. Therefore, it may be concluded that the applied HPSEC method provided adequate MW distributions and can be used for the analysis of DOM from lacustrine pore waters.

3.2 Adsorption of PAs onto soil

3.2.1 Extraction of PAs from soil (Publication III)

The simplicity and convenience of an analytical procedure are crucial for an on-site analysis of nerve agents degradation products. A qualitative determination of CWAs in the field is a top priority task. Therefore, the procedure for extraction and determination of CWA degradation products (which are PAs) should be as convenient, rapid and simple as possible. For PAs analysis in the field a portable CE-CCD instrument with worked out protocol was used[106]. When developing an on-site procedure for extraction of PAs from soil matrices, both adsorption of PAs onto soil particles as well as extraction of all other possible substances from soil should be taken into account. The on-site water extraction procedure for PAs is described in section 2.2.2.2. Apart from PAs a numerous amount of all possible substances was extracted from soil (Figure 5). Therefore, blank soil extracts were examined to reveal all unknown peaks belonging to soil constituents. Comparison of the peaks of soil blanks and soil with those of PAs

extracts revealed that several peaks were derived from the soil matrix. However, the peaks of soil constituents did not overlap with those of PAs making the identification of each PA possible. Most of the soil components migrated slower than PAs (Figure 5A). Some compounds migrated within the range of the migration time of PAs but, they did not disturb the separation. PAs were well separated and migrated in the descending order of their molecule size. Soil components were probably smaller or more charged anions. They might be inorganic anions, such as nitrate and sulfate, or organic residues like aliphatic mono-, di- and tricarboxylates, or other low molecular mass organic components[114]. Soil constituents were not identified as it was not the aim of the study. Water extraction was found to be suitable for a qualitative analysis of PAs on-site. However, the recoveries of the PAs extracted by the developed procedure were very low. If no adsorption occurs, the concentration of PAs (2 mM PAs solution sprayed on the ground) after the extraction with 5 mL of water should be 400 μM. However, the recovery was generally less than 10 %. To enhance the recovery by breaking the adsorption of PAs onto the soil, NaOH could be used. However, the sample solution in even 0.01 M NaOH was unsuitable for CE-CCD analysis because NaOH disturbed the CCD signal significantly due to an enhanced conductivity of the solution. For a quantitative analysis of PAs, a more thorough and, thus, more complicated extraction procedure is needed. This requires several extraction steps and more sophisticated instrumentation (e.g. [85,86,89,91,92,93]), which makes the determination of PAs more difficult and inconvenient for on-site analysis.

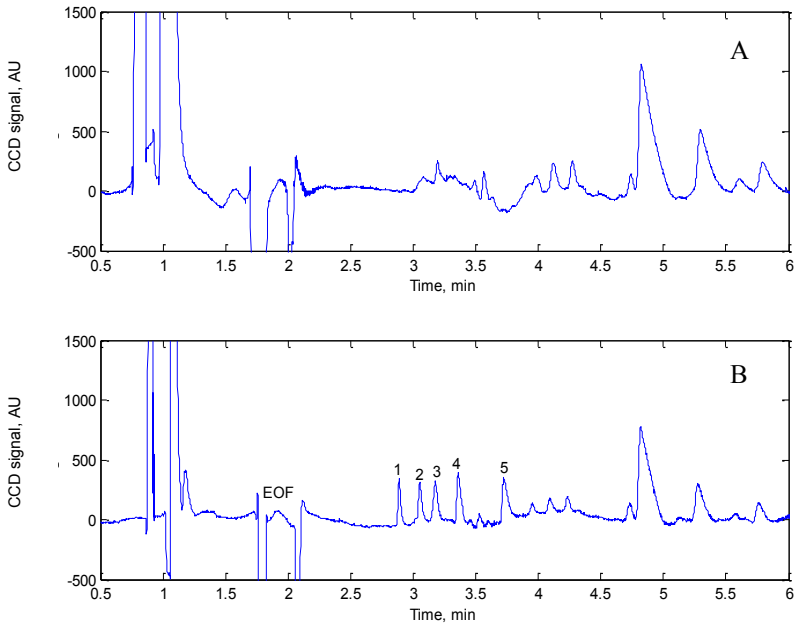

Figure 5. Electropherograms of soil extracts. Samples analysed with a portable CE-CCD instrument, sample injected with a cross-sampler. (A) blank sample, pure soil extract without any standards, (B) PAs extracted from soil. 1 – PMPA, 2 – 1-BPA, 3 – PPA, 4 – EPA, 5 – MPA, EOF – electroosmotic flow. BGE – 15 mM Mes/His, separation voltage – 16 kV, injection volume introduced to the injection device – 0.2 mL. AU – arbitrary units.

3.2.2 Adsorption of PAs onto sand and loam (Publication IV)

To study the adsorption of PAs onto soils depending on soil particle size two types of soils were used, *i.e.* sand and loamy soil. First, possible unknown substances derived from soil during extraction should be separated from PAs. Otherwise, estimation of the degree of adsorption of the acids cannot be made if soil peaks overlap with the peaks of PAs. The water extraction procedure used for adsorption studies in the laboratory differed from that employed on-site. So, in the laboratory, water extracts were centrifuged. Comparison of blank soil extracts with those of soil spiked with PAs revealed two unknown peaks from soil (Figure 6). These peaks were observed in all fractions of sand and loam samples. They were similar to the peaks found in soil extracts on-site (Figure 5A; peaks observed after 4th minute). The unknown compounds migrated slower

than PAs allowing the study of adsorption by CE. The unknown soil constituents were not identified, because it was not the goal of this research.

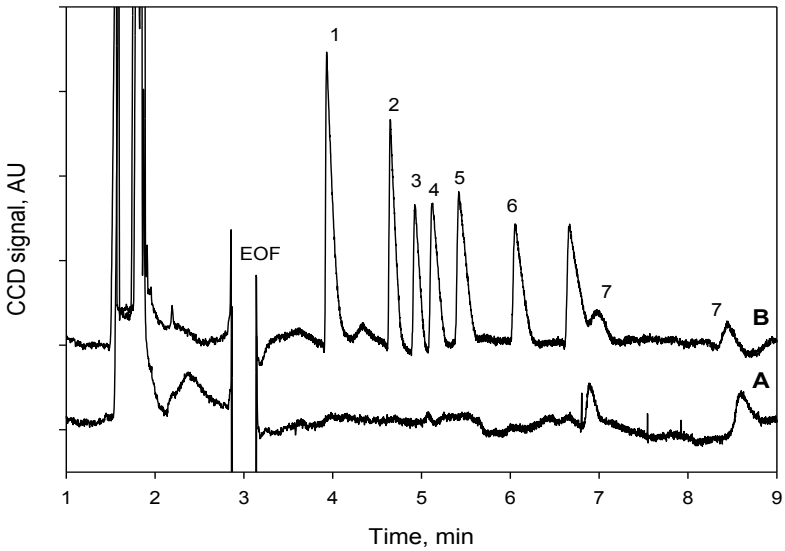

Figure 6. CE separation of blank soil extracts and phosphonic acids: (A) Blank soil extract from sand, size fraction 100–200 μm; (B) extract of 100 μM PAs from sand, size fraction 100–200 μm. EOF – electroosmotic flow; 1 – AEDHP, 2 – PMPA, 3 – 1-BPA, 4 – PPA, 5 – EPA, 6 – MPA, 7 – unknown peaks. AU – arbitrary units.

Performance data for the CE-CCD system are given in Table 5. To maximally improve the performance, an internal standard AEDHP was added to the sample solution. For the system performance data and calibration, ratios between the peak areas of PAs and AEDHP were used. The reproducibility of the system was good, RSD for peak areas varied from 2.6 to 6.9 %. LODs for PAs were in the range of from 1.2 to 7.6 μM. The calibration curves constructed in the range of 10–200 μM demonstrated very good linearity ($R^2 > 0.99$ for all PAs).

Table 5. CE-CCD system performance data for phosphonic acids

	RSD of peak areas (%)[a]	LOD (μM)[b]
PMPA	5.7	7.6
1-BPA	3.5	6.0
PPA	4.2	5.5
EPA	2.6	5.8
MPA	6.9	1.2

[a] The relative standard deviation of peak areas calculated from at least three independent runs
[b] The limit of detection calculated by interpolating calibration curves

3.2.3 Adsorption isotherms of PAs

For adsorption experiments three fractions of soil samples with different particle sizes (<100, 100–200, and 200–400 μm) were spiked with six different concentrations of the PAs mixture. If no adsorption of PAs onto soil had taken place, the concentrations would have been 25, 50, 75, 100, 150, and 200 μM. To estimate the degree of adsorption and construct adsorption isotherms the difference in the added and measured concentrations between PAs was used. Four adsorption isotherms (Langmuir, Freundlich, Redlich-Peterson, and BET) were compared in order to find the one describing closest the performance of real soil. The parameters for the Langmuir (Equation 14) and Freundlich (Equation 15) isotherms were calculated using a least square method in a linearised form. The parameters for the Redlich-Peterson (Equation 17) and BET (Equation 18) isotherms were determined using a nonlinear trial and error procedure using the Solver add-in in Microsoft Excel. The parameters of isotherms are presented in Appendix II.

The Langmuir, Freundlich and Redlich-Peterson isotherms followed a very similar path in the measured concentration region. The Langmuir isotherm levelled out at q_{max} (maximum adsorption on the monolayer) when extrapolated to higher concentrations, while the two other isotherms continued growing. The type of the isotherm fitting best to the adsorption of PAs onto different soil types and fractions varied from sample to sample but were generally all similar. Therefore, the performance of the Langmuir isotherm was considered to be no worse than that of Freundlich and Redlich-Peterson isotherms and its parameter q_{max} could be used for comparison of the adsorptive capacity of soils in this study. Thus, the adsorption of PAs onto sand and loamy soil was described by a simple Langmuir-type curve.

The results demonstrated that the adsorption capacity of sand was higher than that of loam. Smaller sand fractions had a higher adsorption capacity than its largest fraction. However, the difference in adsorption capacity between the finest fractions of sand and loamy soil (<100 μm) was not so significant as in the case of their largest fractions (200–400 μm). The results for PAs adsorption in different soil types may be illustrated on an example of EPA (Figure 7). In sand samples, the adsorption was lowest in the medium-sized fraction (100–200 μm) and highest in the finest fraction (<100 μm). In loamy soil samples, the adsorption behavior was similar in smaller fractions (<100 μm and 100–200 μm), while in the largest fraction (200–400 μm) the adsorption was significantly higher.

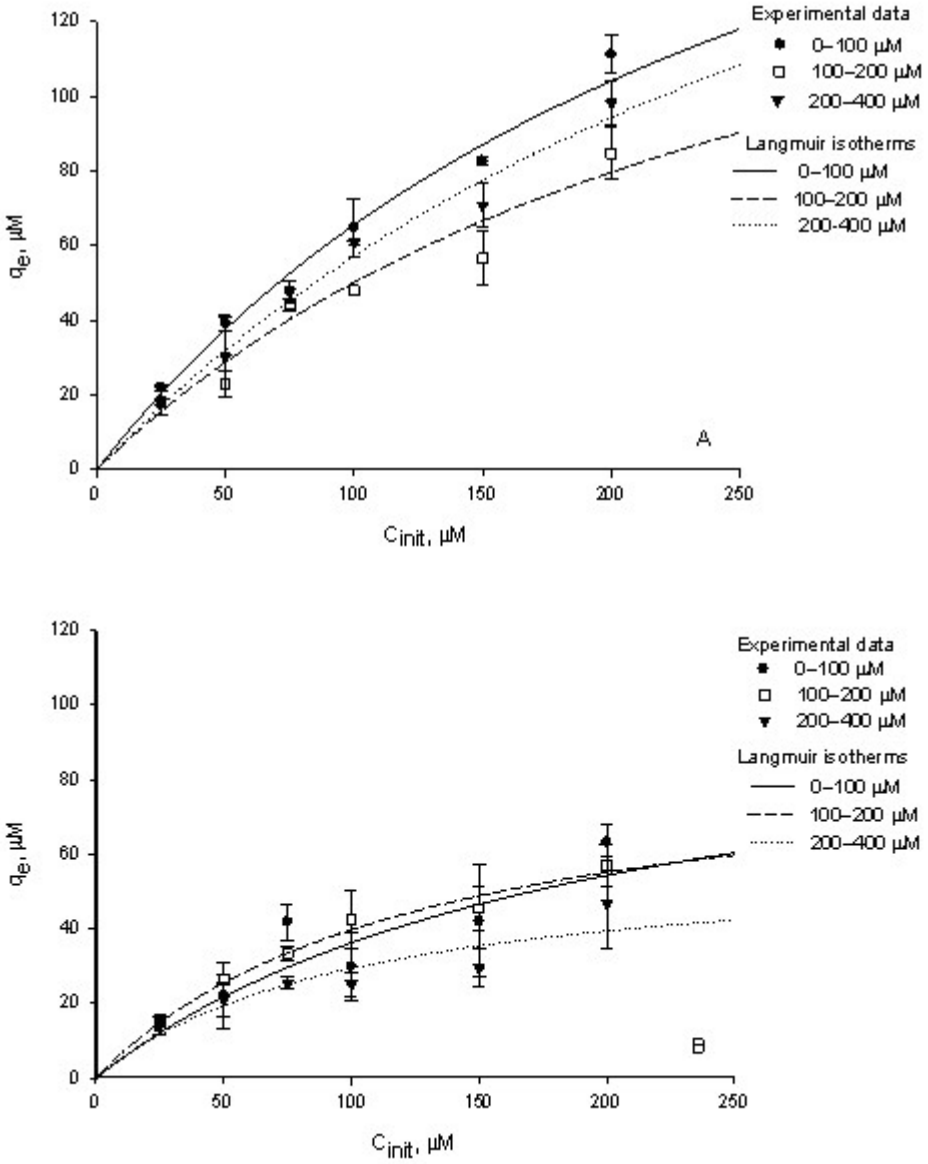

Figure 7. Langmuir adsorption isotherms of EPA in different soil fractions: (A) sand samples, (B) loamy soil samples. C_{init} – initial concentration, q_e – adsorbed amount.

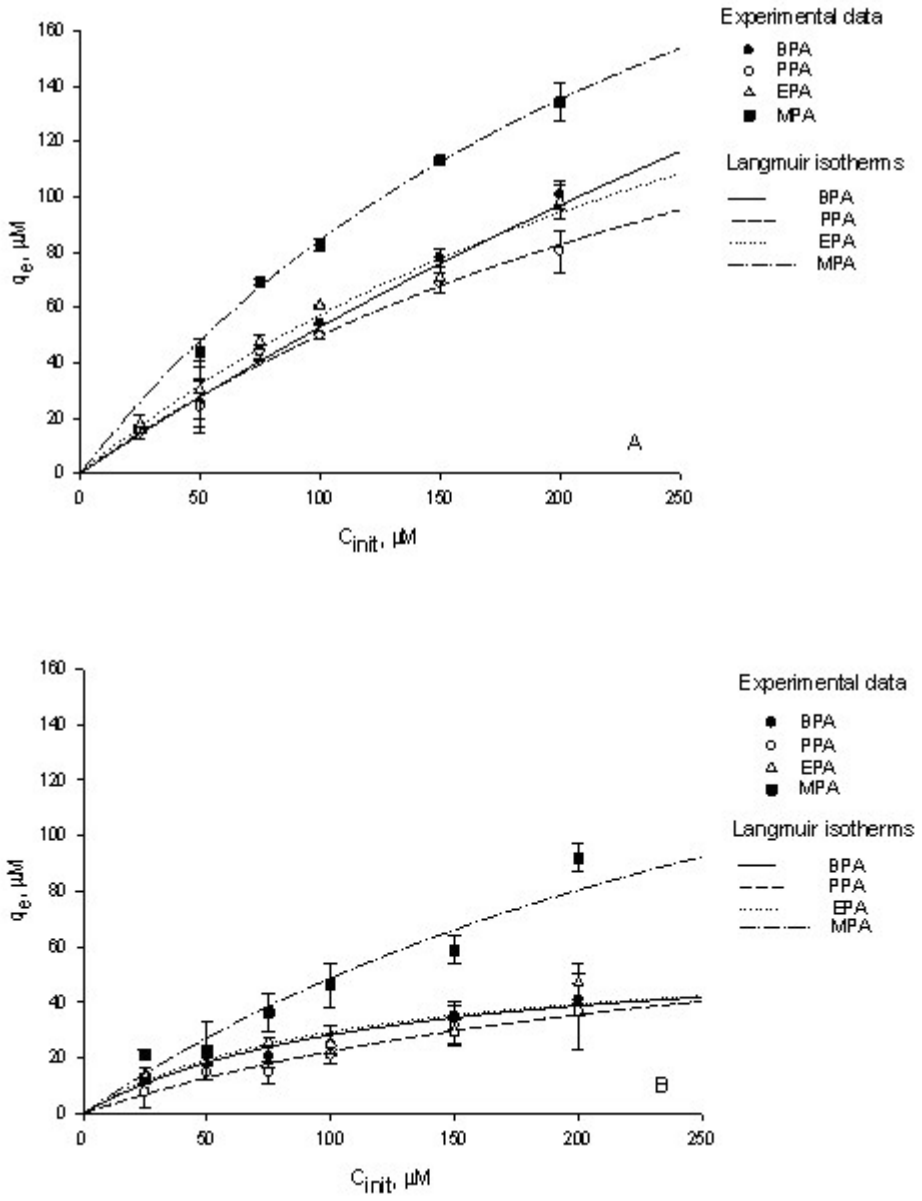

Figure 8. Langmuir adsorption isotherms of different PAs in the largest fraction (200–400 µm): (A) sand sample; (B) loamy soil sample.

The adsorption of PAs in the fractions of sand and loamy soil of different sizes was similar. Figure 8 demonstrates Langmuir adsorption isotherms in the largest sized fraction of sand and loam (200–400 µm). In both samples the adsorption was the highest in the case of MPA. This result confirmed previous

studies on PAs analysis in soils which revealed low recoveries of extracted phosphonates from environmental matrices[85,86]. Moreover, this study also revealed that MPA demonstrated the highest adsorpion resulting in the lowest recoveries during aqueous extraction[87]. This may be accounted for by the size of the phosphonic acids. MPA has the lowest molar mass and, thus, the size. Therefore, more MPA molecules might occupy adsorption sites on the soil particle surface. BPA, PPA, EPA exhibited a similar adsorption behavior in both sand (Figure 8A) and loamy soil samples (Figure 8B)[‡]. The Langmuir adsorption isotherm demonstrated that in the case of the loamy soil sample constant adsorption values were achieved at an analyte concentration of 200 μM, while in the case of the sand sample continuous adsorption was still observed. Thus, it was established that the adsorption of BPA, PPA, EPA, and MPA was more noticeable in sand samples than in the loamy soil ones.

The BET isotherm was used as an alternative to describe the adsorption behavior of PAs in soils. In contrast to the Langmuir adsorption mechanism, the BET isotherm assumes that adsorption takes place in several layers. However, in the present study it was not possible to evaluate which model – Langmuir or BET – was more suitable as the fitting both isotherms to experimental data was almost similar. Both isotherms followed the same path up to the point where the second layer of PAs started to form in the BET isotherm, or the Langmuir isotherm started to level out as the monolayer around the soil particle was filling up (Figure 9). Though the parameter q_{max} in the BET isotherm represents the PAs concentration corresponding to a complete monolayer adsorption, which is the same as for the Langmuir isotherm, these two models describe two different adsorption mechanisms. The q_{max} values of the Langmuir isotherm were 3–10 times higher than those of the BET isotherm (Appendix II). In the PAs concentration range under study the adsorption mechanism was the same because the molecular structures of analytes were very similar. Therefore, it was unlikely that the q_{max} values of one particular PA could vary so much. Hence, the adsorption isotherms of the studied PAs should be either Langmuir's or BET's.

[‡] Experiments for PMPA were not successful, therefore, the results were not presented.

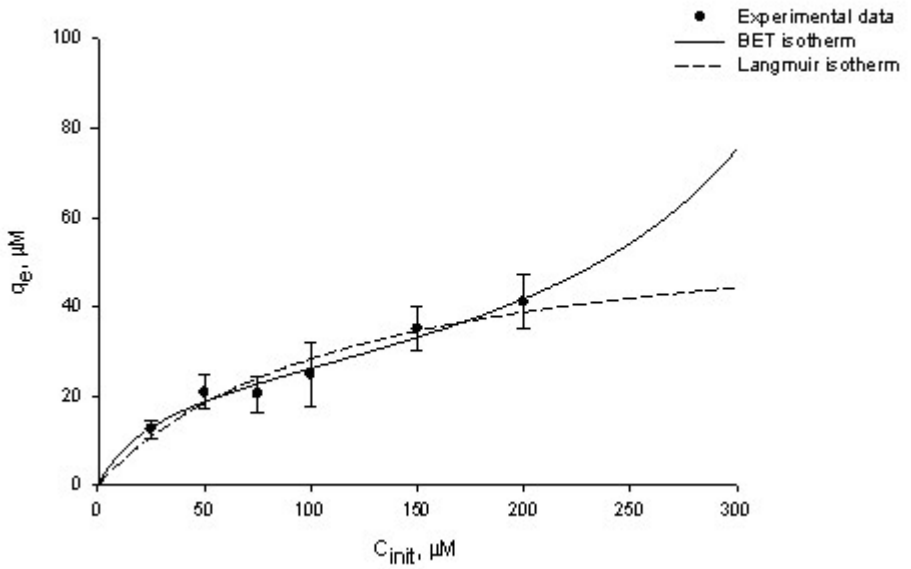

Figure 9. Comparison of Langmuir and BET isotherms in the adsorption of phosphonic acids. Example – BPA in the largest fraction of the loamy soil sample (200–400 µm).

However, the studied concentration range of PAs was too narrow to establish the mechanism according to which adsorption most likely takes place because the measured data were in the range of where only the first monolayer was filled up. Therefore it was not possible to evaluate adsorption at higher concentrations and predict either if PAs adsorption would proceed in one monolayer or several ones. In the BGE system used it was not possible to measure higher concentrations of 5 PAs in a mixture because the separation at higher concentrations was not sufficient.

The CE method with CCD employed in this work allowed fast (less than 6 min) and efficient separation of PAs, as well as evaluation of their adsorption onto sand and loamy soil in the concentration range of 25–200 µM. Minimal or even nonexistent interference from the sample matrix background was observed as the major sand and soil constituents migrated slower than PAs. Although low recoveries of PAs from soil matrices were observed by the authors, no thorough adsorption studies have been done so far. The PAs adsorption in the concentration range of 25–200 µM could be described by a simple Langmuir-type curve.

CONCLUSIONS

The present study demonstrated possibilities of applying HPSEC and CE methods to the analysis of organic molecules in sedimentary pore water and soil extracts, respectively. The general aim of the research was to study the characteristics and properties of these environmental matrices depending on the size of natural matter constituents. The sedimentary pore water DOM was characterized by HPSEC. Phosphonic acids in soil extracts were analysed by a portable CE-CCD instrument. Soil fractions with different particle sizes were studied by CE in respect to the adsorption properties of PAs.
The results of the present thesis can be summarized as follows:

- HPSEC and spectroscopic methods enabled profiling the characteristics of the sedimentary pore water DOM downward through the sediment. The UV detection used in HPSEC limits the amounts of detected organic material, indicating only the content of the DOM fraction (mostly humic) which absorbs UV light. Thus, this method may underestimate the overall content of DOM, but it is suitable for estimation of the content of the humic fraction in pore waters and evaluation of the changes in DOM composition depending on molecular weight. Calibration of the HPSEC column with protein standards has been found to be suitable for the evaluation of molecular weights of DOM. The HPSEC method is non-destructive and for analysis only minimal pore water sample pretreatment is required, providing minimal DOM disturbance or even avoiding it. The spectroscopic analysis supplements the HPSEC analysis and provides information about the origin of DOM.
- The HPSEC method enabled fingerprinting the characteristics of the pore water DOM from Lake Peipsi from two well-dated sediment cores. The age-resolved profiles of pore water DOM characteristics covered 120 and 10,000 years of sediment accumulation. The HPSEC approach was used to evaluate the distribution of UV-absorbing DOM content (computed as a total SEC area), molecular weights, and polydispersity downward through the sediment cores.
- The age-resolved HPSEC and spectroscopic characteristics of DOM demonstrated the dependence of the latter on environmental conditions in Lake Peipsi (lake development, climatic changes, human impacts, eutrophication). DOM characteristics distributions were in agreement with the results of traditional palaeolimnological methods (e.g. sediment diatom assembleges, ostracod assemblages). Thus, DOM characterization by HPSEC with spectroscopic approach can supplement palaeolimnological research and provide valuable information about the changes in DOM composition, origin, and properties.
- Sediment storage conditions affected the characteristics of pore water DOM. Freeze-preservation and subsequent thawing of sediments

resulted in a 2- to 15-fold increase in the MW of the pore water DOM in comparison with that of the pore water DOM from fresh sediments. However, despite that, the content and spectroscopic parameters of UV-absorbing DOM still demonstrated the dependence of DOM characteristics on the environmental conditions in the lake (e.g., DOM characteristics reflected the eutrophication of Lake Peipsi).

- The MWs of pore water DOM from Lake Peipsi did not change downward through the sediment core even at a depth of several meters. The MW values of pore water DOM from fresh sediments were characteristic to aquatic fulvic acids and lacustrine sediment pore water DOM. Also, these were in good agreement with the values obtained by various MS studies of aquatic DOM and fulvic acids.
- A rapid and convenient procedure was developed for the water extraction of phosphonic acids from soil. After the extraction PAs were analysed by a portable CE-CCD instrument.
- The CE-CCD method was successfully used for the investigation of adsorption of 25–200 μM PAs onto sand and loamy soil. The size of sand and loamy soil particles affected the adsorption of PAs but no dependence of adsorption on particle size was observed. For sand the highest adsorption rate was observed for the fraction with a particle size < 100 μm and the lowest for the fraction with a particle size of 100–200 μm. In the case of loamy soil, the adsorption was the highest for 200–400 μm particles, whereas for smaller sized fractions no significant differences were observed.
- The adsorption of PAs onto sand and loamy soil could be modelled by the Langmuir-type curve. However, the concentration range of PAs studied (25–200 μM) was too narrow to predict the adsorption mechanism precisely. At those concentrations only the first monolayer of PAs was filled up, therefore, it was not possible to evaluate if adsorption would take place in one monolayer (described by the Langmuir isotherm) or in several layers (described by the BET isotherm).

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APPENDIX I

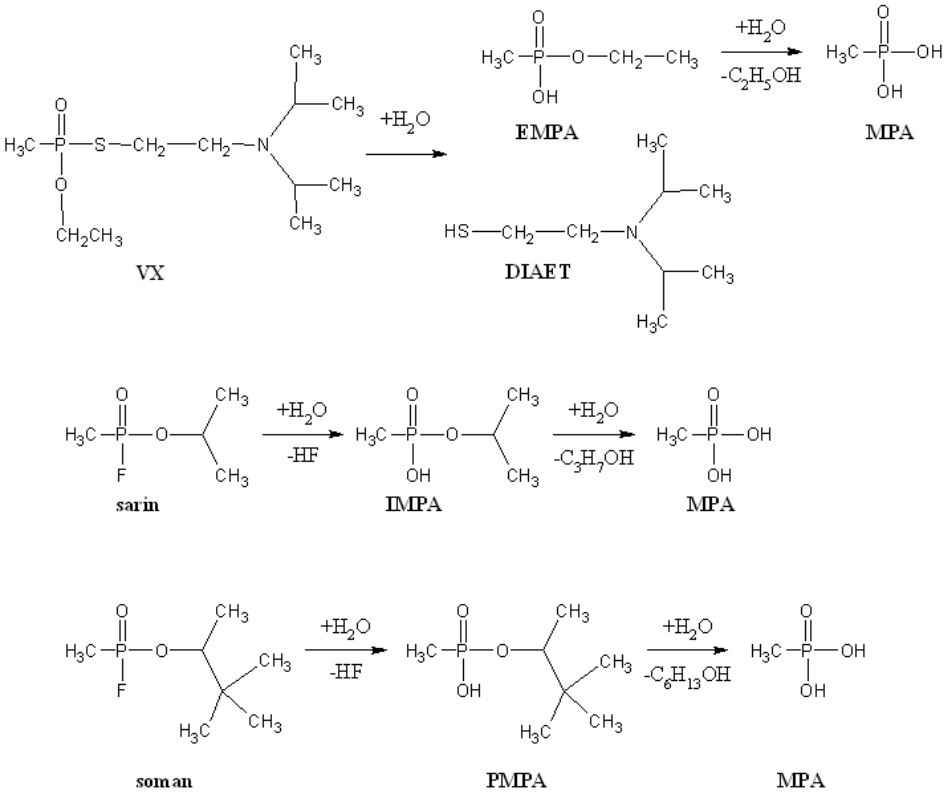

Figure. Hydrolysis of VX, sarin and soman

APPENDIX II

Table. Parameteres of various adsorption isotherms for sand and loamy soil samples

Sand		0–100 µm				100–200 µm				200–400 µm			
		BPA	PPA	EPA	MPA	BPA	PPA	EPA	MPA	BPA	PPA	EPA	MPA
Langmuir	q_{max}	238.3	205.5	252.8	495.2	323.6	62.2	193.4	282.6	581.4	241.7	268.9	339.9
	K_a	0.0036	0.0042	0.0035	0.0022	0.0017	0.0162	0.0035	0.0038	0.001	0.0026	0.0027	0.0033
	r^2	0.9681	0.937	0.9808	0.9837	0.8856	0.9191	0.9376	0.9735	0.9971	0.9867	0.9804	0.9967
Freundlich	n_F	1.9377	2.1648	1.9888	2.0327	1.1653	3.983	1.5924	2.6812	0.8138	1.0074	1.3423	2.1561
	K_F	1.3332	1.4033	1.3302	1.2281	1.2679	2.071	1.3598	1.3877	1.0992	1.1915	1.235	1.2702
	r^2	0.9795	0.9494	0.9912	0.9902	0.8872	0.8938	0.9474	0.9842	0.997	0.9728	0.98	0.9914
Redlich-Peterson	K_R	0.3013	0.3362	0.2941	0.2398	0.2076	0.5458	0.3169	0.3306	0.1360	0.2126	0.2426	0.2657
	a_R	3.9656	4.4092	3.9590	4.2556	1.8591	8.1942	3.3096	5.5674	1.6945	2.0340	2.7244	4.3811
	b_R	1.3516	1.3996	1.3419	1.2711	1.2307	1.7260	1.3729	1.3917	1.1457	1.2369	1.2746	1.3043
	r^2	0.9790	0.9491	0.9912	0.9903	0.8921	0.8970	0.9474	0.9838	0.9970	0.9749	0.9805	0.9924
BET	q_{max}	64.9	58.9	67.2	72.6	35.7	58.8	43.4	66.4	159.9	229.7	153.7	n/a[1]
	K_L	0.0024	0.0023	0.0023	0.0027	0.0032	0.0003	0.0026	0.0023	0.0010	0.0000	0.0007	n/a
	K_S	0.0170	0.0191	0.0167	0.0203	0.0248	0.0165	0.0229	0.0232	0.0041	0.0027	0.0051	n/a
	r^2	0.9862	0.9578	0.9939	0.9488	0.9545	0.9791	0.9634	0.9145	0.9959	0.9876	0.9814	n/a
Loam		Below 100 µm				100 to 200 µm				200 to 400 µm			
		BPA	PPA	EPA	MPA	BPA	PPA	EPA	MPA	BPA	PPA	EPA	MPA
Langmuir	q_{max}	167.7	331.8	108.7	435.3	125.2	199.1	90.0	368.7	61.6	86.6	59.9	229.4
	K_a	0.0028	0.0008	0.005	0.0014	0.0046	0.0013	0.0079	0.002	0.0085	0.0035	0.0096	0.0027
	r^2	0.9871	0.9673	0.8948	0.9805	0.8258	0.9584	0.9797	0.9886	0.9253	0.9643	0.8089	0.9244

Freundlich	n_F	1.0132	0.3921	1.834	1.1518	2.0919	0.3123	1.9376	0.9902	2.2886	0.7218	2.7946	1.7001
	K_F	1.2983	1.1093	1.5672	1.1988	1.6124	1.0709	1.5453	1.1162	1.8659	1.351	1.9951	1.3879
	r^2	0.991	0.9691	0.9171	0.9762	0.8534	0.9342	0.9544	0.9581	0.9583	0.9714	0.8584	0.919
Redlich-Peterson	K_R	0.2844	0.1899	0.4067	0.2224	0.4218	0.1001	0.3956	0.1502	0.4906	0.3117	0.5303	0.3165
	a_R	2.1268	0.9382	3.8715	2.4948	4.3833	0.6155	3.8379	1.9644	4.5442	1.4922	5.8916	3.5245
	b_R	1.3290	1.2091	1.5018	1.2491	1.5247	1.1052	1.4852	1.1621	1.6333	1.3658	1.6995	1.3723
	r^2	0.9911	0.9542	0.9181	0.9788	0.8545	0.9373	0.9582	0.9655	0.9590	0.9713	0.8571	0.9454
BET	q_{max}	40.6	79.4	21.8	45.7	23.4	n/a	n/a	n/a	24.3	37.6	19.5	27.6
	K_L	0.0023	0.0009	0.0033	0.0029	0.0033	n/a	n/a	n/a	0.0023	0.0013	0.0028	0.0034
	K_S	0.0163	0.0039	0.0631	0.0222	0.0586	n/a	n/a	n/a	0.0377	0.0100	0.0762	0.0590
	r^2	0.9949	0.9931	0.9960	0.9740	0.9821	n/a	n/a	n/a	0.9968	0.9965	0.9921	0.9359

[1] Not available, statistically best fitted isotherms do not correspond to real life expectations dut to the negative q_{max}, etc.

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ABSTRACT

Analysis of organic species in sediments and soil samples is an important task in environmental analytical chemistry. High-performance size exclusion chromatography (HPSEC) is a widely used method for the analysis of natural organic polymers in various environmental samples. This method is highly suitable for the characterization of dissolved organic matter (DOM) in natural waters. Capillary electrophoresis (CE) is a well-established method for the analysis of small charged organic molecules in different matrices, including environmental ones such as soil. Both of these methods require minimal sample pretreatment, which does not disturb the structure of analytes, and very low sample volumes, they are cheap, fast and easy to use.

The present PhD thesis has been devoted to the analysis of organic species in complex sediment and soil samples. Firstly, DOM from the sedimentary pore water of Lake Peipsi, Estonia, was characterized by HPSEC to reveal age-resolved changes in the lake and to evaluate the applicability of the method to palaeolimnological research. Secondly, the adsorption of phosphonic acids (PAs) onto sand and loamy soil was investigated using CE.

The pore water DOM from two well-dated sediment cores from Lake Peipsi was studied by HPSEC supported by spectroscopic analysis. The first sediment core was stored frozen, while the second one was stored fresh in cold in order to establish how storage conditions affected the characteristics of pore water DOM. The DOM content, molecular weights (MWs) and polydispersity were determined using HPSEC. The DOM concentration and aromaticity were obtained from spectroscopic measurements. The distribution of these characteristics was profiled downward through the sediment cores to reveal age-related changes covering two time periods – approximately 120 and 10,000 years of sediment accumulation. Comparison of pore water DOM characteristics from two sediment cores showed that the freeze-storage of sediments resulted in a significant increase and great variations in its MW values. MWs of DOM from fresh sediments remained quite stable downward through the core (4 m) showing only minor variations. The reconstructed changes in DOM characteristics were reasonably synchronous with the results of traditional palaeolimnological methods (literature data and simultaneous fossil pigments analysis), confirming that HPSEC combined with spectroscopic approach can add valuable information for palaeolimnological research. The analysis of the short sediment core DOM enabled tracking changes in the trophic state of Lake Peipsi and revealing an eutrophication period in the 1940s–late 1980s caused by anthropogenic impacts. Analysis of the long sediment core DOM allowed reconstructing DOM characteristics profiles related to natural baseline conditions during the Holocene affected mostly by lake system development, climatic conditions and, to some extent, human activity. The HPSEC method combined with spectroscopy may be used to obtain general information about changes in DOM characteristics downward through the sediment core

supplementing palaeolimnological data. However, it should be taken into account that the present approach detects only those DOM components that absorb UV-light (mostly, humic substances which compose the major part of DOM). Generally, this thesis was the first investigation of pore water DOM from Lake Peipsi. Moreover, in this study, pore water DOM from sediments covering 10,000 years of accumulation was analysed for the first time ever.

The CE method is a suitable technique for the separation and investigation of adsorption of 25–200 μM PAs in soil matrices. The adsorption of most common PAs was analysed in sand and loamy soil fractions of different particle size (< 100 μm, 100–200 μm, and 200–400 μm). In case of sand, the finest particles (< 100 μm) adsorbed the PAs to the highest extent, whereas the adsorption was the weakest for the medium-sized fraction (100–200 μm). For loamy soil, the highest adsorption took place in 200–400 μm fraction and two finer fractions behaved similarly. PAs demonstrated similar adsorption behaviour in fractions of all particle sizes. Methylphosphonic acid demonstrated the highest adsorption. The adsorption of PAs can be modelled by a simple Langmuir-like curve. However, CE allowed analysis of PAs in a narrow concentration range only. Therefore, it was not possible to evaluate their precise adsorption mechanism.

KOKKUVÕTE

Keskkonnaanalüütilises keemias on tähtsaks ülesandeks orgaaniliste ainete analüüs settes ja mullas. Eksklusioon kromatograafia (ingl. *high-performance size exclusion chromatography HPSEC*) on levinud meetod orgaaniliste polümeeride analüüsiks erinevates looduslikes proovides. See meetod sobib hästi lahustunud orgaanilise aine põhjalikuks iseloomustamiseks looduslikes vetes. Kapillaarelektroforees (ingl. *capillary electrophoresis*) sobib väikeste laetud orgaaniliste ainete analüüsiks erinevates keskkonnamaatriksites, sellistes, nagu muld. Mõlema meetodi peamised eelised on see, et proovi ettevalmistus analüüsi jaoks on minimaalne, selle käigus uuritavate ainete struktuur ei muutu, vajalik proovimaht on väga väike, analüüsid on odavad, kiired ja lihtsad.

Antud doktoritöös uuriti orgaanilisi ühendeid settes ja mullas. Esiteks, HPSEC meetodi abil iseloomustati lahustunud orgaanilist ainet Peipsi järve settes, et näidata selle ajalisi muutusi setteprofiilis. Samuti, hinnati HPSEC meetodi sobivust paleolimnoloogilisteks uuringuteks. Teiseks, CE abil uuriti fosfoonhapete adsorptsiooni liivas ja savimullas.

Poorivee lahustunud orgaanilist ainet kahest dateeritud Peipsi järve settekernist analüüsiti kromatograafiliselt ja spektroskoopiliselt. Pärast proovivõtmist esimene settekern külmutati ja teine säilitati värskena külmas, et uurida, kuidas säilitamistingimused mõjuvad lahustunud orgaanilise aine karakteristikutele. HPSEC-i abil määrati kaudselt lahustunud orgaanilise aine sisaldus, molekulmassid ning nende jaotus ja polüdisperssus, spektroskoopiliselt leiti lahustunud orgaanilise süsiniku kontsentratsioon ning aromaatsus. Saadud karakteristikud kajastasid setteprofiilis lahustunud orgaanilise aine ajalisi muutusi järves kahe perioodi jooksul – viimase 120 ja 10,000 aasta jooksul. Leitud parameetrite võrdlusest kahe kerni vahel leiti, et lahustunud orgaanilise aine molekulmassid külmutatud kernist olid mitu korda suuremad ja nad oluliselt varieerusid setteprofiilis võrreldes külmutamata kerniga. Poorivee lahustunud orgaanilise aine molekulmassid ei muutunud palju terve setteprofiili ulatuses (4 m), kõikudes väga vähe. Poorivee HPSEC ja spektroskoopiliste karakteristikute muutused langesid kokku teiste näitajatega, mis olid määratud traditsiooniliste paleolimnoloogiliste meetoditega (võrreldud kirjanduse andmete ja fossiilpigmentide analüüsiga). Seega poorivee lahustunud orgaanilise aine HPSEC analüüs koos spektroskoopiaga võib anda kasulikku informatsiooni, mida saab kasutada palaeolimnoloogilistes uuringutes. Lühikese kerni lahustunud orgaanilise aine analüüsist oli võimalik kinnitada Peipsi järve eutrofeerumist 1940–1980 aastatel, mis oli põhjustatud inimtegevusest järve valgalal. Pika kerni lahustunud orgaanilise aine karakteristikute põhjal rekonstrueeriti muutused, mis olid seotud loomulike tingimuste muutustega, nagu järvesüsteemi arengu ja kliimamuutuste ning mingil määral ka antropogeensete faktoritega. Antud analüüsimetoodika puuduseks on see, et sellega saab detekteerida ainult UV-kiirgust neelavaid molekule (enamasti humiinaineid, mis moodustavad suurema osa poorivee lahustunud orgaanilisest

ainest). Antud töö on esimene Peipsi järve poorivee uurimine üldse ning esimene poorivee lahustunud orgaanilise aine analüüs nii pikas setteprofiilis, mis hõlmab 10,000 aastat sette akumuleerimist.

CE meetod sobis hästi 25–200 μM fosfoonhapete adsorptsiooni uurimiseks mullaproovides. Fosfoonhapete adsorptsiooni uuriti liiva ja savimulla erinevate osakeste suurusega fraktsioonides: < 100 μm, 100–200 μm, and 200–400 μm. Liiva puhul saadi kõige tugevam adsorptsioon kõige peenemas fraktsioonis (< 100 μm) ja kõige nõrgem – keskmises fraktsioonis (100–200 μm). Savimulla puhul oli adsorptsioon kõrgem suuremas fraktsioonis (200–400 μm), peenemates fraktsioonides oli adsorptsioon madalam ja omavahel sarnane. Fosfoonhapete hulgas adsorbeerus kõige tugevamini metüülfosfoonhape, teised uuritud fosfoonhaped demonstreerisid sarnast adsorbeerumisvõimet. Fosfoonhapete adsorptsiooni võib modelleerida lihtsa Langmuiri tüüpi kõveraga. Puuduseks oli, et antud metoodikaga oli võimalik lahutada fosfoonhappeid ainult ülalmainitud kitsas kontsentratsioonide vahemikus, seega ei olnud võimalik uurida täpsemat fosfoonhapete adsorptsiooni mehhanismi.

ORIGINAL PUBLICATIONS

PUBLICATION I

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History of anthropogenically mediated eutrophication of Lake Peipsi as revealed by the stratigraphy of fossil pigments and molecular size fractions of pore-water dissolved organic matter

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Abstract We investigated stratigraphic changes in fossil pigments and the molecular structure of the UV-absorbing fraction of pore-water dissolved organic matter in a sedimentary record from Lake Peipsi (Estonia/Russia) temporally covering the 20th century. The aims of the study were to define the onset of eutrophication in the lake and to track its course. An attempt was also made to reconstruct lake conditions before the intensive nutrient loading began. Fossil pigment analysis indicated that the eutrophication of the lake started in the 1960s and accelerated in the 1970s. Sedimentary pigments also indicate a continuing tendency of the lake ecosystem towards eutrophy in the 1980s and 1990s. However, changes in the molecular size structure of pore-water dissolved organic matter indicated that the contribution of autochthonous matter to the organic pool of the lake ecosystem had already started to increase around the end of the 1930s. We conclude that this rise was generated by a coincidence of several anthropogenic and natural factors. The pore-water data also show that a slight relative reduction in the autochthonous organic matter took place in the 1990s. A discordance in the paleodata obtained for the beginning of the 20th century complicates clear conclusions about earlier conditions in the lake. On the one hand, the qualitative characteristics of pore-water dissolved organic matter and the low concentration of chlorophyll *a* indicate that the phytoplankton biomass was low in Lake Peipsi during that period. On the other hand, the concentrations of marker pigments of specific phytoplankton groups are high, comparable with the values in the recent sediments. Possible reasons for the high levels of these pigments in the early 1900s sediments, such as a shift in the preservation conditions of organic substances and their transport from the lake's catchment, are discussed.

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European Large Lakes—Ecosystem changes and their ecological and socioeconomic impacts

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Keywords Paleolimnology · Lake sediments · Sedimentary pigments · Pore water · Dissolved organic matter · Lake Peipsi

Introduction

Increased nutrient loading due to intensified agriculture, industry and urbanization has been one of the forcing factors influencing European lakes during recent centuries. In particular, the period since the Second World War has been characterized by the enrichment of water bodies with biogenic substances. However, initial changes and the course of eutrophication in large water ecosystems are often either poorly documented or information on them is entirely missing. In such cases, a paleolimnological approach is often the only way to reconstruct the history of a lake.

Lake Peipsi is the fourth largest lake in Europe by surface area. The catchment consists of fertile soils; there are several municipalities with up to 200,000 inhabitants and numerous industrial establishments. The lake has been subjected to increasing anthropogenic inputs of nutrients from the 1950s onwards, as demonstrated by paleoecological analyses of diatom assemblages (Heinsalu et al., 2007). Monitoring data collected since the early 1960s reveal subsequent nutrient inputs and changes in the lake ecosystem (Nõges, 2001; Pihu & Haberman, 2001).

In this study, we present a reconstruction of the recent eutrophication history of Lake Peipsi at a high-resolution scale based on fossil pigment records and stratigraphic changes in the molecular size structure of pore-water dissolved organic matter (*p*DOM). Two methods were employed in the analyses of pigments and *p*DOM: first, crude samples were analysed spectrophotometrically; thereafter, compounds in the sample were separated and analysed by high-performance liquid chromatography (HPLC). Although measurements of *p*DOM are not yet widely employed in paleoinvestigations, several studies have demonstrated the capacity of these methods to provide insight into the origin and character of organic matter (OM) (Peuravuori & Pihlaja, 1997; Chin et al., 1998; Lepane et al., 2004). Moreover, results from a recent study have proved that the absorbance characteristics of *p*DOM have high potential for tracking the sources of organic carbon and for assessing the paleoproductivity of a lake (Leeben et al., 2005).

Study site

Lake Peipsi is a large (3,555 km^2), shallow (mean depth 7.1 m, max depth 15.3 m), unstratified and eutrophied water body, which consists of two extensive basins (Peipsi proper and Lake Pihkva) joined by a narrow strait (Lämmijärv). The lake receives its water from a catchment area of 47,800 km^2 and has an outflow into the Gulf of Finland. The mean residence time of the water is about 2 years. A characteristic feature of the lake is the fluctuation of the water level (mean annual range 1.2 m). Approximately 40% of the lake's catchment area is covered by coniferous and mixed forests, and approximately another 40% is used for agricultural purposes. The soils of the catchment are very diverse (loamy Cambisols, Luvisols, Podzoluvisols, sandy Podzols, Planosols, Gleysols, Histosols). During wintertime, the lake is covered by ice for an average of 114 days. A comprehensive overview of the catchment and limnology of Lake Peipsi can be found in Nõges (2001) and Pihu & Haberman (2001).

Materials and methods

Sediment coring and dating

Sediment samples were obtained from the middle of the broadest part of the lake (58°47′14″ N, 27°19′20″ E; water depth 9.2 m) with a freeze corer (Wright, 1980) in winter 2002 and 2006. The cores were sectioned into consecutive 1-cm sub-samples. The 2002 core was subjected to dating and pore-water analyses; pigment analyses were performed on the 2006 core. The two cores were correlated through their loss-on-ignition curves.

For dating, the sediment samples were analysed for ^{210}Pb, ^{226}Ra and ^{137}Cs by gamma spectrometry using a well-type coaxial low background intrinsic germanium detector (Appleby et al., 1986). For the ^{210}Pb chronology, the CRS model (Constant Rate of Supply; Appleby et al., 1986) was applied. The

methodology, results and reliability of the chronology are presented and discussed in detail in Heinsalu et al. (2007).

Analyses of pore-water dissolved organic matter (*p*DOM)

The frozen sediment samples were thawed at 4°C. The pore water was collected and clarified by centrifugation and filtration (pore size 0.2 µm). Absorption spectra were recorded over the range 200–800 nm with a Cadas 100 scanning spectrophotometer (Dr. Lange) using Milli-Q water as a blank. The ratio of the absorbances at 250 and 365 nm was calculated to evaluate the character and molecular structure of *p*DOM: a high ratio indicates organic substances with a low degree of aromaticity and small molecular size (Peuravuori & Pihlaja, 1997). The absorbance values were converted into carbon concentrations using a linear relationship between the absorbance values of *p*DOM at 250 nm and the concentration of dissolved organic carbon (DOC) in the pore water ($r = 0.87$, $n = 10$). The DOC concentration was determined by the standard method ISO 8245 (1999) using a DC-80 Total Organic Carbon Analyzer (Rosemount-Dohrmann): the organic carbon is converted to CO_2 by ultraviolet-promoted persulphate oxidation, and the CO_2 formed is detected in the infrared part of the spectrum.

High-performance size-exclusion chromatography (HPSEC) of pore-water samples was carried out using an HPLC system with a UV detector (Knauer) coupled to a biocompatible BIOSEP-SEC-S2000 column (7.5 × 300 mm, Phenomenex). A 100-µl sample was injected into the column. Compounds were eluted with 0.02 M phosphate buffer (pH 6.8) at a flow rate of 1 ml min^{-1} and detected by absorbance at 254 nm. The HPLC system was calibrated using protein standards, which were run individually; the corresponding elution times were recorded and plotted against their molecular weights. Full details of the HPSEC equipment and procedures are described in Lepane et al. (2004). The total peak areas were calculated from the chromatograms, representing the total UV-absorbing fraction of the DOM in the pore-water sample. To obtain the percentages of molecular fractions, the chromatograms were divided into three molecular size fractions: 0.01–0.2 kDa (low molecular weight, LMW); 0.2–100 kDa (medium molecular weight, MMW) and 100–2,000 kDa (high molecular weight, HMW). The areas of the respective fractions were calculated and divided by the total peak area. Weight-average and number-average molecular weights of *p*DOM (M_w and M_n, respectively) were determined using the formulae

$$M_w = \Sigma(h_i \cdot M_i)/\Sigma h_i \text{ and } M_n = \Sigma(h_i)/\Sigma(h_i/M_i),$$

where h_i is the detector output and M_i is the molecular weight, both at the ith retention time (Mori & Barth, 1999). Thus, M_n is the weight of an 'average' molecule in the mixture, while M_w is the weight of the molecule to which the 'average' atom belongs (Zhou et al., 2000).

Analyses of fossil pigments

Sediment pigments were extracted and analysed following the recommendations of Leavitt & Hodgson (2001). The frozen sediment samples were freeze-dried and pigments were extracted with an acetone-methanol mixture (80:20 v:v) at −20°C for 24 h under a N_2 atmosphere. Thereafter, the extracts were clarified by filtration through a 0.45 µm pore-size filter (Millex LCR, Millipore). The absorption spectra of the pigment extracts were recorded with a Hitachi U-3010 spectrophotometer over the wavelength range 400–800 nm. The ratio between the absorbances at 410 and 665 nm was calculated from the spectra as an indicator of chlorophyll (Chl) *a* preservation. The degradation products of Chl *a* have an absorbance peak at 410 nm, while the absorbance peak around 665 nm is due to Chl *a* and its derivatives (Rowan, 1989).

The pigments were separated with an HPLC installation consisting of two pumps (CE 1100 Cecil Instruments Ltd, UK), a dynamic mixer (Cecil), an injection valve (Rheodyne 7125) with a 100-µl injection loop and a UV detector (CE1200, Cecil). A Spherisorb ODS2 column (4.6 × 150 mm, 3 µm particle size, Waters) was used for the separation. Prior to the HPLC run, an ion-pairing solution was added to the sample extract. The pigments were eluted using a non-linear binary gradient at a constant flow rate of 1.5 ml min^{-1} (Table 1) and identified by their retention times reported in the literature and

Table 1 Elution scheme and solvents used in the separation of pigments by HPLC

Solvent (%)	Time (min)				
	0	2	15	23	25
A	100	50	0	0	100
B	0	50	100	100	0

Solvent A = 80% methanol : 20% 1 M ammonium acetate (pH 7.2) (v:v)

Solvent B = 80% methanol : 20% acetone (v:v)

standards provided by DHI (Denmark). The chromatograms were quantified by absorbance at 450 nm.

Results

Pigment stratigraphy

According to Laugaste et al. (1996), the assemblage of dominant species in Lake Peipsi has not changed during the last 100 years: the main portion of phytoplankton biomass in the lake is formed by diatoms [*Aulacoseira islandica* (O. Müller) Sim., *Aulacoseira. granulata* (Ehr.) Sim., *Stephanodiscus binderanus* (Kütz.) Krieger], followed by cyanobacteria [*Gloeotrichia echinulata* (J. E. Smith) Richter, *Aphanizomenon*, *Microcystis* and *Anabaena* species]. Third come green algae, but their biomass is considerably smaller than that of the two predominant groups. Unfortunately, because the peaks overlap with those of other pigments, the HPLC method we used failed to separate fucoxanthin (a marker pigment for diatoms) and myxoxanthophyll (a marker pigment for cyanobacteria) properly. Therefore, Chl c_2 (for diatoms) and echinenone (for N_2-fixing filamentous cyanobacteria) were chosen to track changes in the dominant phytoplankton groups in the sediments (Leavitt & Hodgson, 2001; Bianchi et al., 2002). We also present zeaxanthin and canthaxanthin data, because the actual pigment composition of cyanobacteria species occurring in Lake Peipsi is not fully known. Zeaxanthin has been detected in *Microcystis* species (Bianchi et al., 2002; Kupperstein & Boyer, 2005), but it is also a common pigment in vascular plants. Canthaxanthin has been used as a marker pigment for colonial and filamentous cyanobacteria (Lami et al., 2000; Leavitt & Hodgson, 2001); however, sometimes herbivore tissues also contain this xanthophyll (Leavitt, 1993). Chlorophyll *b* was chosen as a proxy for green algae and Chl *a* as a proxy for total phytoplankton. However, one should keep in mind that both these are also major pigments in higher plants.

The quantities and profiles of fossil pigments in the sediment record of Lake Peipsi vary greatly. The concentrations of Chl *a* and Chl c_2 in the lower part of the record (50 and 1 nmol g^{-1} OM, respectively) are approximately one quarter of those in the upper part (Fig. 1a, c; Table 2). Their concentrations started to increase in the 1970s. The distribution of canthaxanthin exhibits a reverse pattern, having a slightly higher concentration (around 50 nmol g^{-1} OM) before the 1920s, after which it diminishes somewhat (to 40 nmol g^{-1} OM) and remains stable up to the top of the core (Fig. 1f). The concentrations of echinenone and zeaxanthin vary over the ranges 15–30 and 30–45 nmol g^{-1} OM, respectively (Fig. 1d, e). Their lowest concentrations are detected around the mid-20th century. The concentration of Chl *b* varies over the range 5–10 nmol g^{-1} OM (Fig. 1g) and its stratigraphy appears similar to those of echinenone and zeaxanthin. The lowest concentrations of Chl *b* occur in the 1960s and 1970s.

Temporal changes in the quantity and structure of *p*DOM

The concentration of *p*DOM (expressed as carbon) in the sediment varies from 2 to 3 mM (Fig. 1k, Table 2). Its highest values are around 1900 and at the end of the 20th century. The weight-average molecular weight was less than 7 kDa, varying from 3.5 to 5 kDa (Fig. 1l). The number-average molecular weight of *p*DOM was less than 4 kDa and mainly in the range 1–3 kDa (Fig. 1m). In two sediment layers, one dated to the late 1960s and the other to the very early 1990s, the pore-water organic substances have very high molecular weights (Fig. 1l, m). We would point out that the values for *p*DOM obtained here are not 'real' concentrations and molecular weights of organic substances in the pore water. Freezing and subsequent thawing of organic material in aqueous solution disintegrates particulate OM and disrupts dissolved organic molecules. Comparison of the molecular characteristics of *p*DOM in fresh and frozen samples showed that the molecular weights are approximately 2–3 times higher in unfrozen samples than in frozen samples (Lepane et al., 2006).

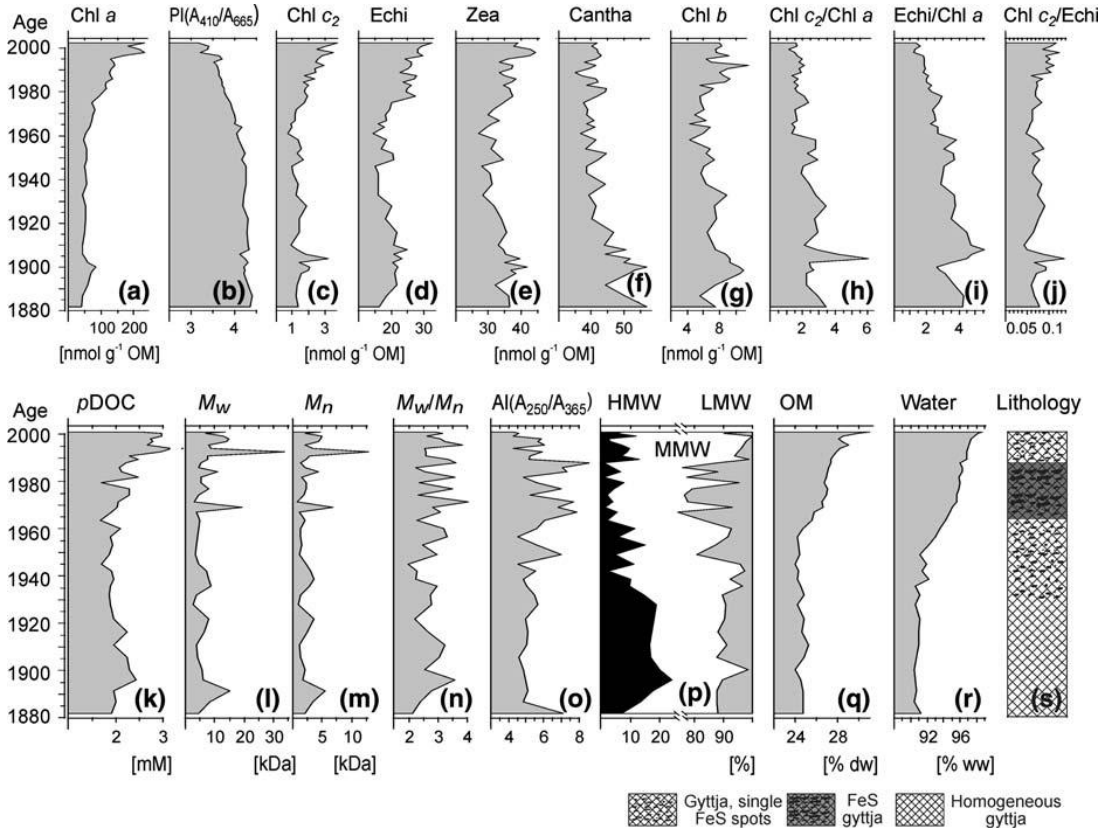

Fig. 1 (*Upper panel*) Age-resolved profiles of the concentrations of selected fossil pigments (**a**, **c**–**g**) and their ratios (**h**–**j**) in the sediments of Lake Peipsi. Preservation index of chlorophyll *a* (PI), calculated as the ratio between absorbances at 410 and 665 nm (A_{410}/A_{665}), is also shown (**b**). (*Lower panel*) Age-related changes in the characteristics of pore-water dissolved organic matter (**k**–**p**). For comparison, the profiles of major sediment constituents (**q**, **r**) and lithology (**s**) (reprinted with permission from Heinsalu et al., 2007) are also shown. Abbreviations: Chl—Chlorophyll; Echi—Echinenone; Zea—Zeaxanthin; Cantha—Canthaxanthin; *p*DOC—Pore-water dissolved organic carbon; M_w and M_n—Weight-average and number-average molecular weight, respectively; AI—Aromaticity index calculated as the ratio between absorbances at 250 and 365 nm (A_{250}/A_{365}); LMW—Low molecular weight; MMW—Medium molecular weight; HMW—High molecular weight; OM—Organic matter; dw and ww—Dry and wet weight of sediments, respectively. For details see Materials and methods

The ratio of M_w to M_n, called polydispersity, has been used as a quantitative measure of the molecular weight distribution of OM in a mixture (Chin et al., 1998). For pure organic substances the ratio is equal to 1; for a mixture of molecules it is greater than 1. In the sediments of Lake Peipsi, the polydispersity of *p*DOM varies over a very narrow range (Fig. 1n), indicating that temporal changes in the molecular weight distribution are small. However, the aromaticity index estimates of *p*DOM demonstrate slightly elevated values from the 1960s to the late 1980s (Fig. 1o, Table 2), implying that the proportion of substances of smaller size and lower aromaticity has increased in the OM accumulated during this period. Ordinarily, lower aromaticity and molecular weight (hence also size) are characteristic of autochthonous OM, while compounds with higher aromaticity and molecular weight predominate in OM derived from terrestrial sources (McKnight et al., 2001). Stratigraphic changes in the distribution of different molecular weight fractions confirm that the proportion of high molecular weight *p*DOM has decreased

Table 2 Spearman rank order correlations for selected characteristics of pore-water dissolved organic matter (*p*DOM) and fossil pigments obtained by different methods (given in parentheses) for the analysed sediments of Lake Peipsi

Pair of characteristics	Spearman	*P*-level
Fossil pigments		
A_{665} (Abs1) and Chlorophyll *a* (HPLC)	0.846	0.000
A_{665} (Abs1) and Chlorophyll *b* (HPLC)	0.247	0.095
A_{665} (Abs1) and Chlorophyll c_2 (HPLC)	0.691	0.000
A_{665} (Abs1) and Echinenone (HPLC)	0.804	0.000
A_{665} (Abs1) and Zeaxanthin (HPLC)	0.484	0.000
A_{665} (Abs1) and Canthaxanthin (HPLC)	−0.257	0.081
*p*DOM		
*p*DOC (PO + Abs2) and Total area (HPSEC)	0.882	0.000
A_{250}/A_{365} (Abs2) and HMW (HPSEC)	−0.545	0.000
A_{250}/A_{365} (Abs2) and MMW (HPSEC)	−0.257	0.109
A_{250}/A_{365} (Abs2) and LMW (HPSEC)	0.274	0.087
M_w/M_n (HPSEC) and HMW (HPSEC)	−0.011	0.947
M_w/M_n (HPSEC) and MMW (HPSEC)	0.440	0.004
M_w/M_n (HPSEC) and LMW (HPSEC)	0.169	0.327

A_{665}—Absorbance of crude pigment extract at 665 nm; *p*DOC—Concentration of pore-water dissolved organic carbon; Total area—Total area of chromatogram; A_{250}/A_{365}—Ratio of absorbances of pore water at 250 nm and 365 nm; HMW, MMW, LMW—Respective areas of the high, medium and low molecular weight fractions in the chromatograms; M_w/M_n—Ratio of weight-average molecular weight and number-average molecular weight; PO—Persulphate oxidation method; Abs1—Absorption spectroscopy of pigment extracts; Abs2—Absorption spectroscopy of pore water; HPLC—High-performance liquid chromatography; HPSEC—High-performance size exclusion chromatography. For details see Materials and methods

somewhat since the 1940s and the proportion of low molecular weight substances has increased (Fig. 1p, Table 2).

Discussion

Onset and course of eutrophication

Qualitative phytoplankton proxies—the concentrations of Chl *a*, Chl c_2, echinenone and zeaxanthin—indicate that a shift of the lake ecosystem towards eutrophy started in the 1970s. From that time onwards the concentrations of the pigments are higher than in earlier years, or are gradually increasing. Statistical analysis reveals that changes in the concentration of Chl *a* correlate well with those of echinenone and Chl c_2 (Table 2), suggesting that the main contributors to the phytoplankton biomass in the lake have been cyanobacteria and diatoms. However, it is difficult to estimate which of these has made the larger contribution. On the one hand, chlorophylls are more photosensitive than carotenoids. On the other, diatom cells sink more rapidly than cyanobacteria. Moreover, many cyanobacteria species form surface blooms and even after the bloom collapse, their colonies continue to float on the surface, prolonging their exposure to sunlight. Different sinking rates of cyanobacteria, and hence different times of exposure to sunlight, could also explain why the concentration of zeaxanthin was higher than that of echinenone in the sediments of Lake Peipsi, whereas according to the monitoring data, the biomass of filamentous cyanobacteria in the lake has been higher than that of unicellular *Microcystis* species (Nõges et al., 1996). The findings of Bianchi et al. (2002) suggest that unicellular cyanobacteria (including picocyanobacteria) may have higher sedimentation rates than the N_2-fixing filamentous cyanobacteria.

Canthaxanthin is the only pigment whose concentration shows no change during the 1970s. As mentioned previously, canthaxanthin has been widely detected also in herbivores. Moreover, when Quibllier-Llobéras et al. (1996) observed the summer planktonic community in an oligo-mesotrophic lake, canthaxanthin was found only in zooplankton, but it

was totally absent from phytoplankton. In the case of Lake Peipsi, further investigations should be conducted to determine whether canthaxantin is a marker pigment of specific cyanobacteria taxa or whether it is confined to herbivores.

The changes in the pigment ratios in the sediment record imply that the ecosystem changed in the 1960s (Fig. 1h–j). The sediment composition data suggest that the sediment deposited since the 1960s clearly differs from the underlying layers: it is richer in OM and less compacted (Fig. 1q, r). The preservation index of Chl *a* declines steadily, simultaneously with changes in the pigment ratios, and this also implies that conditions in the water column or/and sediments have changed since the 1960s (Fig. 1b).

On the basis of long-term investigations of Lake Peipsi, it is generally considered that the lake trophy started to increase in the 1960s. In the 1970s, the process accelerated and a high level was reached in the 1980s. From the early 1990s until 1996 an improvement in the lake water quality was observed. However, since 1997 there has been a continuous and even accelerating deterioration of the lake status (Nõges et al., 1996; Nõges & Nõges, 2006). Changes in fossil pigments accord well with the monitored course of eutrophication, except for the transient recovery of the lake in the 1990s, which is not clear in the fossil pigment stratigraphy. However, limnological data of phytoplankton biomass from that period suggest that despite a lowered nutrient level in 1988–1994 (Loigu & Leisk, 1996), the average phytoplankton biomass remained high (Laugaste et al., 1996).

As shown by the *p*DOM data, changes in the lake OM occurred even before the 1960s. The distributions of molecular weight fractions of *p*DOM indicate a change in the late 1930s to early 1940s (Fig. 1p), suggesting a rise in the proportion of autochthonous matter in the OM pool. The increase in the autochthonous contribution to the bulk of OM becomes more and more evident over the following years. According to the *p*DOM characteristics, the autochthonous component in the sediments was highest during the 1970s and 1980s (Fig. 1o, p). A subsequent reduction in the proportion of the LMW fraction and an increase in the proportion of OM with higher aromaticity in the *p*DOM implies a somewhat decreased contribution of autochthonous matter in the bulk of OM during the 1990s, thus indicating a slight deceleration of eutrophication. The stratigraphic changes in the molecular size structure of *p*DOM (Fig. 1o, p) coincide well with the zonal changes in the sediment lithology (Fig. 1s), demonstrating that the dissolved organic fraction in the sedimentary OM has changed in concert with changes in the particulate fraction.

There is no clear explanation for the autochthonous shift in the character of *p*DOM in the late 1930s to early 1940s. A similar qualitative shift in *p*DOM was observed in the measurements of the fluorescence of *p*DOM from the same core in an earlier study (Heinsalu et al., 2007: Figs. 5, 6c, d). We suppose that the cumulative effects of several anthropogenic and natural factors (pre-war increases in agriculture and urban sewage discharges, intensive fishing, establishment of the Zebra mussel *Dreissena polymorpha* Pallas in the lake, numerous extensive forest fires and low water levels) may have triggered these changes in OM.

According to monitoring observations, very large fluctuations in the summer phytoplankton biomass are characteristic of the Peipsi ecosystem: the interannual differences can be up to 10-fold (Laugaste et al., 1996). Somewhat unexpectedly, we could detect no prominent changes in the productivity proxies in the sediments. However, the two abrupt peaks of *p*DOM molecular weights (Fig. 1l, m) appear to coincide temporally with the two densest phytoplankton bloom events registered by monitoring observations in Lake Pihkva, in 1972 and 1991 (Laugaste et al., 1996: Fig. 9b), when the biomass of cyanobacteria exceeded the mean annual biomass level by factors of 10 and 22, respectively. However, these bloom events are not detectable in other constituents of the sediments. Moreover, we are unable to surmise whether (1) the blooms extended to Lake Peipsi proper and/or (2) the OM produced in Lake Pihkva could be carried to and settle in Lake Peipsi proper. Therefore, the coincidence may be just accidental.

Conditions prior to the 1930s

The paleolimnological inferences about the nutrient loading response in Peipsi over the past 40 years, based on the stratigraphic changes in fossil pigments and molecular structure of *p*DOM, are consistent with

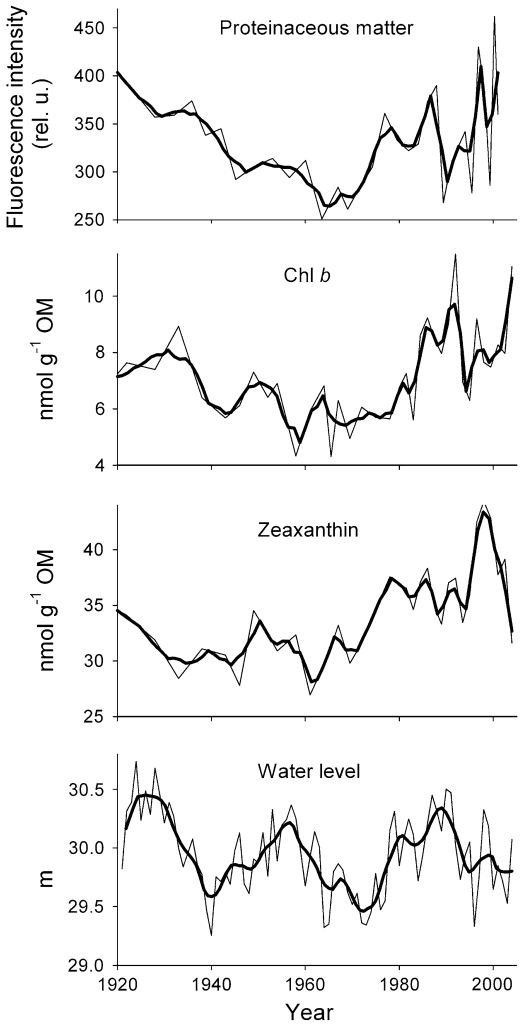

Fig. 2 Comparative curves (fine line—raw data, solid line—smoothed data, Loess curve fitting) of the annual water-level dynamics (elevation, m above sea level) and temporal distribution of proteinaceous matter, zeaxanthin and chlorophyll *b* (Chl *b*) in the settled material of Lake Peipsi. The initial unsmoothed curve of the proteinaceous matter is redrawn with permission from Heinsalu et al. (2007). OM—Organic matter

the monitoring results for the lake. Thus, we conclude that even the pre-eutrophication conditions in Peipsi can be assessed by the sedimentary organics.

Comparison of the fossil pigment chromatograms suggests that the pigment composition of phytoplankton has remained relatively unchanged over the entire time span investigated. This finding coincides well with the results of Laugaste et al. (1996). A rather stable, mesotrophic species composition characterizes the sedimentary diatom assemblage for the period 1880–1950 (Heinsalu et al., 2007). The sedimentary profile and preservation index of Chl *a* obtained in the present study indicate a low algal standing crop and high stability of the lake conditions at that time (Fig. 1a, b).

Besides the low Chl *a* concentrations, the high levels of echinenone, zeaxanthin and Chl *b* in the sediments dated to the 1900–1920s are noteworthy (Fig. 1d, e, g). A slight concurrent increase in the *p*DOC concentration is also discernible (Fig. 1k). Constituents of fluorescent *p*DOM were also present in greater quantities in the lower part of the sediment record than in the sediment layers dated to the mid-20th century (Heinsalu et al., 2007: Fig. 6a, b). This may be explained by the fact that the environmental conditions in the lake during that period were favourable for the preservation of OM and also other pigments, e.g. canthaxanthin (Fig. 1f), diatoxanthin and *β*-carotene (the last two are not shown). Another possible reason is revealed by comparing the temporal changes of these OM constituents with the changes of water level (Fig. 2). Keeping in mind that the dating of the lower part of the core involves a greater error, one could speculate that the runoff of OM from the lake catchment was higher in that period than in previous and subsequent decades. The presence of a greater proportion of large aromatic organic substances supports the hypothesis of intensive terrestrial transport of OM, as they indicate that allochthonous OM predominated in the lake ecosystem before the 1930s. Unfortunately, the water level observation series is too short to detect any further regularities, especially since the influence of water-level fluctuations is severely masked in the upper part of the sediment record by progressive eutrophication of the water body. Therefore, the extent of external loading of OM to the Lake Peipsi ecosystem at the beginning of the 20th century still remains ambiguous.

Conclusions

The first symptoms of environmental disturbance of Lake Peipsi appeared in the settled matter dated to the

late 1930s, where changes in the molecular size composition of *p*DOM indicate an incipient relative increase of autochthonous matter and a corresponding reduction of allochthonous OM. In subsequent decades, this trend becomes more and more obvious. In the fossil pigments, an increase in water column production appears in the 1960s and since then the OM constituents indicate a gradual trend towards eutrophy. The stratigraphic changes in *p*DOM size fractions imply that the nutrient availability for phytoplankton might have declined somewhat in the 1990s. However, these changes are minor and subsequent persistently high concentrations of fossil pigments in the early 2000s do not suggest that the eutrophication level of the lake had been reduced.

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PUBLICATION II

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A 10,000 year record of sediment pore-water dissolved organic matter characteristics from Lake Peipsi as revealed by HPSEC

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In this study, high-performance size-exclusion chromatography (HPSEC) combined with diode-array detection (DAD) was applied for the investigation of dissolved organic matter (DOM) from sediment pore-water from Lake Peipsi, Estonia. The age of the 4 m long sediment core was estimated using the ^{14}C dating method and suggested sediment accumulation for the past 10,000 years. Using the HPSEC approach it was possible to evaluate temporal changes in the content of DOM and its molecular weight characteristics during the Holocene period. The content of dissolved organic carbon (DOC) calculated from spectroscopic data was in good correlation with the total peak areas computed from HPSEC chromatograms. The results revealed that the content of detected DOC in the older samples [10,300 to 2,600 years before present (BP)] was twice as high as in the younger ones (from 2,400 years BP to present). Thus, HPSEC analysis with spectroscopic methods might provide useful information about temporal changes in DOM content and could be used in palaeolimnological research.

Keywords: pore-water dissolved organic matter; sediment; HPSEC; humic substances; palaeolimnology; Lake Peipsi

1. Introduction

Lake sediments represent archives that reflect past environmental conditions. Lacustrine sediments therefore provide an important time perspective on a range of natural processes, such as long- and short-term climatic changes, the development of lake ecosystems and the terrestrial catchment areas that drain into them, as well as prehistoric and modern human impacts on aquatic ecosystems. Lake sediments are generally rich in organic matter (OM), which consists of an extremely heterogeneous mixture of various biomolecules including proteins, carbohydrates, lipids and humic substances (HS), the products of partial breakdown of organic components [1]. The origin of lake sediment OM is different and diverse; it may have biogenic, biochemical and terrestrial, as well as human-induced origins. Roughly, sediment OM can be derived from two sources. Autochthonous OM is generated within the aquatic system as a result of degradation

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processes of organisms that have lived in the lake. Another type is allochthonous OM, which originates outside the lake basin, in the catchment of the lake, and these land-derived organic residues mainly enter the lake via inflowing rivers and streams. Even though the sediment OM is a diverse mixture of components from many sources and with variable degrees of preservation, the abundance and different components of lacustrine sedimentary OM provide evidence of the biota that have lived in the lake and its watershed area, and hence these proxies can be used to reconstruct palaeoenvironmental conditions [2].

Lake Peipsi is the largest transboundary lake in Europe, shared between Estonia and Russia. It is an important economic resource for Estonia and has been exposed to extensive human activity for a long time. The lake and its rivers are mainly used for fishing and water transport. The catchment area has been used for agricultural purposes for several millennia. On the northern side of the lake there are extensive mining areas and several electric power plants operating on oil shale. As a consequence, enhanced delivery of nutrients to Lake Peipsi has induced an increase in primary productivity within the lake and anthropogenic eutrophication during the last few decades [3].

Palaeolimnological studies for the assessment of the eutrophication history of Lake Peipsi are infrequent. A diatom composition study of short sediment core covering the last 130 years revealed that the man-made impact on Lake Peipsi started in the mid-1950s, and the eutrophication reached a maximum in the 1970s and 1980s and showed a slight decrease in the 1990s [4]. Research by Leeben et al. using fossil pigment analysis confirmed those results and also suggested that the contribution of autochthonous organic matter in the lake started to grow in the middle part of the last century [5]. Knowledge of the long-term post-glacial lake development and changing environmental conditions is even more sporadic, and mostly based on a poorly dated diatom and geochemical records with a low time-resolution [6].

However, without knowing the natural baseline conditions of the lake ecosystem it is hard to evaluate its current ecological state. Therefore, more adequate information about the natural development and changes of environmental conditions in Lake Peipsi are essentially needed to evaluate anthropogenic impact on the lake ecosystem. The studies of Leeben and co-authors have demonstrated the applicability of sedimentary pore-water dissolved organic matter (DOM) spectroscopic characteristics as proxies for understanding the origin and properties of OM, and consequently, temporal dynamics of past environmental changes in lakes [5,7–9]. The aim of this research was to investigate stratigraphic changes in the molecular size and quantity of sedimentary DOM from Lake Peipsi over the period of the last 10,000 years. For this purpose, high-performance size-exclusion chromatography (HPSEC) with diode-array detection (DAD) was applied to separate DOM from sediment pore-water. The HPSEC approach has demonstrated a great potential for pore-water DOM investigations [10–12], although it is not yet widely used for palaeolimnological investigations. However, HPSEC analysis may be a suitable approach because it is very simple and non-destructive, and requires minimum sample pretreatment. In addition to HPSEC, the spectroscopic method was used to characterise pore-water DOM.

This study is an attempt to demonstrate that the changes in the content and structure of sedimentary pore-water DOM might be related to environmental and climatic changes in large aquatic ecosystems. Because the HPLC method has shown good results in investigations of small shallow lakes [8,13], it could probably be applied to the examination of larger lakes. However, in the case of small shallow lakes, especially with annually laminated sediments, diagenetic processes are supposed to be minimal, because little or no resuspension occurs and no bioturbation is present [1]. Processes occurring in larger lakes are more complicated because turbulence and bioturbation might affect the deposition of the original organic material.

2. Materials and methods

2.1. *Study site*

Lake Peipsi is the fourth largest lake in Europe by area (3,555 km^2, maximum length ~150 km, and width 42 km). It is a shallow (mean depth 7.1 m, maximum depth 15.3 m), unstratified and eutrophied lake. The lake system consists of two basins (Lake Peipsi sensu stricto and Lake Pihkva) which are joined by a narrow strait (Lake Lämmijärv). It has an outflow into the Gulf of Finland via the Narva River. The basin capacity is >25 km^3, which is fed by more than 240 rivers, brooks and channels [14]. The average residence time of the water is ~2 years [15]. The water of Lake Peipsi has an average pH of 8.1 and a Secchi disk transparency of 1.6 m [16].

The formation of sediments in Lake Peipsi is controlled by hydro-meteorological processes such as waves, wind, water-level fluctuations, drift ice and currents [17]. The bottom topography of Lake Peipsi is monotonous and the composition of the sediments is more or less homogeneous. The shallower areas are mainly represented by sands, silts and late-glacial clays, whereas post-glacial organic-rich gyttja sediments are present in the areas with >9 m of water depth [18], reaching a thickness of 7 m [6].

2.2. *Sediment coring and sample handling*

In March 2007, a 4 m long sediment core was taken on the ice from the central part of the lake (58°47.213′N; 27°19.299′E), using a Russian-type peat corer at a water depth of 9.8 m. The recovered sediment core was immediately packed into 1 m plastic semi-tubes, wrapped in polyethylene film to avoid oxygen exposure and transported to the laboratory. During transportation and before slicing the wrapped core was stored at ~4 °C. The next day the core was sliced into continuous 1 cm thick sub-samples that were immediately packed into small plastic bags, again to maximally avoid oxygen exposure. The sub-samples were stored in the dark at 4 °C.

Additional information on the sample handling procedures can be found in supplementary text S1 (available online only).

2.3. *High-performance size-exclusion chromatography and spectroscopic measurements*

The sediment core was collected and processed with care to avoid oxygen exposure. However, some oxygen exposure might still have occurred during pore-water extraction at ambient atmosphere. That might have introduced some artefacts into the measurements of the abundance and molecular weight (MW) of the DOM. The samples were extracted directly before the HPSEC analysis (12 samples at a time). The sub-samples were centrifuged at 3500 rpm for 30 min and the pore-water obtained was filtered through a 0.45 μm Millipore filter. When they were not being analysed, the samples were stored in the dark at 4 °C to maximally avoid artefact introduction. The samples were measured with 5 cm intervals in the upper sediment layers (from 10.00 to 10.30 m) and further with 10 cm intervals.

Pore-water samples were analysed using a HPSEC system equipped with DAD. HPSEC measurements were carried out on a BioSep-SEC-S 2000 PEEK size-exclusion column filled with glycerol covered silica-based gel (300 × 7.50 mm, Phenomenex). The size of the particles was 5 μm and the size of the pores was 145 Å. The data obtained were analysed using Agilent ChemStation software. The HPSEC system was equipped with a Knauer pump, Rheodyne injector, and Agilent Technologies 1200 Series diode-array detector.

The HPSEC system was calibrated with four protein standards purchased from Phenomenex: bovine thyroglobulin (MW, 670,000), human gamma globulin IgG (MW, 150,000), ovalbumin

(MW, 44,000) and myoglobin (MW, 17,000); also, uridine (MW, 244), Phenomenex, was used to obtain a low molecular range calibration. For the calibration curve standards, elution times were plotted against the logarithm of their molecular weights.

According to the obtained chromatograms total peak areas and areas of separated peaks were calculated. Total peak areas represented the whole amount of ultraviolet (UV)-absorbing biomolecules in pore-water DOM. Also, according to the results, number- and weight-average molecular weights (M_n and M_w, respectively) and their ratios were calculated. For additional information, see supplementary text S2 (available online only).

We used mostly HPSEC peak areas (the total peak area and the HS fraction area) for the evaluation of DOM content in pore-waters, but the DOC concentrations were used to show precise concentrations.

3. Results

The studied sediment core (the core-depth of 13.5 to 9.8 m from the water surface) consisted of greenish grey homogeneous gyttja, whereas the topmost 25 cm of the sequence contained dark coloured gyttja. The age model for the studied Lake Peipsi sediment core showed that the onset of gyttja accumulation at the profile base was dated to 10,400 cal BP (cal BP = calibrated years before present; ^{14}C dates calibrated to calendar years before present, the time scale used with radiocarbon dating in archeology, geology, etc. As the zero point of the age scale, 1 January 1950 is used; e.g. 1500 BP means 1500 years before 1950, that is 450 AD). The age of the upper part of the sediment record was estimated by the correlation of particulate OM content with a core taken in 2002 that was subjected to detailed ^{210}Pb dating [4]. The core depth of 10.0 m was estimated to correspond to approximately AD 1950.

Temporal changes in the content of sediment particulate OM could be roughly divided into three periods: 10,300 to 7,600 cal BP; 7,600 to 2,600 cal BP; and 2,600 cal BP to present (Figure 1). The content of particulate OM in the oldest samples exhibited a distinctly increasing trend. After 7,500 cal BP it became relatively stable. A period of slight increase was observed in 2,600 to 1,000 cal BP, and thereafter the content of particulate OM showed a certain decrease.

The DOM from all pore-water samples was resolved by HPSEC into two main fractions. The first fraction contained compounds with very high MW values (first peak on HPSEC chromatograms, Figure 2). The components of the second fraction (second peak on HPSEC chromatograms, Figure 2) was eluted from the chromatographic column as a broad distribution, sometimes with partially resolved sub-shoulders. The peak of the first fraction was much smaller than the peak of the second fraction. The DAD spectrum of the first fraction revealed that it was typical of proteinaceous material, whereas the second fraction spectrum was characteristic of HS. The position and shape of the first fraction peak were mostly quite stable in almost all samples. By contrast, the shape of the second fraction peak varied temporally (Figure 2), although the retention times remained more or less stable. Poorly resolved sub-shoulders appeared in pore-water samples from older sediment layers. In some samples, the third fraction (third peak) with very low MW appeared (Figure 2(c)), but this appearance did not seem to be regular.

According to the obtained results, temporal trends in the contents of DOC, UV-absorbing DOM (calculated as total peak area), and HS fraction were constructed (Figure 3). In addition, chronological changes of the ratio $A_{250/360}$, indicating aromaticity, and the M_n, M_w and their ratios were constructed (Figure 4).

Unlike the HS fraction, the high MW material eluted from the chromatographic column mostly before the calibrated range (>670,000 Da), and thus it was not possible to estimate its MW distribution adequately. Besides, the amount of the detected high MW fraction was negligible (~5–6% of the total area of all detected DOM). The MW distribution for the HS fraction was

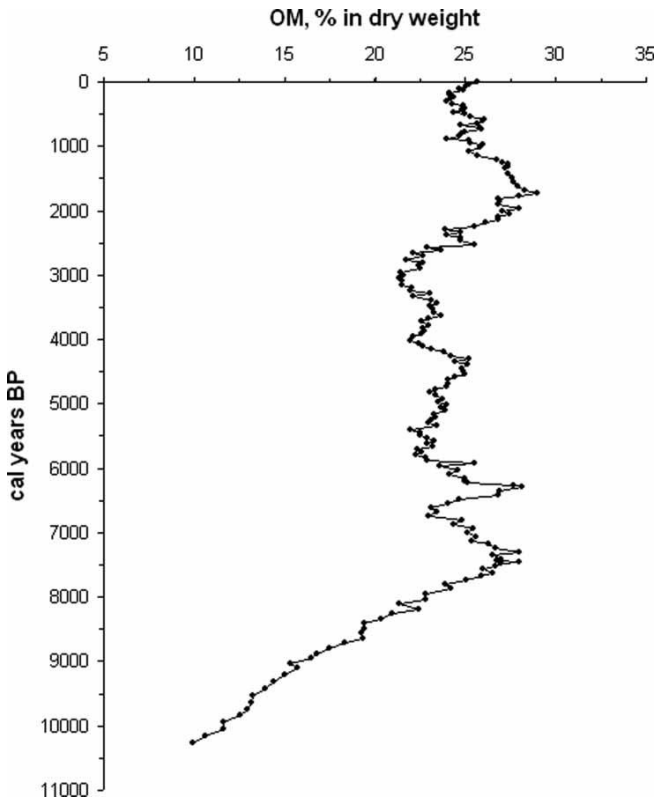

Figure 1. Age-related changes in particulate organic matter content in sediments from Lake Peipsi; cal years BP: calibrated years before present; 0 cal BP = 1950 AD.

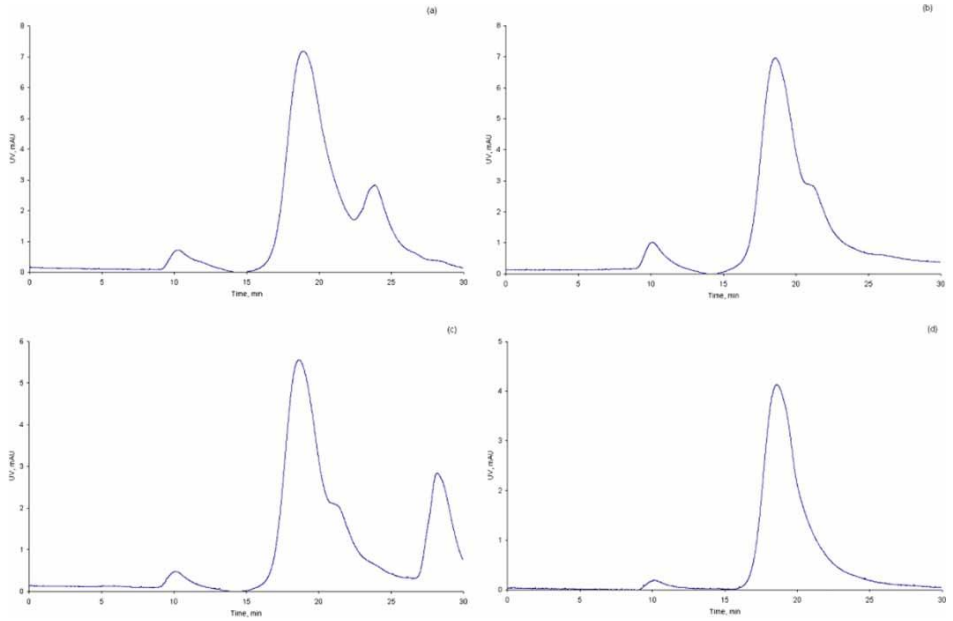

Figure 2. HPSEC chromatograms of pore-waters dated to: (a) 7,200 cal BP; (b) 5,500 cal BP; (c) 4,300 cal BP; (d) 800 cal BP. HPSEC conditions: mobile phase – 100 mM phosphate buffer (pH 6.8) at 0.5 mL · min^{-1}, sample volume 20 μL, detection wavelength 280 nm.

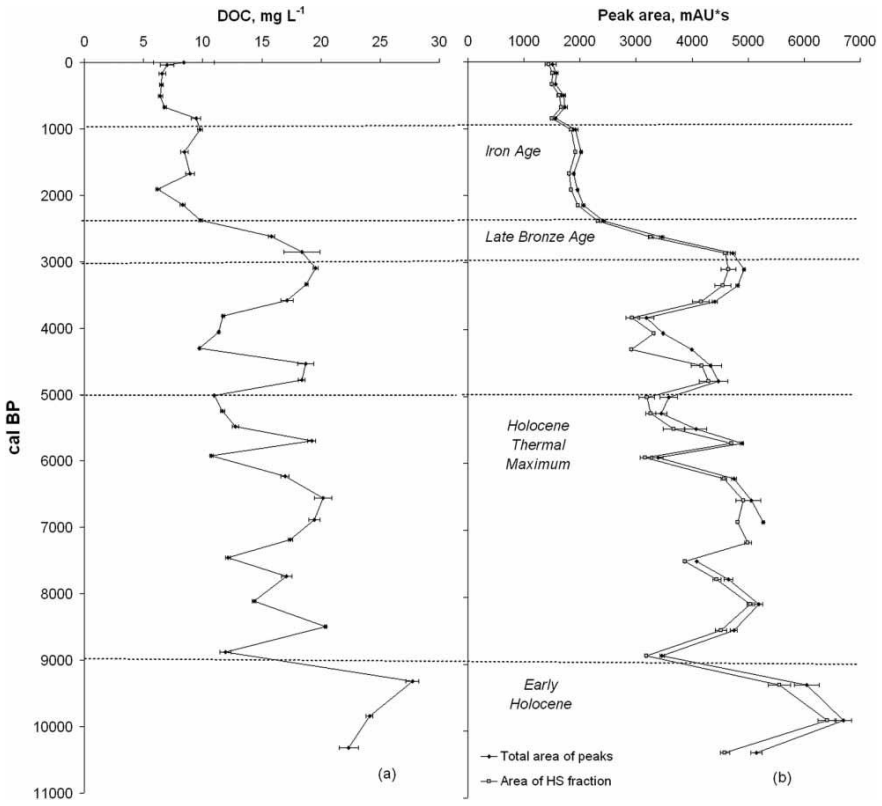

Figure 3. Age-related profiles of: (a) DOC content; (b) total peak area and area of HS fraction.

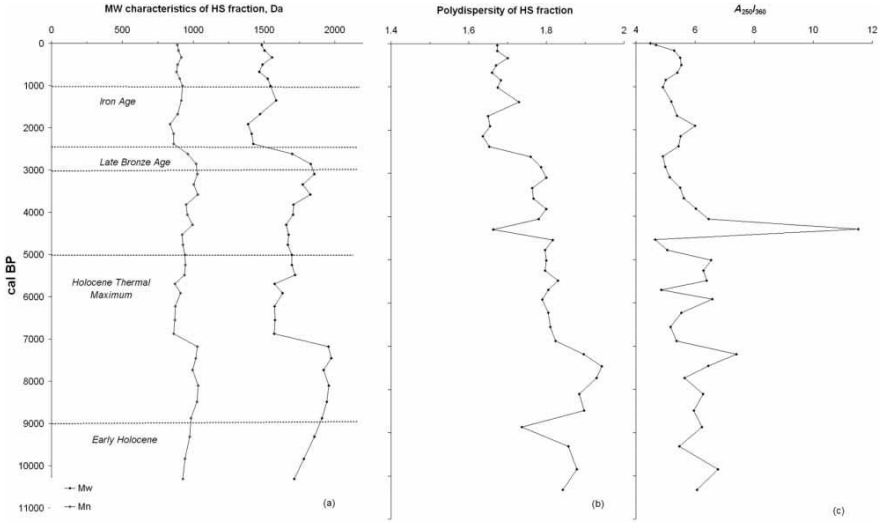

Figure 4. Age-resolved profiles of (a,b) MW characteristics and (c) absorbance ratio $A_{250/360}$ of pore-water DOM.

calculated only for the calibrated range (up to 244 Da). Thus, the MW distribution of HS are probably slightly overestimated. The MW values of the third peak were not calculated, because the peak eluted below the calibrated range.

Temporal distributions of the DOC content, the total area of the peaks (UV-absorbing DOM), and the area of the HS fraction showed similar trends (Figure 3).

Generally, the DOM content in the pore-water decreased over the last 10,000 years – older samples contained amounts of DOM almost twice as high as the ones accumulated recently. According to the characteristics reflecting DOM content (DOC concentration, the total peak area and the HS fraction area), the temporal changes could be roughly divided into two periods:

1. 10,300 to 2,400 cal BP. During that period the content of DOM in pore-water varied greatly with the average DOC content being 16.4 mg L^{-1};
2. 2,400 cal BP to the present. After 2,400 cal BP the content of DOM decreased drastically more than two-fold (average DOC 7.7 mg L^{-1}).

The shapes of the HPSEC chromatograms of the analysed samples varied significantly. The chromatograms of the first period varied greatly in comparison with the second period. The pore-water samples from 10,300–3,800 cal BP were mostly separated into two peaks, one with high MW and the second as a HS peak, usually with a partially resolved low MW sub-shoulder (Figure 2(a),(b)). The major part of this sub-shoulder was usually below the calibrated range (<244 Da). In a few samples, the third peak eluted at longer retention times reflecting extremely low MW compounds (Figure 2(c)).

The components of the high MW fraction were very unstable in the oldest samples (10,300–6,600 cal BP) as the peak varied greatly in height and shape. After 6,200 cal BP the shape of this peak did not change.

The chromatograms of the samples from 3,600 cal BP to the present were all invariant. Components were eluted as two peaks, high MW components and a smooth HS fraction without any sub-shoulder (Figure 2(d)).

According to the protein calibration, the M_w from the HS fraction did not exceed 2,000 Da, varying from 1,400 to 1,900 Da, and the M_n was mostly between 900 and 1,000 Da (Figure 4(a)). In general, both trends were very similar and they did not exhibit any drastic changes. However, several characteristic periods could be distinguished. In the oldest samples (10,300–7,200 cal BP), average MW values increased slightly until 7,200 cal BP. M_w and M_n were ∼1,890 and 990 Da, respectively. The changes occurred around 7,200 cal BP when average MW values decreased. After 2,400 cal BP, M_w and M_n decreased even more (1,490 and 890 Da, respectively).

Polydispersities calculated for the humic-type fraction did not take into consideration components that were below 244 Da. This way, polydispersity values varied between approximately 1.6 and 2.0 and should, in fact, have been higher (Figure 4(b)).

Figure 4(c) shows the temporal changes in pore-water absorbance ratios at 250 and 360 nm. The aromaticity values of the analysed samples varied around ∼4.5–6.5 without any sharp changes. Only one sample, dated to 4,300 cal BP, was differentiated with an extremely high value of 11.52, which might be occasional.

4. Discussion

4.1. *HPSEC analysis of Lake Peipsi pore-water DOM*

The choice of calibration standards for pore-water DOM is crucial in HPSEC. To obtain reliable and adequate results using HPSEC, calibration standards should match the molecular structure of the analysed components as closely as possible [19]. The components of lacustrine pore-water

DOC are very diverse and it is a rather difficult task to find standards which would represent all the compounds. However, according to the literature [12,20,21], HS comprise the main part of the lacustrine DOC. Indeed, in this study, HPSEC analysis also revealed that the dominant components of DOC in Lake Peipsi sediment pore-waters were HS. Also, some compounds of proteinaceous origin were detected. It is not simple to choose appropriate calibration standards for humics because of their unique structure. There is a general agreement that random coil standards such as polystyrene sulphonates should be used for humics MW distribution determination [21]. Globular protein standards could also be used but they were reported to overestimate the MW values of humics by a factor of five or more [21]. In this study, the HPSEC column was calibrated with globular proteins. Despite the statement of caution [21], M_w and M_n values for Lake Peipsi pore-water DOM were in good agreement with MW distributions reported for aquatic fulvic acids [10,12,22] and for freshwater sediment pore-waters [21]. Thus, in our study, the calibration with globular proteins did not lead to over-prediction of HS MW values and might be used for estimation of MW distribution of pore-water DOM.

The presence of proteinaceous matter in pore-water DOM might be caused by the association of undecomposed proteins and humic material into large aggregates. Humic-type molecules could probably encapsulate protein molecules, making them unavailable for bacterial degradation [23].

Light absorbance has usually been used to estimate DOC concentrations in sediment pore-water. However, the exclusive use of UV-absorption for DOC and its MW measurement has a considerable disadvantage because it detects only UV-absorbing molecules. Estimating DOC concentrations by UV-absorbance provides a bias in favour of molecules that contain chromophores and absorb light at the chosen wavelength(s). Components that absorb UV weakly (e.g. polysaccharides) will not be represented properly. Thus, measurements based on UV-absorption may underestimate DOC concentrations. To avoid this, one should use another approach for measuring pore-water DOC concentration (e.g. a combustion-based TOC analyser). Unfortunately, in this study there was no possibility of using a TOC analyser and all DOC contents could be to some extent underestimated. The same applies to the HPSEC analysis with the UV-absorbance detector. As UV-nonabsorbing components are not properly represented, this could also affect MW calculations and introduce a bias in the results. Thus, we should point out that the obtained results concern only UV-absorbing DOM and are discussed in these terms.

In our study, we observed that the temporal distributions of the DOC content, total area of the peaks (UV-absorbing DOM), and the area of the HS fraction showed similar periods which had a good correlation between themselves (the correlations between DOC concentration and total peak area and between DOC concentration and HS fraction area were both $R^2 = 0.86$). Thus, the total peak area calculated from HPSEC chromatograms may be a good predictor for DOC concentrations in sediment pore-waters.

4.2. *Temporal changes of sediment pore-water DOM characteristics*

In this study, sediment pore-water DOM variables from Lake Peipsi were employed to track changes in a sediment core representing ~10,400 years of accumulation. HPSEC analysis of pore-water samples revealed several periods of structural and quantitative DOM changes. These changes were probably dependent on post-glacial changes of lake environmental conditions. However, OM diagenesis might also have affected the nature and distribution of DOM in the upper sediment layers.

Several earlier investigations indicate that the beginning of the Holocene coincided with the shallow water level of Lake Peipsi. According to Hang et al. [24], at the onset of the Holocene a low-level period occurred during which ‘Small Lake Peipsi’, with a water level 8 m lower than today, existed. In addition, the presence of lake lime layers in between late glacial clays and gyttja

beds in several locations of the basin has been associated with an early Holocene lake level low stand [6,25,26].

Therefore, we attribute the up-core transition from low to high sediment particulate OM content to a progressively deepening water body. A shallower water depth likely induced fine-grained mineral sediment redistribution through wave turbulence and deposition of sediment with low OM content, whereas deeper conditions allowed accumulation of OM-rich gyttja. Also different sediment pore-water DOM variables indicate variations that might be related to changing environmental conditions in Lake Peipsi. The content of DOM in pore-waters was highest during that period. In addition, the shapes of the pore-water DOM chromatograms varied. The variation occurred in the area of high MW fraction, probably due to the diverse aggregation of proteinaceous matter with HS. The elution profile of the HS fraction also varied greatly in that period, resulting in partially resolved compounds with different degrees of resolution. These variations during that period might reflect the fact that they contained a very complex mixture of components of different origin. The sources of the OM could be different, being the increased inflow of terrestrial OM and elevated lake biological productivity. The relatively higher polydispersity of the HS fraction in these samples was in agreement with this assumption (Figure 4(c)). This evidence might suggest that as the lake level rose rapidly, forcing the coastline to retreat, large areas of coastal soils, terrestrial plants and marginal peatlands were eventually flooded, abraded and transported to the basin, and thus land-derived material may have provided a source of sediment OM. However, the increased release of nutrients from intensively abraded and drowned shoreline, combined with increased run-off to the lake, possibly brought abundant dissolved nutrients to the lake and stimulated higher rates of primary in-lake production.

However, the real sources of OM could be difficult to identify. The absorbance ratio $A_{250/360}$ did not vary drastically, and thus it did not allow the dominance of allochthonous or autochthonous OM to be differentiated. Probably both sources (in-lake bioproductivity and land-derived influx) were responsible for formation of the sediment OM. As average MW values were slightly higher compared with the younger samples (M_w was ~1,900 Da and M_n was ~1,000 Da), it might be possible that OM derived from terrigenous sources could even dominate during the early Holocene. Although the changes in average MW values were not great, it is possible that they were also related to temporal changes in DOM content and structure. The polydispersity of the samples was 1.80–1.90, suggesting relatively homogeneous OM.

In the early stages of Lake Peipsi's development (10,300–7,600 cal BP), when the water level was low, OM in the upper sediment layers could also have been slightly resuspended by turbulence events. When the water level rose, turbulence no longer caused OM resuspension as waves could not reach the lake bottom.

The content of particulate OM showed steady values at 7,600 cal BP, suggesting that the lake-level of Lake Peipsi may have stabilised. Thereafter, variations in the data were mostly minor gradual oscillations, additionally indicating relatively uniform environmental conditions. This possibly implies that by approximately 7,000 cal BP, Lake Peipsi had attained a size and morphology comparable with that of today. However, the content and structure of DOM were still not stable. Chromatographic elution profiles of HS components dissolved in pore-water were still variable until 3,800 BP. Mostly compounds with MW values $<$800 Da varied. As the lake system and its water level were stable by that time, the changes in DOM content and structure were probably related to the lake inner processes like changing bioproduction. In addition, climatic change might have influenced the accumulation of OM in the lake. The period in Estonia between 9,000 and 5,000 cal BP, the Holocene thermal maximum, had an annual mean temperature 2.5 °C higher than today [27]. This may have affected the higher DOM content several thousand years ago compared with the present time.

Chromatograms of the DOM accumulated after 3,600 cal BP were very similar. Thus, the composition of OM in Lake Peipsi was probably more or less stable during the last 3,600 years.

In addition, after 2,400 cal BP a sharp decrease in the DOM content of pore-water was observed. As could be seen from the results, the content of DOM including HS in the early Holocene was almost twice as high as during the late Holocene. At the same time, the proportion of particulate OM increased. These changes temporally coincided with the beginning of human activity in the area around Lake Peipsi. The earliest traces of the adoption of cereal farming in Lake Peipsi catchment area, defined as the start of continuous cereal pollen grains in sediment pollen curves, are dated to the Late Bronze Age at 3,000 to 2,500 cal BP, whereas the overall adoption of cereal cultivation and onset of the formation of an agrarian landscape throughout Estonia took place during the Iron Age from 2,500 cal BP [28]. Extensive agricultural land use would lead to deforestation. The opening up of catchment essentially might lead to topsoil disturbances and increased intensities of erosion, and could produce an outbreak of soil-derived nutrients to the basin, subsequently stimulating the increase in productivity of the lake. However, pore-water DOM variables indicate decreasing trends. The possible explanation for this might be due to the changes in the nature of DOM in the sediments from 2,400 cal BP to the present, because during this period OM content increased in the particulate phase, but decreased in the dissolved phase (Figures 1 and 3). Leeben et al. [5] reported that Lake Peipsi had become eutrophic in the past few decades. This might have led to a greater input of microbially derived OM. Microbially derived OM has a more aliphatic character because of large amounts of lipids and polysaccharides which do not absorb UV light. Thus, this part of DOM is likely to have remained undetected by UV detection. According to Meyers and Ishiwatari [1], OM buried in the deeper sediment layers undergoes anaerobic degradation. Material derived from aquatic sources is generally more sensitive to biodegradation than the OM derived from terrestrial sources. Thus, land-derived OM (HS and lipids) becomes dominant as the components from aquatic sources are converted by microbial activity (carbohydrates, amino acids). Owing to diagenesis, only the most resistant components of original organic material remain. Microbially reprocessed OM also contributes to the sediment material. Thus, the amount and structure of OM buried in the sediments might be different to a certain extent from the original deposited OM. So, on the one hand, the higher DOC concentrations in the older pore-water samples from Lake Peipsi (10,300 to 2,600 cal BP) might to some degree reflect a typical OM diagenesis, leading to more humic-like DOC which would be detected by UV absorption-based techniques. But on the other hand, because during the water level rise in the lake, land-derived humic material would have been dominant, the higher DOC concentrations would more likely correspond with the depositional history.

5. Conclusions

The HPSEC method combined with spectroscopic data provides valuable information about DOM properties in lacustrine sediment pore-water.

In this study, DOC concentrations calculated from absorbance spectra correlated very well with the total areas of the peaks and HS fraction areas computed from the HPSEC chromatograms. Thus, HPSEC analysis allows the DOM content in sediment pore-waters to be evaluated and might be useful as an alternative method when there is no possibility of measuring DOC concentration. Also, HPSEC analysis combined with spectroscopic methods might provide general information about temporal changes in lacustrine sediment DOM characteristics. This approach could be used in palaeolimnological research, especially in research on small shallow lakes. Processes in the sediments of large lakes are diverse and the interpretation of the results might be more sophisticated.

The advantages of the HPSEC method used with UV detection were its simplicity and minimal sample pretreatment. However, the potential disadvantage of UV absorption-based techniques

was that the UV-nonabsorbing fraction remained unrepresented and a part of the potential DOM was underestimated.

Acknowledgements

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PUBLICATION III

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Analysis of the degradation products of chemical warfare agents using a portable capillary electrophoresis instrument with various sample injection devices

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Abstract

In the present research, the performance of three sample injection devices in a portable capillary electrophoresis (CE) instrument was examined. These were the so-called cross-sampler, horizontal injection channel and vertical injection channel. All the three showed a good reproducibility of migration times (the relative standard deviation (RSD) was 4.3% in the case of the cross-sampler, 6.0% in the case of the horizontal injection channel and 1.7% in the case of the vertical channel). However, the reproducibility of peak areas was not sufficient. Hence, this study was mainly focused on qualitative analysis. The cross-sampler injection device was used in the portable CE instrument to analyse the composition of degradation products of chemical warfare agents (CWA). For the analysis of CWA degradation products simple procedures for the extraction of phosphonic acids from different surfaces, such as soil, concrete and granite blocks, tile floor, were developed.

Keywords: chemical warfare agents; phosphonic acids; portable CE instrument; sample injection

1. Introduction

The development and miniaturization of portable instrumentation is becoming more and more important in analytical chemistry research. In field analysis its total time or cost may greatly benefit from the use of portable instruments. This way it is possible to skip sample storage and transportation from the sampling site to the lab and obtain results for fast decisions. Portable instruments find mostly use in environmental applications, forensic and clinical analyses as well as detection of chemical warfare agents (CWA). At present the portable instruments are usually made only for some specific task. The detection techniques employed are often based on electrochemical sensors [1], photometry [2] and voltammetry [3,4]. The portable instruments based on separation methods enable a wider range of analyses to be carried out. Nowadays there exist portable versions of almost all separation methods,

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like gas chromatography (GC) [5,6], high-performance liquid chromatography (HPLC) [7], ion chromatography (IC) [8] or capillary electrophoresis (CE) [9-15].

In terms of a potential use in portable instruments CE has one main advantage over the other separation methods. CE does not require high pressure pumps for operating as liquid chromatography does. Separation is done by high voltage (HV). The simplicity of generating HV in portable devices is beyond comparison while the pressure needed for chromatographic techniques can only be achieved using complicated mechanical high-pressure pumps.

Today most portative CE instruments are based on contactless conductivity detection (CCD) [16-18]. CCD has sufficient sensitivity and can easily be coupled to portative instruments as its power consumption is very low. Also, because of its very simple detection cell construction, CCD is much more convenient than the other electrochemical detection techniques like amperometry, potentiometry and conductometry This is especially important in the case of CE considering the small dimensions of the separation capillary. The CCD detectors are basically constructed of two axially placed tubular electrodes which encompass the separation capillary. This means that the signal of CCD is gathered longitudinally along the capillary, instead of a transversal mode of conventional absorbance detection schemes. One of the two electrodes is excited with AC signal and the other electrode is used to register the same signal after passing through the cell.

So far portable CE instruments have mostly been used in traditional electrokinetic or hydrodynamic injection techniques that involve a lot of repeated operations with a background electrolyte (BGE) and sample vials. In commercial bench-top instruments these operations are automated by an auto-sampler, but in portable field instruments they must be carried out manually. In portable instruments the use of sampling techniques with as low a number of manual operations as possible should be considered. Therefore, the development of new sample injection approaches is highly welcome. The present contribution tests several different types of injection devices that could be operated using conventional plastic syringes.

2. Materials and methods

2.1. Chemicals

For the analysis of the degradation products of chemical warfare agents the following phosphonic acids were used: methylphosphonic acid (MPA), ethylphosphonic acid (EPA), 1-butylphosphonic acid (1-BPA), propylphosphonic acid (PPA), and pinacolyl metylphosphonic acid (PMPA). MPA, EPA and 1-BPA were purchased from Alfa Aesar, Lancaster Synthesis (Windham, NH, USA) and PPA and PMPA from Sigma-Aldrich (Steinheim, Germany). L-Histidine (His) and 2-(*N*-morpholino)ethanesulfonic acid hydrate (MES hydrate) were also purchased from Sigma-Aldrich. Sodium hydroxide was purchased from Chemapol (Prague, Czech Republic).

Stock solutions were prepared by dissolving an exact amount of each phosphonic acid in MilliQ water to a concentration of 10 mM. This was followed by a further mixing of all five analytes into a standard solution of different concentrations.

BGE was prepared by dissolving an exact amount of His and MES in MilliQ water.

2.2. Instrumentation

The in-house made portable CE instrument was equipped with a CCD detector and various injection devices. The dimensions of the instrument were 330×180×130 mm. The system ran on 10 common AA-type rechargeable batteries with an overall ouput of 15 V. Its operating time running on batteries was up to 4 hours. The system high-voltage output was up to 25 kV. The cell of the CCD detector was made of a rectangular piece of aluminium. There were milled three holes for two tubular electrodes and an operational amplifier. 8 mm electodes made of syringe needles were placed in separate chambers with a gap of 0.8 mm between them. One of the electodes was excited with a peak-to-peak sine wave at 60 V which can oscillate in a frequency range of 50-300 kHz. The second electrode picked up and amplified the signal after it had passed the capillary walls and the liquid in the capillary. The detection process was controlled by a computer with in-house written software. In the present work, all expreriments were carried out at a detector oscillation frequency of 200 kHz.

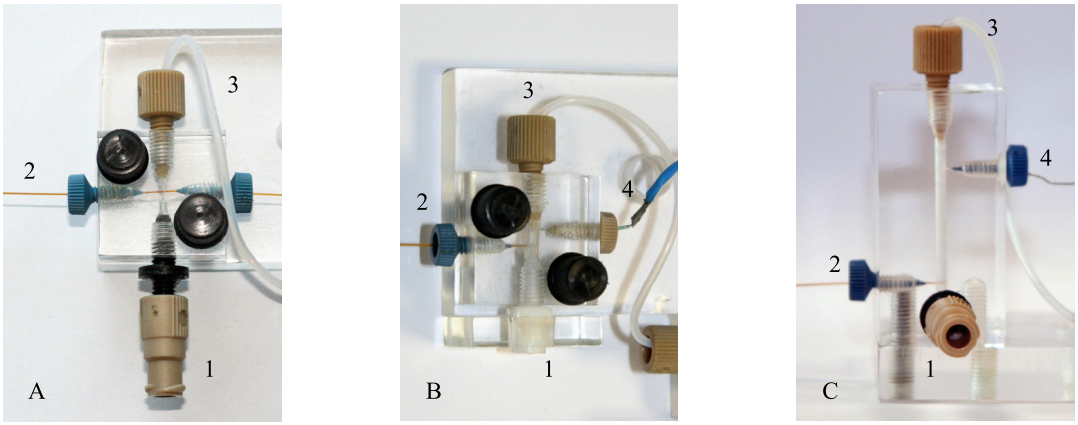

Fig. 1. The construction of various injection devices for a portable CE instrument: (a) cross-sampler; (b) horizontal injection channel; (c) vertical injection channel. 1 – sample injection socket for syringe, 2 – separation capillary, 3 – waste channel, 4 – grounding electrode.

The use in the portable CE system of three different injection devices was examined. The aim was to replace plastic vials commonly used in CE with a more convenient system and to minimize the number of manual operations in the injection procedure. For this purpose the cross-sampler, so-called horizontal channel and vertical channel were tested. The construction of the cross-sampler (Fig. 1a) was similar to that of the devices used in the microchip electrophoresis. It consisted of two perpendicular channels, one was for injection and the other, for separation. At the crossing point of both channels the ends of two capillaries were inserted. The analytes were separated in the longer capillary (a total length 44 cm, an effective length 36 cm), the shorter capillary (6 cm) was connected to the vial containing BGE. A small amount of the sample was introduced into the cross-sampler and then the voltage was applied to carry out the experiment. A detailed description of the cross-sampler and the whole portable CE equipment used in this work has been presented by Seiman *et al.* [15]. The amount of the sample introduced into the cross-sampler was 0.02 ml.

The second injection device was the horizontral channel (Fig. 1b) made of the polymethylmethacrylate (PMMA) block with dimensions of 10×25×25 mm. The length of the injection channel was 10 mm. The channel served as an inlet vial. The inlet end of the separation capillary was connected with the injection channel, while the electrode was placed on the opposite side. For the analysis 0.1 ml of the sample solution was introduced with the syringe into the injection channel. After the sample injection the inlet channel was filled with 0.25 ml of BGE. Capillary electrophoresis injection device with this type of construction was reported first by Kuban *et al.* [19]. It was used for flow injection analysis combined with CE. Injection device was operated by two peristaltic pumps. In our case simplier approach is used and injections are made manually using plastic syringes.

The operation of the vertical channel device with the dimensions of 25×25×47 mm (the length of the injection channel 33 mm) was based on the same principle as that of the horizontal channel (Fig. 1c). The difference between the two injection devices was in sample inlet channel direction. In both injection devices the separation capillaries with a total length of 55 cm and effective length of 45 cm were used. For the injection 0.25 ml of the sample solution was injected into the sample inlet channel which was later filled with 0.5 ml of BGE.

The fused silica capillary (i.d. 75 μm and o.d. 360 μm) was purchased from Agilent Technologies (Santa Clara, CA, US). In all experiments the BGE used was a 15 mM MES/His solution, the separation voltage was 16 kV.

2.3. Extraction procedure for CWA degradation products

To analyse CWA degradation products a simple procedure for their extraction from different surfaces was developed. First, for sample preparation 5 ml of the stock solution of five phosphonic acids with a concentration of 2 mM was sprayed on a small ground area and exposed for approximately 1.5 h. 2 g of sample was taken from the upper layer of soil and placed into 50 ml plastic vials. For the extraction 10 ml of MilliQ water was added to the soil sample. The sample was sonicated for 30 minutes, and filtered through a medium fast paper filter (Whatman, Maidstone, UK) and 0.45 μm Millipore filter (Sarstetd, Germany).

For the extraction of CWA degradation products from varoius surfaces (granite blocks, concrete blocks, asphalt, tile floor, etc) 5 ml of a 2 mM 5 phosphonic acid solution was sprayed. After complete drying the surface was wiped with a filter paper (medium fast paper filter, Aldrich, USA) moistened in 2 ml of MilliQ water. The filter was then introduced into a 50 ml vial and 8 ml MilliQ water was added. The sample was sonicated for 30 minutes and after that filtered through a 0.45 μm Millipore filter.

3. Results and Discussion

3.1. Repeatability of the injection devices tested

The convenience of the sample injection procedure is crucial in portable CE instruments. Replacing plastic vials with sample and BGE in the field may be unhandy. The injection devices requiring no operations with sample and BGE vials should be preferred.

As a detailed description of the work dedicated to the cross-sampler has been presented in [15], this study compared advantages and disadvantages of the sampler over the other injection devices. Sample injection in all three samplers is performed by pushing a certain amount of the sample into the channel with a syringe. In horizontal and vertical channels the sample is then washed out with a certain amount of BGE. Theoretically, the injected volume should be dependent only on the sample volume introduced into the channel and configuration of the injection device. However, as all these manipulations are made by hand, it is difficult to control the pushing force and make always reproducible injections. By pushing harder, more sample is introduced into the capillary. In the case of horizontal and vertical channels, the pushing force also plays a certain role when washing with BGE as then the sample zone is pushed further into the separation capillary. By pushing the syringe with BGE too hard or using too much liquid for washing, it is possible to push the sample so far into the separation capillary that it will affect migration times.

The performance of the samplers was estimated on a standard mixture of five phosphonic acids with a concentration of 100 μM. The results are given in Table 1.

Table 1. The migration time reproducibility for five phosphonic acids

Injection device	Relative standard deviation (RSD, %)
Cross-sampler	4.3*
Horizontal injection channel	6.0*
Vertical injection channel	1.7**

*at least three-day reproducibility

** one-day reproducibility

The vertical sampler had a better reproducibility of migration times than the other two injection devices. Though, the RSD data for the vertical channel was calculated only during one day, as it was found to suit less for routine analysis than the horizontal channel. At the same time, the cross-sampler was found to be more convenient to be used in the portable CE instrument as it required a minimum number of operations and so was used for the analysis of CWA degradation products in the portable CE instrument.

3.2. Analysis of the phosphonic acids extracted from different surfaces

As the reproducibility of peak areas was not sufficient for all the injection devices investigated, we focused only on the qualitative analysis of CWA samples. A simple and fast procedure for the exraction of CWA degradation products from different surfaces was worked out. A 2 mM phosphonic acid mixture was sprayed on the ground (and other surfaces, like asphalt or concrete blocks) outside, but CE experiments with the portable CE instrument were carried out in the laboratory, not on-site. These were preliminary experiments for the newly-built portable instrument which was first tested in laboratory conditions.

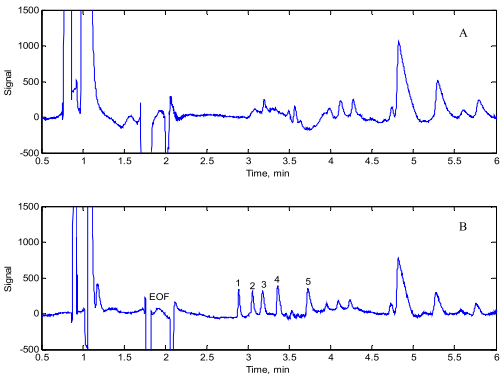

Fig. 2. The electropherograms of soil extracts (sample injected with the cross-sampler). The BGE solution is 15 mM MES/His. Separation voltage: 16 kV. A – blank sample, pure soil exctract without any standards, B – phosphonic acids extracted from soil. 1 – PMPA, 2 – 1-BPA, 3 – PPA, 4 – EPA, 5 – MPA, EOF – electroosmotic flow.

Soil extracts were prepared as described in section 2.3. The extraction experiments were carried out using soil samples collected from different places. In all cases it was possible to extract all five phosphonic acids (Fig. 2). During the extraction, some components of the soil were also extracted, but their peaks did not overlap with those of phosphonic acids. Thus, the procedure for extraction of CWA degradation products from soil might be suitable for the qualitative analysis. During this stage of the study the recovery of phosphonic acid standards was not measured.

A universal procedure of extraction of CWA degradation components from different hard surfaces such as granite, concrete blocks and tile floor was also successfully worked out. The results are presented in Figs. 3 and 4. Using this procedure all five phosphonic acids were extracted from the surfaces investigated. Some unknown compounds were also extracted and separated from phosphonic acids peaks. Again, their peaks did not overlap with those of phoshonic acids.

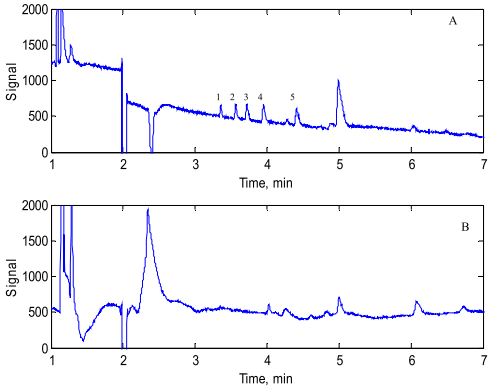

Figure 3. The electropherograms of extracts from concrete blocks (sample injected with the cross-sampler). The BGE solution is 15 mM MES/His. Separation voltage: 16 kV. A – phosphonic acids extracted from concrete blocks. 1 – PMPA, 2 – 1-BPA, 3 – PPA, 4 – EPA, 5 – MPA, EOF – electroosmotic flow; B – blank sample, pure exctract from concrete blocks without any standards.

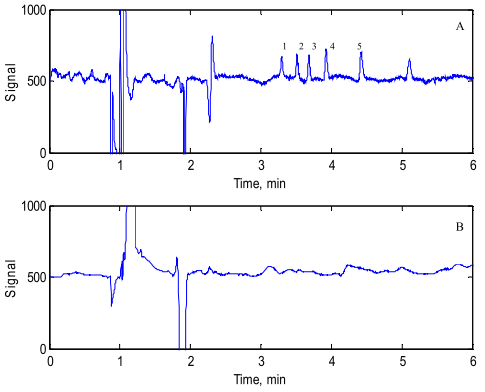

Figure 4. The electropherograms of extracts from tile floor (sample injected with the cross-sampler). The BGE solution is 15 mM MES/His. Separation voltage: 16 kV. A – phosphonic acids extracted from soil. 1 – PMPA, 2 – 1-BPA, 3 – PPA, 4 – EPA, 5 – MPA, EOF – electroosmotic flow; B – blank sample, pure exctract from tile floor without any standards.

4. Conclusions

The present study demonstrated that all three injection devices investigated were suitable for the qualitative analysis of compounds using a portable CE instrument. However, as the cross-sampler required fewer manual operations, it was more convenient to be used in field experiments. In laboratory analysis horizontal and vertical injection channels might also be used as an alternative to plastic vials. Unfortunately, it was not possible to obtain good reproducibility for peak areas with these injection devices and only qualitative analysis was done in the present study. To achieve better peak area reproducibility, further improvements and investigations are needed.

The developed procedures were suitable for the extraction of all five phosphonic acids from soil and various hard surfaces. Though, during the extraction several unknown components were revealed. Their peaks did not overlap with those of phosphonic acids. So, it would be possible to identify CWA degradation products from different surfaces using a simple water extraction procedure.

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PUBLICATION IV

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The detection of nerve agent degradation products in different soil fractions using capillary electrophoresis with contactless conductivity detection

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The adsorption of various phosphonic acids in sand and loam was studied. Samples of both soil types were sieved into three different fractions according to particle size. The fractions used were in the range 0–100, 100–200 and 200–400 μm. The performance of the capillary electrophoresis equipped with contactless conductivity detection was investigated. The limit of detection for the phosphonic acids tested was in the range 0.11–1.4 ppm. Different isotherms were constructed for all adsorption curves. Adsorption was found to be higher in sand than in loam when the Langmuir adsorption isotherm was used. The adsorption of methylphosphonic acid was higher than that of other phosphonic acids due to the smaller molecular size of the former.

Keywords: adsorption; capillary electrophoresis; chemical warfare agents; contactless conductivity detection; soil fraction

1. Introduction

Organophosphorous nerve agents are one of the most toxic substances ever synthesised. Although having found limited use so far, determination of these substances or their degradation products is still an important field of research, especially in the last 10 years, which have seen terrorist activity increase. Therefore, there is a continuous need for rapid and reliable methods for the detection of nerve agents and their degradation products. Nerve agents are categorised according to structure into two groups: G- and V-type. Both types have several similar structural features, such as the existence of a double bond between the terminal oxygen and phosphorous or two lipophilic groups and one leaving group bound to the phosphorous. These features make nerve agents unique and distinct from the large group of organophosphates, such as the herbicides, pesticides, and insecticides widely found in soil due to their use in agriculture. In aqueous environments, these organophosphorous nerve agents hydrolyse more or less easily to produce non-toxic and more stable compounds. The most important degradation products of nerve agents are alkyl alkylphosphonic acids, which are suitable for verifying the presence or use of organophosphorous

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nerve agents. Moreover, being specific to particular nerve agents, these acids can be used to identify their parental nerve agents.

Various separation methods such as gas (GC) [1] and liquid chromatography [2] and capillary electrophoresis (CE) [3] can be used to analyse the degradation products of nerve agents. Nowadays, the chromatographic methods developed for this purpose are mostly coupled to mass spectrometry [4,5]. GC is a suitable tool for analysing easily volatile nerve agents whose degradation products, however, cannot be directly applied to GC analysis because of their high polarity and low water solubility. Therefore, a derivatisation procedure for conversion of the degradation products into more volatile compounds is needed. A detailed review of possible separation techniques for the analysis of nerve agents is given in Hooijschuur et al. [6]. In view of the fact that phosphonic acids need no derivatisation, the fast, simple and relatively inexpensive CE is often preferred. CE analysis requires simple sample preparation involving dilution and filtration, and easy equipment operations and maintenance. CE is less sensitive to sample matrix and, therefore, real samples such as aqueous soil extracts and river water can be subjected to CE analysis [3,7,8]. It is possible to use direct [3] and indirect UV [9] to detect nerve agents and their degradation products in CE. The indirect mode of UV detection is preferred as it is more sensitive because phosphonic acids do not contain any chromophoric groups. Lately, a contactless conductivity detection (CCD) has also been used to detect nerve agent degradation products [10–12].

Basic principles of CCD and a more detailed description of the current set-up are available as supplementary material S1 (available online only). In this work, a CCD–CE system was used to study the adsorption of phosphonic acids in different fractions of soil.

The adsorption curves can be fitted to different types of isotherms, the Langmuir and Freundlich types being the most common. Kothawala et al. fitted their experimental data for the adsorption of organic carbon onto mineral soils to four different types of adsorption isotherms [13]. The Langmuir isotherm was found to produce more robust results. Fitting the Freundlich and Langmuir isotherms to the same data has been compared in Vasanth Kumar and Sivanesan [14]. Again, the latter was more advantageous. In this study, a linear least squares method was used to estimate the performance of the Langmuir and Freundlich adsorption isotherms. In addition to the Langmuir and Freundlich adsorption isotherms, the Redlich–Peterson and BET-isotherms were also calculated. An introduction to the theoretical background of these adsorption isotherms is available as supplementary material S2 (available online only).

2. Materials and methods

2.1. *Soil samples*

Environmental soil samples were collected from two different locations in Estonia. The sand sample was taken from a park in the city of Tallinn (latitude: 59°23′42.13″, longitude: 24°40′37.02″) and the loam sample from a forest in the Kõpu rural municipality, Viljandi county (latitude: 58°19′34.72″, longitude: 25°17′45.19″). Samples were collected from the surface layer of soil at a maximum depth of 5 cm. Sand and loam samples had not been exposed to nerve agents or their degradation products before. The sampling sites were chosen far away from agricultural areas to avoid possible contamination of samples with the other types of organophosphates used in agriculture, such as herbicides or insecticides.

Gravimetric analysis was used to determine the organic content of the soil samples. First, the crucibles used for the analysis were heated to 550 °C for 4 h in a muffle furnace to gain constant weight. Second, after cooling for 30 min, the crucibles were weighed and ~ 1 g of a particular soil sample was added to the crucibles. The soil samples were treated for 4 h at a temperature of 550 °C. After a 30 min cooling period, the crucibles with temperature-treated samples were

Table 1. Organic matter in different soil fractions.

Size of fraction, μm	Amount of organic matter, %	
	Sand	Loam
<100	1.24	5.64
100–200	0.55	7.03
200–400	0.50	6.05

weighed again. The organic content of the soil samples was calculated using the difference between the two masses. Four hours was long enough for all the samples to lose their organic part.

The adsorption of phosphonic acids in soil may be considered as a sum of physicosorption and chemisorption. A simple explanation for this could be as follows. Phosphonic acids are adsorbed onto inorganic soil particles by undergoing physicosorption, whereas their adsorption onto the organic part of a soil sample takes place under the mechanism of chemisorption. This simplified theory may be applied to explaining the adsorptive behaviour of phosphonic acids in different types of soil. Therefore, the organic content of soil samples was determined using gravimetric analysis and muffle furnaces.

Data on the organic content of soil samples are presented in Table 1. The organic content of the loam samples (5.64–7.03%) was 10 times as high as that of the sand samples (0.50–1.24%). The organic content of the finest fraction of the sand sample was ~ 2.5 times as high as that of the other two fractions. This means that the organic matter of the sample contained small fractions of clay, silt and very fine sand. However, in the case of loamy soil, the respective figures were slightly different. So, the organic content of its medium fraction was the highest and that of the finest fraction the lowest.

2.2. *Preparation of soil extracts*

Loam and sand samples were first dried at room temperature until the mass of both samples was constant. The procedure took three to four days. After that, the samples were fractionated by particle size using three sieves with different hole sizes. The sand and loam samples were sieved into three fractions: <100, 100–200 and 200–400 μm. Samples with a particle size >400 μm were disposed of. Basically, the fractions represented very fine, fine and medium-grained sand. Clay and silt were not thoroughly dealt with because Estonian soils are mainly sand-based. Silt and clay were the components of the finest fraction of samples.

For sample preparation, 0.5 g of the fractionated soil material was weighed into 2 mL plastic vials. The samples were spiked with a 2 mM stock solution of five phosphonic acids. The added amounts of phosphonic acids were 12.5, 25, 37.5, 50, 75 or 100 μL. After a 50 min exposure to phosphonic acids, MilliQ water was added to the soil samples to obtain a total volume of 1 mL. This means that the concentration of phosphonic acids in the samples was 25, 50, 75, 100, 150 or 200 μM, respectively. The samples were then shaken for 10 min and also centrifuged for 10 min. A 500 μL aliquot of an unfiltered supernatant was placed into 0.5 mL plastic vials and 2-aminoethyl dihydrogenphosphate (AEDHP) was added as an internal standard. The concentration of the internal standard was 500 μM. The unfiltered samples were subjected to CE analysis.

2.3. *CCD–CE experiments*

2.3.1. *Instrumentation*

All experiments were carried out using a commercially available Agilent Technologies CE instrument (Waldbronn, Germany) equipped with a diode array detector. For detection a CCD detector

was used instead of a UV detector. The CCD detector was made in-house. A detailed description of this detector is given in Seiman et al. [15] and supplementary material S1 (available online only). The combination of the in-house CCD detector and the conventional bench-top Agilent instrument enabled application of the detection schemes developed for CCD to a large number of samples because the Agilent instrument provides possibilities for programming long sequences of separate analyses.

The uncoated fused-silica capillary (i.d., 75 μm; o.d., 360 μm) with a total length of 55 cm was purchased from Agilent Technologies (Santa Clara, CA, US). The length of the capillary to the CCD cell was 45 cm and to the DAD cell, 49 cm.

2.3.2. *Standards*

For analysis of the degradation products of toxic organophosphates the following phosphonic acids were used: methylphosphonic acid (MPA), ethylphosphonic acid (EPA), 1-butylphosphonic acid (1-BPA), propylphosphonic acid (PPA) and pinacolyl metylphosphonic acid (PMPA). MPA, EPA and 1-BPA were purchased from Alfa Aesar, Lancaster Synthesis (Windham, NH, USA) and PPA and PMPA were from Sigma-Aldrich (Steinheim, Germany). AEDHP used as the internal standard, and BGE components, L-histidine (His) and 2-(*N*-morpholino)ethanesulphonic acid hydrate (MES hydrate), were also purchased from Sigma-Aldrich (Steinheim, Germany). Sodium hydroxide was purchased from Chemapol (Prague, Czech Republic).

Standard stock solutions were prepared by dissolving an exact amount of each phosphonic acid in MilliQ water to a concentration of 10 mM. This was followed by a further mixing of all five analytes into a standard solution of a total concentration of 2 mM.

BGE for capillary electrophoresis analysis was prepared by dissolving an exact amount of His and MES in MilliQ water.

2.3.3. *Procedures*

A new capillary was flushed with a 1 M NaOH for 10 min and then with water for 10 min and with BGE for 10 min. Before starting the experiments, the capillary was flushed with a 0.1 M NaOH for 3 min, with water for 10 min and with BGE for 10 min every day. Between each run it was rinsed with water for 2 min and with BGE for 3 min. The BGE was a 15 mM Mes/His buffer. The sample was injected hydrodynamically for 10 s (50 mBar). In all experiments, the cartridge with the separation capillary was thermostated at 25 °C. The separation voltage was 20 kV.

3. Results and discussion

3.1. *Performance of the CCD-CE system*

For the analysis of adsorption of phosphonic acids in soil, first, calibration curves had to be constructed. This was a suitable opportunity to evaluate the performance, including the reproducibility, sensitivity, etc., of the combined CCD–CE equipment. The calibration curve of seven points was constructed in the region 10–200 μM. An internal standard AEDHP was added to every sample at a concentration of 500 μM. The constructed calibration curve demonstrated good linearity in the measured range as the coefficient of determination between experimental data and the constructed calibration curve was close to one ($R^2 > 0.99$ for all phosphonic acids). Multiple repetitive analyses needed for the construction of the calibration curve were performed to calculate the reproducibility of the CCD–CE system. Detailed performance data are given in Table 2. The reproducibility of the system was acceptable as the relative standard deviations (RSD) of

Table 2. Performance data for phosphonic acids.

	LOD* (μM)	RSD† (%)	$b_0^{\ddagger}$	$b_1^{\ddagger}$	$(R^2)^{\S}$
PMPA	7.56	5.68	−0.0514	0.0068	0.9915
1-BPA	5.96	3.48	−0.0572	0.0096	0.9977
PPA	5.45	4.23	−0.0605	0.0111	0.9974
EPA	5.84	2.58	−0.0847	0.0145	0.9952
MPA	1.20	6.92	−0.0236	0.0196	0.9984

Notes: *Limit of detection. †Relative standard deviation of peak areas. ‡Calibration line equation $y = b_0 + b_1 c$ parameters: b_0, intercept; b_1, slope; y, detector response; c, concentration. §Square of correlation coeficient of calibration line.

peak areas were in the region 2.6–6.9%. To estimate the sensitivity of the CCD detector, LODs for all the phosphonic acids analysed were calculated by interpolating the calibration curves. The LODs were in the range of 1.2 (0.11) and 7.6 (1.4) μM (ppm).

3.2. *Analysis of blank soil extracts*

When developing procedures for the extraction of nerve agent degradation products from soil, all possible unknown substances that could have been extracted from soil had to be separated from phosphonic acids using CCD–CE analysis. Otherwise it would have been impossible to estimate the adsorption of nerve agent degradation products in soil. Blank extracts of loam and sand samples were examined first to discover all unknown peaks belonging to substances which extracted from soil under the current extraction procedures. A comparison of blank soil extracts and extracts containing phosphonic acids was necessary to find out if peaks of the latter were separated from each other and from all unknown peaks belonging to soil extracts.

Blank samples (Figure 1(B)) had two peaks that were observed in case of all loam and sand samples. Only the height of the peaks in different fractions varied. All unknown peaks migrated slower

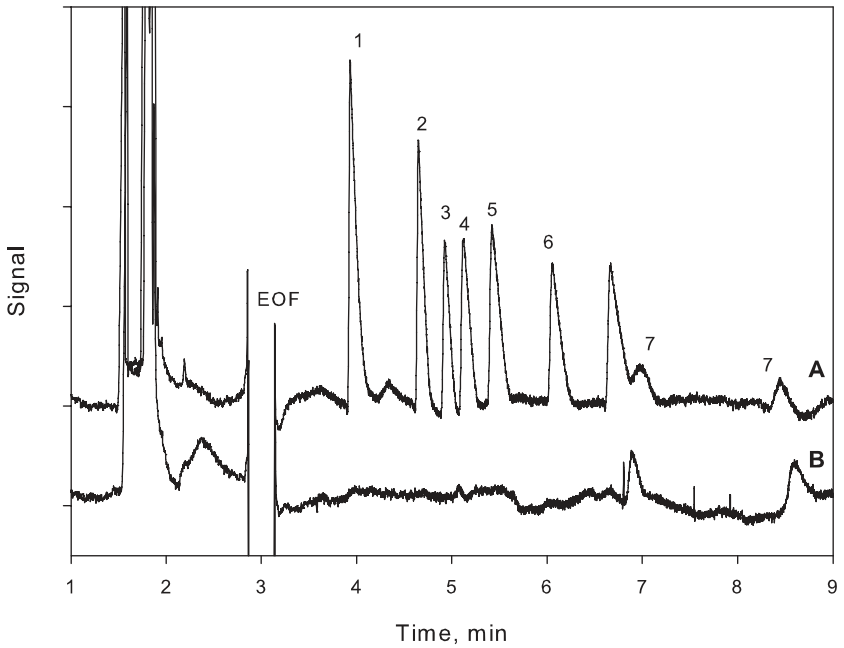

Figure 1. Separation of blank soil extract and phosphonic acids. (A) Blank extract from sand, fraction 100–200 μm; (B) extract of 100 μM phosphonic acids from sand, fraction 100–200 μm. EOF, electroosmotic flow; 1, AEDHP; 2, PMPA; 3, BPA; 4, MPA; 5, EPA; 6, MPA; 7, unknown peaks.

than phosphonic acids (Figure 1(A)). It may be assumed that these unknown peaks corresponded to some small anions, though they were not identified as it was not the goal of this article.

3.3. *Adsorption isotherms of different soil samples*

Sand and loam extracts were prepared using six different concentrations of phosphonic acids by spiking a particular sample with a certain amount of the standard mixture of phosphonic acids. Spiked samples were extracted with water after a certain time. With no adsorption at all the concentration of phosphonic acids in these extracts would have been 25, 50, 75, 100, 150 and 200 μM. In reality, all these concentrations were lower as some amount of phosphonic acids was adsorbed into the soil. The difference between these two concentrations was well suited for estimating the degree of adsorption of phosphonic acids in a particular soil sample. All together, adsorption curves were constructed for two types of soil and three fractions of each. Six concentrations of phosphonic acids were measured to construct one adsorption curve. With three parallel experiments for every sample, 108 soil extracts were analysed in total.

On the basis of the experimental data, four different types of adsorption isotherms were constructed. A comparison of the determination coefficients of experimental data and fitted isotherms enables an assumption to be made about which isotherm of the four is the closest to the performance of real soil. The parameters and determination coefficients for all calculated isotherms are given in Table 3. The parameters of the Langmuir and Freundlich isotherms were calculated using a least square method in a linearised form. The parameters of the Redlich–Peterson and BET-isotherms could not be found using a linear form of the method because they contain three unknown parameters. Instead, a nonlinear trial and error procedure was used to determine all three parameters. The coefficients of determination of calculated isotherms and experimental data were used to compare different adsorption models.

A comparison shows no significant difference to exist in determination coefficients between the Langmuir, Freundlich and Redlich–Peterson isotherms in the measured concentration region. The isotherms follow closely each other's path when plotted on the same graph. When extrapolated to higher concentrations, the Langmuir isotherm levels out at q_{max}, while the other two isotherms continue to grow. The Redlich–Peterson isotherm would have been expected to have superior fitting because it is described with the aid of three adjustable parameters, instead of two as is the case with the Langmuir and Freundlich isotherms. Apparently, there is no significant difference in determination coefficients (R^2) between the above adsorption isotherms, and the type of the best fitting isotherm varies from sample to sample. Therefore, the Langmuir isotherm may be considered to be no worse in performance than the other two and its parameter, q_{max}, which is a maximum adsorption on the monolayer coverage, could be used to compare the adsorptive capacity of various samples.

At a low concentration of phosphonic acids, the Langmuir adsorption isotherm is linear because no molecules were adsorbed on the surface. At higher acid concentrations, the adsorption curve decreases until it reaches a constant value, the maximum adsorption, when all adsorption sites are filled and the monolayer of phosphonic acids is formed on the surface of soil particles.

The results obtained demonstrate the adsorption capacity of sand to be higher than that of loam. The adsorption capacity of smaller fractions of sand samples is higher. However, the difference in adsorption capacity between the smallest fractions (below 100 μm) of sand and loam samples is not as significant as that between their larger fractions (200–400 μm).

Figure 2 illustrates the adsorption of EPA in different soil samples. EPA was chosen as an example to illustrate the adsorptive behaviour of all the phosphonic acids tested in this work, with the exception of PMPA. In case of the sand sample, adsorption was lowest in the medium-sized fraction and highest in the smallest fraction. In case of the loam sample, two smaller fractions

Table 3. Parameters of various adsorption isotherms for sand and loam samples.

		< 100 μm				100–200 μm				200–400 μm			
		BPA	PPA	EPA	MPA	BPA	PPA	EPA	MPA	BPA	PPA	EPA	MPA
Sand													
Langmuir	q_{max}	238.3	205.5	252.8	495.2	323.6	62.2	193.4	282.6	581.4	241.7	268.9	339.9
	K_a	0.0036	0.0042	0.0035	0.0022	0.0017	0.0162	0.0035	0.0038	0.001	0.0026	0.0027	0.0033
	r^2	0.9681	0.937	0.9808	0.9837	0.8856	0.9191	0.9376	0.9735	0.9971	0.9867	0.9804	0.9967
Freundlich	n_F	1.9377	2.1648	1.9888	2.0327	1.1653	3.983	1.5924	2.6812	0.8138	1.0074	1.3423	2.1561
	K_F	1.3332	1.4033	1.3302	1.2281	1.2679	2.071	1.3598	1.3877	1.0992	1.1915	1.235	1.2702
	r^2	0.9795	0.9494	0.9912	0.9902	0.8872	0.8938	0.9474	0.9842	0.997	0.9728	0.98	0.9914
Redlich–Peterson	K_R	0.3013	0.3362	0.2941	0.2398	0.2076	0.5458	0.3169	0.3306	0.1360	0.2126	0.2426	0.2657
	a_R	3.9656	4.4092	3.9590	4.2556	1.8591	8.1942	3.3096	5.5674	1.6945	2.0340	2.7244	4.3811
	b_R	1.3516	1.3996	1.3419	1.2711	1.2307	1.7260	1.3729	1.3917	1.1457	1.2369	1.2746	1.3043
	r^2	0.9790	0.9491	0.9912	0.9903	0.8921	0.8970	0.9474	0.9838	0.9970	0.9749	0.9805	0.9924
BET	q_{max}	64.9	58.9	67.2	72.6	35.7	58.8	43.4	66.4	159.9	229.7	153.7	n.a.*
	K_L	0.0024	0.0023	0.0023	0.0027	0.0032	0.0003	0.0026	0.0023	0.0010	0.0000	0.0007	n.a.
	K_S	0.0170	0.0191	0.0167	0.0203	0.0248	0.0165	0.0229	0.0232	0.0041	0.0027	0.0051	n.a.
	r^2	0.9862	0.9578	0.9939	0.9488	0.9545	0.9791	0.9634	0.9145	0.9959	0.9876	0.9814	n.a.
Loam													
Langmuir	q_{max}	167.7	331.8	108.7	435.3	125.2	199.1	90.0	368.7	61.6	86.6	59.9	229.4
	K_a	0.0028	0.0008	0.005	0.0014	0.0046	0.0013	0.0079	0.002	0.0085	0.0035	0.0096	0.0027
	r^2	0.9871	0.9673	0.8948	0.9805	0.8258	0.9584	0.9797	0.9886	0.9253	0.9643	0.8089	0.9244
Freundlich	n_F	1.0132	0.3921	1.834	1.1518	2.0919	0.3123	1.9376	0.9902	2.2886	0.7218	2.7946	1.7001
	K_F	1.2983	1.1093	1.5672	1.1988	1.6124	1.0709	1.5453	1.1162	1.8659	1.351	1.9951	1.3879
	r^2	0.991	0.9691	0.9171	0.9762	0.8534	0.9342	0.9544	0.9581	0.9583	0.9714	0.8584	0.919
Redlich–Peterson	K_R	0.2844	0.1899	0.4067	0.2224	0.4218	0.1001	0.3956	0.1502	0.4906	0.3117	0.5303	0.3165
	a_R	2.1268	0.9382	3.8715	2.4948	4.3833	0.6155	3.8379	1.9644	4.5442	1.4922	5.8916	3.5245
	b_R	1.3290	1.2091	1.5018	1.2491	1.5247	1.1052	1.4852	1.1621	1.6333	1.3658	1.6995	1.3723
	r^2	0.9911	0.9542	0.9181	0.9788	0.8545	0.9373	0.9582	0.9655	0.9590	0.9713	0.8571	0.9454
BET	q_{max}	40.6	79.4	21.8	45.7	23.4	n.a.	n.a.	n.a.	24.3	37.6	19.5	27.6
	K_L	0.0023	0.0009	0.0033	0.0029	0.0033	n.a.	n.a.	n.a.	0.0023	0.0013	0.0028	0.0034
	K_S	0.0163	0.0039	0.0631	0.0222	0.0586	n.a.	n.a.	n.a.	0.0377	0.0100	0.0762	0.0590
	r^2	0.9949	0.9931	0.9960	0.9740	0.9821	n.a.	n.a.	n.a.	0.9968	0.9965	0.9921	0.9359

Note: *Not available, statistically best fitted isotherm does not correspond to real life expectations having negative q_{max}, etc.

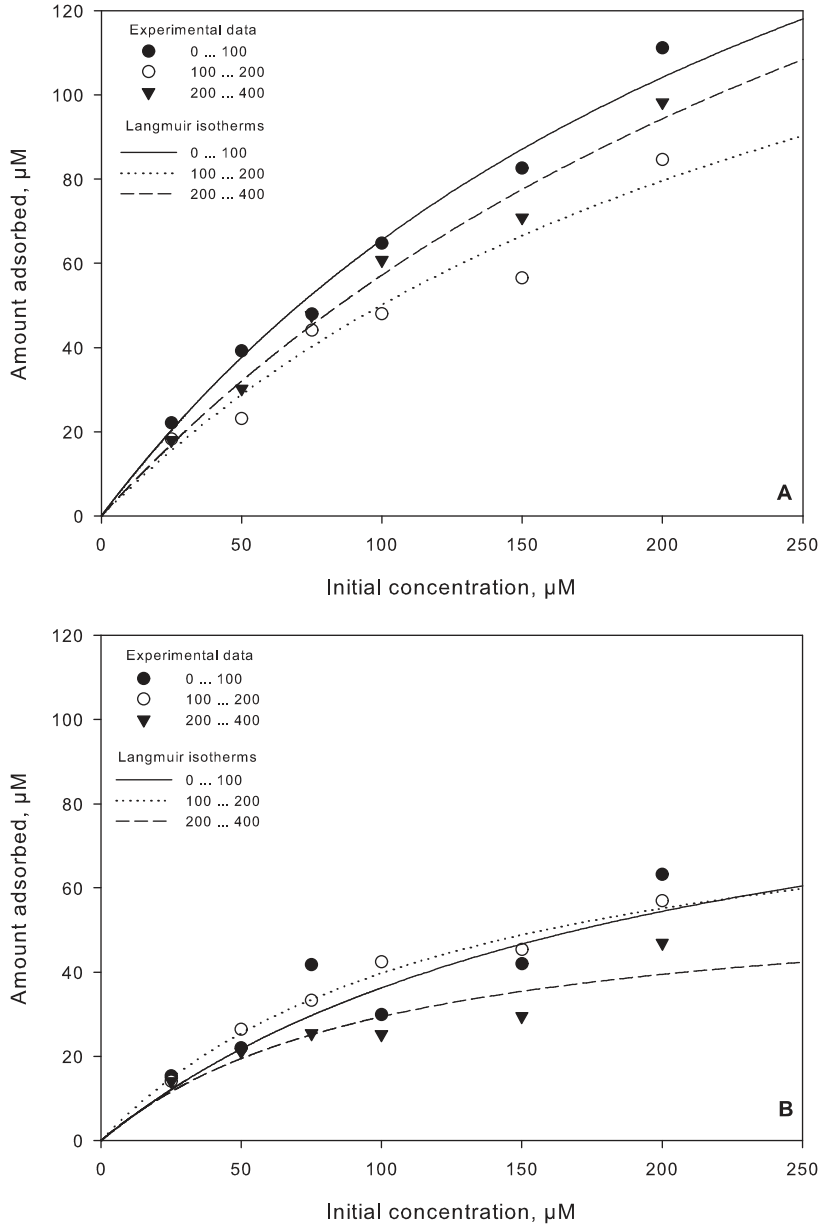

Figure 2. Adsorption data with fitted Langmuir isotherm of EPA in different soil fractions. (A) Sand samples, (B) loamy soil samples.

demonstrated a similar adsorption, while the largest fraction had an adsorption isotherm which was significantly higher than that of the other fractions.

The adsorption of different phosphonic acids in different fractions of sand and loamy soil samples was rather similar. On the basis of the adsorption isotherms of 200–400 µm fractions (Figure 3), some general conclusions can be drawn. First of all, adsorption was highest in the case of MPA. This could be explained by the different sizes of the various phosphonic acid molecules. Of the four phosphonic acids, the molar mass of MPA was by far the lowest. The other three acids had a similar adsorption rate in both sand (Figure 3(A)) and loamy soil (Figure 3(B)). From the two figures it can be seen that the Langmuir adsorption isotherm in the case of the loam sample

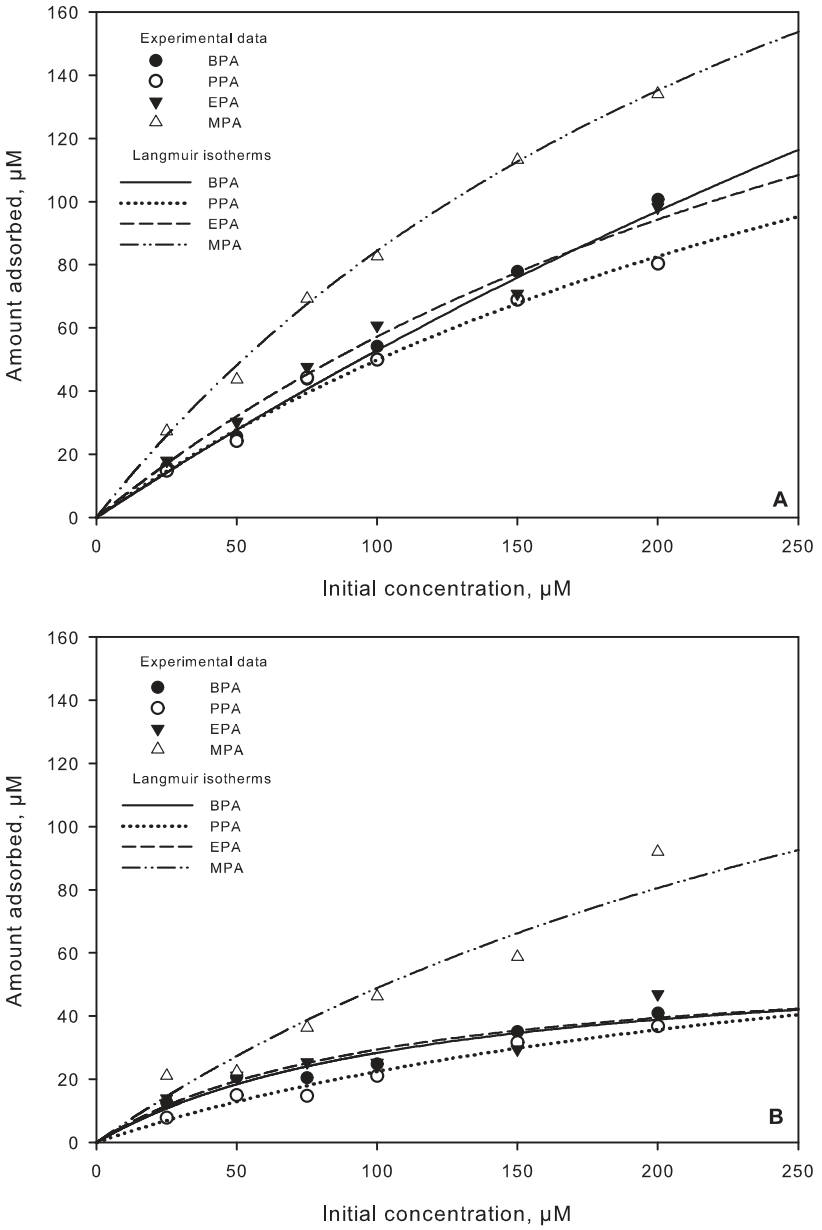

Figure 3. Adsorption data with fitted Langmuir isotherm of different phosphonic acids in soil fractions. (A) Phosphonic acids in sand, fraction 200–400 µm; (B) phosphonic acids in loamy soil, fraction 200– 400 µm.

achieved its constant value at an acid concentration of 200 mM, whereas in the case of the sand sample, the adsorption isotherm at this concentration, except for MPA, was still in the linear region. Therefore, one could expect that the adsorption of phosphonic acids in the sand sample would be higher, which was also confirmed by q_{max} values.

An alternative explanation for the adsorptive behaviour of nerve agent degradation products in soil could be given using the BET-isotherm. While the Langmuir isotherm treats adsorption in one monolayer, the BET-isotherm, on the contrary, assumes that the adsorption of phosphonic acids on the surface of soil particles takes place in several layers. Statistically it is not possible to tell which

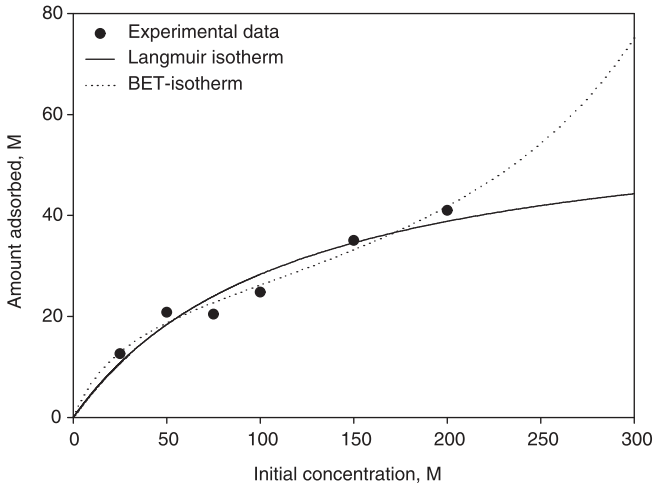

Figure 4. Comparison of fitting Langmuir and BET-isotherm to the same experimental data. Experimental conditions: MPA in soil fraction sized 200–400 μm.

model is more likely as there is no significant difference in determination coefficients between the Langmuir and BET-isotherms, i.e. the quality of fitting both isotherms to experimental data is almost similar. A comparison of two isotherms is given in Figure 4. Both isotherms follow the same path until the point where the second layer of the adsorbent starts to form in the BET-isotherm or the Langmuir isotherm starts to level as the monolayer around the soil particle is filling up. One of the three parameters describing the BET-isotherm is q_{max}, which is the concentration corresponding to a complete monolayer adsorption. The definition of this parameter is exactly the same as in the case of the Langmuir isotherm equation. Apparently, the values of q_{max} of the Langmuir isotherm are 3–10 times higher than those of the BET-isotherm.

For all the phosphonic acids tested, the adsorption mechanism is the same because their molecular structures are very similar. Therefore, it is unlikely that the adsorption concentration corresponding to a complete monolayer of one particular phosphonic acid could vary in such a wide range. Hence, the adsorption mechanism of nerve agent degradation products must be either Langmuir's or BET's. The concentration range under study is apparently too narrow to tell us which of the two mechanisms is more likely, because the measured points are in the range where only the first monolayer is filling up. Unfortunately, in a particular BGE system, much higher concentrations cannot be measured with CE because separation between high concentration phosphonic acid peaks is not sufficient. It is possible to use CE to measure the adsorption of every phosphonic acid separately at higher concentrations, but this may lead to different results compared with the situation when they are detected together because it is very likely that phosphonic acids with highly similar molecular structures compete for the same adsorption sites around a soil particle.

4. Conclusions

Phosphonic acids as degradation products of their parental nerve agents may serve as excellent fingerprint markers for the verification of the use of nerve agents. The adsorption of different phosphonic acids in different fractions of loam and sand samples was studied. The results demonstrated that the difference in adsorption between loam and sand samples was more significant than that between individual fractions. A comparison showed the adsorption of MPA in different soil samples to be relatively higher than that of other phosphonic acids.

CE has proven to be a suitable tool for separating phosphonic acids and could therefore easily be used for the analysis of adsorption. Moreover, it enables analysis of the adsorption of several components on the same adsorbent simultaneously. This offers a great opportunity to study a competitive adsorption of similar molecules on the same adsorption sites. In CE, the use of minimum concentrations of substances is restricted by the sensitivity of CCD–CE, while application of maximum concentrations is restricted by the separation system because separation is insufficient above certain concentrations.

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2007, master thesis – "Humic substances analysis in aquatic samples by HPSEC and MALDI-TOF MS"

7. Research directions

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8. List of original publications

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ELULOOKIRJELDUS

1. Isikuandmed

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3. Hariduskäik

2001, Tallinna Tõnismäe Reaalkool, keskharidus
2005, Tallinna Tehnikaülikool, keemia- ja keskkonnakaitse tehnoloogia, tehnikateaduste bakalaureus
2007, Tallinna Tehnikaülikool, keemia- ja materjaliteadus, loodusteaduste magister

4. Täiendusõpe

September – detsember 2006	Masaryki Ülikool, Brno, Tšehhi, Loodusliku orgaanilise aine analüüs MALDI-TOF MS meetodiga
2007 – 2008	Uute tootmistehnoloogiate ja –protsesside doktorikool
2009 – 2011	Doktorikool “Funktsionaalsed materjalid ja tehnoloogiad”
26.-27. märts 2009	projekti “FLAVOURE“ kursus “Food and Feed Quality and Safety”
Oktoober 2009 – jaanuar 2010	Masaryki Ülikool, Brno, Tšehhi, metallide analüüs ICP-MS meetodiga
10., 11., 15. veebruar 2010	kursus “Kaasaegsed AAS analüüsimeetodid”

5. Teenistuskäik

Jaanuar – detsember 2007	Intertek Eurolab OÜ, keemik
Jaanuar 2008 – jaanuar 2009	TTÜ Keemiainstituut, erakorraline teadur
2008 – 2011	TTÜ, Analüütilise keemia õppetool, teadusuurimistöö EV Kaitseministeeriumi projektide täitmise raames

6. Kaitstud lõputööd

2005, bakalaureusetöö – "Humiinainete ekstraktsioon ja iseloomustamine kõrgsurve eksklusioonkromatograafilise meetodiga"

2007, magistritöö – "Humiinainete iseloomustamine kõrgsurve eksklusioonkromatograafia ja MALDI-TOF MS mass-spektromeetria meetoditega"

7. Teadustöö põhisuunad

Vedelikkromatograafia, eksklusioonkromatograafia, humiinained, kapillaarelektroforees

DISSERTATIONS DEFENDED AT TALLINN UNIVERSITY OF TECHNOLOGY ON *NATURAL AND EXACT SCIENCES*

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