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Stücheli, Patrick E.; Niggemann, Jutta; Schubert, Carsten J.

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Comparison of different solid phase extraction sorbents for the qualitative assessment of dissolved organic nitrogen in freshwater samples using FT-ICR-MS

Patrick E. STÜCHELI,^{1,2} Jutta NIGGEMANN,³ Carsten J. SCHUBERT^{1*}

¹Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Surface Waters – Research and Management, Seestrasse 79, 6047 Kastanienbaum, Switzerland; ²Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Universitätstrasse 16, 8092 Zürich, Switzerland; ³University of Oldenburg, Institute for Chemistry and Biology of the Marine Environment, Research Group for Marine Geochemistry (ICBM-MPI Bridging Group), 26129 Oldenburg, Germany

*Corresponding author: carsten.schubert@eawag.ch

ABSTRACT

Fate and reactivity of dissolved organic matter (DOM) is directly linked to its chemical composition. Therefore, molecular characterisation, for example using Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), is used for a better understanding of those factors. To study organic compounds in the water column, an efficient extraction method is important. The commonly used extraction method for FT-ICR-MS is solid phase extraction (SPE) using a reversed-phase sorbent (BondElut PPL). But this method, to the best of our knowledge, was not evaluated for its ability to extract organic nitrogen compounds which are important building blocks of life and therefore an important fraction of DOM. In this study, several solid phase sorbents were tested for their ability to extract organic nitrogen compounds from water samples of natural aqueous environments. Different cartridges concerning their retention mechanism and pore size were tested. Three cartridges with different extraction mechanism (reversed phase, cation exchange or a mixture of both) or different pore size were tested. Except for one sorbent type, which heavily contaminated the samples with organic molecules, the tested cartridges leached neither a significant amount of dissolved organic carbon (DOC) nor dissolved organic nitrogen (DON). The sorbents were tested with lake water to be able to investigate their functionality in real conditions. It could be shown, that the molecular composition of the sample should be considered for the choice of the sorbent material. Additionally, it was shown that a mixed-bed sorbent is a valuable complementary SPE sorbent for the molecular characterisation of lacustrine samples using FT-ICR-MS and it might also be useful for a quantitative extraction. Furthermore, it could be shown that HyperSep Retain CX sorbent allows to extract a broader range of organic nitrogen compounds leading to a more comprehensive data set for investigating organic nitrogen compounds in lakes using FT-ICR-MS.

Key words: Solid phase extraction; organic nitrogen; limnology; cation exchange; Fourier transform ion cyclotron resonance mass spectrometry.

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INTRODUCTION

Dissolved organic matter (DOM) in the ocean is a huge reservoir of fixed carbon and even bigger than the reservoir of carbon in the form of CO₂ in the atmosphere (Ogawa *et al.*, 2001). This is especially true for river mouths, where rivers transport around 2.5x10¹⁴ g of dissolved organic carbon (<0.5 μm) from the continent into the ocean each year (Hedges *et al.*, 1997; Kim *et al.*, 2015). In lacustrine systems, the burial of organic carbon is estimated to be in the same range or even higher than in the oceans (Mendonca *et al.*, 2017). Taking into account that the area of lakes is about 35 times smaller than that of the oceans, the burial of organic material in lakes is extraordinarily high. Since the burial and reworking of DOM by bacteria is linked to its chemical composition, the molecular structure of the DOM needs to be known (Sun *et al.*, 1997a). The chemical

composition of DOM in a lacustrine system may vary because it is dependent on the hydrology, broad-scale landscape and climate variables (Kellerman *et al.*, 2014; Kothawala *et al.*, 2014). A possible method for the molecular characterisation is Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) which can be used *e.g.* to reveal degradation processes both in marine (Tremblay *et al.*, 2007; Sleighter and Hatcher, 2008; Lechtenfeld *et al.*, 2014; Medeiros *et al.*, 2016) and freshwater systems (Kujawinski *et al.*, 2004; Kim *et al.*, 2006; Kellerman *et al.*, 2014) or to determine the bioavailability of DOM (Sun *et al.*, 1997b). Therefore, FT-ICR-MS measurements can help to better understand environmental process such as degradation and preservation as well as diagenetic pathways of organic matter in aquatic systems (Kim *et al.*, 2003a; Kujawinski *et al.*, 2004, Kim *et al.*, 2006) and might also lead to “new markers for biogeochemical processes” (Kujawinski *et al.*, 2009). It can also be applied to

estimate the origin of DOM in aquatic systems such as terrestrial contribution (Medeiros *et al.*, 2015, 2016). Additionally, molecular characterisation of DOM in lakes might also help to design experiments or choosing the best study sites for a given research question (Kujawinski *et al.*, 2004). To be able to characterise DOM with the means of a FT-ICR-MS, an as complete as possible DOM fraction should be extracted. Tangential flow filtration is an often-used method for the extraction of organic molecules (Amon and Benner *et al.*, 1996; Roland *et al.*, 2008) down to a minimum size of 1 kDa. However, more than 70% of the DOM is smaller than 1 kDa, which could lead to an incomplete molecular characterisation (Benner *et al.*, 1997; Dittmar and Kattner, 2003). To be able to characterise also the low molecular weight molecules, solid phase extraction (SPE) is a valuable extraction method. SPE with XAD (Aiken *et al.*, 1979; Dittmar and Kattner, 2003) and C18 (Kim *et al.*, 2003b) resins were used. In 2008, Dittmar *et al.* showed that the non-polar reversed phase sorbent Bond Elut PPL (Agilent) has extraction efficiencies up to 70% of total DOM which is significantly higher than for C18 cartridges.

So far, most studies about the PPL resin did only concentrate on its ability to retain the carbon fraction of the DOM. The retention of the organic nitrogen compounds, which are indeed a subset of dissolved organic carbon (DOC) but may be differently retained, were not especially evaluated. However, dissolved organic nitrogen (DON) is an important component of the DOM fraction because the DON consists of building blocks of life such as amino acids or peptides. (McCarthy *et al.*, 1997, 2004). The nitrogen compounds are also involved in the bacterial loop (Amon and Benner 1996) and are suggested to be the limiting factor for heterotrophic growth in benthic ecology (Tenore, 1988). Hence, the extraction of DON molecules must be taken into account. However, Arellano *et al.* (2017) indicated a low extraction efficiency for DON in coastal samples using the PPL sorbent. On the other side, Spanik *et al.* (2007) showed that strong cation exchange resin can be used for the pre-concentration of amino acids in water samples. Therefore, in this study cation exchange resins as well as reversed phase resins with different pore sizes (Tab. 1) were tested for the extraction of DON and peptides in water samples. To evaluate the usability for a quantitative extraction for each sorbent type, the carbon and nitrogen content of each extract was determined. The qualitative evaluation of the extracts, that means the number of different identified molecular formulae extracted per sorbent type, was done using FT-ICR-MS. Additionally, the molecular composition based on FT-ICR-MS measurements on DOM of Lake Lucerne and Lake Rotsee were compared.

METHODS

Sampling

Samples from Lake Lucerne (LL) and Lake Rotsee (LR) were collected for this study. The two lakes were chosen based on their different environmental properties. This allowed to characterise the influence of the molecular composition of a sample on the SPE. LL is a prealpine oligotrophic lake in the centre of Switzerland with a permanent oxic water column and a maximum depth of 206 m. The total phosphorous (TP) of LL is below 5 mgP m⁻³ (Finger *et al.*, 2013). The water samples of LL were collected at a depth of 50 m in the Gersauer basin. LR is a small eutrophic lake near the city of Lucerne in Switzerland with TP concentrations in the epilimnion between 36 and 94 mgP m⁻³ (Bloesch *et al.*, 1974). It has a maximum depth of 16 m and is monomictic. The water column is mixed annually in late fall/winter (Schubert *et al.*, 2010) and is stratified during summer time. The oxycline is located between 7 and 11 m and was at 7.3 m during sampling. The samples of LR were collected at a depth of 10 m in the anoxic area of the water column to ensure highly different properties of the water samples and therefore also a different chemical composition of DOM.

Sample preparation

All samples were collected using a 25 L Niskin bottle. While the samples from LL were filtered using combusted Whatman GF/F filters, LR samples were filtered using several tangential flow filtration steps. After filtration, the samples were acidified to pH=2 with HCl (30% suprapur, Merck). TOC and TN of each sample were measured after each filtration step using a Shimadzu TOC-L-CSH total organic carbon analyser equipped with a TNM-L total nitrogen measurement unit and an ASI-L autosampler.

Solid phase extraction

Five cartridges with different retention mechanisms and pore sizes were tested (Tab. 1). For LL, two replicates of the same sample were performed whereas only one sample of LR was examined (see Tab. 2). All cartridges were rinsed and conditioned following the procedure described by Pohlabein *et al.* (2015). All sorbent types were treated the same way to increase the comparability of the results. In brief, the cartridges were soaked in MeOH overnight, rinsed with two cartridge volume of MilliQ water followed by two cartridge volumes of MeOH and subsequently rinsed with acidified MilliQ (pH=2). Around 0.5L to 1L (dependent on the sorbent volume) of acidified sample passed the cartridges using gravity. The exact volume was determined by the mass

difference of the bottle before and after the sample passed the cartridge. The cartridges were desalted with one bed volume of 0.01M HCl and dried with a stream of nitrogen (5.0 grade). The organic compounds were eluted with 8 mL methanol and the cation exchange cartridges were eluted a second time with 1mM KOH in methanol to be able to elute also the strongly bound positively charged molecules. For each cartridge, at least one blank consisting of 0.01M HCl in MilliQ was performed. 500 μ L aliquots of each extract were transferred to a combusted DOC vial, dried over night at 40°C and redissolved in 0.01M HCl (10mL) and DOC and TN were determined (see section “sample preparation”). The extraction efficiencies for DOC were determined as the ratio of the

extracted carbon and the total carbon present in the sample prior to the extraction. The concentration of DON was calculated as the difference of the measured TN concentration and the concentration for the inorganic nitrogen (IN). The IN concentration was measured with a Skalar San++ flow injection analyser. The IN concentration of the SPE extracts were below detection limit (LOD) (NO_3^- 5 $\mu\text{gN L}^{-1}$, NO_2^- 2 $\mu\text{gN L}^{-1}$) and therefore, the value for TN was assumed to be the concentration for DON. The extraction efficiencies for DON were then calculated as the ratio of the DON concentrations in the extracts and the DON concentrations in the sample prior to the extraction. All samples were then stored at -18°C until FT-ICR-MS measurement.

Tab. 1. Properties of the tested SPE resins. Asterisks mark the values not explicitly specified by the supplier for the lot used in this study. In this case, the catalogue values are given. All sorbent types are functionalised polymers. The Bond Elut PPL sorbent type was chosen because it is the most common used sorbent type. Evolute ABN and Isolute ENV+ are similar to Bond Elut PPL with the same retention mechanism but with different pore size. This allowed to test influence of the pore size on the retention of nitrogen containing molecules which are often found in area of higher MW. SupelSelect SCX was chosen because it is a strong cation exchange sorbent and was therefore expected to be able to retain the at low pH positively charged nitrogen containing molecules. HyperSep Retain CX unite both extraction mechanisms.

Sorbent	Extraction mechanism	Sorbent weight (mg)	Average pore diameter (Å)	Specific surface area ($\text{m}^2 \text{g}^{-1}$)	Supplier	Cat. No.
Bond Elut-PPL	Reversed phase, non-polar surface	1000	0.90	640	Agilent	12255002
Evolute ABN	Reversed phase, non-polar surface	500	42	505	Biotage	610-0050-CXG
Isolute ENV+	Reversed phase, non-polar surface	1000	800*	1000	Biotage	915-0100-C
SupelSelect SCX	Strong cation exchange (sulfonic acid groups)	1000	80-200*	160-420*	Supelco	54245-U
HyperSep Retain CX	Mixed phase (cation exchange and reversed phase)	2000	83	716	Thermo Scientific	60107-312

Tab. 2. Number of identified molecular formulae of each cartridge for Lake Lucerne and Lake Rotsee as well as the average molecular weight (intensity weighted), the extraction efficiencies of DOC and DON (blank corrected) and H/C, O/C and N/C ratios.

	PPL		ENV+		SupelSelect		HyperSep	
LAKE LUCERNE								
# molecular formulae	3756	3985	3346	3182	3837	3900	5901	5674
Average molecular weight	350	359	385	384	365	378	401	383
H/C	1.23	1.20	1.23	1.23	1.30	1.30	1.28	1.29
O/C	0.35	0.38	0.42	0.43	0.34	0.35	0.38	0.38
N/C	0.110	0.123	0.118	0.116	0.127	0.121	0.131	0.135
DOC extraction efficiency	56.0	53.5	37.3	41.3	30.3	25.5	55.7	45.1
DON extraction efficiency	43.0	41.2	41.4	56.5	17.5	18.7	23.9	17.6
LAKE ROTSEE								
# molecular formulae	4243		3752		3812		5990	
Average molecular weight	345		349		330		343	
H/C	1.29		1.3		1.26		1.31	
O/C	0.37		0.39		0.35		0.38	
N/C	0.125		0.126		0.118		0.136	
DOC extraction efficiency	24.2		42.5		30.9		40.4	
DON extraction efficiency	10.4		21.8		10.1		12.8	

FT-ICR-MS

This method is useful for the molecular characterisation of environmental samples (Riedel and Dittmar, 2014) because it allows the assignment of molecular formulae to each mass-to-charge ratio (m/z) which allows to characterise the number of different nitrogen compounds for each extract. The samples were diluted with methanol and MilliQ water to a DOC concentration of approximately 10 ppm in methanol and water (1:1 v/v). The amount of extract used for the dilution was calculated using the DOC concentrations measured with the Shimadzu TOC/TN analyser. The blanks, which did not contain enough DOC to achieve a 10 ppm concentration in methanol and water (1:1 v/v), were diluted with water to obtain a 1:1 (v/v) mixture of MeOH and water which is optimal for the instrument. All samples were passed through a Teflon filter (0.2 μm) to avoid clogging of the capillaries of the instrument. The Teflon filter was rinsed 10 times with a 1:1 (v/v) mixture of water and MeOH prior to the filtration of the samples to avoid contaminations by the filter. The measurements were done with a Bruker Solarix 15 Tesla FT-ICR-MS in negative mode at the University of Oldenburg. The detailed measurement routine can be found in Rossel *et al.* (2015). In brief, the spectra were internally calibrated using a list of >50 known molecular formulae over the whole mass range which naturally occur in environmental samples. Only molecular formulae which had an error of the mass accuracy smaller than 100 ppb were used. The standard deviation for the samples was <0.04 ppm and for the blanks <0.05 ppm. To remove false positives, peaks with an intensity smaller than the method detection level (MDL) were neglected (Riedel and Dittmar, 2014). Molecular formulae were assigned to each peak applying the method of Koch *et al.* (2007) by using an in-house Matlab routine which considered possible combinations of C, H, O, N, S and P with an error <0.5 ppm (Rossel *et al.*, 2015). Unlikely DOM molecular formulae (such as N4S2 or N3P) were neglected. To increase the comparability of the samples, peaks smaller than the standardised detection limit (SDL) were removed as well (Rossel *et al.*, 2015). All sample spectra were blank corrected using the corresponding blank spectrum.

However, molecular formulae which showed an at least 20 times higher intensity than the corresponding peak in the blank spectrum were used. Each molecular formula was assigned to a compound group dependent on the correspondent H/C and O/C ratio as well as the aromaticity index and the number of nitrogen atoms using the classification of Santl-Temkiv *et al.* (2013) (see supplementary information Tab. S1). A modified version of the aromaticity index was used (Koch and Dittmar, 2006). Please note, that the molecular formulae are assigned into compound groups due to the similarity of elemental ratios, aromaticity index and # nitrogen atoms with known compounds of the corresponding compound class.

RESULTS AND DISCUSSION

Blanks

Blanks for every sorbent type were obtained by extracting MilliQ water with the same procedure used for the samples. Non-purgeable organic carbon (NPOC) and TN measurements were carried out to characterise the total amount of leaching. The number of molecules and the molecular formulae were determined using FT-ICR-MS measurements with the same method as for the samples. DOC values are given as the amount of carbon leached (in μg) from the sorbent per litre water extracted and are the mean values of three measurements. The LOD for the TN measurement was 5 $\mu\text{g L}^{-1}$ and detection limit for DOC was 4 $\mu\text{g L}^{-1}$.

The ABN cartridge showed an especially high amount of DOC leaching with 0.5 mg C per litre of MilliQ extracted which made the ABN cartridge not suitable for the extraction of DOC from environmental samples because the amount of DOC in the blank was around the same amount of DOC extracted from the samples (see Tab. 3). Therefore, this cartridge type was neglected in the following discussion. The DOC leaching of the other four tested cartridges was tolerable and could be corrected by blank-subtraction. The concentration of organic nitrogen was below detection limit for all cartridge types making all cartridges suitable for the extraction of DON concerning the blank perspective. The FT-ICR-MS

Tab. 3. Blank values for the different cartridges. A detailed description can be found in the text.

Cartridge	DOC ($\mu\text{g/L}$)	# molecules	Peptides	CHON	CHO	CHOS	CHONS
Bond Elut-PPL	8.4 \pm 2.7	341	115	52	42	44	92
Evolute ABN	502.7 \pm 10.1	496	42	47	260	101	42
Isolute ENV+	21.8 \pm 3.7	922	150	89	366	267	125
SupelSelect SCX	18.3 \pm 1.4	409	72	53	207	46	68
HyperSep Retain CX	21.4 \pm 1.8	1965	20	52	1286	554	27

measurements were carried out without further dilution with methanol as the DOC concentrations were already below 10 ppm. The blank of the HyperSep Retain CX cartridge showed a high diversity of CHO and CHOS compounds. Nevertheless, because the absolute amount of contamination was not higher than with the other cartridges, HyperSep Retain CX was still found to be suitable for the extraction. This statement is also valid for the molecular characterisation with FT-ICR-MS since the samples can be corrected by blanks. Additionally, this cartridge type had the lowest amount of contaminations from nitrogen compounds, especially of peptides, making this cartridge especially suitable for the extraction of peptides and nitrogen compounds.

Extraction efficiency

The extraction efficiencies of HyperSep Retain and SupelSelect are given as the combined extraction efficiency of both extracts. The concentration of DON was calculated as the difference of the measured TN concentration and the concentration of IN. IN and TN concentrations for LL samples were $612 \pm 15 \mu\text{g L}^{-1}$ and $660 \pm 24 \mu\text{g L}^{-1}$, respectively. For LR, the IN concentration was $1290 \mu\text{g L}^{-1}$ and the TN value $1400 \pm 34 \mu\text{g L}^{-1}$. Because the standard deviation of IN and TN are in the range of the expected DON concentration, the true total value for DON might be different than the calculated one. However, the error remains the same for every cartridge as the maximum DON value is the same for all samples. Hence, a qualitative evaluation of the nitrogen extraction for the cartridges is possible.

For LL, the PPL cartridge had the highest DOC extraction efficiency (56%) which is equal to the extraction efficiency reported by Dittmar *et al.* (2008). The sorbent HyperSep Retain CX had a similar extraction efficiency for DOC (see Tab. 2). This is also the sorbent type which was able to extract the highest amount of different molecules which may be due to the mixed retention mechanism. The other three cartridge types had a significant lower extraction efficiency. Especially the cation exchange cartridge SupelSelect had a low DOC extraction efficiency around 30%. Interestingly, the total amount of molecules found in this cartridge type is similar to the one of the PPL cartridge indicating that the cation exchange mechanism is able to extract a similar number of molecular formulae but with a lower efficiency. The detected molecule formulae gave only a low signal. Therefore, it seems that the cation exchange mechanism may extract additional molecules but only in low concentration. This could be due to the extraction conditions which were the optimal conditions for the PPL cartridge. The same extraction conditions were used for the cation exchange sorbents to improve the comparability of the cartridges. An improvement of the extraction

conditions (*e.g.* optimization of sample pH) for the cation exchange sorbents should therefore lead to higher extraction efficiency of the cation exchange sorbents. This influence of the extraction conditions might be lower for the HyperSep sorbent because of its mixed retention mechanism. The reversed phase mechanism of the HyperSep sorbent is most probably compensating the lower DOC extraction efficiency of the fraction which is susceptible towards cation exchange mechanism while the cation exchange mechanism is still leading to a higher total amount of detected molecular formulae in the extract. The DON extraction efficiency of the cation exchange sorbents might be low due to the low sample pH which might have led to a high degree of protonated cation exchange sites preventing the charged organic nitrogen molecules from binding to the sites. Therefore, a slightly higher pH (*e.g.* pH=3) could lead to a higher extraction efficiency because less cation exchange sites get occupied by H^+ . On the other site, less organic nitrogen molecules are in the protonated form at a higher pH. However, proteins, which are a major fraction of DON (Tuschall and Brezonik, 1980), typically have an isoelectric point between 4 and 10 (Schwartz *et al.*, 2001). Therefore, most proteins should still be positively charged at a pH of 3.

The ENV sorbent has the same extraction mechanism like the PPL sorbent. Therefore, the extraction conditions are also favourable for the ENV sorbent which explains the higher extraction efficiency of ENV compared to SupelSelect. Especially the DON extraction efficiency is high compared to the other sorbent types. Interestingly, the DOC extraction efficiency of the ENV sorbent does not drop in LR. This might be due to the bigger pore sizes which retained the molecules better than the PPL cartridge which shows a decreased DOC extraction efficiency for LR samples. This drop in DOC extraction efficiency is also not distinct in the extracts of the cation exchange sorbents. Therefore, the DOM of LR seems to contain higher concentrations of molecules which are more selective towards cation exchange mechanism (30.9% of SupelSelect compared to 24.2% of the PPL sorbent) and/or bigger pore sizes (ENV+: 42.5%). The cation exchange mechanism of the HyperSep cartridge might compensate the lower extraction efficiency for the reversed phase mechanism leading to the conclusion, that a mixed phase sorbent is less dependent on the molecular composition of the sample compared to the other tested cartridges. On the other hand, the total amount of found molecular formulae of LR is higher for the PPL sorbent compared to the SupelSelect sorbent. This implies that many compounds in LR have a high affinity towards the reversed phase extraction mechanism but those molecules exist only in low concentration. Another reason for this phenomenon might be that molecular formulae found in

an extract of a cation exchange sorbent correspond to a higher amount of different isomeric compounds.

Extraction mechanism

When comparing the nitrogen compounds extracted by the PPL sorbent and the SupelSelect sorbent, it can be seen that they extract a common set of around 50% of the total nitrogen compounds found in both cartridges (see Fig. 1). But each cartridge has an own set of nitrogen molecules which were only extracted by a specific sorbent type (SupelSelect around 20% and PPL around 30%). Using HyperSep Retain CX, a sorbent with mixed retention mechanism it is possible to extract both individual sets of nitrogen compounds (see Fig. 1). The same is true when comparing the ENV+ sorbent with the HyperSep sorbent. The PPL and the ENV+ sorbent have the highest common set of extracted nitrogen compounds. This is not surprising giving the fact that both have the same retention mechanism (reversed-phase) and the two sorbents only differ in pore size. However, the bigger pore size leads to a small set of additional compounds (295) but this set is still smaller than the one of PPL (511) which leads to the conclusion that the PPL sorbent is the more efficient reversed-phase sorbent for a qualitative extraction. The HyperSep Retain sorbent leads to the

highest set of identified nitrogen containing molecular formulae of all four cartridges with an average of 97% of all identified nitrogen compounds. However, a combination of two cartridges with different extraction mechanism, *e.g.* SupelSelect and PPL, might also be a promising option. Therefore, the extracts of PPL, SupelSelect and HyperSep were compared using a Venn diagram (see Fig. 2). For a better overview, ENV+ was not used for the Venn diagram (Fig. 2) because the PPL resin showed overall a better retention than ENV+ and therefore, PPL was used to represent the reversed phase extraction mechanism. Looking at the total amount of extracted compounds, the combination of SupelSelect and PPL seems reasonable because this combination covered a total set of 75% for LL and 71% for LR of the total amount of identified compounds. In comparison, HyperSep Retain led to a total set of 86% (LL) and 88% (LR) of all identified compounds. Therefore, the combination of SupelSelect and PPL would lead to an exclusive set of 14% (LL) and 12% (LR) of compounds which cannot be extracted by the HyperSep sorbent whereas 62% (for both lakes) of these are compounds without any further heteroatom despite oxygen (CHO compounds). On the other side, only a minor set of nitrogen containing compounds can exclusively be extracted by a combination of PPL and SupelSelect

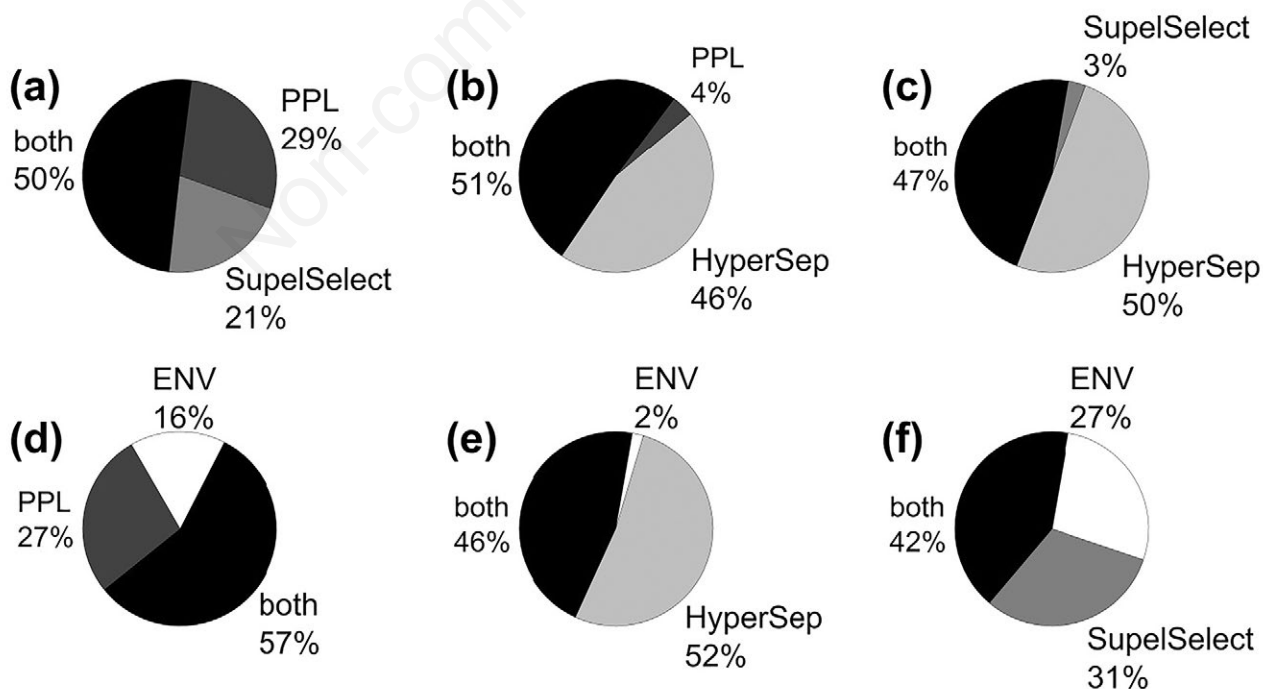


Fig. 1. Comparison of the nitrogen compounds extracted by different sorbent types for Lake Lucerne. The PPL sorbent was compared with the SupelSelect sorbent (a) and with the HyperSep sorbent (b). The SupelSelect sorbent was compared with the HyperSep sorbent (c). The ENV+ sorbent was compared with PPL (d), HyperSep (e) and SupelSelect (f).

whereas almost all identified nitrogen containing compounds could be found in the extract of HyperSep retain (95% for LL and 96% for LR, see Figs. 2c and 2d).

Therefore, the HyperSep Retain sorbent leads to a higher set of all extracted compounds as well as an especially high set of nitrogen containing molecules. This is also true for almost every assigned compound classes except black carbon molecules or oxygen-poor polyphenols (see Fig. 3b).

However, keeping in mind that the PPL sorbent is one of the best sorbents for a quantitative extraction and a

combination with another cartridge would certainly even increase the extraction efficiency, the gain in extraction efficiency might compensate the lower amount of extracted molecules, especially if nitrogen containing molecules are not of special interest. But for a qualitative assessment of nitrogen containing molecules, the usage of HyperSep Retain as sorbent seems to be recommendable.

Looking at a specific nitrogen containing compound class, the peptide-like molecules, the hypothesis for the better extraction with cation exchange mechanism is confirmed (see Fig. 4). In LL for example, SupelSelect,

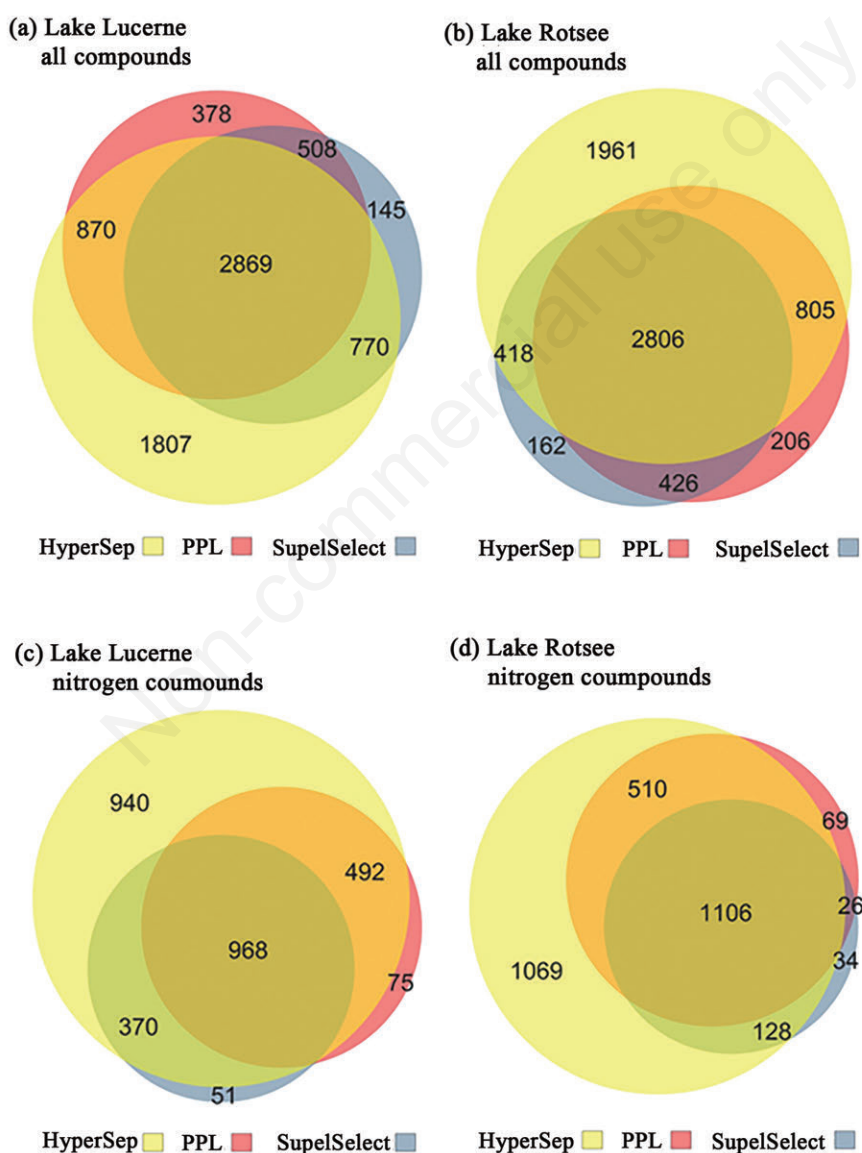


Fig. 2. Venn diagram comparing the extracts of the PPL, SupelSelect (SS) and HyperSep Retain (HS) sorbents for both lakes. The total amount of identified compounds for Lake Lucerne (a) and Lake Rotsee (b) as well as only the nitrogen containing compounds for Lake Lucerne (c) and Lake Rotsee (d) were compared. PPL and SupelSelect have a common set of 31 nitrogen containing compounds for Lake Lucerne but unfortunately, it was impossible to draw this intersection while keeping the correct relationship (Fig. 5).

the only cartridge with pure cation extraction mechanism, had the second highest amount of peptides extracted. Only the HyperSep cartridge, which has a mixed extraction mechanism, showed a higher amount of peptide-like molecular formulae. This could be due to the alkaline character of peptides which should be therefore positively charged at the applied pH. Dividing the molecular formulae of the peptides into the groups CHON (peptides without further heteroatoms), CHONS (peptides containing sulphur) and CHONP (peptides containing phosphorous), it can be seen that the cartridge SupelSelect is especially efficient for the extraction of sulphur containing peptides. By comparing the sulphur containing peptides found in the HyperSep extract of LR and LL it becomes clear that LR contains less peptides of this kind (93 in LR compared to 145 in LL), which explains the lower amount of peptides extracted in LR by SupelSelect.

On the other hand, PPL is in particular able to retain peptides without any further heteroatom. This kind of peptide was found more often in LR (262) than in LL (146), therefore, more peptide-like molecules can be found in the PPL extract of LR. This observation reveals, that the choice of the sorbent type depends on the molecules of interest and is also dependent on the molecular composition of the lacustrine DOM. Since almost all detected peptide-like molecular formulae (around 90%), independent on the class of peptide, could be found in the HyperSep Retain CX extract, this sorbent can be recommended for the qualitative assessment of peptides. Additionally, this cartridge type is less susceptible to different molecular composition of DOM due to its mixed retention mechanism which makes this cartridge especially useful for the qualitative assessment of DOM for different lake systems.

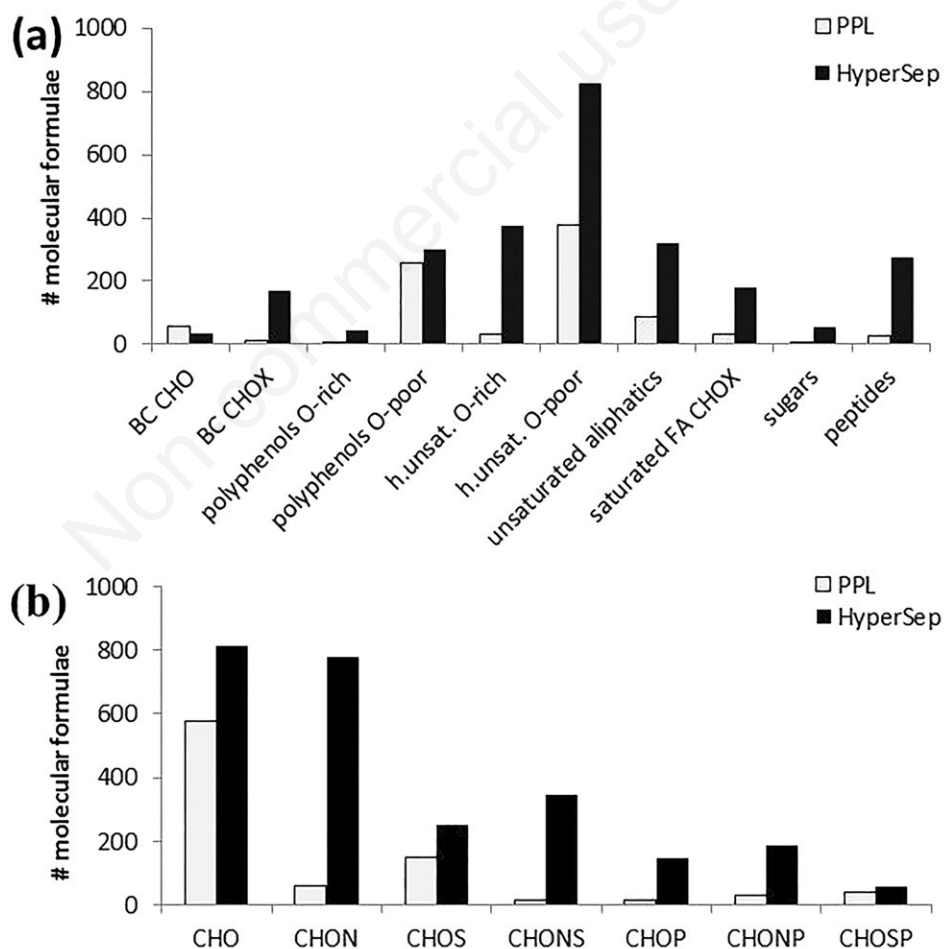


Fig. 3. Comparison of the PPL extract with the HyperSep extract for LL. Compounds found in both sorbent types are not shown. The data for LR can be found in the supplementary information (see Fig. S4). The compounds were then classified after Santl-Temkiv (a) and according to their molecular formula (b). Abbreviations used are BC for black carbon, FA for fatty acids and h. unsat. for highly unsaturated. CHOX means that the compound contains an additional heteroatom despite of oxygen.

Molecular composition of Lake Lucerne and Lake Rotsee

The difference in molecular composition of both Lake Lucerne and Lake Rotsee will be discussed referring to the difference of the HyperSep Retain extracts of both lakes because these extracts were the most complete ones as could be shown before. Like previously done, the compounds for both lakes were compared and classified whether they appear only in one lake or in both (see Fig. 5).

Only the compounds which appeared solely in one lake were taken into account for the discussion to be able to

highlight the differences of the two lakes (see Figs. 5 a+b). The different filtering techniques (GF/F and ultrafiltration) prior to the SPE are expected to have only a minor influence on these results because only compounds smaller than 1 kDa were taken into account for the comparison. These compounds should neither be removed by GF/F nor by the ultrafiltration which had a molecular weight cut-off at 1 kDa. In total, 7918 different molecular formulae could be determined and around 50% of the formulae occurred in both lakes. Around 25% appeared uniquely in LL and LR (1928 and 2017 respectively).

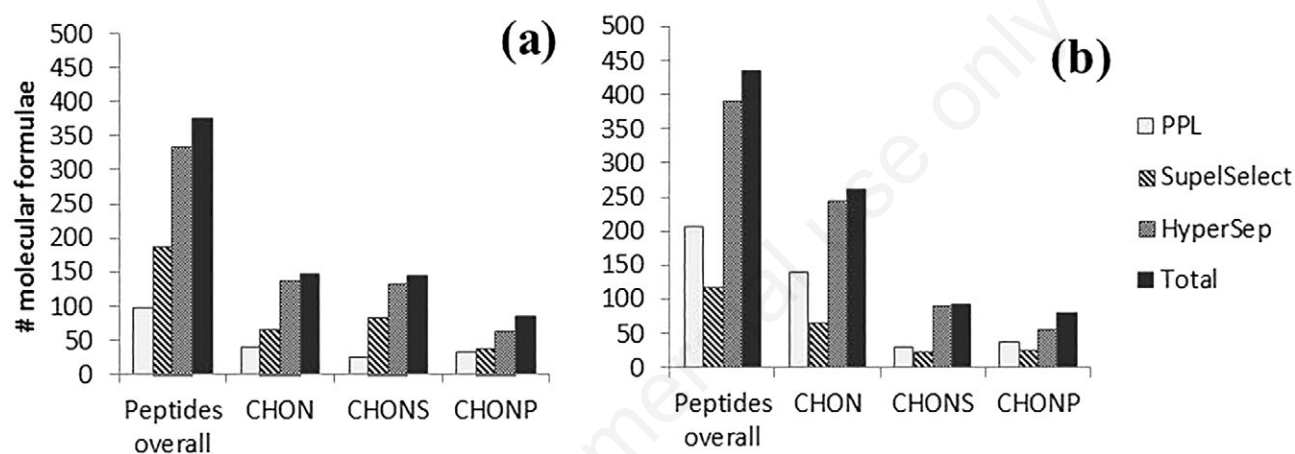


Fig. 4. Number of peptide-like molecular formulae found in the extracts of the cartridges PPL, SupelSelect and HyperSep Retain in (a) Lake Lucerne and (b) Lake Rotsee. The total amount of peptides was divided into three subgroups: (1) peptides without any further heteroatoms, (2) peptides with sulphur and (3) peptides with phosphorus.

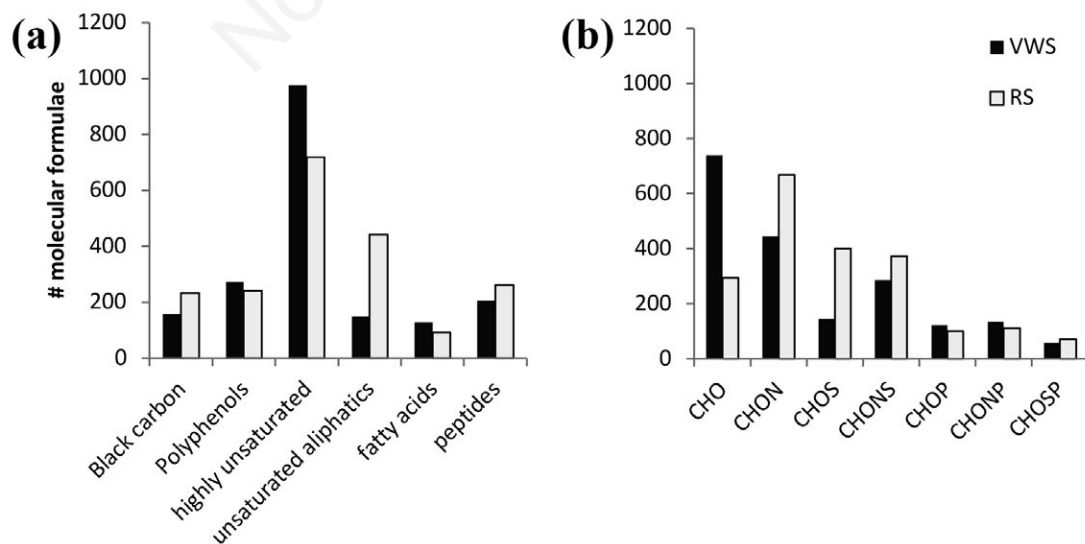


Fig. 5. Comparison of the molecular formulae uniquely found in only one of the two lakes.

LR shows an increased abundance of proteins and aliphatic compounds compared to LL. This indicates a higher contribution of autochthonous material compared to LL because proteins and aliphatic substances are supposed to be produced by organisms living in the lake (Kellerman *et al.* 2014). Additionally, the abundance of nitrogen containing compounds is much higher in LR indicating a high amount of autochthonous production in the lake since nitrogen compounds are important building blocks of life (McCarthy *et al.* 1997, 2004). This observation also fits with the eutrophic state of LR. The oligotrophic LL has an increased abundance of polyphenols and highly unsaturated compounds which are typical for tannins and lignin found in higher plants (Kellerman *et al.* 2014, Santl-Temkiv *et al.* 2013). This higher occurrence of allochthonous material in LL compared to LR is related to the high input of rivers entering the Gersauer basin. Nevertheless, LR shows also many polyphenols and highly unsaturated compounds indicating the terrestrial influence of trees and grass surrounding the lake. The compounds uniquely found in LR also showed a slightly higher N/C ratio (0.151) compared to the compounds unique to LL (0.142) which indicates a slightly increased bioavailability of the organic matter for LR (Sun *et al.*, 1997). This can be explained by the higher terrestrial influence of DOM in LL because terrestrial OM is usually more recalcitrant (Aller *et al.*, 1996; Prahl *et al.*, 1997; Burdige, 2007). Additionally, the increased abundance of sulphur-containing molecules in LR hints towards incorporation of inorganic sulphur by bacteria. For example, sulphide is prone to oxidation at the chemocline by phototrophic sulphur bacteria (Jørgensen *et al.*, 1991). Subsequently, assimilatory sulphate or sulphite reduction by methanotrophic bacteria could lead to the relatively high abundance of organic sulphur compounds in LR (Daniels *et al.*, 1986). Therefore, this finding could lead to the design of a limnological experiment which examines the sulphur cycle in LR.

CONCLUSIONS

Five different sorbent types with different extraction properties and extraction mechanisms were tested in this study to find a suitable sorbent type for the extraction of DOM from lacustrine water for a qualitative analysis using FT-ICR-MS. The commonly used PPL sorbent was used as a reference sorbent for the comparison of the four new sorbents (Evolute ABN, Isolute ENV+, SupelSelect SCX and HyperSep Retain CX). Whereas the Evolute ABN sorbent is not suitable for the extraction of bulk samples due to its high leaching of organic substances (>0.5 mg per litre MilliQ water) all other cartridges are suitable for the extraction of DOM

with DOC leaching <22 µg/L MilliQ water. The HyperSep Retain CX sorbent is especially suitable for nitrogen compounds due to the low leaching of organic nitrogen compounds and due to the good retention ability towards organic nitrogen molecules. Using the HyperSep sorbent, around twice as much nitrogen containing molecular formulae and up to a factor of four more peptide-like formulae could be assigned compared to the often used PPL cartridge. It could be shown that the retention ability of a sorbent is dependent on the molecular composition of an environmental sample. A different retention mechanism is therefore leading to a different set of compounds as can be seen in Fig. 4. Therefore, a mixed-bed sorbent brings the advantage of being less dependent on the molecular composition of a lake due to the combination of two extraction mechanisms. By comparing the extract of the HyperSep Retain sorbent to those of the PPL and the SupelSelect sorbents, it becomes obvious that the HyperSep Retain sorbent is able to retain around 97% of the nitrogen compounds found in the extracts of those three sorbent types (see Fig. 1). The HyperSep Retain sorbent did not only show good retention ability towards nitrogen molecules but an overall good retention ability. For both investigated lakes, HyperSep Retain was able to retain more molecules for each different type of molecular formula (see Fig. 3b) and almost every compound class (see Fig. 3a). In some cases it might be useful to combine a pure reversed-phase sorbent (such as PPL) and a cation exchange sorbent (or mixed-phase), especially for a quantitative extraction. The HyperSep Retain CX cartridge is possibly also useful for marine samples but due to the higher amount of cations in marine samples the cation exchange sites may get occupied by inorganic cations leading to an inorganic fraction in the methanol extracts and maybe also resulting in lower extraction efficiencies. To summarize, it was shown that the HyperSep Retain CX sorbent is a valuable complementary SPE sorbent to the normally used PPL cartridge. Due to the combination of two extraction mechanisms, a more complete set of DOM molecules can be extracted from lacustrine water samples. It especially allows to extract a more complete compound spectra of DON of lake samples leading to a more comprehensive data set for a qualitative analysis of DON with FT-ICR-MS in lacustrine systems. For example, using the HyperSep Retain sorbent instead of PPL sorbent allows to extract compounds with a higher N/C ratio indicative for a higher bioavailability of the organic matter (Sun *et al.*, 1997b). Furthermore, it was shown that FT-ICR-MS is a useful method to characterise organic matter in lakes and hence opens a wide field of designing new experiments and answer research questions.

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