BIOGEOCHEMIE GELÖSTER KOHLENHYDRATE IN DER ARKTIS

BIOGEOCHEMISTRY OF DISSOLVED CARBOHYDRATES IN THE ARCTIC

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List of Frequently Used Abbreviations

Approx.	Approximately
Chl *a*	Chlorophyll *a*
Conc.	Concentrated
Da	Dalton (relative atomic weight)
Dil.	Diluted
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
MilliQ	Water obtained by the MILLIPORE$_{185}$ plus system
N.d.	Not determined
POC	Particulate organic carbon
POM	Particulate organic matter
St.	Station
TCHO	Total carbohydrates
THNS	Total hydrolysable neutral sugars
TFNS	Total free neutral sugars
UDOM	Ultrafiltrated dissolved organic matter

Neutral sugars:

Fuc	Fucose
DORib	Deoxyribose
Rha	Rhamnose
Ara	Arabinose
Gal	Galactose
Glc	Glucose
Man	Mannose
Xyl	Xylose
Frc	Fructose
Rib	Ribose

Zusammenfassung

In dieser Arbeit wird eine umfassende Betrachtung von gelösten Kohlenhydraten als einer der größten Bestandteile der gelösten organischen Substanzen (DOM) im Arktischen Ozean präsentiert. Ausgehend von den terrestrischen Quellen des eurasischen Kontinentes wurde der Eintrag von Kohlenhydraten durch die Flüsse in das nördliche Polarmeer untersucht. Im Arktischen Ozean wurden gelöste Kohlenhydrate der sibirischen Schelfgebiete, der Karasee, der Laptevsee, des zentralen Arktischen Ozeans, der Framstraße sowie der Grönlandsee bestimmt.

Die Bestimmung der Kohlenhydrate erfolgte mit zwei Meßverfahren. Die Gesamtkonzentration von Kohlenhydraten wurde mit einer kolorimetrischen Methode und die Zusammensetzung von individuellen, neutralen Zuckern mit einem flüssigkeitschromatographischen Verfahren mit gepulster amperometrischer Detektion (HPAEC-PAD) bestimmt. Die hohe Empfindlichkeit beider Methoden ermöglichte eine Bestimmung in Fluß- und Meerwasser ohne eine Aufkonzentrierung des organischen Materials.

Die Kohlenhydratkonzentrationen zeigten, je nach Untersuchungsgebiet, hohe Variationen. Sehr hohe Konzentrationen, mit Werten zwischen 10 and 41 µM C (im Mittel 25 µM C), wiesen die nordrussischen Flüsse auf. Eine starke Abnahme der Kohlenhydratkonzentration trat beim Übergang in die Randmeere des Arktischen Ozeans auf, wo die Werte zwischen 2.6 and 9.7 µM C (im Mittel 5.3 µM C) lagen. Der prozentuale Anteil der Kohlenhydrate am Gesamtkohlenstoff (DOC) verringerte sich dabei weniger stark von durchschnittlich 4.7 auf 3.9%. Mit bis zu 20% vom DOC (durchschnittlich ca. 8%) wurden weitaus höhere Kohlenhydratanteile im offenen Arktischen Ozean gefunden. Dies deutete auf einen starken Einfluß des Phytoplanktons auf die Kohlenhydratkonzentration hin. Im Tiefenwasser lagen die Kohlenhydratkonzentrationen teilweise unter 2 µM C (entspricht ca. 1% von DOC). Eine regelmäßige Abnahme des Kohlenhydratgehaltes des Tiefenwassers vom Sibirischen Schelf bis ins Kanadische Becken war vermutlich auf die wachsende Entfernung zu den terrestrischen Quellen zurückzuführen.

Detailliertere Aussagen ließen sich mittels der Zusammensetzung der individuellen zusammengesetzten Zucker treffen. Von den erfaßten Zuckern Fucose, Rhamnose, Arabinose, Galactose, Glucose, Mannose, Xylose, Fructose und Ribose gab keine Komponente, die ausschließlich im Probenmaterial eines speziellen Gebietes vorkam. Es zeigten sich aber systematische Tendenzen, anhand derer die verschiedenen Wassermassen charakterisiert werden konnten. Als Indikator für organisches Material marinem Ursprungs eignete sich Ribose, deren prozentualer Anteil an den Kohlenhydraten in den Flußproben sehr niedrig war, im marinen Milieu aber bis zu 20% erreichte.

Genereller Hauptbestandteil der Kohlenhydrate war Glucose, deren Anteil an den Gesamtkohlenhydraten sich zudem sehr gut für eine Charakterisierung des diagenetischen Grades des DOM eignete. Terrigenes, diagenetisch altes DOM aus den

Flüssen hatte sehr niedrige Glucoseanteile zwischen 25 und 28%, ebenso wie diagenetisch altes DOM aus Tiefseeproben (ca. 20%). Hohe Glucoseanteile von bis zu 86% deuteten auf frisch produziertes marines DOM in Oberflächenwasser und Meereis hin. Die geringen Unterschiede in den Glucoseanteilen der Flußproben deuteten auf einen Einfluß der Vegetationsformen der jeweiligen Einzugsgebiete hin. Gelöstes Material, welches aus Tundragebieten eingetragen wurde, wies etwas höhere Glucoseanteile und daher eine geringere diagenetische Veränderung auf als jenes aus Taigagebieten.

Ebenfalls als Indikatoren für die diagenetische Überarbeitung von DOM eigneten sich die prozentualen Anteile der Deoxyzucker, Fucose und Rhamnose, und Arabinose. Deren jeweilige Anteile nahmen mit dem Grad der Diagenese von frisch produziertem DOM (maximal 5%) zu altem DOM (bis zu 40%) zu.

Im Zusammenhang mit altem DOM wurde die Zusammensetzung refraktärer Kohlenhydrate untersucht. Von Phytoplankton frisch produzierte Kohlenhydrate liegen größtenteils in Form von Polysacchariden vor. Diese Polysaccharide sind wiederum leicht bioverfügbar, d.h., sie werden schnell, z.B. von Bakterien, aus dem DOM entfernt. Allerdings sind teilweise mehr als die Hälfte der Kohlenhydrate, vor allem im Tiefenwasser, refraktär und mikrobiellem Abbau weitestgehend entzogen. Mittels einer Kombination aus XAD-2 und -4 Adsorptionsharzen wurde der als refraktär angesehene Anteil des DOM, die sog. Huminstoffe, aus der Wasserprobe extrahiert und in vier verschiedene Fraktionen nach hoher bzw. niedriger Molekülgröße und -polarität aufgetrennt.

Mit zunehmendem Alter des DOM stieg der Anteil der extrahierbaren Huminstoffe am DOC von durchschnittlich 45% in Oberflächenproben auf bis zu 67% in Tiefseeproben an. Ähnliche Werte wurden für die Gesamtkohlenhydrate erzielt, während für die Summe der einzelnen, neutralen Zucker erheblich niedrigere Extraktionsausbeuten erreicht wurden. Aber auch hierbei stieg der Anteil der extrahierbaren Zucker mit der Tiefe von 8 auf 24% an. Diese Unterschiede wurden auf die chemische Zusammensetzung der Kohlenhydrate zurückgeführt. Vermutlich bestehen die refraktären Kohlenhydrate aus chemisch modifizierten Zuckern, wie z.B. geladenen Uronsäuren oder Aminozuckern, die mit dem angewandten Meßverfahren nicht zugänglich waren. Allerdings müssen ihre Anteile in der unfraktionierten Seewasserprobe nur von untergeordneter Bedeutung sein, da in diesen Proben die Konzentrationsunterschiede zwischen den beiden Meßverfahren nur gering waren. Zudem lagen ca. die Hälfte der mit XAD extrahierten Zucker aus oberflächennahen Proben und mehr als 70% der aus Tiefenwasserproben extrahierten Zucker in einer unpolaren, mit Methanol gewonnen Fraktion vor. Da sich zudem mit zunehmendem Grad der Diagenese insbesondere die Molekülgröße der zuckerenthaltenden Verbindungen verringerte, wurde vermutet, daß ein Großteil der refraktären Zucker in niedermolekularen, unpolaren Verbindungen eingebunden ist.

Generell nahm der Anteil der extrahierbaren Deoxyzucker mit zunehmender Tiefe und Diagenese ab, während die extrahierbaren Anteile von Arabinose und Xylose zunahmen. Das Verhältnis aus der Summe der molaren Anteile der Deoxyzucker zu der Summe der

Anteile von Arabinose und Xylose charakterisierte die Huminstoffe hinsichtlich ihres diagenetischen Grades. Dementsprechend konnte extrahiertes DOM einer Eisscholle als mikrobiell sehr frisch produziertes DOM, welches auch frischer als das aus Oberflächenwasser extrahierte DOM war, charakterisiert werden. Am stärksten mikrobiell überarbeitet war erwartungsgemäß das refraktäre Material der Tiefseeproben.

Summary

A detailed characterization of dissolved carbohydrates as one of the largest pools of dissolved organic matter (DOM) in the Arctic Ocean is presented. From the terrestrial sources on the Eurasian continent, the riverine inflow and the distribution of carbohydrates in the Arctic Ocean were investigated. Dissolved carbohydrates of the Siberian rivers and shelf areas, the Kara and Laptev Seas, the central Arctic Ocean, Fram Strait, and Greenland Sea were determined.

Dissolved carbohydrates were measured by two methods. The concentrations of bulk carbohydrates were determined by the colorimetric L-tryptophan / sulphuric acid method, and individual, neutral sugars of free and combined carbohydrates by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The high sensitivity of both methods allowed detection in freshwater and seawater samples without pre-concentration.

The concentration of total carbohydrates showed large variations. Highest values, between 10 and 41 μM C (25 μM C on average) were measured in the samples of the Russian rivers. A strong decrease of the carbohydrate concentrations occurred during the transition into the marine environment, where the values ranged from 2.6 to 9.7 μM C (5.3 μM C on average). The proportion of dissolved carbohydrates of total dissolved organic carbon (DOC) decreased much less from 4.7% in the rivers to 3.9% in the shelf areas. Higher proportions of carbohydrates of DOC (up to 20%, 8% on average) were found in surface waters of the Arctic Ocean. This suggests a strong influence of phytoplankton on the occurrence of carbohydrates. In deep waters, the total carbohydrate concentrations were partly below 2 μM C, corresponding to approx. 1% of DOC. A continuous decrease of the carbohydrate content of deep waters from the Siberian shelf to the Canadian Basin was ascribed to the increasing distance of the terrestrial sources.

Detailed information was obtained from the composition of the individual combined neutral sugars. Fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, fructose, and ribose could be determined and occurred in all water masses and matrices. The main component of dissolved carbohydrates was glucose, which was well suited for the characterization of the diagenetic state of DOM. Terrigenous, diagenetic old DOM of the rivers had very low glucose proportions between 25 and 28% of total carbohydrates, similar to diagenetic old DOM of deep water samples (approx. 20%). In contrast, freshly produced DOM of surface waters and sea ice was characterized by high glucose proportions of up to 86%. Small variations of the glucose proportions between the samples of the different rivers suggested an influence of the vegetation forms on dissolved carbohydrates. Dissolved organic matter, drained from tundra areas, exhibited higher glucose proportions and hence a lower diagenetic modification than DOM from tundra areas. Indicators for diagenetic modifications were also the deoxysugars, fucose and rhamnose, and arabinose. Their proportions increased with increasing diagenetic

degree from 5 to approx. 40% each. Ribose, whose proportion was very low in the rivers, but up to 20% in marine environments, evolved as a good indicator of marine DOM.

The composition of refractory carbohydrates was determined by extraction of recalcitrant organic matter from the seawater using a combination of XAD-2 and-4 adsorption resins and separation into four fractions according to molecular size and polarity. These carbohydrates, components of so-called humic substances, are remnants from the carbohydrates, which are freshly produced and rapidly modified.

With increasing age of DOM, the proportion of the humic substances of the total DOC increased from 45% in surface samples to 67% in the deep sea. Similar proportions were obtained for the total carbohydrates. The extraction efficiencies of the total sugars as sum of the individual neutral sugars was much lower, but increased also with depth from 8 to 24%. These significant differences were attributed to the chemical composition of the pool of carbohydrates. Part of refractory carbohydrates may be composed of chemically modified carbohydrates, e.g., charged uronic acids or amino sugars, which are not accessible with the chromatographic method. However, their contribution to the total carbohydrates of the seawater is only small, as the differences between the two detection methods were only marginal in the samples. More than the half of the XAD-extractable sugars of surface samples and more than 70% of deep sea samples were found in the unpolar fraction, eluted with methanol from the XAD resins. Since the molecular size of the carbohydrates decreased also with increasing state of diagenesis, it is concluded that the majority of refractory carbohydrates were bound or associated to unpolar compounds of small molecular size.

As a general trend, the proportion of the XAD-extractable deoxysugars decreased with increasing state of diagenesis, while the corresponding proportions of arabinose and xylose increased. Using the ratio of the sum of the molar proportions of the deoxysugars and the sum of arabinose and xylose, the extracted humic substances were characterized with respect to their diagenetic degree. DOM from fresh material of an ice floe exhibited a lower diagenetic degree than DOM of surface waters, and, as expected, the most refractory DOM was extracted from deep sea waters.

1 Introduction

1.1 Dissolved Organic Matter in the Ocean

Almost all water of the world's hydrosphere (97% by volume) is found in the oceans, which cover more than 70% of the world's surface. The giant ice caps of Antarctica and Greenland account for further 2% and other reservoirs like rivers, lakes, groundwater, snow and vapour contribute less than 1% to the global hydrosphere (Berner and Berner, 1987). The oceans are also by far the largest reservoir of carbon, containing approx. 50 times more carbon than the atmosphere (Benner et al., 1992; Siegenthaler and Sarmiento, 1993). The vast majority are the dissolved carbonate species, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}). Only a fraction, which amounts the atmosphere's carbon dioxide, are dissolved and suspended organic substances (total organic carbon, TOC). Besides the uncertainty concerning the composition of this carbon pool, even the estimates about the amount of organic carbon have been revised in the last few years, and are still matter of discussion (Sharp, 1997). The organic matter is biogeochemically cycled both in particulate and dissolved form (Figure 1). This classification is operationally defined: organic substances passing a filter of 0.45 µm are considered as dissolved (dissolved organic carbon, DOC). DOC is the major reactive component of the global carbon cycle and contributes more than 90% to TOC, the remaining 10% are particulate organic carbon (POC). The proportion of living biomass to POC is only approx. 1% (Cauwet, 1978).

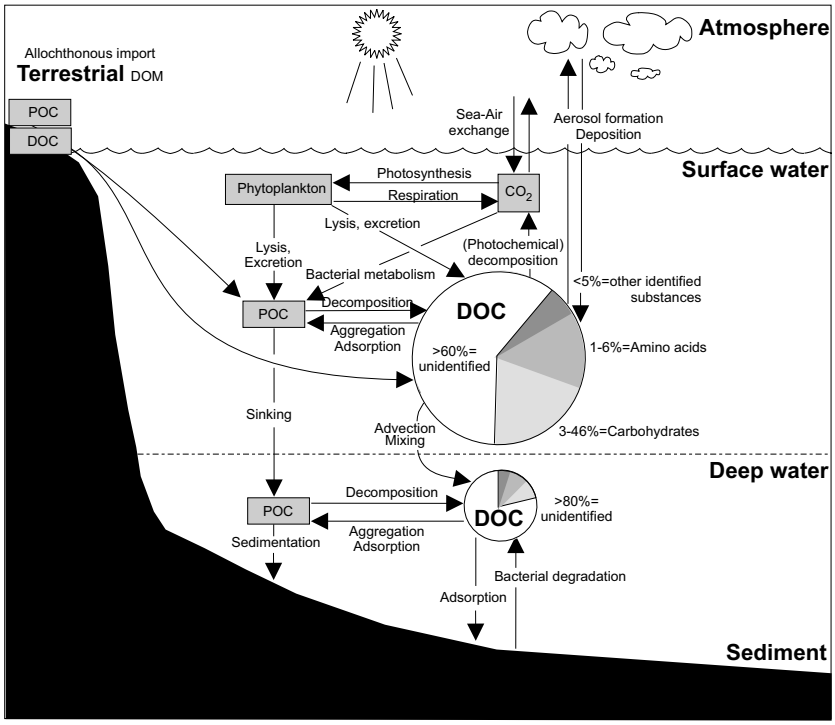


Figure 1: Biogeochemical cycle of organic carbon in the ocean (modified after Wangersky, 1972).

Any uncertainties about concentrations, composition, and geochemical or biological activity like consumption and production limit the ability to include DOC in biogeochemical models.

DOC is not homogenously distributed in the oceans. The large water reservoirs of the deep ocean contain less than 10% of DOC, the rest can be found in the surface-mixed layer (above 100 m), which is in permanent contact with the atmosphere and participates in the active carbon cycle. Most marine DOC is autochthonous organic carbon, derived from photosynthetic microorganisms. Allochthonous import, which accounts only for less than 5% of the total annual import, is supplied by river discharge or rain precipitation. Terrigenous organic matter comprises only a small fraction (0.7 to 2.4%) of dissolved organic matter (DOM), is reactive and cycles more rapidly than marine DOM. The remineralization of terrigenous DOM contributes to high rates of primary production in coastal regions, where some terrigenous DOM also mixes conservatively and remains unaltered (Opsahl and Benner, 1997).

The mean residence time for organic carbon in the ocean is calculated to be 16 years (Hedges, 1992a), indicating a relatively rapid cycling of most marine organic matter. In contrast, the apparent radiocarbon age of deep sea DOC is 4000 to 6000 years (Williams and Druffel, 1987; Bauer et al., 1992), indicating a very long residence time of some organic compounds. This DOC is considered to be refractory, i.e., resistent to microbial degradation (Barber, 1968) in corresponding time scales. In limnic and terrestrial ecosystems, these recalcitrant substances are called “humic substances”. These are macromolecular compounds of unspecific composition with a molecular weight of up to 500 kDa. Terrigenous humic substances are secondary condensation products of smaller molecules released during degradation of detritus (Tranvik, 1992). Their composition is very heterogeneous and depends on the biological and chemical modifications during their formation. Due to their aromatic character, they can be extracted from aquatic matrices with adsorption resins like Amberlite® XAD (Malcolm, 1989; Thurman and Malcolm, 1981). Hence, the definition of humic substances is primarily operational. In marine ecosystems, the terrestrial organic matter undergoes degradational rearrangements and the aromatic character of DOM is very low (Malcolm, 1990). Marine “humic substances” therefore comprise only the fraction of DOC, which can be isolated with XAD-resins, and their molecular and structural properties may not be representative for bulk DOC. The microbial utilization of marine “humic substances” was studied by Bussmann (1999). Surface humic substances showed the same quality in bioavailability as seawater DOC and were significantly better utilized than humic substances from deep waters. Amon and Benner (1994) and Benner et al. (1992) have pointed out that high-molecular weight compounds (molecules larger than 1 kDa) isolated by ultrafiltration, mainly freshly produced carbohydrates, are rapidly consumed in surface waters. Low-molecular weight organic matter persists in the deeper oceans. The molecular size is suggested to correspond with chemical alteration or diagenetic status (Amon and Benner, 1994).

About 40% of total DOC is characterized. The literature data describing the amount and chemical composition of dissolved organic matter are more or less a reflection of methodological capabilities and analytical techniques at that time. In the past decades, since the earliest investigations of Pütter (1909) considerable progress in the characterization of DOM was made. Carbohydrates account for the largest fraction with highly variable proportions between 3 and 46% (e.g., Pakulski and Benner, 1994). According to Ernst (1983), amino acids represent the second largest class of compounds (6%), followed by uronic acids (1.8%), aldehydes and ketones (1.5%), urea and fatty acids (1% each), hydrocarbons, phenols, sterols, and vitamins (all less than 1%). These values depend on the source of DOM. In polar waters, the contributions of carbohydrates and amino acids are much lower than these average data, although the DOC concentrations are comparable. Rich et al. (1997) and Engbrodt (1997) measured mean carbohydrate concentrations of 4.5 and 4.8 µM C, respectively. They comprised up to 5% of DOC, while amino acids comprised only up to 2.6% of DOC (Fitznar, 1999).

1.2 Carbohydrates

The chemical class of carbohydrates consists of a multiplicity of substances. It is a generic term for polyhydroxycarbonyl compounds like aldoses (with an aldehyde as functional group) and ketoses (with a keto group). Further, the term includes high molecular weight compounds, which could be transferred (e.g., via hydrolysis) into their constituting monomers. Normally, carbohydrates are described with a total formula $C_nH_{2n}O_n$ or $C_n(H_2O)_n$ and are therefore called “hydrates of carbon”, leading to the term carbohydrates. This notation was maintained, even though it is not correct in the case of, e.g, rhamnose (6-deoxy-mannose, $C_6H_{12}O_5$) and nitrogen- or sulphur-containing substances (e.g., amino sugars and sugars carrying sulphate ester groups). The names of the monomeric carbohydrates are marked by the suffix *“ose”* (e.g., glucose, fructose).

Whereas “carbohydrates” has a more general meaning, “sugars” and “saccharides” specify destinct compounds. These terms are of historical origins, and there is no strict distinction between sugars and saccharides, which are used as synonyms. The monomeric sugars detected by the high pressure anion exchange chromatography with pulsed amperometric detection method used in this study are defined as “neutral sugars”, a definition due to their chemical properties. The bulk determination of the colorimetric method leads to “total carbohydrates”.

1.2.1 Abundance and Distribution of Marine Dissolved Carbohydrates

Produced in the Calvin-Benson-Cycle, carbohydrates comprise about 10 to 70% of organic matter in phytoplankton cells (e.g., Romankevich, 1984). They originate in the dissolved phase from direct exudation by healthy organisms, decomposition of dead

organisms (e.g., Hellebust, 1965; Ittekkot et al., 1981), and viral lysis (Fuhrmann and Suttle, 1993). Dissolved and particulate carbohydrates are metabolized in the food web, i.e., taken up and released by heterotrophic organisms (e.g., Strom, 1997; Cowie and Hedges, 1994).

The importance of carbohydrates is based on the ubiquitous abundance as structural cell components (e.g., cellulose, pectins, and chitin, Romankevich, 1984), storage polymers (e.g., starch), algal and bacterial exudates (e.g., uronic acids and sulphate esters, Decho and Lopez, 1993), and other biopolymers like lipopolysaccharides. These are all combined polysaccharides, which provide the major proportion of dissolved carbohydrates. Monomeric carbohydrates can be directly utilized by heterotrophic organisms and are hence abundant only in low concentration. The functional diversity is reflected in the chemical composition. Varying sequences of homo- or heteropolymeric chains and side-chains of pentose and hexose monomers lead to a manifold of different structures. Derivatisation of the saccharides leads, e.g., to amino- and deoxysugars.

The marine environment has to be distinguished in at least two different ecosystems: the euphotic zone influenced by sunlight, including the estuaries, and the dark intermediate and deep waters. While plankton production and rapid cycling predominantly influence the first one, the latter ones are regions of microbial remineralization and consumption. In order to understand the biological processes, most studies focused on the phytoplankton-derived carbohydrates.

The production and release of dissolved polysaccharides by phytoplankton is variable and depends on the physiological and nutritional state of the growth. Generally, polysaccharides are released, especially under conditions of nutrient deficiency. Ittekkot et al. (1981) monitored the carbohydrate composition of a phytoplankton bloom in temperate waters (North Sea), where during the exponential growth phase the excretion products were dominated by fructose and glucose. Mannose and galactose were not only present in diatom cell constituents but also in their released dissolved products (Myklestad et al., 1972). Rhamnose, fucose, and arabinose, which are components of algal cell walls (e.g., Haug and Myklestad, 1976), increased in the dissolved fraction during phases of decay and were presumably released after cell wall destruction by bacteria. Free dissolved carbohydrates occurred at the end of the bloom and are supposed to be released by cleavage of the polysaccharides (Ittekkot et al., 1981).

Excretion products of diatoms were studied in enclosure experiments (Mopper et al., 1995). These exudates were rich in fucose, rhamnose, and galactose throughout the diatom bloom and showed a different molecular composition compared to the residual material being rich in glucose (glucans, i.e., storage products). Depending on the dominating species and the phase of the phytoplankton bloom, the spectrum of the dissolved carbohydrates can vary within wide ranges (e.g., Ittekkot et al., 1981; Ittekkot et al., 1982; Mopper et al., 1995).

The concentrations of carbohydrates in the surface layer of the open ocean are much higher and show greater regional or diurnal variations than in deeper layers. Maximum values of more than 30 µM C were measured in the Gulf of Mexico, while the values for

the North Atlantic and Antarctic waters were only in the range of 7 to 11 µM C and 14 to 20 µM C, respectively. With greater depth, the concentrations decreased to 5 to 7 µM C (Pakulski and Benner, 1994). The strongest decrease occurred within the euphotic zone, indicating rapid consumption of the labile carbohydrates. Below the euphotic zone, concentrations are rather uniform. In surface waters, the main component is glucose with approx. 25% (e.g., Skoog and Benner, 1997), followed by galactose, mannose, xylose, fucose, arabinose and rhamnose. In deep waters of the Pacific Ocean, glucose dominates with more than 60%. In North Atlantic deep waters, glucose is less dominant, while the proportions of fucose, rhamnose, and arabinose are higher than at the surface (Mopper et al., 1980).

1.2.2 Determination of Carbohydrates in Seawater

The analytical challenges of seawater analysis are low concentrations of organic substances in the high saline matrix. Extraction with organic solvents is inapplicable due to the high solubility of the polar carbohydrates in water. Hence, detection has to be done in the aqueous medium, or water and salt have to be removed completely. Until recently, investigations of carbohydrates in seawater focused on the bulk determination of carbohydrates and related compounds since analytical techniques on molecular level were not available so far. Analytical methods for the determination of carbohydrates in aqueous solutions were developed in clinical research in order to measure glucose in blood and urine and have been used for decades. Established in the first half of 19th century, most methods are based on colorimetrical reactions of carbohydrate functional groups with a dye. A disadvantage of these colorimetrical methods is that the functional groups of sugars, e.g., aldehyde or ketone groups, are not specific only to carbohydrates, especially in natural sample matrices. Nevertheless, they serve well for a bulk determination of carbohydrates and carbohydrate-analogues. From these methods the L-tryptophan / sulphuric acid method (Josefsson et al., 1972) is most sensitive, can also be applied in saline waters and is therefore used in this work. However, distinction between mono- and polysaccharides is not possible. This can be achieved with more extensive methods like the method using 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) as reagent (Johnson and Sieburth, 1977). This method allows analysis with or without hydrolysis prior to a derivatization of monosaccharide-derived alditols with MBTH. Hence, mono- and polysaccharides can be analyzed separately. Highly sensitive colorimetric methods use fluorometric reagents but require preceding desalting (e.g., Hirajama, 1974).

Bulk determination of carbohydrates is not sufficient for the understanding of the role of sugars in aquatic environments. Therefore, liquid and gas chromatographic methods were developed in the late 1970's for the determination on a molecular level (Mopper, 1977; 1978a; 1978b). These methods require appropriate derivatization and enrichment steps, which represent possible sources of contamination. Besides the difficulties of

enrichment and chromatographic separation of carbohydrates, detection has been a major problem. In 1990, a rapid and reproducible liquid chromatographic method with electrochemical detection of mono- and oligosaccharides was introduced (Johnson and LaCourse, 1990). This technique was adapted for seawater samples without enrichment steps (Mopper et al., 1992), advanced to a standard method, and was thus used in this study. This sophisticated technique is still refined. Recently, the method has been applied for the determination of amino sugars in seawater (Kaiser and Benner, 2000). A disadvantage of this chromatographic method is, that only monosaccharides can be detected in seawater and thus polymeric compounds have to be hydrolyzed prior to chromatographic separation. Only few methods have yet been developed for direct analysis of polysaccharides, which are mass spectrometric techniques like direct temperature-resolved ammonia chemical ionization mass spectrometry (Boon et al., 1998) or matrix-assisted laser desorption and ionisation technique keeping the molecules undestructed (Kazmaier et al., 1998). However, the variety of naturally occurring organic compounds leads to very complex spectra and definite molecular structures are exceedingly difficult to obtain.

Common procedures are acidic hydrolysis of polymeric compounds and analysis of the released monomers. Hydrolysis conditions vary depending on the purpose and material of interest. Generally, hydrolysis with sulphuric acid gives similar or higher yields than other acids such as hydrochloric or trifluoracetic acid (e.g., Mopper, 1977; Pakulski and Benner, 1992; Borch and Kirchman, 1997). It has to be considered that acidic hydrolysis is a combination of cleavage of glycosidic bonds on one hand, and destruction of the released monomers on the other. For instance, to increase hydrolytic efficiency, lyophilizated ultrafiltration powder pretreated with 12 M sulphuric acid is applied prior to hydrolysis with 1.2 M sulphuric acid (Skoog and Benner, 1997). Hydrolysis with 0.85 M sulphuric acid gives high yields without considerable destructive losses (Borch and Kirchman, 1997). Ion chromatography requires neutralization and desalting after hydrolysis. Hereby, considerable losses of carbohydrates have been reported. For instance, uronic acids or amino sugars are lost. Hydrolysis with hydrochloric acid yields less monomers but facilitates neutralization and desalting and is therefore preferred by some authors (Borch and Kirchman, 1997).

1.3 Extraction of Refractory Dissolved Organic Matter

In marine aquatic science, methods and techniques from limnology have been adopted. In soil chemistry, humic substances can be easily extracted with acidic and alkaline solutions. This technique is inappropriate with aquatic samples. Therefore, solid-phase extraction procedures were established. A common procedure in limnology is the adsorption on XAD-resins with subsequent elution with sodium hydroxide solution. A common combination is XAD-4 and -8, but XAD-1, -2, and -7 have also been used (Aiken et al., 1979), depending on the composition and origin of the organic matter. Application

of XAD techniques to seawater samples results in significantly lower yields than for freshwater (Druffel et al., 1992). Other isolation techniques, particularly ultrafiltration, are used to concentrate DOM in seawater and are reported to collect large amounts of DOC (Benner et al., 1992). Due to their different fractionation criteria, XAD-resins and ultraflitration extract different partitions of the DOM pool. Tangential-flow ultrafiltration with a 1 kDa cut-off filter yields selectively macromolecules larger than 1 kDa, primarily freshly produced carbohydrates (Benner et al., 1992; Skoog and Benner, 1997) and proteins (McCarthy et al., 1996). Isolation of marine humic substances for the analysis of refractory substances and carbohydrates, which might be part of this fraction, is therefore best suitable with XAD-extraction (Malcolm and MacCarthy, 1992). The characteristics of XAD isolation exclude free polysaccharides and make carbohydrates selectively accessible, which are associated or even incorporated in the complex and highly condensed structures of humic substances.

1.4 The Arctic Ocean

The Arctic Ocean receives, relative to its size, the largest freshwater flux from rivers compared to other oceans. The major freshwater inflow reaches a total of 3300 km^3yr^{-1} or 0.11 Sv, which is about 10% of the global river discharge. Major contributors are the Yenisey (603 km^3yr^{-1}), the Ob (530 km^3yr^{-1}), and the Lena (520 km^3yr^{-1}) (Figure 2). The largest other Russian rivers discharge 504 km^3yr^{-1} in sum, among them Kolyma, Indigirka, and Moroyyakha. The MacKenzie River (340 km^3yr^{-1}) almost exclusively drains the Canadian side (all data from Aagaard and Carmack, 1989). The annual water discharge is highly seasonal. The maximum is reached during the snow melting period from April to May in the European part (west of Ob and Kara Sea, respectively) and from late May to June in the eastern Asian part of Siberia (Telang et al., 1991). More than 80% of the annual discharge occurs in this period (Gordeev et al., 1996).

Besides its high riverine input, the permanent ice cover is the most striking feature of the central Arctic Ocean, making it unique amongst the other oceans. Both results in a strong stratification of the water column (Figure 3). The intermediate water is dominated by the inflow of Atlantic waters from the Norwegian and Greenland Seas. One branch of this warm and saline Atlantic water passes the Fram Strait and follows the continental slope to the East (Jones et al., 1995). The other branch overflows the Barents Sea shelf (Blindheim, 1989), cools down due to heat loss to the atmosphere, flows from the northern Kara Sea shelf through the Santa Anna Trough into the Nansen Basin and forms a cold tongue of characteristic water between the continental slope and the Fram Strait inflow (Rudels et al., 1994). Thus, the salinity of the Kara Sea shelf is high.

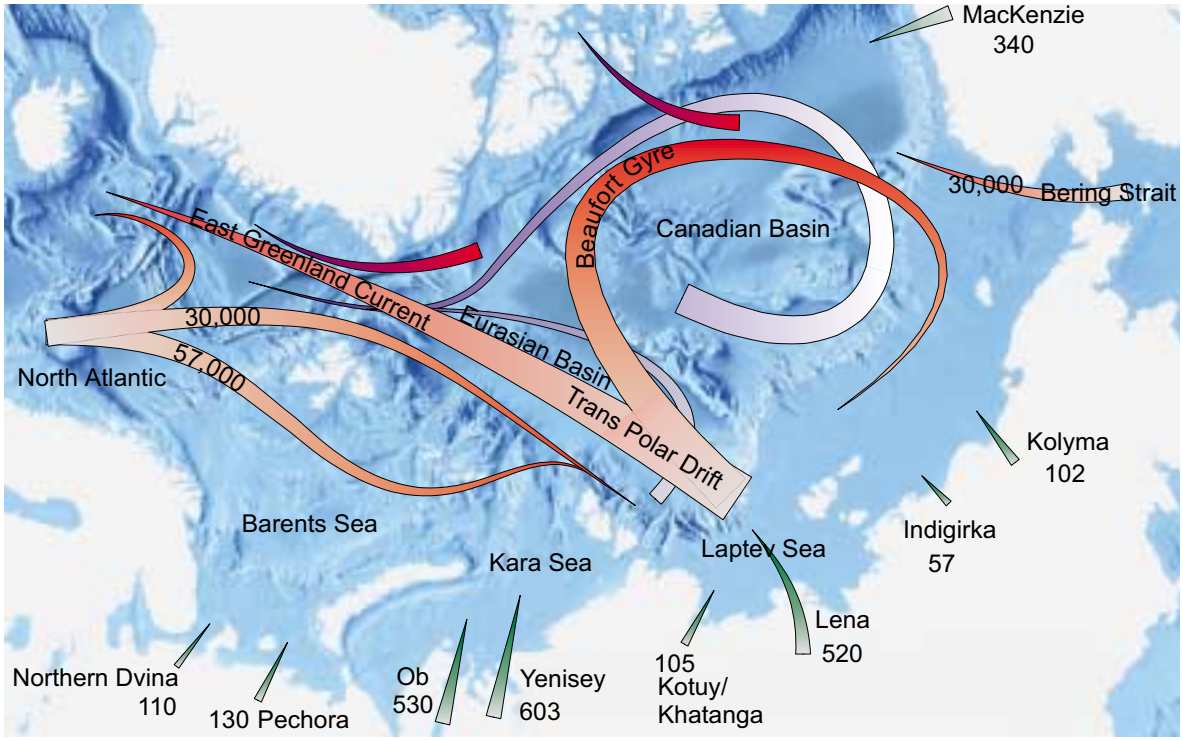


Figure 2: Hydrographic currents and water inflow of the Arctic Ocean. Flow rates are given in km^3yr^{-1}; (modified after Aagaard et al., 1995; Aagaard and Carmack, 1989; Jones et al., 1998);
green arrows: river discharge;
red arrows: surface currents;
red-blue arrows: subsurface currents;
blue arrows: deep water currents.

In the Arctic Ocean, the sharp halocline in approx. 200 m depth isolates the Arctic Ocean interior from non-particulate vertical exchange with the upper waters. The waters in the halocline are formed during winter freezing in the shelf areas and are advected into the central Arctic Ocean (Schlosser et al., 1995). Atlantic water, which is traditionally defined as the layer between the 0.0°C isotherms, extends down to 800 m depth (Bauch, 1995) (Figure 3). Below, Arctic Intermediate Water, deep water and finally the bottom water are located. The deep waters of the Eurasian and Canadian Basin differ in their oceanographic signature. Canadian Basin Deep Water (CBDW), crossing the Lomonosov and Alpha Ridges, enters the Fram Strait at approx. 1800 m depth.

Through the Bering Strait, water of Pacific origin enters the Arctic Ocean. The Bering Strait sill is only about 50 m deep, and the Pacific water flows as a subsurface current through the Arctic Ocean. With its typical high silicate and phosphate concentration, it can be traced through the Canadian Arctic Archipelago and along the Greenland slope as part of the East Greenland Current (Jones et al., 1998) (Figure 2).

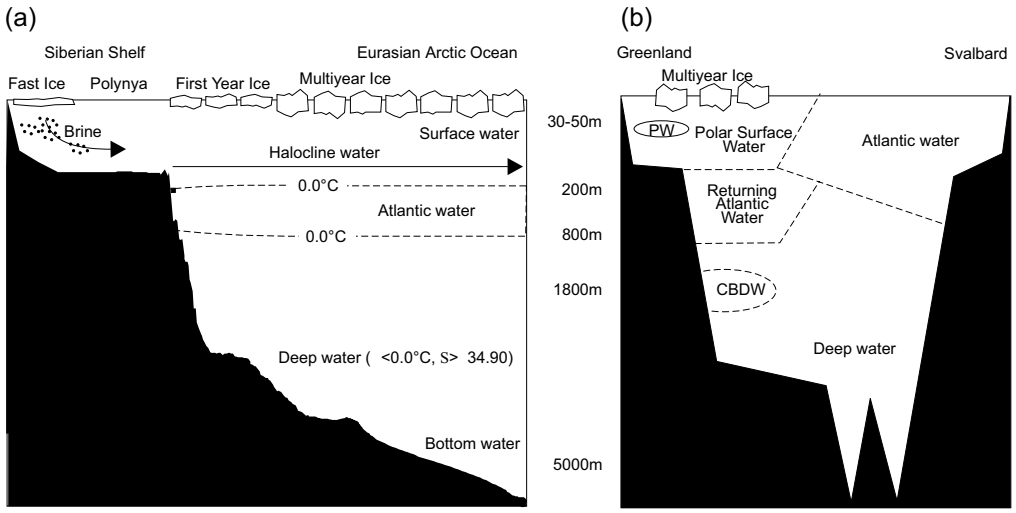


Figure 3: Water mass distribution in (a) the central Arctic Ocean and (b) in Fram Strait (modifed after Schlosser et al., 1995; Rudels, 1989);
PW = water of Pacific origin; CBDW = Canadian Basin Deep Water;
θ = potential temperature; S = salinitiy.

The presence of the Severnaya Zemlya islands favours creation of lee polynyas. Polynyas are small-scale ice-free regions, which are created by wind and surface currents. Here, large ice generation and strong brine rejection occurs (Cavalieri and Martin, 1994), which are supposed to drain downwards into the Nansen Basin and presumably allow deep slope convection and hence vertical transport of DOM.

The importance of the Arctic has been assigned to its role in influencing the global climate, by regulation of the global heat budget and deep water formation by thermohaline convection (e.g., Rudels et al., 1994; Aagaard and Carmack, 1994). On the other hand, the Arctic as a sensitive climate system itself could react to climate changes, which will also affect the amount and composition of DOM in the Arctic Ocean. Little is known about the seasonal and annual variability of biogeochemical processes, and possible consequences of climate variations like enhanced carbon release from thawing permafrost soils are still obscure. Sea ice dynamics have the potential to affect DOM distribution by its release from melting ice floes, which may be coupled to increased primary production. Thus, it is of great importance to improve our knowledge about the DOM constituents, which inhere a central role in the global biogeochemical cycles.

2 Objectives

Aim of this work is to investigate the distribution of dissolved organic matter in polar Arctic environment with emphasis on carbohydrates. Carbohydrates, which play a key role in biogeochemical processes, are fairly characterized in the Arctic Ocean. For their assessment, two analytical techniques are applied. One determination method of bulk carbohydrates is available yet, while the other method for the determination of combined and individual neutral sugar species has to be established. At the time the thesis was begun, no neutral sugar data of the Arctic Ocean were available, the data set of bulk carbohydrate was scarce, and basic knowledge about their occurrence is to be raised.

The focus of this work is laid on the following topics:

- The influence of terrestrial and phytoplanktonic sources to the carbohydrate composition at the threshold between the Eurasian continent and the adjacent marine environment.
- The changes in composition and distribution of dissolved carbohydrates, which characterize different environments and are coupled to specific oceanographic regimes of the Arctic Ocean.
- The compositional differences between freshly produced DOM and aged DOM of high diagenetic degree.
- The content of carbohydrates and their association in and to the structures of marine humic substances, which are selectively separated from total DOM.

3 Material and Methods

3.1 Characterization of the Sampling Areas

Sampling sites were chosen which represent different aquatic Arctic environments. Russian river samples were collected for the determination of organic matter of terrestrial origin. To follow mixing processes with the marine and saline environment, inshore shelf samples were taken. Transport of organic matter towards the central Arctic was traced using samples from shelf stations of Kara and Laptev Seas.

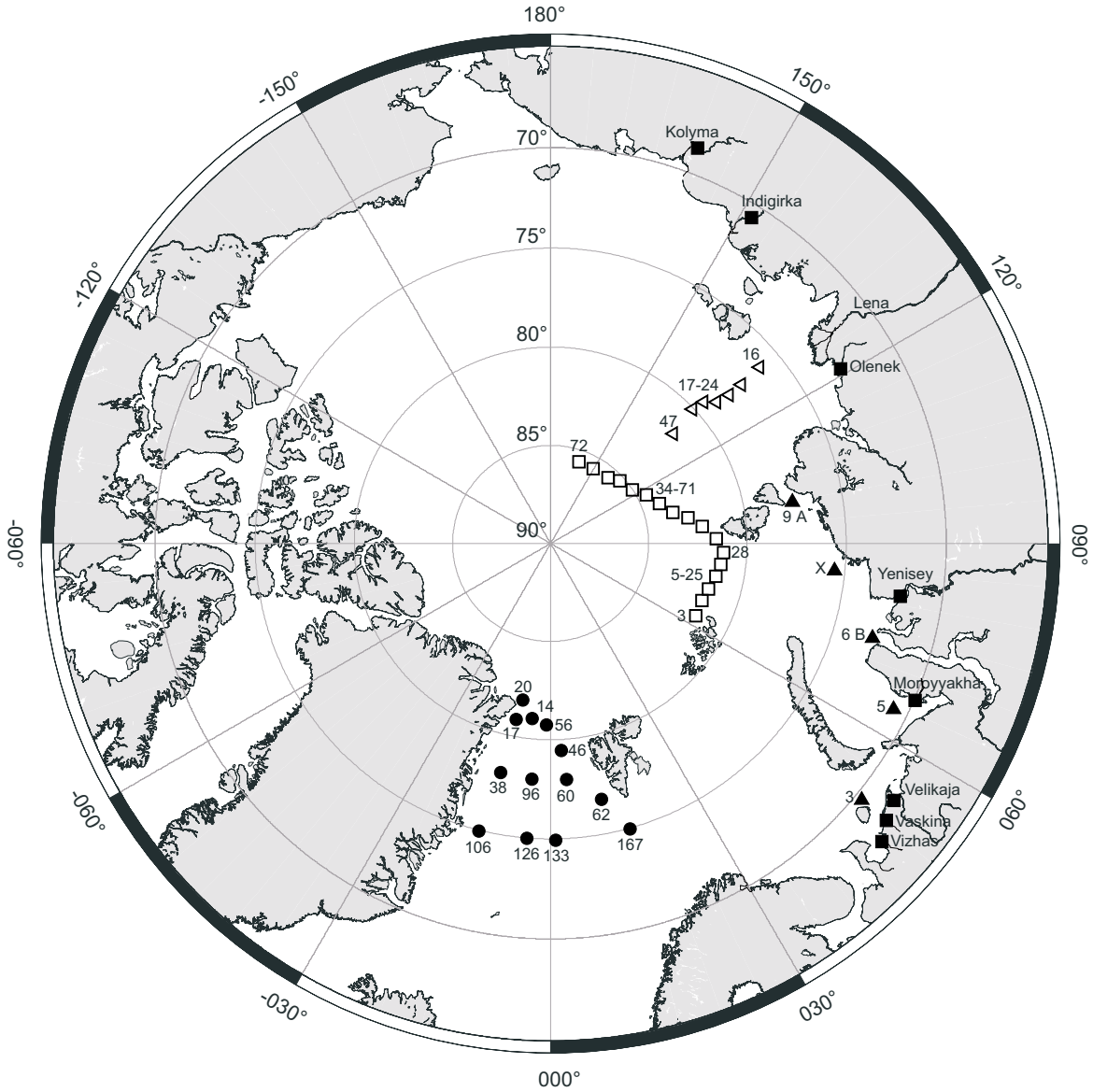


Figure 4: Arctic and Russian sampling sites: ◁ ARK XI/1, □ ARK XII, ● ARK XIII/3, ▲ SWEDARCTIC TE 94 shelf stations and ■ SWEDARCTIC TE-94 river stations; station numbers and sample names are the original identifiers of each cruise.

Further changes during transport were determined partly in the Canadian Basin, the central Arctic Ocean, and the North Atlantic Ocean via Fram Strait. Modifications of dissolved organic matter with depth were analyzed in deep water samples in the central Eurasian and Canadian Basins. For all samples, salinity, temperature and nutrient data are available, and many samples have been analyzed for dissolved organic nitrogen, chlorophyll *a*, amino acids, and lignin.

The samples from the Russian rivers and inshore areas were collected during the Swedish Tundra Expedition SWEDARCTIC Tundra Ecology 94 (TE-94) with the research and supply vessel “Akademik Fedorov” organized by the Swedish Polar Research Secretariat in summer 1994 (for sampling sites and locations, see Figure 4). Most inshore samples were taken of 10 m depth, except stations X (20 m) and 9A (50 m). All riverine sampling sites are located close to the river mouths in the estuaries and the samples were taken from surface water, except Yenisey (10 m), during or directly after the maximum summer water discharge. The composition of the riverine DOM is widely determined by the vegetation of the drainage areas (Lobbes et al., 2000). Taiga is the prevailing vegetation of central, and tundra of northern Siberia. Taiga is characterized by coniferous woods and bogs, and tundra is a treeless steppe. The shortest rivers Vaskina, Velikaja, and Moroyyakha drain exclusively tundra. Most of the other rivers drain tundra and taiga, while the longest rivers are more influenced by taiga than tundra (Strasburger, 1983; Times Atlas, 1997).

Shelf and open ocean samples were obtained during three expeditions with the research ice breaker “Polarstern” in the summers of 1995 (ARK XI/1, Laptev Sea), 1996 (ARK XII, Kara Sea and central Arctic Ocean) and 1997 (ARK XIII/3, Fram Strait and Greenland Sea) (Figure 4). During the latter cruise, samples of an ice floe were collected.

All expeditions took place in summer, hence ice conditions show minimal annual extent and ice cover changes little. The central Arctic Ocean was generally covered with pack-ice up to 100%, except the southernmost stations 16 and 17 in the Laptev Sea and the westernmost St. 3 near Franz Josef Land, which were ice-free. The eastern Fram Strait with warm Atlantic water was ice-free, while the ice concentrations in the East Greenland Current were up to 100%.

3.2 Sampling and Preservation

Water samples were collected with CTD-rosettes (ARK XI/1, ARK XII, ARK XIII/3), 5 l Niskin bottles (SWEDARCTIC TE-94), or onboard seawater supply (ARK XIII/3, St. 62). Sampling containers were handled with gloves to avoid contamination. Dissolved organic matter was obtained by filtration of samples through GF/F glass fibre filters (nominal pore size 0.7 µm; WHATMAN INT., UK; precombusted for 5 h at 550°C). Samples for neutral sugars analyses and DOC were filtered directly into glass ampoules (precombusted for 5 h at 550°C) and immediately frozen at -30°C. Samples for nutrients and total carbohydrates were filtered into new 100 ml polyethylene bottles and fixed with 3.5%

mercuric chloride solution (300 µl / 100 ml sample) and stored in the dark at 4°C until analysis (Kattner, 1999). Samples from the SWEDARCTIC expedition were GF/F filtered and stored at -30°C in 1 l polyethylene containers. Samples for the adsorption with XAD resins were prepared by filtering 25 l of water with acid washed (1 M hydrochloric acid) cellulose acetate filters (0.8 µm and 0.2 µm in sequence, SCHLEICHER UND SCHUELL, Dassel) by gravity for 20 hours. The filtrate was used within 6 hours for XAD-adsorption.

The ice floe (approx. 250 kg) was sampled during ARK XIII/3 directly from the ship with a crane trailered iron basket. To avoid contamination, the box was washed in the surrounding water. Onboard, with a minimum amount of handling, the ice was put in a large acid rinsed polyethylene container and melted during the next 2 days at room temperature. The melted ice was filtered through a 0.6 µm polycarbonate filter cartridge and immediately adsorbed on resin.

3.3 Resin-Based Fractionation of Dissolved Organic Matter

Fractionation of DOM from seawater samples was performed with Amberlite® XAD-2 and XAD-4 adsorption resins (commercial Amberlite®, practical grade quality, ROHM & HAAS Corp., Philadelphia). Two different resins were used to maximize the adsorption efficiency for DOM. They are unspecific adsorbers on polystyrene basis, cross-linked with divinylbenzene. Both have a strong hydrophobic character and differ in their degree of cross-linkage, i.e., the content of divinylbenzene. XAD-4 has a larger active surface, a higher adsorption capacity and smaller pore sizes than XAD-2. The pore sizes of XAD-4 are approx. 9 nm and of XAD-4 only half of it (5 nm). This results in a higher adsorption capability of XAD-4 for smaller molecules than XAD-2, which extracts preferentially larger molecules.

Contamination from external sources as well as internal collapse may strongly affect adsorption procedures with organic extraction resins. External contamination may occur all time during handling. Internal breaking of the resin structure is thought to be caused by strong pH changes during extraction procedure. The two used resins XAD-2 and -4 are rather insensitive to internal breaking (Aiken et al., 1979; Malcolm, 1989). Problems with severe organic contamination of the XAD resins occurred during earlier expeditions, even though the resins were cleaned carefully onboard directly prior to use. To overcome any difficulties during experiments aboard, all resins were cleaned and checked at the home laboratory.

Besides XAD-2 and -4, XAD-7 is a common solid phase extraction resin in aquatic biogeochemistry and was used on cruises before. It adsorbs even polar molecules, as it is based on an acrylic acid polymer structure. XAD-7 is very sensitive to pH changes resulting in enormous carbon release (Aiken et al., 1979). As there is a high risk of systematical contamination of the samples, XAD-7 was not used for the extraction procedure.

The XAD resins were purified successively with dichloromethane and acetonitrile followed by methanol in a Soxhlet extractor (according to Malcolm, 1989). Each extraction took 24 h and was performed five times. To control the purification process, a subsample of 10 ml of the resin was taken out of the extractor after the last extraction with methanol. In an acid-rinsed glass chromatography column, the resin sample was washed with 1000 ml of MilliQ water (a MILLIPORE$_{185}$ plus system was used during this work), 100 ml of 0.1 M sodium hydroxide and 100 ml of 0.1 M hydrochloric acid solution (Suprapur® grade, MERCK, Darmstadt) with a flow rate of 10 ml min^{-1}. DOC was determined in the final 10 ml of the acid. If the sample was DOC-free, the resin was carefully transferred into acid-rinsed 30 ml chromatography columns (300 mm x 14 mm inner diameter) with a P2-frit and a teflon stopcock. 23 pairs of XAD-2 and -4 columns were prepared. These columns were carefully topped and sealed with parafilm until utilization aboard. The resin was kept in methanol to avoid gas bubble formation and to maintain sterile resins.

Aboard the ship, the methanol was completely removed: The columns were mounted under a dropping funnel and washed with 2 l of MilliQ water, followed by acidic and alkaline solutions (100 ml of 2 M hydrochloric acid and 100 ml of 0.1 M sodium hydroxide solution, with a flow rate of 2 ml min^{-1}).

For DOM extraction, the two resins were installed in sequence (Figure 5): To avoid blocking of the resin pores by macromolecules, which would lead to reduced adsorption capacity, the sample passed the coarser XAD-2 first and then XAD-4. The fittings between the sample container and the columns were either acid rinsed teflon tubing or glass connectors. The samples were acidified to pH=2 prior to extraction with 20 ml of conc. hydrochloric acid solution to protonate acidic groups and reduce polarity, leading to increased adsorption efficiency. At a flow rate of 1 drop per second, the extraction of a 20 l sample took between 21 and 24 h. After 10 l, a 100 ml subsample was taken from the effluent, which is the hydrophilic fraction (HI).

After the sample has completely passed the columns, they were rinsed with 250 ml of 0.1 M hydrochloric acid to remove the rest of the saline sample from the resin. The resins were eluted separately. Polar molecules (hydrophobic acid fraction, HbA) were eluted with 100 ml sodium hydroxide solution (p.a. quality grade, MERCK, Darmstadt). Less polar substances (hydrophobic neutral fraction, HbN) were eluted with 100 ml of methanol (LiChrosolv® grade, MERCK, Darmstadt). The fractions were collected in acid-rinsed polyethylene bottles and stored at -30°C until analysis in the home laboratory (Figure 5).

Prior to further analysis, the methanol in the HbN-fraction was removed in a vacuum rotary evaporator. To avoid destruction of DOM, water bath temperature did not exceed 40°C and methanol was not evaporated until dryness. Complete removal of methanol was ensured by triple addition of 50 ml MilliQ water and repeated evaporation. The sample was then adjusted to 100 ml with MilliQ water and kept at –30°C until analysis.

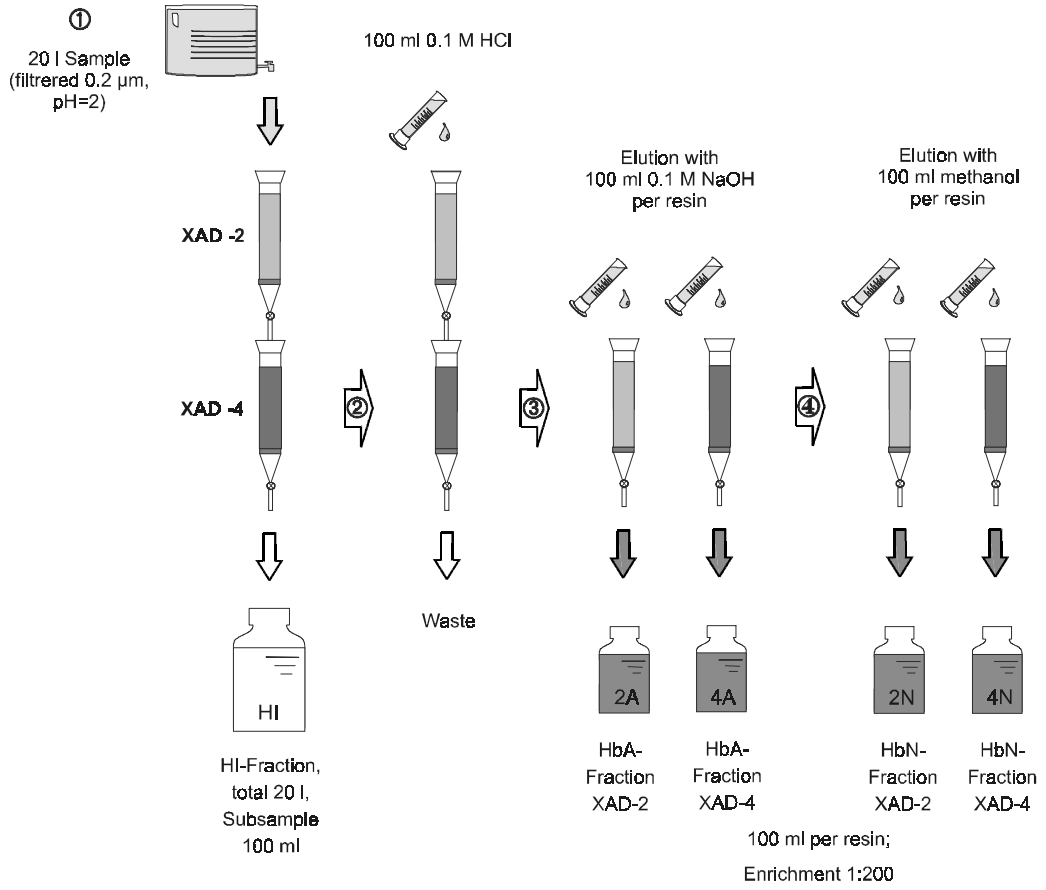


Figure 5: Sequence of seawater extraction with XAD resins and fractionation of DOM.

The ratio of the carbon and carbohydrate concentrations of the summed up fractions of XAD-2 and XAD-4 (referred as XAD-2:XAD-4) is introduced as a measure for the size-distribution of the extracted organic matter. The nominal pore size of the XAD-2 resin is approx. as twice as large as that of XAD-4. Hence the resins exhibit different adsorptive capabilities for small and large organic compounds (Malcolm, 1989). The higher the ratio, the larger should be the molecular size. The ratio of the carbon and carbohydrate concentrations of the HbN to HbA fractions of both resins (referred as HbN:HbA) is introduced as a measure for the polarity of the organic matter. Methanol and sodium hydroxide solution exhibit different elution capabilities for marine humic substances. The higher the HbN to HbA ratio, the less polar the molecules should be. A detailed description of the isolation of marine humic substances is presented by Malcolm (1989).

Special attention was paid to a quantitative, reproducible, and practicable procedure for aboard operation. To certify that the extraction of DOM with XAD resins is applicable on DOC-measurements, a series of blank studies was carried out. To assure that no carbon was released from the resins, 20 l of acidified MilliQ water were passed through the resin columns in blank experiments. These experiments were handled as described before. In Figure 6a, the DOC concentrations during the course of one of these blank

experiments is shown. The blank value of the acidified sample in the polyethylene container was less than 5 µM. At the beginning of the blank experiment, the DOC concentration in the HI-fraction was 12 µM, which decreased to 6 µM after all the MilliQ water had passed the columns. After washing with 250 ml of hydrochloric acid, the DOC concentration in the effluent was 8 µM. The concentrations of the HbA- and HbN-fractions were all less than 10 µM C (Figure 6a).

Lara and Thomas (1994) studied the adsorption of freshly produced DOM from algal cultures with ^{14}C labelled DOC and reported high irreversible adsorption of DOM if eluted only with sodium hydroxide solution. However, about half of this DOM can be removed with methanol. In experiments with seawater, 99% of the initial DOC content were recovered in the hydrophilic and hydrophobic fractions of the fractionation process and hence no irreversible adsorption at the resins was observed (Figure 6b).

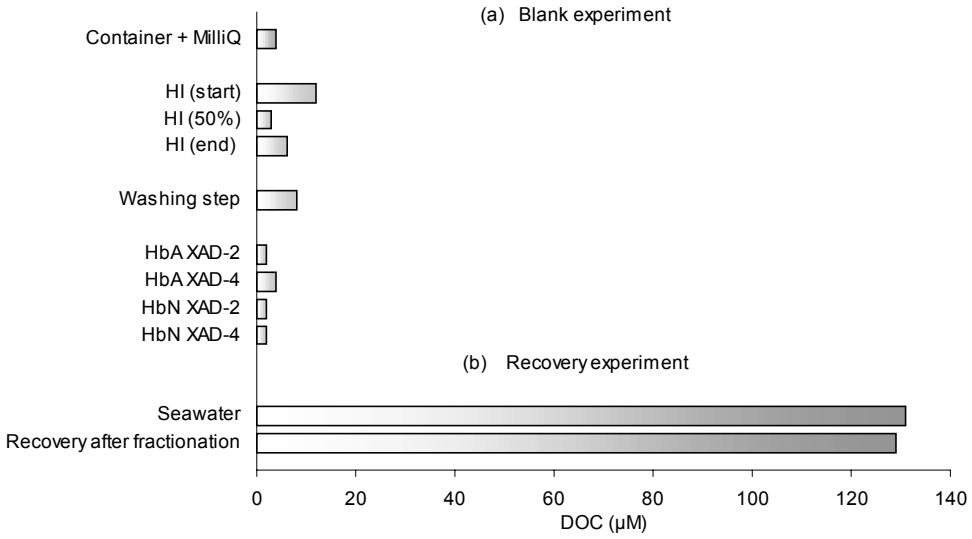


Figure 6: (a) DOC concentrations in the blank experiment;
(b) 99% of DOC of a seawater sample were recovered by XAD fractionation.

3.4 Analysis of Dissolved Organic Carbon

Dissolved organic carbon was determined by high-temperature catalytic oxidation using a SHIMADZU TOC-5000 analyzer at 680°C with the standard platinum catalyst and non-dispersive infrared detection (NDIR) of the generated carbon dioxide. The high-sensitivity catalyst provided by the manufacturer is unsuitable for seawater analysis due to rapid destruction after a few injections. Samples (approx. 10 ml) were acidified with 100 µl of 2 M hydrochloric acid (Suprapur® grade, MERCK, Darmstadt) in the SHIMADZU autosampler vials and purged with oxygen prior to analysis to remove inorganic carbon. The vials were cleaned for 24 h in chromosulphuric acid to oxidize all organic contaminants and rinsed thoroughly with MilliQ water. Samples were measured in duplicates at a series of 5 to 7 injections per measurement. Standards were run with potassium hydrogen phthalate ($C_8H_5O_4K$, provided by SHIMADZU, KANTO CHEMICALS, Tokyo, Japan) at appropriate concentrations. While seawater samples were run without dilution, most of the XAD-extracts were diluted by 1:10. For further details see Benner and Strom (1993) and Skoog et al. (1997).

3.5 Automated Colorimetric Determination of Total Dissolved Carbohydrates

The method used within this work is based on the reaction of hydrolyzed carbohydrates with L-tryptophan in conc. sulphuric acid with boric acid at 100°C. Besides the hydrolytic cleavage of the glycosidic bonds of oligo- and polysaccharides, many degradation products form in the strong acidic and dehydrative medium. Pentoses form furfural, hexoses the corresponding 5-(hydroxymethyl)-derivative. Even γ-pyrones and benzene derivatives are known to occur. These condensation products react with the amino group of L-tryptophan like a Maillard-reaction. Through a stepwise reaction with intermediate products (e.g., Amadori rearrangement), intense coloured complexes are formed, the melanoidines (Ledl and Schleicher, 1990) (Figure 7). These complexes have an absorption maximum at approx. 500 to 530 nm. Measurements were carried out at 525 nm. Unfortunately, the signal height is different for individual carbohydrates. In Table 1 the response factors for different carbohydrates and derivatives compared to the calibration standard glucose are listed (Eberlein and Hammer, 1980; Engbrodt, 1997). For instance, 2-deoxysugars are degraded in strong acidic environments. In consequence, the lower signal responses lead to a partial underestimate of the real carbohydrate concentration. Even unmodified hexoses and pentoses exhibit a lower response than glucose.

Table 1: Comparison of the response signal of carbohydrates (in % relative to glucose) for the L-tryptophan / sulphuric acid method (Eberlein and Hammer, 1980; Engbrodt, 1997).

Compound	Component class	Signal (%)
Glucose	Aldohexose	100
Galactose	Aldohexose	88
Mannose	Aldohexose	87
Fructose	Ketohexose	72
Arabinose	Aldopentose	85
Xylose	Aldopentose	92
2-Deoxyribose	2-Deoxypentose	26
Rhamnose	6-Deoxyhexose	106
Fucose	6-Deoxyhexose	93
Galacturonic acid	Uronic acid	84
α-Glucosamine	2-Hexosamine	≈ 0
Sucrose	Disaccharide	173
Cellobiose	Disaccharide	184

All chemicals were obtained by MERCK (Darmstadt). All aqueous solutions were prepared with artificial seawater (67.6 g sodium chloride in 2000 ml MilliQ water). For the reagent, 2.5 g L-tryptophan (biochemical grade) and 12.5 g boric acid (p.a. grade) were dissolved in 500 ml conc. sulphuric acid (p.a. grade) under ice cooling. As a strong degradation of the reagent under light influence and warmth was observed, the mixed reagent was kept in a brown glass bottle and protected against direct sunlight during analysis. It was stored overnight at -30°C and replaced every second day. Nitrate standards were prepared of a stock solution of 1.000 ± 0.0002 g l^{-1} NO_3 ($^1/_{62}$ mol N l^{-1}) in artificial seawater. Carbohydrate standards were made of exsiccator-dried glucose (biochemical grade).

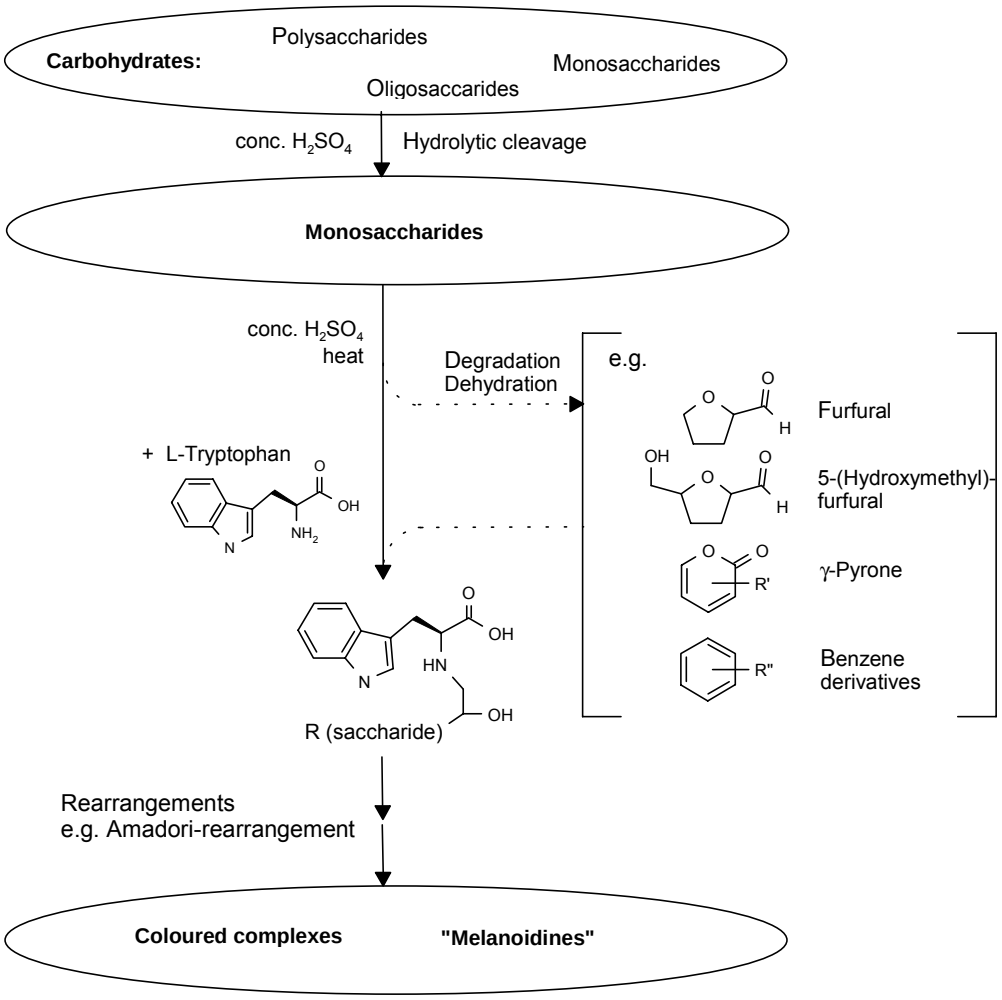


Figure 7: Degradation of carbohydrates and their reaction with L-tryptophan.

The analysis was processed with an AutoAnalyzer II System from Technicon (BRAN+LUEBBE, Norderstedt; Figure 8). For the determination 1.33 ml of the sample were required. Alternating, sample and artificial seawater were taken by the sampler, so that the system was washed. The timing intervals were adjusted to a ratio of 2.3 : 1 (250 s for sample : 110 s for artificial seawater). The polystyrene sampling cups were covered with parafilm against evaporation and contamination, which could easily be punctured by the sampling needle. A peristaltic proportioning pump delivered the solutions via a reaction coil, in which reagents and sample were well mixed, to an encapsulated oil heating bath (104 °C ± 0,1°C, G-type, coil length 2.5 m, inner diameter 2.0 mm). The flow rate was adjusted to a residence time of the reagent mixture of 15 min in the heating bath. After passing the bath, the yellow to violet solution was pumped directly to a single-channel photometer. The adsorption of the colorimetric complex was measured in a 50 mm x 1.5 mm (inner diameter) flowcell at 525 nm against air as reference. The signals were processed in a multi-channel recorder (series R-60, RIKADENKI, Freiburg i. Br.) on three recording ranges. This allowed covering a range from very low to maximal adsorption without any changes in recording set-up. Due to the

strong corrosive reagent, all tubing were made of Acidflex® (BRAN+LUEBBE, Norderstedt) or Viton® (ALLIANCE, Friedrichsdorf/Ts.) and all fittings made of glass.

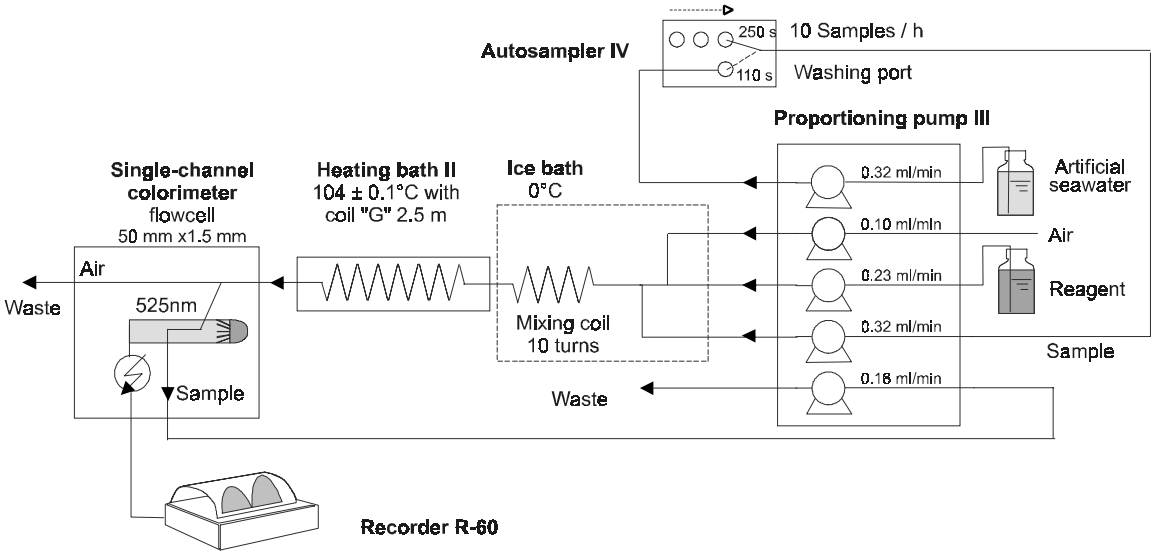


Figure 8: Flow scheme of the AutoAnalyzer II system for the determination of TCHO in seawater (modified after Eberlein et al., 1985). The heating bath is used for determination of carbohydrates, the ice bath for determination of nitrate plus nitrite.

Besides carbohydrates, nitrate and nitrite form coloured complexes with the reagent (Eberlein et al., 1985; Eberlein and Schütt, 1986). Even a nitrate concentration of 1 μmol l^{-1} affects the determination of carbohydrates. At ambient temperature and below, there is no reaction of tryptophan with carbohydrates, but with nitrate and nitrite. The error introduced by nitrate and nitrite can therefore easily be determined in a second measurement with the heating bath off-state. The mixing coil had to be placed in an ice bath at 0°C to avoid heating of the mixture between the aqueous sample and the sulphuric acid of the reagent. In the oxidative environment of sulphuric acid, all nitrite is oxidized to nitrate. This nitrate concentration is subtracted from the carbohydrate-plus-nitrate signal in the “warm“ run to obtain the correct carbohydrate concentration. For maximal analytical performance, the system has to be run continuously to avoid deposits of precipitated reagents or salts in the tubes or fittings. The system was washed with MilliQ water and 2 M sodium hydroxide solution at least once a week. Pump tubes had to be replaced at regular intervals. The determination limit was lower than 0.3 μM C, the precision at that level was better than 10% (CV, n=5).

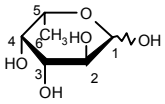
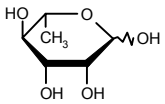
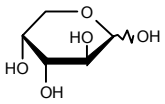
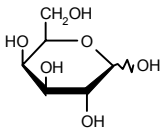
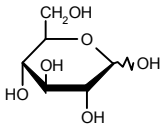
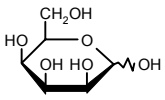
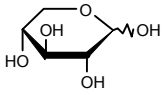
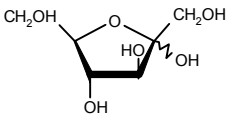
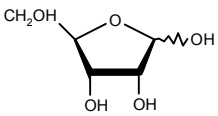
3.6 High Performance Amperometric Analysis of Free and Combined Neutral Sugars

The determination of total carbohydrates (TCHO) is a rough bulk determination. Reactions of functional groups (e.g., condensation of a carbonyl group with the amino group of tryptophan) may lead to under- or overestimates of the carbohydrate concentration of the sample.

For a high precision determination of the concentration of carbohydrates including their composition, different methods have been developed. Most of them were mass spectrometric or liquid chromatographic techniques. Since the late 1980's, a well understood and applied method is the high pressure anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Johnson and LaCourse, 1990). For analyses in marine environments, this procedure was later refined (Mopper et al., 1992). After chromatographic separation on a strong, neutral sugar specific anion exchange resin with a minimum of pH=13, monosaccharides are detected with an electrochemical detector.

Fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glc), mannose (Man), xylose (Xyl), fructose (Frc), and ribose (Rib) are the natural occurring monomeric saccharides being detected by this method (Table 2). Without prior hydrolysis, these sugars are detected as total free neutral sugars (TFNS). If oligo- and polysaccharides are hydrolyzed with strong acid treatment, the composition of total hydrolyzable neutral sugars (THNS) can be measured. Besides hydrolytic cleavage of glycosidic bonded oligo- and polysaccharides, destruction of monomers like the complete destruction of 2-deoxysugars is a critical point. 6-Deoxysugars, i.e., monosaccharides with no hydroxyl group at C-6, as fucose (6-deoxygalactose) and rhamnose (6-deoxymannose), are not degraded during hydrolysis. Little is known about the destruction of other sugar compounds, e.g., degree of deglycosidation (deacetylation) or dehydration (as described for the mechanism of the L-tryptophan / sulphuric acid method) leading to underestimates of carbohydrate concentration and wrong composition. The occurrence of lyxose as a minor constituent of the sugar spectrum has been described which could be an epimerization product of xylose during hydrolysis (McCarthy et al., 1993).

Table 2: Neutral sugar monomers which can be determined with the HPAEC-PAD method; the D- or L- form can be alternatively used.

Component	Total formula	Structural formula
L-Fucose (Fuc), 6-Deoxy-L-galactose	$C_6H_{12}O_5$	
L-Rhamnose (Rha), 6-Deoxy-L-mannose	$C_6H_{12}O_5$	
D-Arabinose (Ara)	$C_5H_{10}O_5$	
D-Galactose (Gal)	$C_6H_{12}O_6$	
D-Glucose (Glc)	$C_6H_{12}O_6$	
D-Mannose (Man)	$C_6H_{12}O_6$	
D-Xylose (Xyl)	$C_5H_{10}O_5$	
D-Fructose (Frc)	$C_6H_{12}O_6$	
D-Ribose (Rib)	$C_5H_{10}O_5$	

3.6.1 Sample Preparation

Prior to the chromatographic determination of combined neutral sugars, the sample was hydrolyzed under strong acidic conditions. If not otherwise stated, all chemicals were obtained by MERCK, Darmstadt. 4.0 ml of the sample were treated with 0.2 ml of conc. sulphuric acid (p.a. grade) and heated for 24 h at 110°C in sealed ampoules, which were

precombusted for 6 h at 550°C. Hydrolysis was stopped by immediate chilling to -30°C. At this temperature, samples were stored for several weeks.

Before chromatography of hydrolyzed samples, the acidic solution was neutralized and ions, which interfered the chromatographic separation and detection, were removed. A sample preparation slightly modified after Borch and Kirchman (1997) was applied. For neutralization 2.5 ml hydrolyzed sample were carefully added in small aliquots to 0.3 g calcium carbonate (precipitated, p.a. grade, precombusted 550°C, 9 h) to reduce effervescence. Then, the suspension was centrifuged for 5 min at 4000 g. The supernatant was carefully transferred to the ion exchange column. To quantify operational losses during the subsequent procedure, 2-deoxyribose (DORib, 0.1 ml of a 10 mM stock solution, all carbohydrate standards were biochemical grade) was added as internal standard after neutralization. This standard was used since 2-deoxysugars in the sample are destroyed completely during hydrolysis. During chromatography, deoxyribose elutes between fucose and rhamnose and interferes with no other neutral sugar.

Ions and particularly multiple charged ions such as sulphate, borate, carbonate and bicarbonate interfere with the chromatographic separation competing with the carbohydrate-anions for the ion exchange sites of the column. In consequence, peak resolution decreases with each run. Therefore, these ions were removed from the neutralized hydrolysate by ion exchange. As cation exchange resin, AG-MP 50 and as anion exchange resin, AG-1 X8 (BIO RAD, Hercules, U.S.A.) were used. They are polystyrene / divinylbenzene cross-linked resins with sulphonic acid or quaternary ammonium functional groups, respectively. The resins were purified by Soxhlet extraction with dichloromethane, methanol and MilliQ water in sequence for 24 h each. Afterwards, they were converted to the active ion exchange form. AG-MP 50 was flushed with 10 resin volumes of 2 M hydrochloric acid (Suprapur® grade) and AG-1 with the same volume of saturated sodium bicarbonate solution (p.a. grade). Finally, the resins were rinsed with 4 resin volumes of MilliQ water. The ion exchange was performed in an acid rinsed glass chromatography column of 4 ml volume with a P2 frit and a teflon stopcock. 1 ml of each resin were well mixed and pipetted in the column. The resin bed was rinsed with 5 column volumes of MilliQ water and with 200 µl of the sample, which was driven out by an argon stream and then discarded. Then a sample volume covering the resins was added for deionization. After gas stopped evolving (approx. 4 min), the sample was blown in an analysis vial with argon.

For regeneration of the ion exchange resin mixture, its exchange capacities were saturated with a saturated sodium chloride (p.a. grade) solution and the resins were separated. The resin AG-1 is less dense than AG-MP 50 and can be simply pipetted from the latter. The resins were regenerated with 10 resin volumes of 2 M hydrochloric acid and saturated sodium bicarbonate solution, respectively. Soxhlet extraction was not necessary, since there was no contamination of the resins during sample preparation.

Immediately before analysis, the sample was degassed with helium, since oxygen led to a negative peak during detection, which appeared exactly in place of fructose and ribose. A degassing time of 4 min was found to be best. If degassing takes too long, a

positive helium peak might appear. After degassing, the sample was transferred directly into the autosampler, since oxygen would resolve again within a few minutes. The autosampler was used as an automated washing and injection system to ensure precise injection with high reproducibility of retention times.

3.6.2 Anion-Exchange Chromatography

The separation of carbohydrates is based on the weak acid behaviour in a strong alkaline medium (Figure 9a), where the hydrogen at the C-1 hydroxyl group becomes acidic, induced by the (-I)-effect of the ring-oxygen. Glucose, for example, has a pK_a value of 12.28 (in aqueous solution at 25°C). The pK_a values of other carbohydrates is between 12.00 and 13.60. For partially deprotonation for anion exchange chromatography, a pH-value of 13 must be achieved, otherwise separation is not sufficient. Substituted carbohydrates show also acidic behaviour. In particular, sugars substituted with (-I)-effect groups like aminosugars and acetylated glycosides could easier be deprotonated (Figure 9b). Glycosides with (+I)-effect substituents show weaker acidity and have poor chromatographic properties. C-1 glycosylated saccharides and oligo- and polysaccharides show no acidic behaviour at their non-reducing carbon (Figure 9c).

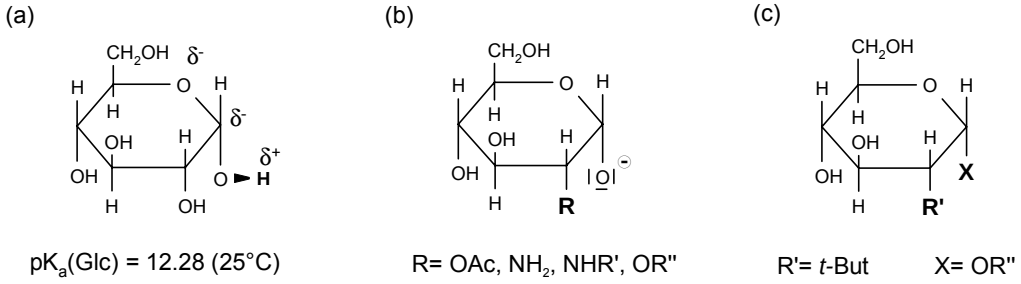


Figure 9: (a) Weak acidic behaviour of glucose in strong alkaline environment;
(b) (-I)–substituents strengthen acidity while (+I)-substituents weaken acidity;
(c) C-1 substituted saccharides show no acidity.

The high pH-value makes high demands on the technical equipment and column materials. Classical silica-based columns are dissolving at high pH, thus base-stable polymer anion exchange columns were used. They consist of 10 µm diameter polystyrene / divinylbenzene cross-linked (5%) pellicular resin (DIONEX TN20) with quaternary amine anion exchange sites and can be operated at pH values up to 14 and up to a pressure of 4000 PSI (280 bar).

Nearly all metals are corroded at this high pH-value and are therefore unsuitable. Thus, eluent containers, tubing, fittings, column package are made of non-corrosive materials. The pump housing is polyether-etherketone (PEEK), the piston is zircon dioxide or sapphire, and the checking valves are also sapphire, although the mechanical

properties of these non-metals are inferior to metals. In particular, pistons and their seals tend to break and age rapidly.

A concentration of the eluate of 24 mM sodium hydroxide is recommended (e.g., DIONEX TN20). A slight adjustment of the concentration was necessary, since mannose and xylose were not always separated, due to slightly varying temperature in the laboratory or aging of the anion exchange column. Therefore, the eluent was prepared with a concentration of 28 mM, so that concentrations between 19 and 28 mM could be adjusted by dilution with helium degassed MilliQ water. If the concentration adjustment of the eluent did not improve the separation, the flow rate was lowered from 1.00 ml min^{-1} to 0.94 ml min^{-1}. This fine-tuning led to a good separation also of mannose and xylose. If the separation of all peaks deteriorated, the column was washed with 200 mM sodium hydroxide solution for at least 10 min. Afterwards, the eluent was allowed to equilibrate for half an hour. In times of high sample throughput, this procedure was performed each day before analysis. Piston seals were regularly washed with MilliQ water to remove salt residues. Overnight, the pump was kept running, so that no salt could crystallize in the system. Flow rate was kept low at 0.04 ml min^{-1} and the eluent had the same concentration as during analyses. During analyses of free neutral sugars, the oligo- and polysaccharides were not hydrolyzed and eluted later than the monosaccharides. The column was then cleaned with higher concentrated sodium hydroxide solution (300 mM) after each run at a washing interval of 5 min. The chromatographic equilibrium was restored after 20 min of flushing with the analytical eluent.

A DIONEX DX-500 chromatography system with a GP-40 two piston gradient pump, DIONEX PA-1 guard column and analytical 4x250 anion exchange columns and a DIONEX ED-40 electrochemical detector were used. For system control and data processing DIONEX PeakNet software 5.1 was used. A MERCK-HITACHI AS-4000 autosampler was used for automated sample injection. The flow scheme is shown in Figure 10.

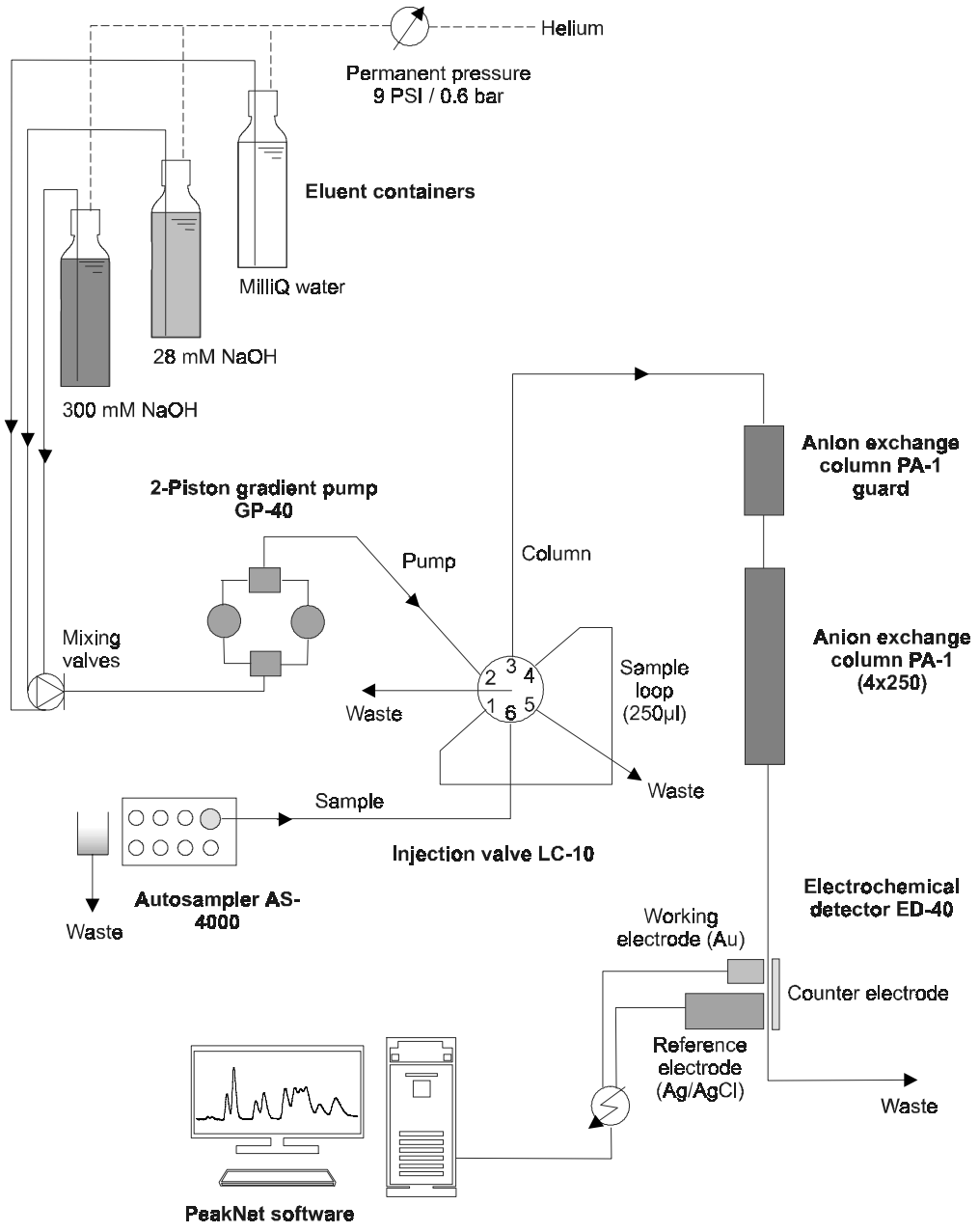


Figure 10: Flow scheme of the HPAEC-PAD system.

3.6.3 Eluent Preparation

The most crucial point of HPAEC-PAD is the sensitivity to carbon dioxide. Even traces react with the sodium hydroxide from the eluent leading to the formation of sodium carbonate. This carbonate reduces the anion exchange capability of the chromatography columns leading to reduced peak resolution. Sodium hydroxide pellets are hence unsuitable. To minimize any risk of contamination with carbon dioxide, the sodium hydroxide eluent solution was prepared of 50% aqueous solution (19.25 M NaOH, Baker

Analyzed, MALLINCKRODT BAKER B.V; The Netherlands), in which sodium carbonate precipitates. Strong sodium hydroxide solutions leach borate and other anions from glassware. They occupy, like carbonate, the anion-exchange-sites of the column. Therefore, no glassware was used and pipetting was done with acid washed polypropylene pipettes or Eppendorf tips.

For the main eluent, 3 ml of sodium hydroxide solution were transferred directly from the surface under argon gas shield into the eluent container with 2000 ml of helium degassed MilliQ water. 1 ml of 1 M barium acetate solution was added to remove even traces of carbon dioxide. MilliQ water always contains dissolved oxygen and carbon dioxide. To remove these gases, the water was purged with high purity helium for 5 to 10 min (quality grade He 5.6, MESSER-GRIESHEIM, Krefeld; helium of less quality contains contaminants and high carbon dioxide amounts). The 28 mM sodium hydroxide solution was purged with helium for additional 10 min. The eluent was pressurized with helium all time to avoid trapping of carbon dioxide from air. The eluent could be stored or used for more than a week, while without addition of barium acetate the eluent had to be renewed after a few days.

The higher concentrated eluents for column washing were prepared by adding 20 or 30 ml of sodium hydroxide solution and 1 ml of barium acetate solution to 1900 ml of MilliQ water resulting in 200 and 300 mM solutions, respectively.

A further critical point was the purity of the MilliQ water used for eluent preparation. Occasionally, the MILLIPORE system produced MilliQ water of lower quality resulting in an erratic and noisy baseline during chromatography. Thus, the MilliQ water was further purified by oxidative distillation. 4 l of MilliQ water were treated with 5 g of potassium persulphate (p.a. grade) and 5 ml of conc. sulphuric acid (p.a. grade), heated to reflux for 3 h and then distilled over a 0.5 m distillation column filled with glass Raschig rings at a rate of ca. 0.5 l h^{-1}. The glassware was either acid rinsed or precombusted at 500°C for 3 h. The first 1 l was discarded. All fittings were Teflon sealed, no silicon sleeve grease was used and the distillate was retained in a brown glass bottle. A cleaning distillation preceded every distillation. Chromatography improved significantly compared to MilliQ water. Commercially available HPLC-grade water (HPLC/Spectro grade, PIERCE, Rockford) showed no improvement compared to MilliQ water. It is low in organic contaminants, but higher than desired in inorganic ions (DIONEX, TN20).

3.6.4 Electrochemical Detection

The most suitable method to detect carbohydrates is the electrochemical pulsed amperometric method, which permits detection of sugars of approx. 1 picomole without derivatization. Furthermore, this method cannot distinguish between α– and β–anomers of sugars. An electric potential at the working electrode made of inert material oxidizes organic molecules if the potential is high enough. The induced electric current is amplified and processed. The current is proportional to the concentration of the organic molecules

passing the detector. In Figure 11, the mechanism of electrochemical carbohydrate oxidation in alkaline aqueous solution is shown (DIONEX, TN20).

First, the aldehyde group is oxidized rapidly releasing two electrons. Then, by carbon-carbon bond cleavage, formic acid separates and six electrons are released. Further separation of formic acid gives two additional electrons, which, however, is a slow reaction and therefore dependent on the residence time at the electrode. Overall, a maximum gain of 10 mole electrons per mole carbohydrate is attainable. Only a minority of molecules in the cell is oxidized.

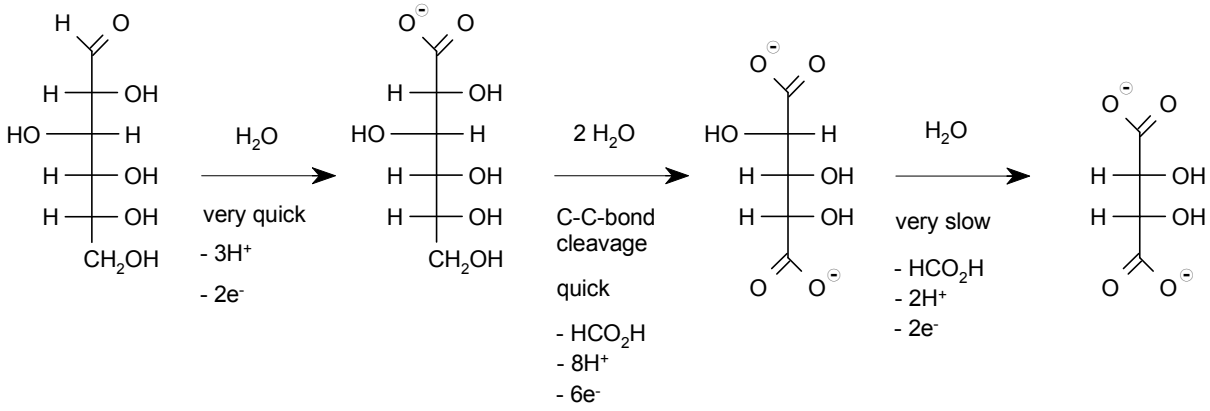


Figure 11: Mechanism of electrochemical oxidation of carbohydrates with PAD.

For optimal signal-to-noise ratio, the oxidation current is measured only for a short period while the oxidation potential is applied. Electric charge is integrated over time and measured in coulombs (C). For the analyses of free and combined neutral sugars in seawater samples, detection range was from 0.05 to 10 nC.

The products of the oxidation reaction poison the electrode surface. To maintain optimal electrode surface activity, the electrode was cleaned between the measurements. This was done by rising the oxidation potential high enough to oxidize the gold surface. Simultaneously, the oxidation products are desorbed from the surface. To reduce the electrode surface back to gold, the potential is lowered. The sequence of the three potentials is a waveform characteristic for pulsed amperometric detection. Standard settings for waveform and potentials provided by the manufacturer (DIONEX TN21) were proved to be optimal and therefore used in this work (see Appendix).

Due to its high sensitivity, the electrochemical detection is influenced by external electric currents. Although the electrode is shielded by a Faraday's cage, the sodium hydroxide eluent may serve as a conductor. If the cell outflow has liquid connection to the drainage container, this results in an enormous negative signal larger than any carbohydrate signal.

The working electrode was regularly checked and tuned up according to the manufacturer's manual. By time, oxidation products accumulated on the surface of the electrode or gold from the electrode was removed leaving a small cavity, which

broadened the peaks and decreased the sensitivity. Small amounts of residues could be easily wiped away with an eraser. To maintain the gold and surrounding surface, careful grinding with wet grinding paper (granulation 1000) and final polishing with the manufacturer's fine polishing kit was necessary. This procedure was carried out at least every two weeks or if detection sensitivity decreased drastically. This was mostly the case after measuring free neutral sugars when oxidation products of polymeric substances precipitated and could not be swept away by the eluent stream.

3.7 Separation and Detection Characteristics of the HPAEC-PAD System

The hydrolysis of the samples was quantitative, since no oligo- and polysaccharides were detected. Additionally, no further substances had retention times greater than 20 min. Thus, chromatographic separations could be run in sequence without column-cleaning step.

It is inevitable that neutral sugars are lost during sample preparation and these losses have to be quantified since the recoveries vary from sample to sample. Deoxyribose was completely lost during hydrolysis of the sample, which makes it possible to use it as an internal standard for quantification of neutral sugar recoveries. Addition of deoxyribose after hydrolysis therefore enables to correct for the neutral sugar losses, which were calculated in consideration of the signal of a standard with known deoxyribose concentration that did not pass through the preparation steps. The overall recoveries were in the range between 30 to 50% (Table 3). During neutralization, losses of carbohydrates ranged from 20 to 30%. The ion exchange procedure caused the greatest losses among the sample preparation steps (up to 50%). No monosaccharide losses were detected during degassing.

Table 3: Efficiency of the preparation procedure. Data are given in concentrations of poly- and monomeric sugars after each step (in % of the total content) and losses per step (in %).

Preparation step	Concentration of polysaccharides (% total)	Concentration of monosaccharides (% total)	Losses of neutral sugars per step (%)
Original	100	0[1]	
Hydrolysis	0	≈100	≈0
Neutralization	0	>70	<30
Ion exchange	0	>35	<50
Degassing	0	>35	0
Overall	0	>35	<65

[1]: free monosaccharides had not to be hydrolyzed

After optimizing the method, the 10 sugars were clearly separated within about 14 min. All peaks were highly symmetrical, except for the late eluting fructose and ribose,

which tended to tail. Fucose eluted first after 3.5 min. In natural samples, fucose was sometimes only a small peak on a shoulder. The internal standard, 2-deoxyribose, eluted after 4.6 min and did not interfere with other sugars. The last and most broadened peak was ribose eluting at about 14 min. For standard runs the coefficient of variation (CV) of the retention times was 0 to 2% and for the peak areas 0 to 5%. In natural samples, CV was 0 to 10% and 1 to 6%, respectively. The elution order never changed and identification was therefore unequivocal. The chromatographic separation is sensitive to incomplete ion-exchange after hydrolysis. If neutralization was not quantitative and pH still low, retention times decreased rapidly, but resolution of the peaks was still adequate as peaks sharpened.

A good control of the quality of a chromatographic separation is the resolution factor R_S, the degree of disengagement of two bands. R_S is defined as the ratio of the difference of the retention times (t_{Ri}) to their baseline width (w_i; Figure 12):

Equation 1:
$$R_S = 2\frac{t_{R2} - t_{R1}}{w_1 + w_2}$$

t_{Ri} = retention time of substance i;
w_i = baseline width of substance i.

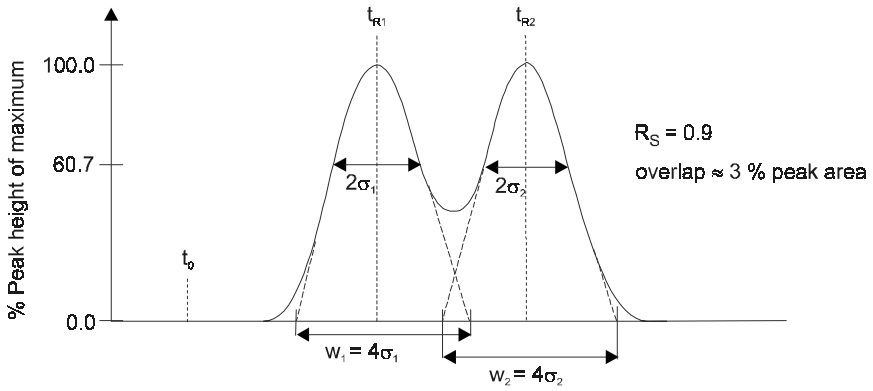


Figure 12: Measurements for idealized (Gaussian) peak curves (Snyder et al., 1988).

The width of a “real” peak at its base is difficult to determine. But, for an ideal (Gaussian) peak, the base width is about $4\sigma_i$, and $2\sigma_i$ is the peak width at 60.7% of its height. Peaks that strongly overlap have values of lower than 0.6. For standard separation runs, R_S varied between 0.7 (mannose and xylose) and 4.5 (deoxyribose and rhamnose) (Table 4). Even for lowest observed peak resolution, i.e., the separation of mannose and xylose, the maximum peak overlap was less than 8% (Snyder and Kirkland, 1979; Snyder et al., 1988).

Besides R_S, another measure for chromatographic separations is the column plate number N. Columns with large N values will produce narrow peaks and good resolution. N is calculated as follows:

Equation 2: $$N = \frac{L}{H}$$

L = length of the chromatographic column;
H = plate height.

The term “column plate” is semiempiric and based on the theory that the (physical) column consists of many (theoretical) column plates at which liquid and stationary phase equilibrate. Since this equilibrium is individual for substances, the larger the column plate number, the better the separation of peaks will be. The column plate height (H) is the minimum particle size of the column material. In case of the used DIONEX PA-1, the column length is 250 mm plus 50 mm for the PA-1 guard column, and the particle diameter is 20 to 50 µm (DIONEX, TN20). This results in a maximum column plate number of about 15000, and a minimum of 6000. The practical column plate height is determined as follows:

Equation 3: $$H = \frac{\sigma_i^2}{L}$$

σ_i = half peak width in 60.7% of total peak height.

The resulting practical column plate numbers for the individual sugars are given in Table 4. For glucose, for example, the practical column plate height is H = 48 µm. The column plate number of N = 6250 is relatively low due to the slightly broadened peaks.

Table 4: Chromatographic characteristics for the separation of a standard mixture. Deoxyribose was added as internal standard. Peak separation was estimated after Snyder et al. (1988)

Peak no.	Compound	Retention time t_R (min)	Column plate number N	Resolution factor R_s	Peak separation (%)
1	Fucose	4.03	16000		
				1.65	100
2	Deoxyribose	4.55	13223		
				4.50	100
3	Rhamnose	6.08	11755		
				1.80	100
4	Arabinose	6.72	11111		
				3.88	100
5	Galactose	8.27	7438		
				1.41	>99
6	Glucose	8.92	6250		
				0.87	>96
7	Mannose	9.37	4592		
				0.70	>92
8	Xylose	9.77	4281		
				1.72	100
9	Fructose	10.80	3746		
				1.50	100
10	Ribose	11.89	2142		

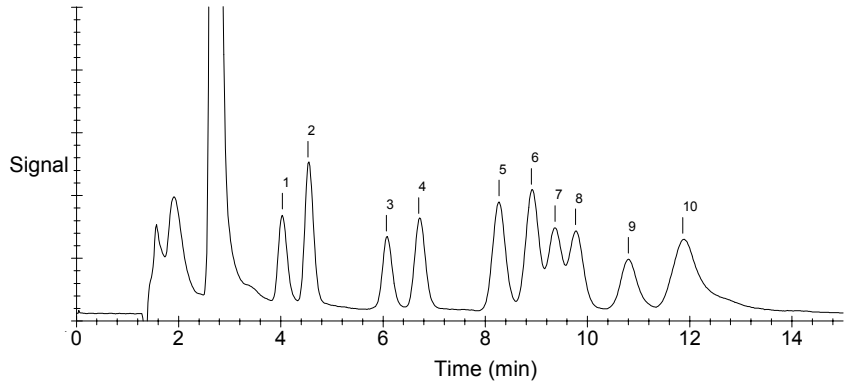


Figure 13: Chromatogram of a standard; for peak numbers refer to Table 4.

The chromatogram of sugars from a hydrolyzed sample is given in Figure 14. The retention time is probably affected by the complex sample matrix and sample preparation procedure. Except for fructose, all compounds were present in this sample. Resolution factors were between 0.6 and 5.05 (Table 5). Separation of mannose and xylose was poor due to the small amount of xylose in this sample, but peak area separation was still better than 90%. Column plate numbers ranged between 3000 and 14000 indicating good resolution.

Table 5: Chromatographic characteristics for the separation of a natural sample. Deoxyribose was added as internal standard.

Peak no.	Compound	Retention time t_R (min)	Column plate number N	Resolution factor R_s	Peak separation (%)
1	Fucose	4.20	14063		
				1.43	>99
2	Deoxyribose	4.70	9972		
				5.05	100
3	Rhamnose	6.67	9000		
				1.50	100
4	Arabinose	7.30	7438		
				3.84	100
5	Galactose	9.03	6805		
				1.54	100
6	Glucose	9.77	5760		
				1.19	>99
7	Mannose	10.47	3114		
				0.60	>90
8	Xylose	10.88	3025		
				3.93	100
10	Ribose	13.63	2939		

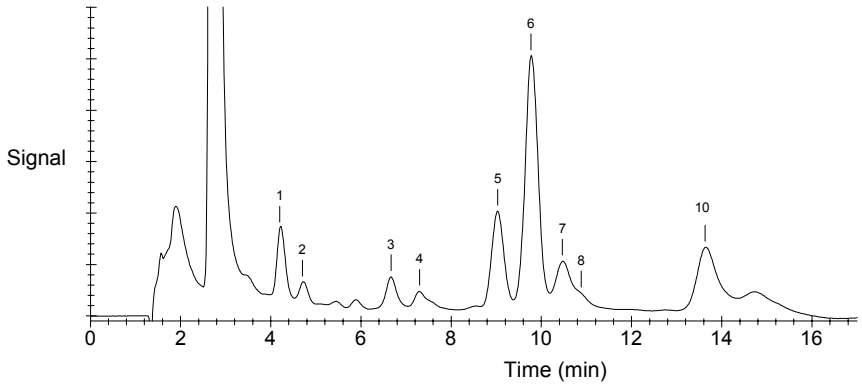


Figure 14: Chromatogram of a sample; for peak numbers refer to Table 5.

Detection limits varied during this work, depending on the conditions of columns and detector. Minimum detection was also limited by the amount of interfering substances in samples resulting in an erratic baseline and overlay with peaks of carbohydrates. For standards, detection limit was always below 10 nM C for each sugar. For natural samples, detection limit was higher but always below 20 nM C, due to baseline irregularities at that high sensitivity. The precision at that level was better than 20% (CV, n=6) for separate injections of the same sample. Efficiency of hydrolysis and losses during the sample preparation were thoroughly quantified.

4 Results

4.1 Composition of Dissolved Organic Matter in Arctic Environments

4.1.1 Dissolved Organic Carbon

DOC data of the Russian estuaries and the Laptev Sea (Lobbes, 1998), the central Arctic Ocean, and the adjacent northern Kara Sea (Bussmann and Kattner, 2000) comprise a wide range of concentrations. In the river samples, DOC concentrations ranged from 387 µM (Kolyma) to 912 µM (Vizhas). The average of all river samples was 626 µM, that is the fourfold of the DOC values of the inshore stations, which ranged from 84 µM (St. 3) to 214 µM (St. X). Surface DOC concentrations in the Arctic Ocean, i.e., samples of the light influenced euphotic zone (above 100 m), were between 150 µM in the southern Laptev Sea (ARK XI/1, St. 16) and 70 µM in the ice-covered central Arctic Ocean (ARK XII, St. 43). The DOC concentrations of deep sea samples, i.e., all samples taken below 100 m depth, increased from West (50 µM in the Nansen Basin, 54 µM in the Amundsen Basin, and 56 µM in the Makarov Basin) to East reflecting the different DOC signatures of the deep basins (Bussmann and Kattner, 2000).

In the Fram Strait and Greenland Sea, there are the same water masses as in the central Arctic Ocean (cf. section 1.4). Polar surface water samples had DOC concentrations of 81 to 86 µM. The Atlantic water at the eastern side of the Fram Strait exhibited lower surface values of 62 µM. Low values were also determined in all deep sea samples, ranging from 47 to 50 µM. Additionally, two water masses were sampled: Canadian Basin-derived deep water, overflowing the Lomonosov Ridge at 1800 m depth and water of Pacific Ocean origin at 30 m depth, both east of the Greenland shelf slope. The Canadian Basin deep water had a very low DOC concentration of 39 µM and the Pacific water with 81 µM was in the range of surface and near-surface waters.

4.1.2 Total Carbohydrates

Total carbohydrates (TCHO) were determined throughout the Arctic Ocean. Samples through the oceanic basins were taken in Laptev Sea, central Arctic Ocean and its boundary to Kara Sea, Fram Strait, and Greenland Sea. All relevant water masses of the Arctic Ocean were sampled. TCHO was not determined in the Siberian rivers and inshore stations because of the complete coverage of these stations with THNS analyses.

Highest TCHO concentrations were measured in surface samples of the Laptev Sea and the central Arctic (Figure 15). Near the Lena River plume, at about 350 km distance off the Lena delta, maximum concentrations of up to 10.8 µM C were found.

With greater distance, TCHO decreased gradually to 5 µM C. With increasing depth, TCHO concentrations decreased rapidly to about 3 µM C in 30 to 50 m.

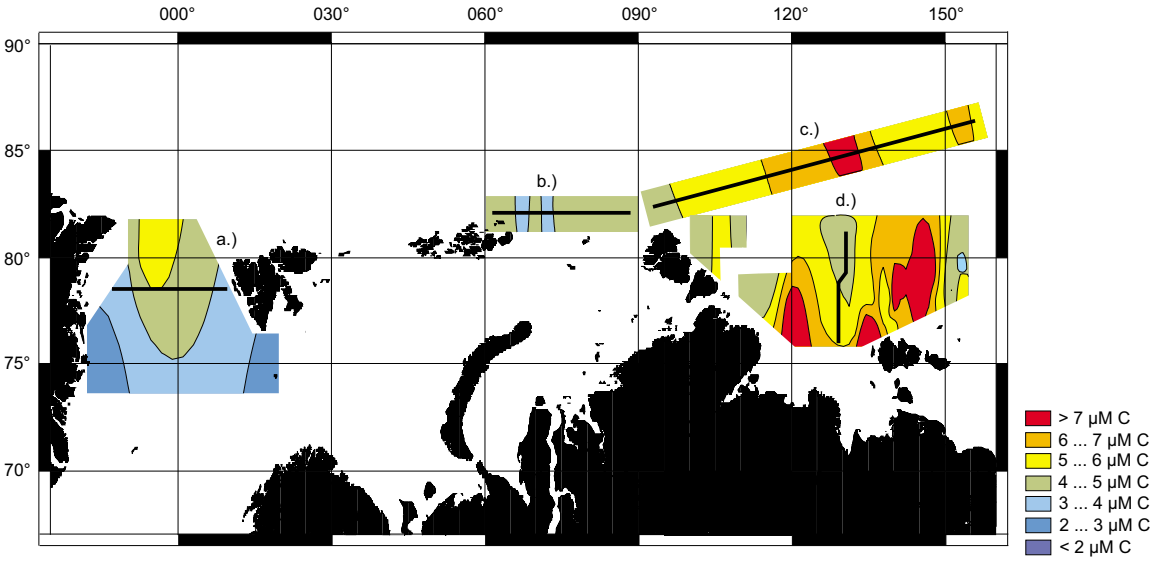


Figure 15: Surface TCHO distribution in the Arctic Ocean. The depth profiles of transects (a) to (d) are shown in Figure 16.

The TCHO concentrations during the Laptev Sea transect (Figure 16a) did not decrease uniformly with depth. At the near-shelf stations (stations 21 and 23), TCHO concentrations decreased in the upper 1000 m to 2.5 µM C, but then increased further downward to more than 4.4 µM C. The distant stations (stations 24 and 47), on the other hand, showed a continuous decrease of TCHO with depth to less than 2 µM C.

In the northern Kara Sea, at a distance of about 1000 km off the Ob and Yenisey River estuaries, surface carbohydrate concentrations were slightly lower than in the Laptev Sea (3 to 5 µM C). The transect along the continental slope crossed two deeper troughs (Figure 16b), in which TCHO was inhomogenously distributed. In the westerly Santa Anna Trough, TCHO decreased continuously from surface (4.9 µM C) to bottom (2.3 µM C). In the Voronin Trough, TCHO decreased near the surface from 4.6 to 3.1 µM C, but then increased again to 5.0 µM C towards the bottom.

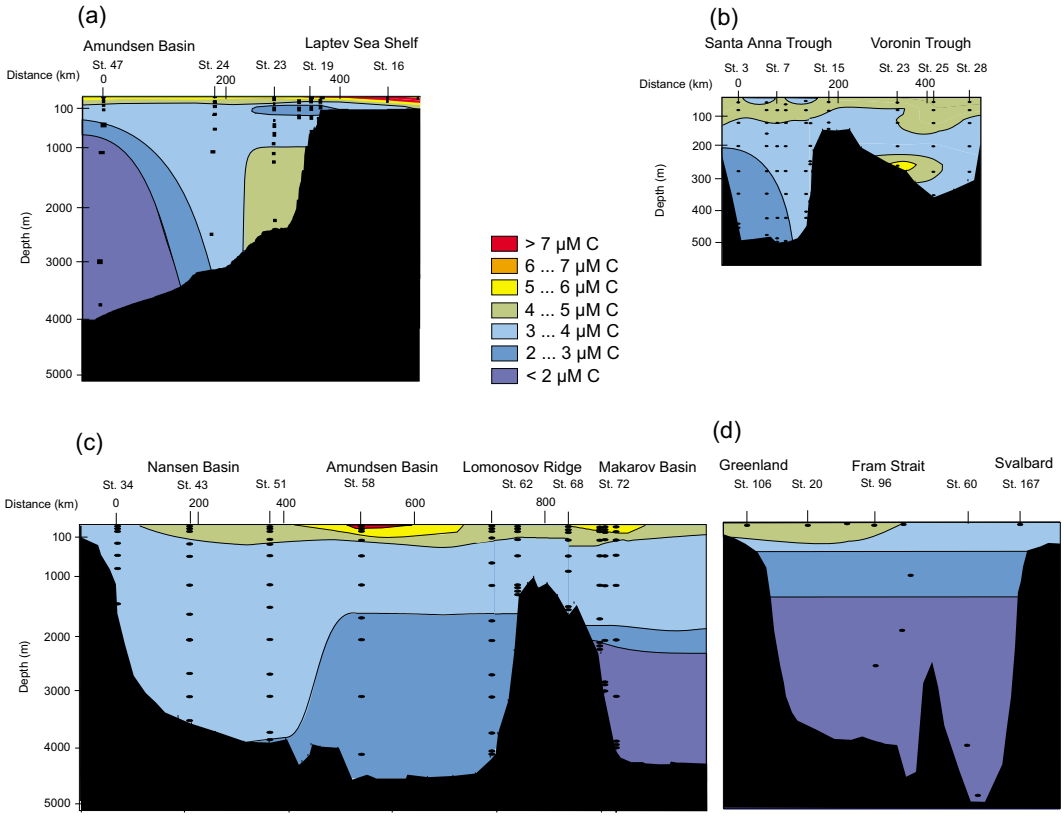


Figure 16: Depth profiles of TCHO concentrations in the Arctic Ocean; transects through
(a) Laptev Sea towards Lena River delta;
(b) Santa Anna and Voronin troughs (northern Kara Sea);
(c) Amundsen Basin towards Makarov Basin;
(d) Fram Strait.

During the transect crossing the Eurasian Basin towards the Lomonosov Ridge (Figure 16c), no increase of carbohydrates towards the bottom was detected. In the Nansen Basin, TCHO concentrations were generally lower and decreased from surface to depth from 7.4 µM C (at St. 58) to 3.4 µM C. In the Amundsen Basin, TCHO decreased to even lower concentrations of 2.0 µM C. Crossing the Lomonosov Ridge into the Makarov Basin, a similar distribution of TCHO with very low values (1.6 µM C) at depth exceeding 3000 m was found.

In the Fram Strait (Figure 16d), TCHO concentrations decreased continuously with depth. Surface values were in the range from 2.0 µM C (St. 106) to 4.2 µM C (St. 17). Highest concentrations were measured in the northern marginal ice zone. Deep sea samples did not exhibit concentrations higher than 1.5 µM C, similar to the deep sea samples of the central Arctic Ocean. The TCHO concentration in the ice floe (St. 56E, 4.8 µM C) was higher than in surface waters.

4.1.3 Total Free and Hydrolysable Neutral Sugars

Molecular level analyses of neutral sugars were performed on selected seawater and river samples. Samples from the northern Kara Sea (ARK XII, stations 7 to 25), the Fram Strait (ARK XIII/3), and the shelves and rivers (SWEDARCTIC TE-94) were analyzed for free (TFNS) and combined neutral sugars (THNS).

Concentrations of THNS were always much higher than TFNS, which were detected only in a few river samples (Figure 17). In the Arctic Ocean concentrations of free sugars were below the detection limit.

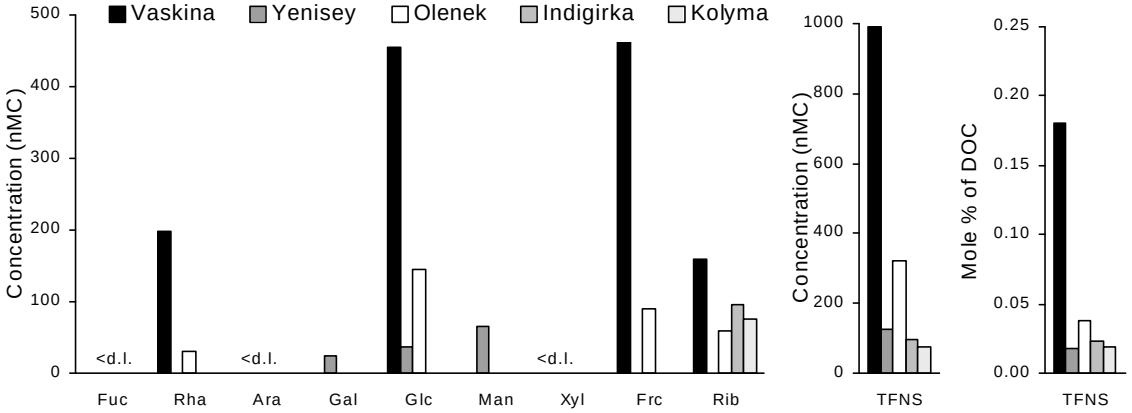


Figure 17: Concentrations of free neutral sugars and proportions of DOC in the rivers; <d.l. = below detection limit.

In the river samples, TFNS concentrations were very low, despite very high DOC concentrations. Their proportion to DOC was maximal 0.23%, and less than 4% of THNS. Distribution of TFNS and concentrations of individual sugars were very heterogeneous. Glucose, fructose and ribose were the most abundant neutral sugars. In the Vaskina River, where TFNS concentrations were maximal, even free rhamnose was determined.

The distribution of THNS in the Siberian river samples, on the contrary, was very similar in all samples (Figure 18a). Main components were the hexoses, glucose, mannose, and galactose, followed by the deoxysugars rhamnose and fucose, and the pentoses, arabinose and xylose. Ribose and fructose were very low concentrated. The organic carbon normalized yield of THNS varied between 2.0 and 9.9%, with a mean of 4.7%. Concentrations of THNS decreased eastwards. Highest concentrations were found in all samples west of Yenisey, lowest concentrations in the rivers Olenek (120°E), Indigirka (151°E) and Kolyma (155°E). Sampling began in the West in early June and continued until end of June at the stations in the East. The stations in the West were therefore more influenced by early summer conditions. The proportions of the individual neutral sugars to the total were nearly constant in all samples (Figure 18b).

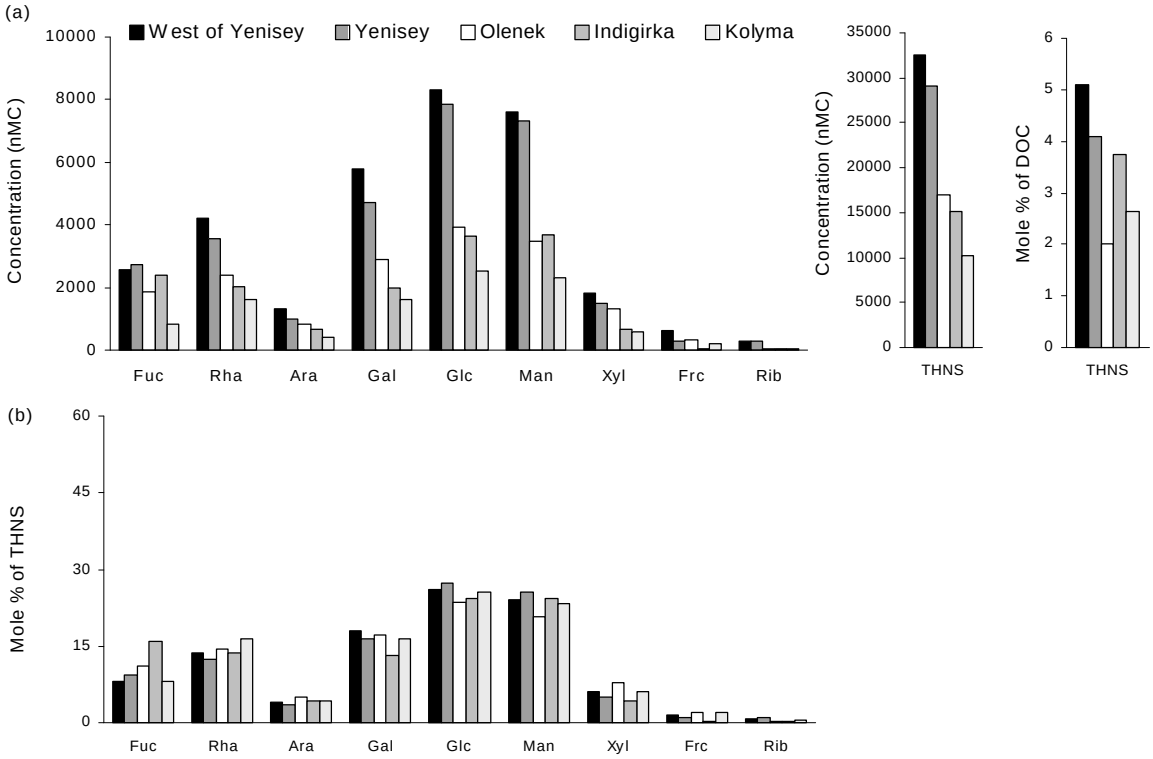


Figure 18: (a) Concentrations of combined neutral sugars and proportions to DOC and (b) proportions of individual neutral sugars in the rivers; data of the small rivers west of Yenisey (Vizhas, Vaskina, Velikaja and Moroyyakha) were averaged.

At the inshore shelf stations off the Siberian coast, the THNS contribution to DOC showed a more heterogeneous pattern than that of the rivers (Figure 19a). The carbohydrate composition of these marine samples was completely different. Most abundant neutral sugars were glucose, mannose, galactose, and, in contrast to the rivers, the deoxysugars and ribose. Highest THNS concentrations were detected in the sample south of Severnaya Zemlya (St. 9A) between the Kara and Laptev Seas. Second highest concentrations were found at St. 3, the most westerly station at about 050°E located in the eastern part of the Barents Sea near the mouths of the rivers Vaskina and Velikaja. At the vicinal St. 5 concentrations were lower, but the composition was very similar to the one of St. 3, except for galactose. Samples of St. 6B and St. X had similar THNS proportions of DOC. The THNS concentration at St. X was nearly half the concentration of the sample from St. 9A, which exhibited a different carbohydrate pattern with exceptional high mannose and xylose concentrations (Figure 19b). Highest proportions of glucose were found at stations 5 and 6B. Mannose, fucose, and arabinose proportions increased from West to East.

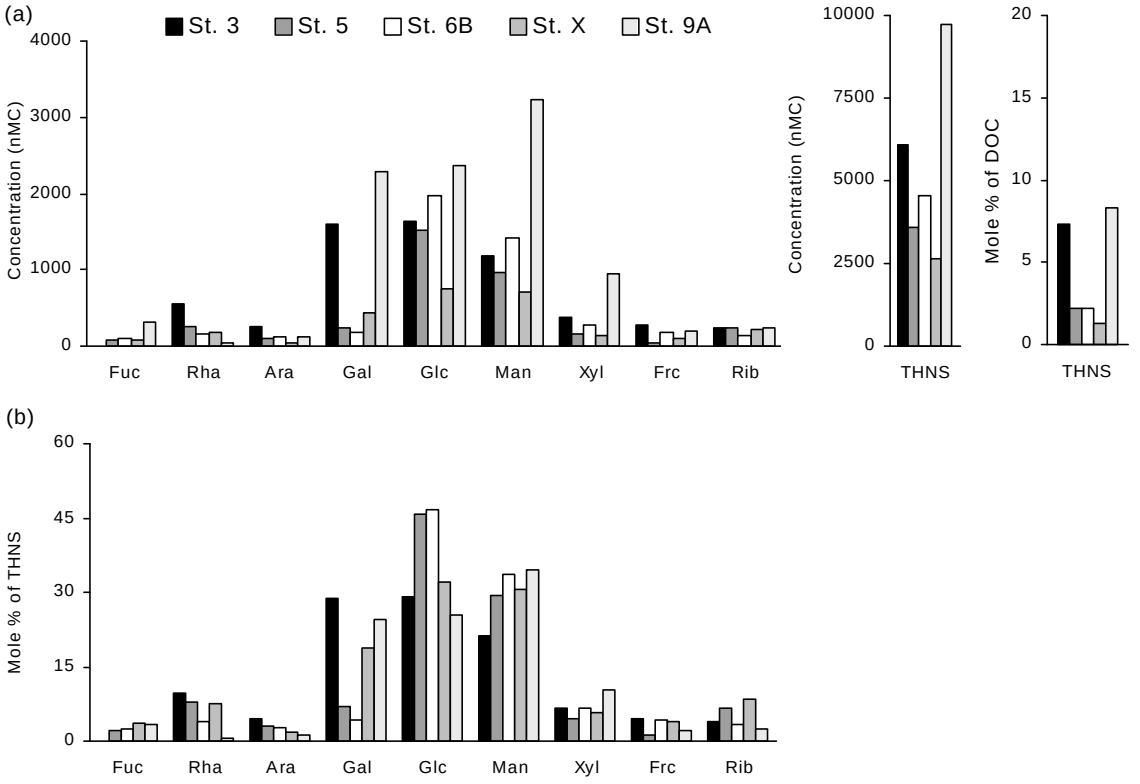


Figure 19: (a) Concentrations of combined neutral sugars and proportions to DOC and (b) proportions of individual neutral sugars at the inshore stations.

In the northern Kara Sea (stations 7, 15, 23 and 25), the THNS composition differed between the Santa Anna and Voronin Troughs. In the Santa Anna Trough (stations 7 and 15) (Figure 20a), main components of the surface samples were ribose, glucose, and mannose. The proportions of ribose and mannose of the total carbohydrates decreased clearly with depth, while glucose increased (Figure 20b). Galactose, which was much less abundant, exhibited higher proportions at the surface than in deeper waters. At St. 15, where the Barents Sea branch of Atlantic water was sampled, THNS concentrations decreased continuously towards the bottom. Galactose, fructose, and rhamnose were more abundant than at St. 7, where the Fram Strait branch of Atlantic water was sampled. Here, THNS concentrations and its contribution to DOC did not considerably differ with depth. Rhamnose, galactose, and xylose contributed much less to THNS than at St. 15 (Figure 20b).

In the Voronin Trough (stations 23 and 25), the concentration of THNS was clearly higher than in Santa Anna Trough (Figure 20c). The neutral sugar distribution exhibited also a different composition. Main components were glucose (up to 86% of THNS) and mannose, but ribose was less concentrated than in the Santa Anna Trough samples, especially in surface samples, where its contribution to the total was only 10% (Figure 20d). The deoxysugars, arabinose, and galactose were lower and xylose much higher concentrated than in the Santa Anna Trough.

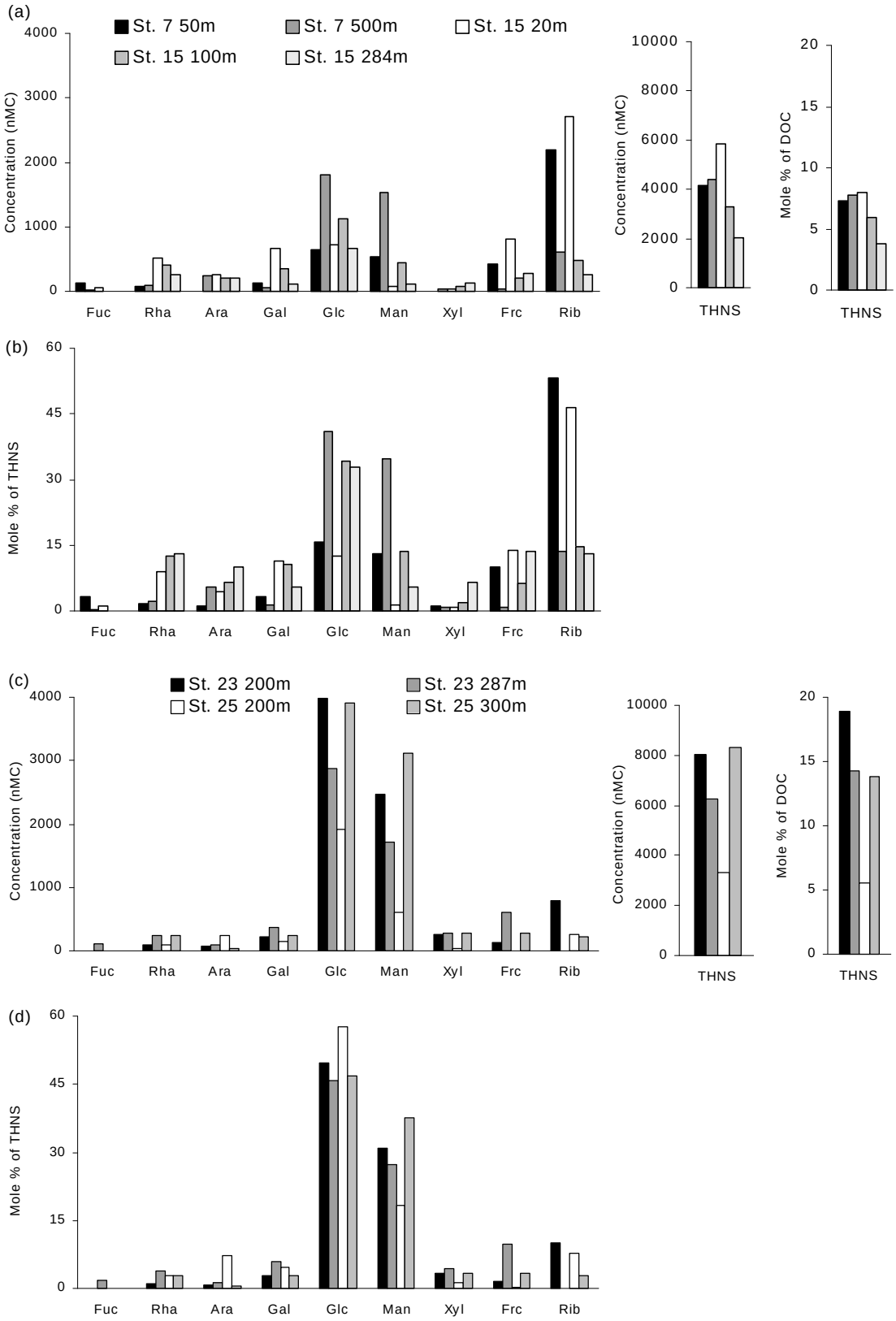


Figure 20: (a) Concentrations of combined neutral sugars and proportions to DOC and (b) proportions of individual neutral sugars in the Santa Anna Trough; (c) Concentrations of combined neutral sugars and proportions to DOC and (d) proportions of individual neutral sugars in the Voronin Trough.

In the Fram Strait and Greenland Sea, the gradient of neutral sugar concentrations between surface and deep sea, was higher than in other areas (Figure 21a). The neutral sugar distribution also changed with depth (Figure 21b). Even surface samples differed significantly in THNS distribution. The proportions of THNS of DOC were the lowest values measured in the Arctic Ocean. The maximum was measured in the ice floe sample (7.7%, St. 56E) and the minimum in deep water (1.0%, St. 46, 4500 m). According to these data, the samples can be divided into three categories.

Surface water of the East Greenland Current (St. 96) and the ice floe (St. 56E) exhibited THNS concentrations of 4.0 and 8.5 µM C, respectively, and THNS proportions of DOC of 5.4% and 7.7%. These samples were characterized by visible algal activity (e.g., *Melosira sp.* strands in the ice floe). The low THNS-concentrated deep water samples (stations 38, 1800 m, and 46, 4500 m) had proportions of 1.3 and 1.0%, respectively, and THNS concentrations of 1.4 and 1.8 µM. The surface samples (stations 17, 50 m and 167, 20 m) exhibited intermediate THNS concentrations and -proportions. At St. 17 in the East Greenland Current, where water of Pacific Ocean origin was sampled, the THNS concentration was 2.6 µM C, and the THNS proportion 3.1% of DOC.

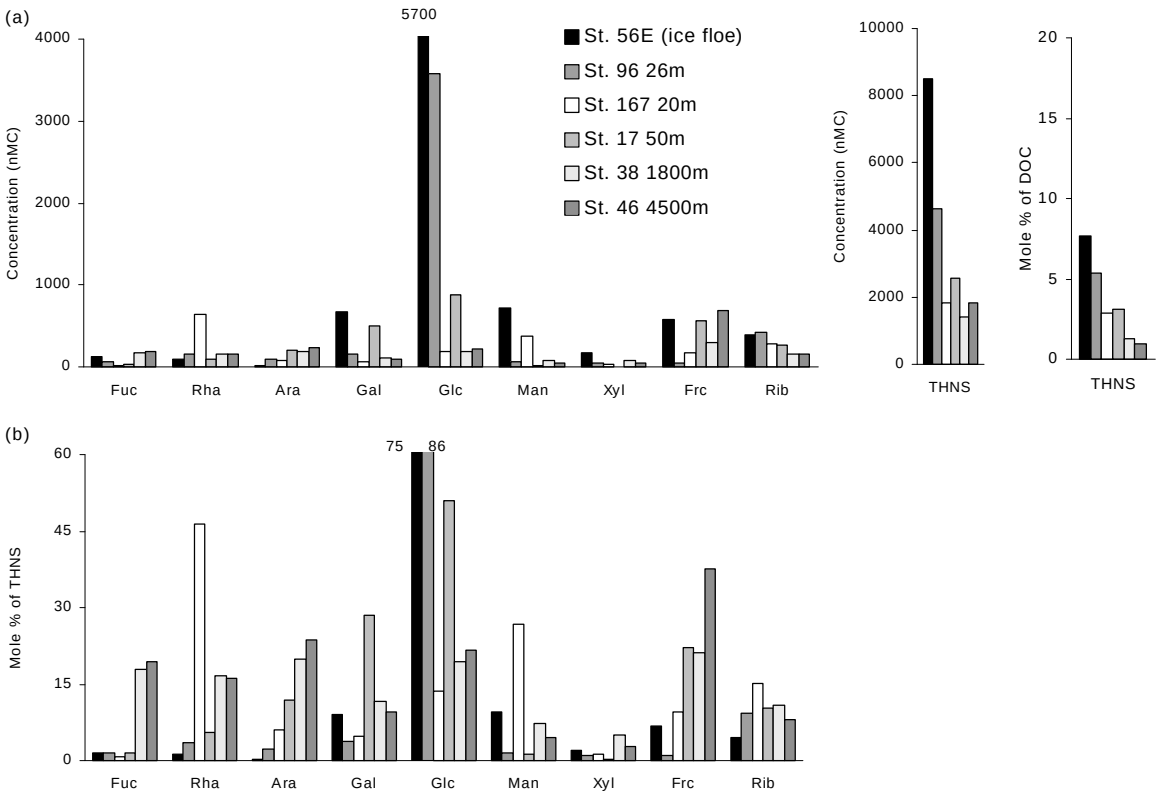


Figure 21: (a) Concentrations of combined neutral sugars and proportions to DOC and (b) proportions of individual neutral sugars in the Fram Strait and Greenland Sea samples.

At St. 167, the Barents Sea branch of Atlantic water was sampled which exhibited the lowest THNS concentrations (1.8 µM C) and also the lowest THNS proportion of the surface samples (2.9% of DOC). This water mass floes eastward, undergoes changes on the Barents shelf and was sampled in the Santa Anna Trough.

The carbohydrates of the ice floe (St. 56E) and the Polar Surface Water (St. 96) were predominated by glucose, which contributed approx. 80% to THNS (Figure 21b). Other important neutral sugars in the ice floe were mannose, galactose, ribose, and fructose. The deoxysugars were only low concentrated. In the Polar Surface Water (St. 96), glucose and ribose contributed more to THNS than the other neutral sugars. The Atlantic water (St. 167) had a completely different carbohydrate composition. The major components were rhamnose, mannose and ribose. The proportion of glucose of THNS was only 14%, which were the lowest value of all measured surface samples. In contrast, the surface water of St. 17 was dominated by glucose, galactose, and fructose, whereas the deoxysugars occurred only in trace amounts. Deep waters of stations 38 and 46 exhibited homogenous carbohydrate compositions. Glucose, fructose, and arabinose were most abundant. In the Fram Strait and Greenland Sea samples, arabinose and fructose showed a clear trend towards higher proportions with increasing depth (Figure 21b).

4.2 Composition of Fractionated Organic Matter

4.2.1 Dissolved Organic Carbon

Fractions of DOM isolated with XAD from Fram Strait and Greenland Sea samples were examined for DOC, TCHO, and THNS. Table 6 and Figure 22 show the carbon yields for the extraction procedures. Generally, between 29% (St. 56E, ice floe) and 67% (St. 46, 4560 m) of total DOC could be extracted, and the extraction efficiency was higher in deep (i.e., deeper than 100 m) than in surface waters. Extraction of deep water samples resulted in a recovery between 45 to 67% (58% on average). Lower recovery rates with 45% on average were obtained in the Polar Surface Water samples. Extraction was lowest (29%) in the ice sample (St. 56E). The maximum recovery of a surface sample was achieved from water of Pacific origin (St. 17) with 62%. All other surface samples exhibited extraction efficiencies between 42 and 48%. It was observed, that the HbN and HbA fractions of XAD-2 at the stations 17, 96, and 106 were yellowish coloured, in contrast to all other eluates, which were colourless.

Table 6: Extraction efficiency of DOC from seawater with XAD-2 plus-4, ratio between DOC extracted with XAD-2 and XAD-4 (XAD-2:-4 ratio), and ratio between HbN and HbA fractions of both resins (HbN:HbA ratio); n.d. = not determined.

St.	Depth (m)	Description	Extraction efficiency (% total DOC)	XAD-2:-4 ratio	HbN:HbA ratio
56E	0	Ice floe	29	2.2	3.0
56W	1	Polar Surface Water	47	n.d.	1.2
20	20	Polar Surface Water	45	2.4	1.5
167	20	Atlantic water	48	1.0	2.8
96	26	Polar Surface Water	42	2.6	1.6
106	45	Polar Surface Water	46	3.9	1.8
17	50	Water of Pacific origin	60	2.1	1.5
133	1000	Deep water	51	n.d.	1.3
126	1800	Deep water	45	1.9	1.3
38	1800	Canadian Basin Deep Water	56	1.2	2.7
14	2900	Deep water	62	0.6	1.8
46	4500	Deep water	67	1.3	1.7
60	5650	Deep water	64	1.0	2.2
Mean of Polar Surface Waters			45±2	3.0±0.8	1.8±0.5
Mean of deep waters			58±8	1.5±0.8	1.5±0.3

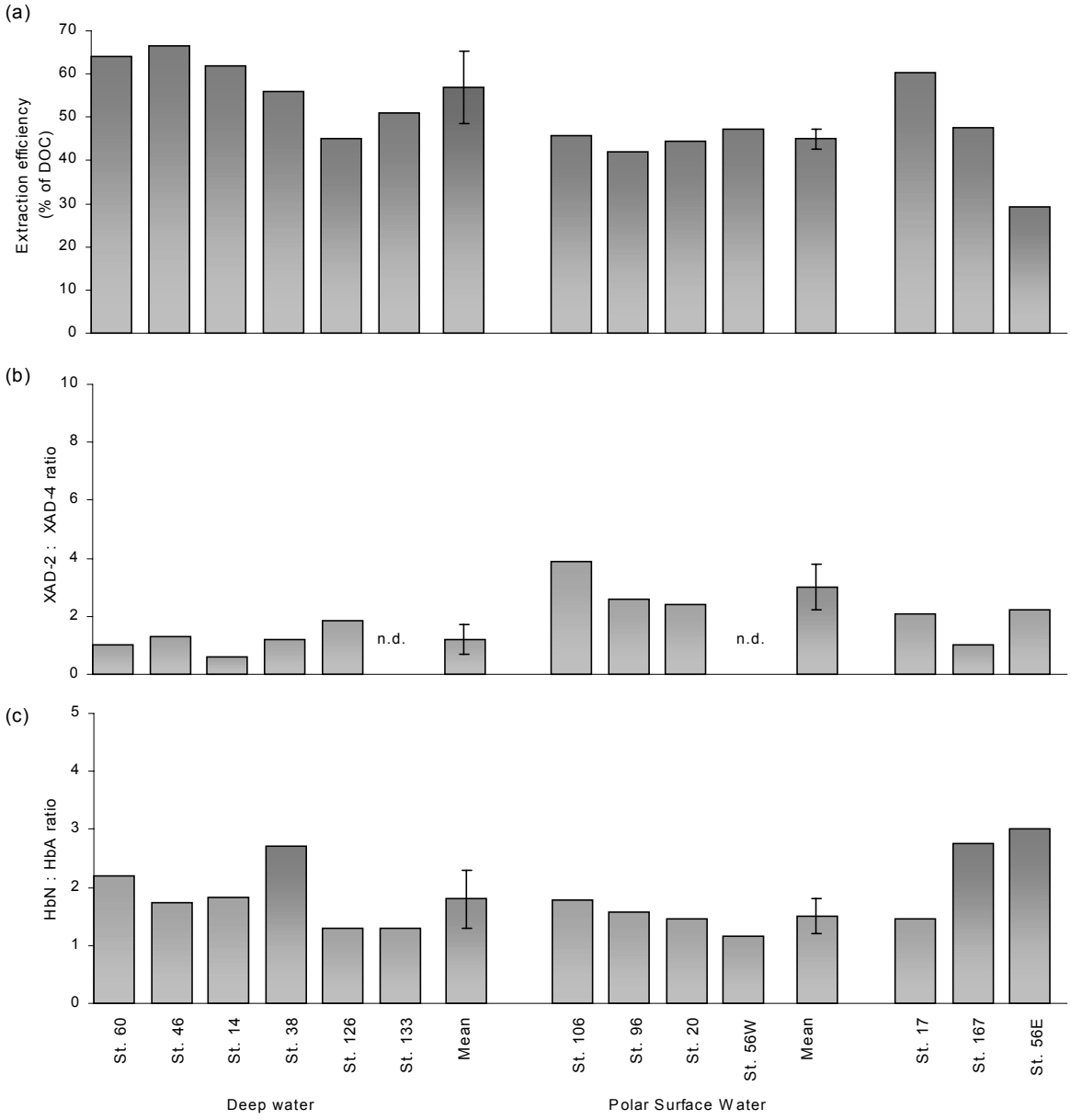


Figure 22: (a) Extraction efficiency of DOC from seawater with XAD-2 plus-4;
(b) ratio between DOC extracted with XAD-2 and XAD-4 (XAD-2:-4 ratio);
(c) ratio between HbN and HbA fractions of both resins (HbN:HbA ratio);
n.d. = not determined.

The molar ratio of the extraction efficiencies of XAD-2 and XAD-4 fractions (referred as XAD-2:XAD-4) is introduced as a measure for the size-distribution of the extracted organic matter, since XAD-2 has larger pore sizes than XAD-4, which results in different adsorptive capabilities for small and large organic compounds. The higher the ratio, the larger should be the molecular size. Furthermore, the ratio of the extraction efficiencies of the HbN to HbA fractions (referred as HbN:HbA) is used as a measure for the polarity of the extracted organic matter. The XAD-2 and XAD-4 fractions of both

eluates were summed up and their molar ratios calculated. A higher HbN to HbA ratio characterizes fractionated DOM of low polarity.

The distribution of DOC in the different XAD-fractions showed also depth-dependent trends. The XAD-2:XAD-4 ratio of the DOC concentrations decreased from 3.0 on average in surface samples to only 1.5 in deep sea samples. Only at St. 167, the surface samples had a low ratio of 1.0. Changes in HbN:HbA ratios were only marginal in the water column (1.8 to 1.5 towards greater depths). High HbN:HbA ratios were found at the stations 38, 60, 167, and the ice floe without clear trend.

4.2.2 Total Carbohydrates

Carbohydrates accounted for 3 to 10% of total DOC in seawater samples. A portion of TCHO could be isolated with XAD. For the characterization of the XAD-extracted carbohydrates, various proportions and ratios were calculated (Table 7, Figure 23). The extraction efficiency of carbohydrates, i.e., the proportion of the extracted carbohydrates to the total carbohydrates of the samples, ranged from 29 to 79%, which is similar to the carbon yields. In Polar Surface Water samples, 41% of total carbohydrates could be extracted on average, and in deep sea samples 59%. The proportions of XAD-extractable carbohydrates of total DOC ranged from 0.9 to 4.1%. Since surface samples contained much more carbohydrates, their proportion of XAD-extractable carbohydrates is higher (1.8%) than in deep sea samples (1.3%).

The XAD-2:XAD-4 ratio of the TCHO concentrations showed high variations with values ranging from 0.2 (St. 14) to 12.6 (St. 96). For the average ratios of surface and deep water samples, a significant depth-dependency was evident, similar to fractionated DOC. The surface samples exhibited a value of 9.9 and the deep sea samples of 2.3 on average. These values were significantly higher than for DOC, except in the ice floe sample (St. 56E), which had a ratio smaller than the fractionated DOC. The variation of the HbN:HbA ratio was smaller. It varied between 0.7 and 4.7. Equivalent to fractionated DOC, no significant depth-dependency was found, and the values were approx. as high as calculated for fractionated DOC. In contrast to DOC, the Atlantic water and the ice floe samples did not show extraordinarily high HbN:HbA ratios.

Table 7: Extraction efficiency of TCHO (in % of total TCHO) from seawater with XAD-2 plus-4, total TCHO proportion of DOC, proportion of extracted TCHO of total carbon and of extracted carbon, ratio between TCHO extracted with XAD-2 and XAD-4 (XAD-2:-4 ratio), and ratio between HbN and HbA fractions of both resins (HbN:HbA ratio);
extr. = XAD-extractable.

St.	Depth (m)	Description	Extraction efficiency (%TCHO)	TCHO (%DOC)	Extr. TCHO (%DOC)	Extr. TCHO (%extr. DOC)	XAD-2:-4 ratio	HbN: HbA ratio
56E	0	Ice floe	28	4.3	1.3	4.3	1.7	1.3
20	20	Polar Surface Water	45	5.2	2.3	5.2	8.4	3.0
167	20	Atlantic water	49	3.3	1.6	3.4	5.9	1.3
96	26	Polar Surface Water	47	4.4	1.8	4.4	12.6	1.8
106	45	Polar Surface Water	47	2.4	1.1	2.4	8.7	1.4
17	50	Water of Pacific origin	79	5.2	4.1	6.8	3.9	3.3
133	1000	Deep water	50	3.9	2.0	3.8	3.4	0.7
38	1800	Canadian Basin Deep Water	50	2.6	1.3	2.3	1.2	1.0
14	2900	Deep water	59	2.0	1.2	1.9	0.2	2.6
46	4500	Deep water	70	1.3	1.0	1.4	5.3	1.2
60	5600	Deep water	64	1.4	0.9	1.4	1.8	4.7
Mean of Polar Surface Water			41±8	4.1±1.1	1.8±0.7	4.5±1.2	9.9±2.3	1.8±0.8
Mean of deep water			59±9	2.2±1.1	1.3±0.4	2.2±1.0	2.3±1.9	2.0±1.7

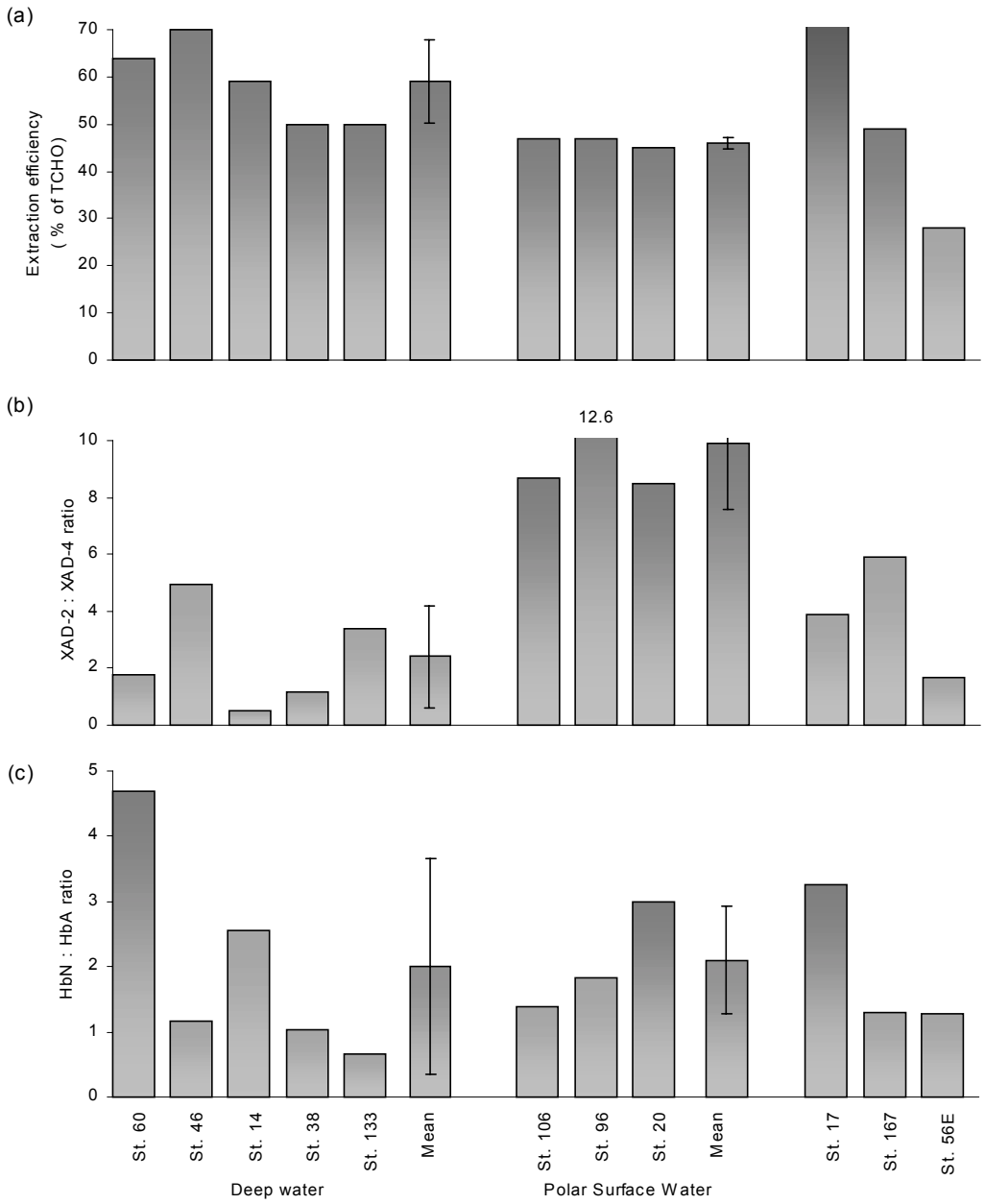


Figure 23: (a) Extraction efficiency of TCHO (in % of total TCHO) from seawater with XAD-2 plus-4;
(b) ratio between TCHO extracted with XAD-2 and XAD-4 (XAD-2:-4 ratio);
(c) ratio between HbN and HbA fractions of both resins (HbN:HbA ratio).

4.2.3 Combined Neutral Sugars

Carbon yields for THNS isolated with XAD were calculated as for TCHO (Table 8 and Figure 24). Highest yields for THNS were found in the deep sea samples with 27 and 21%, and in the ice floe sample (25%, St. 56E). Yields from other stations varied between 6 and 11%. Surface samples (without the ice sample) contained 8% XAD-extractable THNS and deep sea samples 24% on average. The discrepancy of neutral sugar yields between surface and deep sea samples was larger than the yields for fractionated DOC and TCHO. The neutral sugar yield of St. 56E was similar to the yields of fractionated DOC (29%) and fractionated TCHO (28%).

Table 8: Extraction efficiency of THNS (in % of total THNS) from seawater with XAD-2 plus –4, proportion of total THNS of DOC, proportion of extracted THNS of total and of extracted DOC, ratio between THNS extracted with XAD-2 and XAD-4 (XAD-2:-4 ratio), and ratio between HbN and HbA fractions of both resins (HbN:HbA ratio);
extr. = XAD-extracted, n.d. = not determined.

St.	Depth (m)	Description	Extraction efficiency (%THNS)	THNS (%DOC)	Extr. THNS (%DOC)	Extr. THNS (%extr. DOC)	XAD-2:-4 ratio	HbN: HbA ratio
56E	0	Ice floe	25	7.7	1.94	6.7	2.6	1.3
96	26	Polar Surface Water	11	5.4	0.58	1.4	1.8	0.8
167	20	Atlantic water	6	2.9	0.16	0.3	1.6	1.7
106	45	Polar Surface Water	n.d.	n.d.	0.35	0.8	7.0	2.3
17	50	Pacific water	9	6.8	0.62	1.0	2.1	1.9
38	1800	Canadian Basin Deep Water	21	3.7	0.80	1.4	1.9	4.5
46	4500	Deep water	27	4.0	1.00	1.6	1.0	n.d.
Mean of surface water			8±3[1]	5.7±2.0	0.4±0.2[1]	0.9±0.4[1]	3.0±2.3	1.6±0.6
Mean of deep water			24±4	3.9±0.2	0.9±0.1	1.1±0.4	1.5±0.6	4.5

[1]: without St. 56E

The proportions of extracted THNS ranged from 0.16 to 1.94% of DOC. The surface water samples showed lower THNS proportions (0.4% on average) than the deep sea samples (0.9% on average). These values were lower than the proportions of fractionated TCHO of DOC (1.8 and 1.3), and the depth-dependency is directly opposed. The high extraction efficiency measured in the ice sample lead to THNS proportions higher than found in the case of refractory TCHO. The proportion of refractory THNS to refractory DOC exhibited no depth-dependency.

The XAD-2:XAD-4 ratios ranged from 1.0 to 7.0. A depth-dependency could be observed, similar to fractionated DOC and TCHO. In the deep sea samples, the ratios were only half of the surface sample ratios (1.5 and 3.0, respectively). The HbN:HbA

ratios of the surface samples ranged from 0.8 to 2.3 (1.6 on average) and were significantly lower than the value of the deep sea sample (4.5).

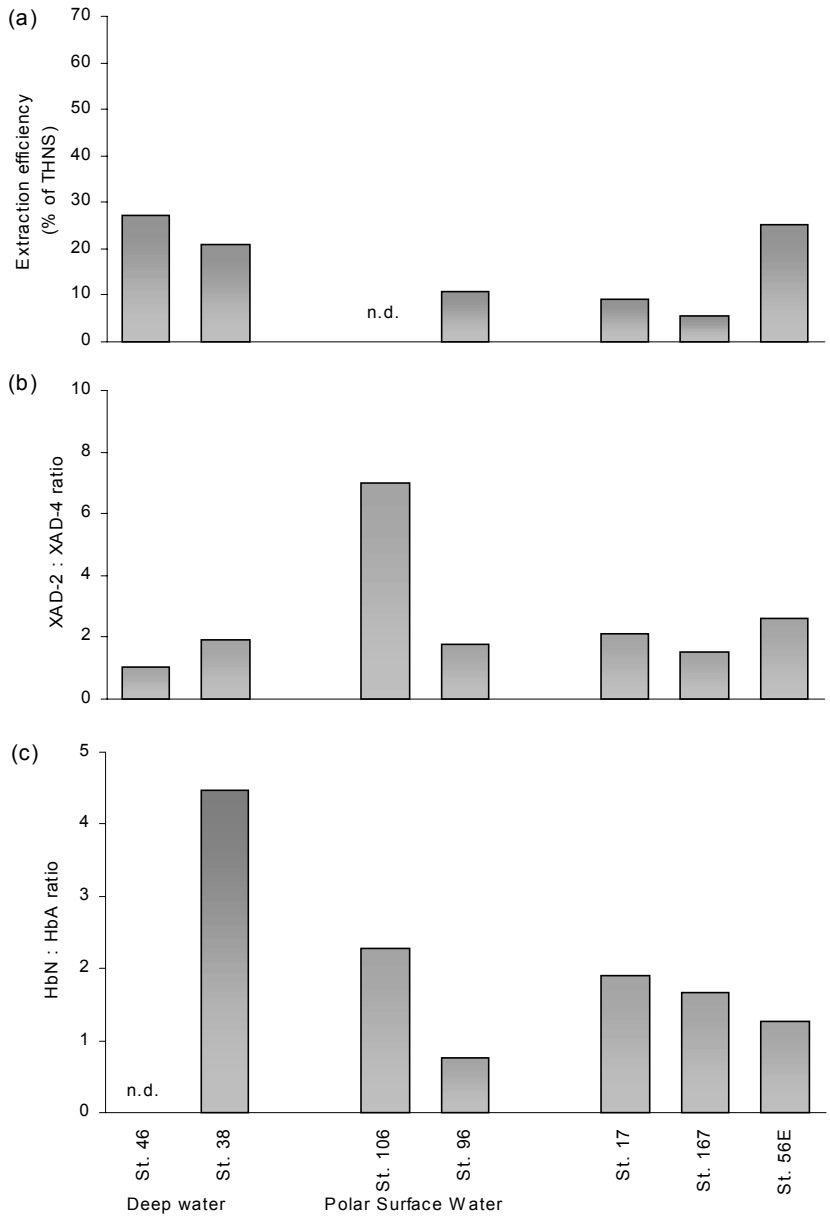


Figure 24: (a) Extraction efficiency of THNS (in % of total THNS) from seawater with XAD-2 plus-4;
(b) ratio between THNS extracted with XAD-2 and XAD-4 (XAD-2:-4 ratio);
(c) ratio between HbN and HbA fractions of both resins (HbN:HbA ratio);
n.d. = not determined.

In Figure 25a, the concentrations of individual neutral sugars in the hydrophilic and hydrophobic XAD-fractions, and in Figure 25b the distribution of the neutral sugars among the 4 fractions is shown. Their individual extraction efficiencies, i.e., the proportion of an individual neutral sugar extracted from THNS with XAD, varied within wide ranges. Corresponding to the extraction efficiencies of THNS, most of the neutral sugars were found in the hydrophilic fraction.

The major part of the fractionated sugars of the ice floe (St. 56E) was found in the HbN and HbA fractions of XAD-2, except for glucose, fructose, and ribose. Approx. one third of these sugars were found in the HbN and HbA fractions of XAD-4. The sugars in the XAD-2 eluates were homogenously distributed among the acidic and neutral fractions, whereas the sugars in XAD-4 varied widely in the fractions. Fucose, rhamnose, and arabinose were not found in the HI fraction, in which the overwhelming amount of glucose, xylose, and ribose was detected.

The two Polar Surface Water samples St. 96 and St. 106 were very similar in the composition of extracted THNS and their distribution among the fractions, with the exception of glucose, which was present in extraordinary high amounts at St. 106. The overall concentration at St. 106, however, was nearly the half of St. 96. Compared to the ice floe, the proportions of deoxysugars and arabinose were higher relative to the other sugars, while galactose, glucose, mannose, and xylose remained nearly constant. The proportions of the XAD-4 fractions also increased, being nevertheless lower than 20%. Fucose was still not found in the HI fraction, whereas only 20 to 30% of rhamnose and arabinose could be extracted with XAD.

The spectrum of fractionated THNS of the Atlantic water (St. 167) was completely different. Main component was ribose, while the amount of glucose was relatively low. Xylose and fructose were near the detection limit and only found in the hydrophilic fraction. Almost half of the sugars were found in the XAD-4 fractions. In contrast to the polar surface water, major part was found in the HbN fractions. The extraction efficiencies were generally low, being 20% in maximum. Again, only fucose was completely extracted with XAD.

The Pacific water (St. 17) was dominated by fructose. The distribution of the other extractable neutral sugars was very similar to the Polar Surface Waters. Almost all carbohydrates were recovered in the XAD-2 fractions, apart from fructose, which was also present in XAD-4. All components in the acidic fractions were very low. The extraction efficiencies for the individual sugars were little higher than in the Atlantic water. Besides of fucose, mannose and xylose were completely extracted.

In the deep water sample (St. 38), THNS distribution was completely different. In contrast to all other samples, the extraction yield of fucose was lower than 10%. The corresponding yields for rhamnose, arabinose, fructose, and ribose ranged between 4 and 8% only. The proportions of mannose and xylose increased, reaching concentrations similar to galactose and glucose. Additionally, their extraction yields ranged from 33 to 45%. The proportion of the XAD-4 fractions was higher than in the other samples.

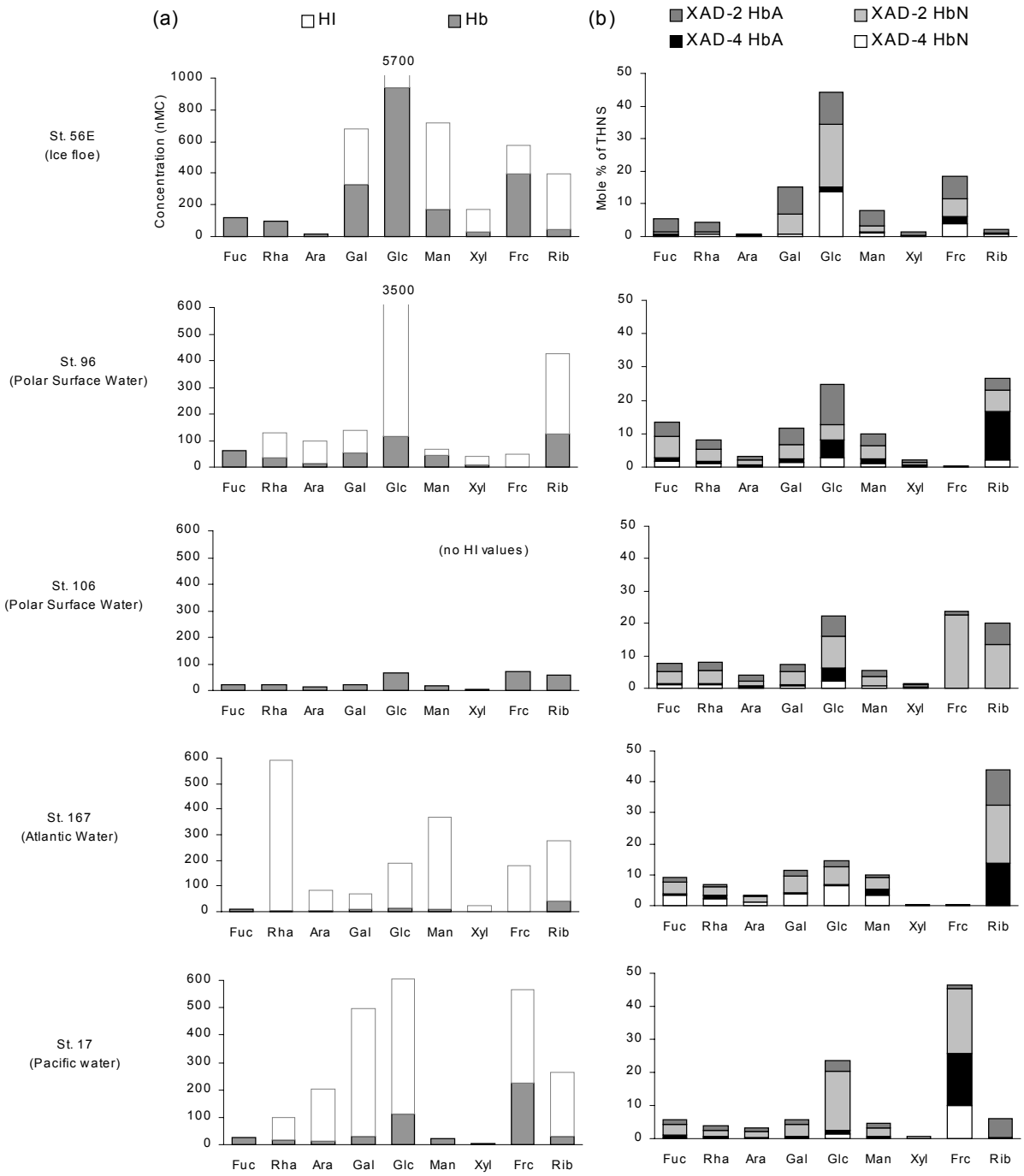


Figure 25: (a) Concentrations of neutral sugars in hydrophilic (HI) and hydrophobic (Hb) fractions of XAD-extraction of seawater samples;
(b) neutral sugar composition (in % of extracted THNS) of the XAD-fractions.

Generally, the extraction yields for individual sugars differed among the samples. The increase of extraction efficiencies with depths, which was observed for fractionated DOC, TCHO, and THNS, is not reflected by all individual neutral sugars. A common feature of all surface samples was the complete removal of fucose with XAD. The yield of rhamnose and arabinose decreased with depth. Galactose exhibited, with the exception of the Pacific and Atlantic water, rather constant extraction efficiencies. The

extraction of glucose and ribose yields low amounts in all samples, in contrast to mannose and xylose. Their extraction was most efficient in the Pacific and deep waters. Glucose, fructose, and ribose, if present, made largest contribution to the HbA fraction of XAD-4. For the XAD-2 fractions, the distribution was more homogenously, with larger contributions of galactose, glucose, mannose, and ribose. The deoxysugars, in contrast, were mainly present in the HbN fractions. The coloured fractions were rich in fructose or ribose dominating the neutral sugars.

4.3 Comparison between XAD and Ultrafiltration

For comparative purpose, a surface sample of Fram Strait (St. 62), taken with the on-board seawater supply system, was ultrafiltrated (for details of the ultrafiltration procedure, see Benner et al., 1997) and XAD-fractionated. To get more structural information about the extracted organic matter, the concentrate and permeate of the ultrafiltration were XAD-extracted each. In the ultrafiltration concentrate, all molecules with a molecular mass larger than 1 kDa are retained. The permeate contained all smaller molecules. Furthermore, the acidic and neutral fractions of the XAD-extraction of the original sample were both ultrafiltrated. The obtained fractions were analyzed for DOC, and partly for TCHO and THNS. The molar content of organic matter in the fractions was related to the molar content of the original sample. Accordingly, percentage values refer to original concentration.

4.3.1 Distribution of Dissolved Organic Carbon

The extraction efficiencies of DOC of the surface sample were almost identical for the XAD and the ultrafiltration extraction method. With ultrafiltration, 29% of total DOC were recovered in the concentrate and 71% found in the permeate (Figure 26). 32.6% of the permeate's DOC and 54.5% of the concentrate's DOC could further be extracted with the XAD resins. Summing up the extraction efficiencies of ultrafiltration and of XAD-extraction of the permeate, nearly the half (45.1%) of the sample's total DOC could be extracted by the sequence ultrafiltration - XAD. With the XAD method, 25.9% of the total DOC was extracted. Total recovery of 45.2% could be achieved by subsequent ultrafiltration of the XAD-fractions. This yield was identical to that of the other operational sequence. The total amount of extractable organic matter was therefore independent on the extraction order. Furthermore, the results of the overall extraction recoveries of both sequences imply that both methods are quantitative.

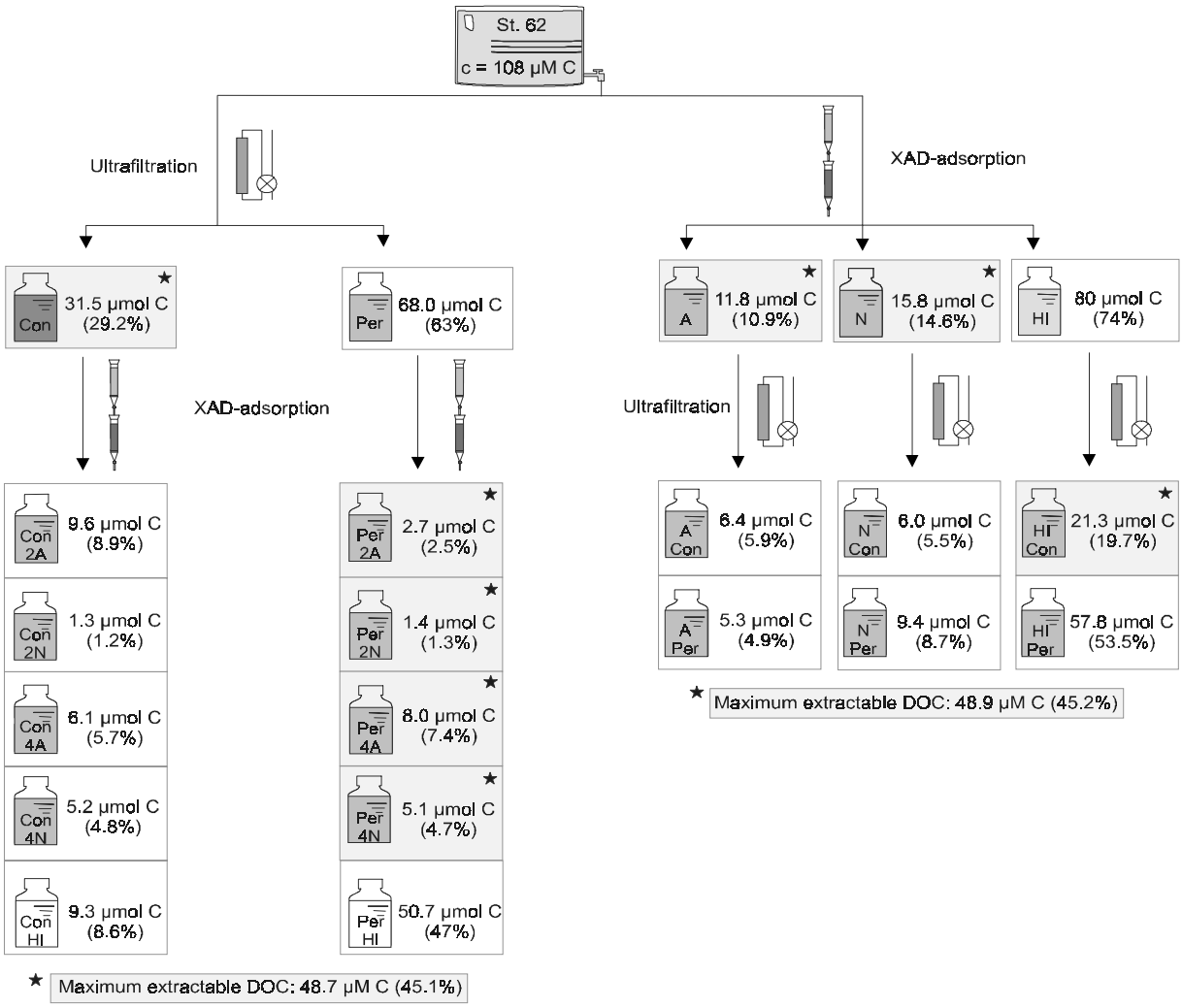


Figure 26: Comparison of XAD and ultrafiltration methods: DOC concentrations in the fractions in % of total DOC, for notation of the fractions cf. Figure 5; Con = concentrate and Per = permeate of ultrafiltration.

The size-fractionation of the ultrafiltration affected the distribution of XAD-extractable DOM on the XAD-2 and XAD-4 fractions. The XAD-2:XAD-4 ratio (where the concentrations of HbN and HbA fractions of each resin were added, see also section 4.2.1) of the ultrafiltration concentrate was much higher (1.0) than that of the ultrafiltration permeate (0.3). This finding supports the assumption, that the two resins, differing in their pore size, fractionate extracted DOM by size. The HbN:HbA ratios (where the respective concentrations of XAD-2 and XAD-4 were added) of the concentrate and the permeate were low and did not differ much (0.4 and 0.6).

For the original sample XAD-2 and XAD-4 elutes were not differentiated due to operational reasons. The HbN:HbA ratio of the original sample (1.3) is splitted into a lower ratio in the XAD-concentrate (0.9) and into a higher ratio in the XAD-permeate (1.7).

4.3.2 Distribution of Total Carbohydrates

Total carbohydrate analyses were performed on the most important fractions. The ultrafiltrated fractions of the XAD-adsorption were not analyzed for TCHO. In contrast to the DOC distribution, both methods extracted different amounts of carbohydrates. If first was ultrafiltrated, 21.4% of the TCHO of the original sample could be extracted, which was less than the DOC yield (Figure 27). If the original sample was extracted with the XAD resins first, about 7% of TCHO were recovered.

No substantial enhancement of the carbohydrate yield obtained by the ultrafiltration method could be achieved by XAD-extraction of the permeate. Thus, most resin-active carbohydrate compounds are larger than 1 kDa and are therefore extracted by ultrafiltration. The overall recovery of the extraction sequence ultrafiltration - XAD was 21.4%, nearly the half of the efficiency of the DOC extraction (45.1%). The XAD-extracts of the ultrafiltrate concentrate reflected the larger molecular size. Carbohydrates were much higher concentrated in the XAD-2 fractions than in XAD-4, the XAD-2:XAD-4 ratio was about 8. The HbN:HbA ratio was with 0.6 similar to the ratio of the DOC concentrations. In the ultrafiltrate permeate, the XAD-2:XAD-4 ratio of 1.6 reflected the extraction of smaller molecules. The HbN:HbA ratio of 3.0 was much higher than in the ultrafiltrate concentrate and also higher than the ratio of the DOC concentrations.

4.3.3 Distribution of Combined Neutral Sugars

To obtain detailed information about the pool of carbohydrate compounds, the original material, the XAD fractions, and the ultrafiltrated original sample were analyzed for THNS. The difference between the bulk determination of TCHO and THNS showed molecular and structural properties of carbohydrates in general. Fructose and ribose were not included due to uncertainties by quantification of these late eluting monomers. The original sample showed high concentrations of glucose, mannose, galactose, and arabinose (Figure 27). Neither xylose nor deoxysugars were abundant. THNS accounted for 1.2 µM C in total. With XAD-fractionation, approx. 10% of the total THNS were extracted. Contrary to TCHO, THNS was higher enriched in the acidic fraction than the neutral fraction. The HbN:HbA ratio was 0.5 and lower than the ratio of the DOC concentrations. This reflected the hydrophilic nature of the sugars. The composition of both fractions was, however, very similar and reflected the original composition except for arabinose, which was reduced in the XAD eluates. Comparable to the HI fraction, arabinose and glucose were the major components of the ultrafiltration permeate. The XAD resins could extract early no sugars out of this permeate. The ultrafiltration concentrate, the original sample, and the XAD-fractions had similar monomeric compositions, even though the absolute recovery of the ultrafiltrate was much higher (30.9%). About 6.5% of the THNS could only be extracted

from the ultrafiltration concentrate by XAD-treatment, which did not affect in particular its neutral sugar composition. Only rhamnose was slightly retained by XAD.

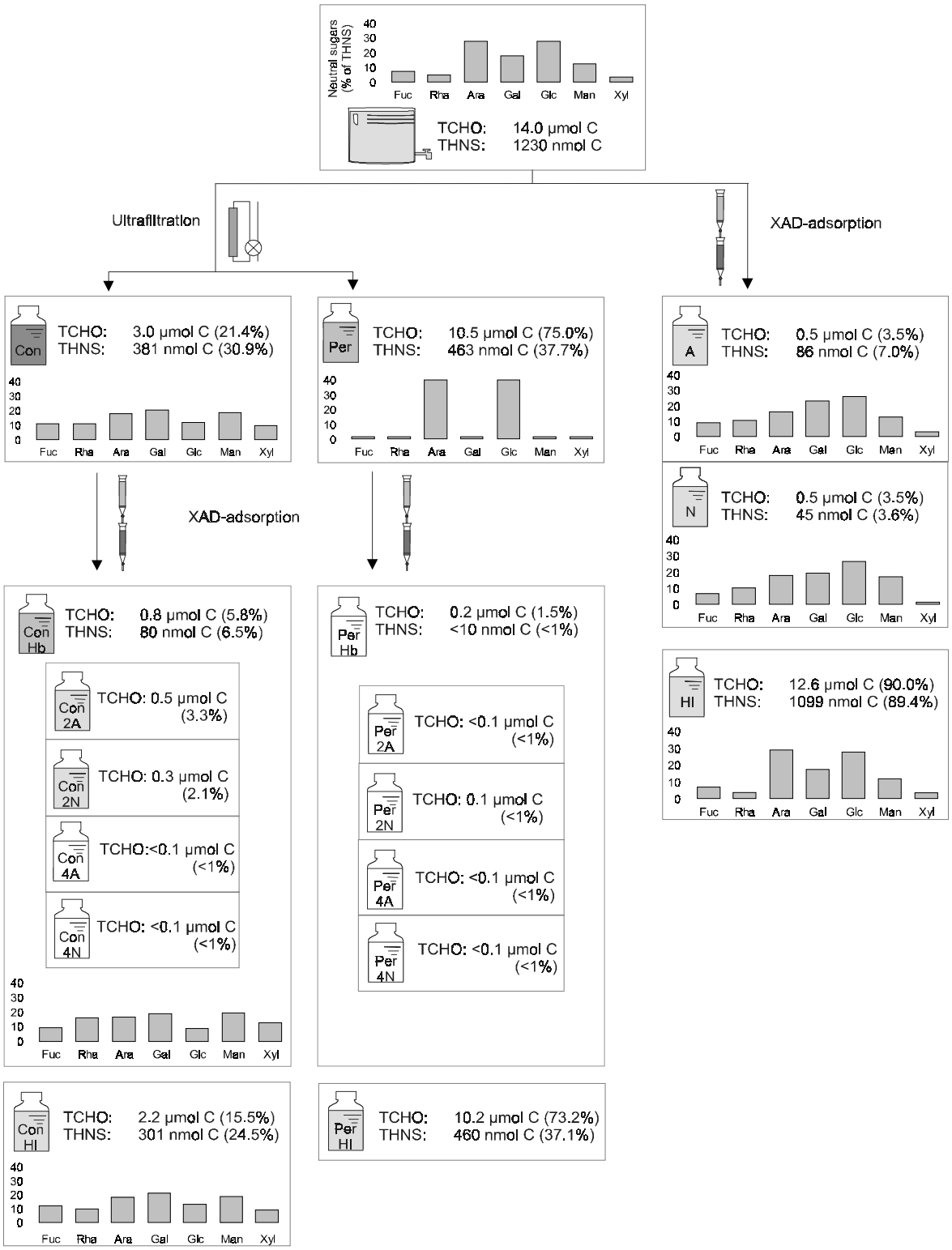


Figure 27: TCHO and THNS content in the fractions and THNS patterns (individual neutral sugars in % of THNS): comparison of XAD and ultrafiltration methods; TCHO and THNS concentrations in the fractions in % of total TCHO and THNS, respectively; for nomenclature of the fractions cf. Figure 5; Con = concentrate and Per = permeate of ultrafiltration.

4.4 Comparison between the TCHO and THNS-Concentrations

The concentrations of dissolved carbohydrates were determined by two methods. In Figure 28, the TCHO concentrations are compared with the THNS concentrations. In the seawater samples, the carbohydrate concentrations are mostly distributed near the 1:1 fit (Figure 28a). Greatest differences emerged in samples with high glucose proportions (St. 23, 200 m, St. 25, 300 m, St. 56E). In these samples, the carbohydrate concentrations measured with the THNS method were approx. 100% higher than the concentrations measured with the TCHO method. Contrasting to this, the carbohydrate concentrations of the XAD-fractionated DOM were mostly higher if measured with the TCHO method than with the THNS method (Figure 28b). Here, only the XAD-fractions of the ice floe sample (St. 56E) had higher THNS-concentrations than TCHO-concentrations.

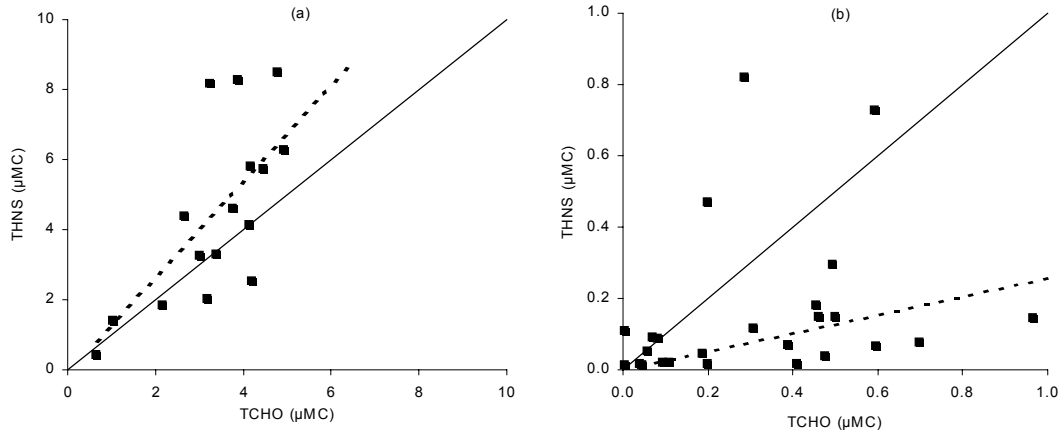


Figure 28: TCHO versus THNS carbohydrate concentrations;
(a) seawater samples and
(b) XAD-fractions;
solid line depicts the 1:1 fit and dotted line the regression fit.

Similar results were found by Mopper et al. (1995) for seawater samples, who obtained only half of the sugars by bulk determination compared to the THNS method. One reason for these differences may be the lower TCHO response signals of most carbohydrates than the calibration standard glucose (Table 1), which will lead to underestimates of TCHO concentrations. However, in the samples with the highest discrepancies, glucose accounted for the overwhelming part of the individual sugars. This suggests that the lower response factors of the other sugars might have little influence on the total concentration or that their influence is overlaid by other factors. If the lower response signals were not the reason for the differences in carbohydrate concentrations, presumably the different hydrolysis conditions accounted for that, since the hydrolysis of the THNS method takes much longer (24 h) than the one of the TCHO method (approx. 15 min). The hydrolysis of the THNS method has to be considered as

nearly quantitative, since no hydrolysis-resistant polysaccharides could be detected during chromatography (section 3.7). This finding is supported by the results of Borch and Kirchman (1997), who investigated hydrolysis efficiencies of various polysaccharide standards under similar conditions as were used for the THNS method. They found a hydrolysis efficiency of >95% for such polysaccharides as, e.g., starch. Furthermore, Benner et al. (1992) confirmed TCHO concentrations of fresh polysaccharide-rich UDOM, which were obtained by a modified MBTH method using a hydrolysis procedure similar to the THNS method of this study, by ^{13}C-NMR carbohydrate estimation. Their results of both methods were in good agreement. It can therefore be assumed, that in the case of the seawater samples (Figure 28a), high amounts of hydrolysis-resistant glucose-rich polysaccharides caused the higher THNS- than TCHO-concentrations.

This may also be the reason for the observed high THNS-concentrations of the extracted DOM of the ice floe. In the case of the other XAD-fractions, neither the lower response factors nor high amounts of hydrolysis-resistant polysaccharides can account for the mostly higher carbohydrate concentrations obtained by the TCHO method. Lower THNS- than TCHO concentrations can thus only be attributed to the composition of the individual monosaccharides. These differences suggest reasons on a molecular basis, since distinct individual neutral sugar concentrations are summed up for the THNS concentration and other non-neutral and substituted sugars are not included. In consequence, high amounts of those carbohydrates lead to underestimates of the carbohydrate concentrations obtained by the THNS method in the humic substances. In Table 9, the carbohydrate concentrations of the two detection methods are compared.

Table 9: Comparison between carbohydrate concentrations obtained by TCHO and THNS detection method.

		TCHO method	THNS method
Detected sugars		All	Neutral sugars
Concentrations in	Seawater	≈ Equal	
	Seawater (high glucose proportion)	Low	High
	XAD-fraction	High	Low
	XAD-fraction (high glucose proportion)	Low	High

5 Discussion

5.1 Diagenetic Modification of Dissolved Carbohydrates in the River-Shelf-Ocean System

A major problem for the differentiation of the various marine and terrestrial organic matter sources is that marine organisms are commonly more variable in carbohydrate composition than vascular plants (Barker and Somers, 1970; Percival, 1979). In consequence, it is difficult to distinguish between carbohydrates originating from different plankton categories (zooplankton, phytoplankton) and bacteria. Freshly produced DOM is characterized by high levels of glucose and ribose. Ribose, which is a component of RNA and present as a metabolite in small organisms (Cowie and Hedges, 1984a), might be useful to distinguish between marine and vascular terrestrial sources, since in plant tissues ribose is superimposed by structural polysaccharides. However, due to analytical difficulties, only few marine ribose data are available. Furthermore, there have been several attempts to distinguish between terrestrial sources like angiosperms and gymnosperms, which differ in xylose and mannose content or between woody and non-woody plants, which differ in arabinose content (e.g., Cowie and Hedges, 1984a). Decomposition processes in detritus liberate, for instance, algal cell wall components such as fucose and rhamnose (e.g., Cowie and Hedges, 1984a; Cowie and Hedges, 1994). Since deep sea DOM has a high radiocarbon age of 4000 to 6000 years (Williams and Druffel, 1987), even slow processes are important for the transformation of marine DOM. In the following, diagenetic trends were deduced from the distribution of neutral sugars, such as a decrease of glucose and an increase of deoxysugar proportions during diagenesis.

5.1.1 The Russian Rivers

The composition of riverine DOM depends on the origin and diagenetic state of the material. Autochthonous material is produced by algae in the rivers, while allochthonous material is pedogenous and introduced via ground water and surface run-off.

The available data set on carbohydrate distribution in the Russian rivers in literature is scarce and so far no neutral sugars data are published. The TFNS and THNS concentrations of this study were very high with values up to 1.3 and 40 µM C, respectively, compared to other world rivers. TFNS contributed to a maximum of 4% to total carbohydrates. In a French estuary, Senior and Chevolot (1991) found maximum TFNS and THNS values of 2.5 and 5.4 µM C, respectively. Maximum concentrations of 7.6 µM C TCHO were measured by Volk et al. (1997) in a North American white water creek and 9.6 µM C TCHO in the Lena River delta in northern Siberia by Lara et al. (1998). Hedges et al. (1994) investigated the THNS distribution of ultrafiltrated DOM of the Amazon River and some major tributaries. The composition is very similar to the Russian rivers and consistent with the results of Ittekkot and Arain (1986) for POM from

the Indus River and with results from Sweet and Perdue (1982) for POM from a small temperate river. In all these samples, glucose dominated and galactose, mannose, xylose, arabinose, and the deoxysugars were present in similar amounts. Opsahl and Benner (1999) compared the distribution of particulate and dissolved neutral sugar in several tropical rivers. They proposed a diagenetic sequence for vascular plant-derived organic matter, which is based on the mole percentage of glucose, which decreased from plants, to riverine POM, and riverine DOM (Figure 29). According to their results, rivers can be classified into two categories: one with high glucose proportion of approx. 50% (low diagenetic degree), and another with low glucose proportion of approx. 25% (high diagenetic degree). The MacKenzie River, which drains into the Canadian Basin of the Arctic Ocean, belongs to the latter category. The same attribution was found for the rivers in this study with proportions of glucose between 24 and 28% indicating highly degraded DOM. It is therefore likely that the riverine DOM was constituted primarily of refractory DOM from soil material.

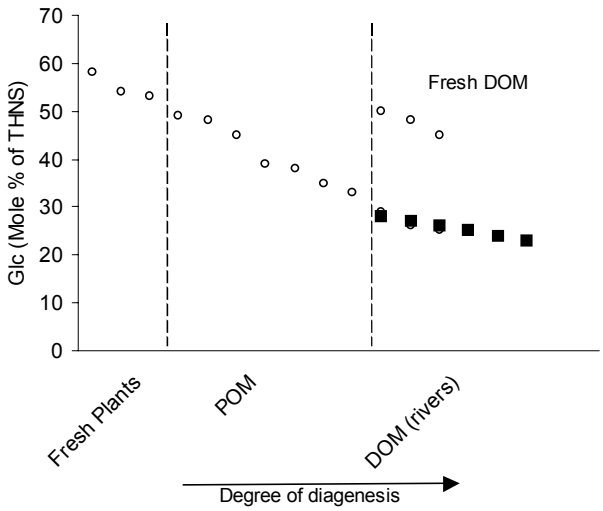


Figure 29: Diagenetic sequence of terrestrial and riverine organic matter based on the mole percentage of glucose to THNS;
■ this thesis; o Opsahl and Benner (1999).

The carbohydrate composition of the rivers may also be influenced by autochthonous production of phytoplankton. However, in the Ob, phytoplankton plays only a minor role in the carbon budget (Telang et al., 1991). In the Yenisey, autochthonous production was also low (Amon and Spitzy, 1999). Due to the high turbidity, the productive zone is limited to the upper few decimetres. Using lignin as molecular tracer for vascular plant organic matter, Lobbes et al. (2000) have shown that phytoplankton-derived DOM is low compared to soil-derived DOM in the major Russian rivers.

The carbohydrate composition of the riverine DOM might be therefore dependent on the prevailing vegetation of the catchment areas of the rivers (Figure 30). Tundra and taiga are the main vegetations of northern and central Eurasia. Coniferous woods and bogs characterize the taiga. Adjacent to the north, the tundra is a treeless permafrost

steppe. In consequence, all smaller rivers located in the north, like Moroyyakha, Vaskina, and Velikaja, drain almost 100% tundra. The largest rivers Yenisey and Kolyma flow primarily through taiga, while the rivers Indigirka, Olenek, and Vizhas flow through both vegetation forms.

The reason for different THNS yields might be different remineralization rates in the prevailing soils. In the taiga and temperate regions, carbon accumulates less in soils due to higher remineralization rates. Easily degradable substances, such as carbohydrates, are rapidly depleted during humification (Opsahl and Benner, 1999). Their concentrations in the taiga-dominated rivers were therefore low (Figure 30). In the Siberian tundra, however, accumulation is three times higher (Dixon et al., 1994) and remineralization rates are much lower. A mixture of diagenetic fresh and old organic matter is drained into the rivers, and, therefore, carbohydrate proportions were comparatively high.

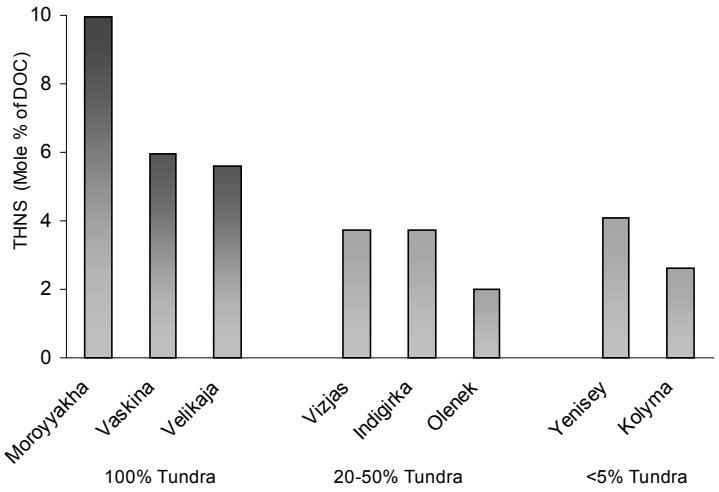


Figure 30: Influence of the vegetation of the catchment areas of the rivers on the proportion of THNS to DOC; vegetation estimated from the Times Atlas (1997).

The proportion of glucose to THNS also varied between the high-latitude and other tundra rivers (Figure 31). The rivers with intermediate tundra influence (between 20 and 50%) exhibited the lowest glucose proportions (23.5 to 25.0%) of all rivers. Both, the rivers with solely or none tundra influence exhibited clearly higher glucose proportions of 25.6 to 27.8%. The other neutral sugars did not show such tendency.

These results suggest the dominance of different diagenetic processes in the different areas. Tundra-derived DOM is characterized by a low diagenetic degree, indicated by high THNS yields and glucose proportions. Taiga-derived DOM is generally of high diagenetic degree, as evident from low THNS yields and low glucose proportions. In solely taiga-derived areas, also glucose-rich DOM was detected.

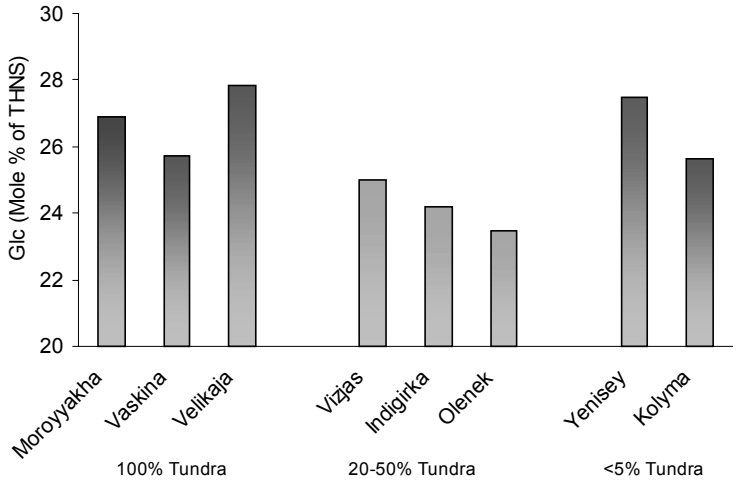


Figure 31: Influence of the vegetation of the catchment areas of the Siberian rivers on the proportion of glucose to THNS; vegetation estimated from the Times Atlas (1997).

This diagenetic signature is confirmed by Fitznar (1999) who investigated the concentrations and D/L-ratios of dissolved amino acids in the same rivers. Although of low statistical significance, the data indicate two tendencies. First, glycine and glutamic acid contributed little to total amino acids in the <5%-tundra category, compared to the other rivers. Second, the proportion of D-amino acids decreased from rivers with low tundra to high tundra proportion. The elevated D/L-ratios and low glycine proportions both indicated bacterial activity and elevated degradational state (Fitznar, 1999). Therefore, dissolved amino acids reflect “fresher” material with increasing tundra proportion. This is in good agreement with the results of the carbohydrate distribution.

5.1.2 The Russian Shelf Areas

During transition from the rivers to the marine environment, extensive biogeochemical modifications of DOM may occur due to strongly changing conditions such as electrolyte content, pH-value, and biological diversity. Conservative mixing is the simplest scenario for the mixing of riverine DOM in the ocean. Salinity can be used as conservative tracer for this mixing process, since the discharge of the Russian rivers is the major freshwater source of the Arctic Ocean. Sea ice melting and formation play only a minor role for the salt balance. In the Laptev Sea, conservative mixing of DOC with respect to salinity was observed (Kattner et al., 1999).

At the inshore stations DOC concentrations were about four times lower than in the rivers (580 and 150 µM, respectively, Cauwet and Sidorov, 1996; Lobbes, 1998). A conservative mixing behaviour could be detected neither for DOC nor for THNS (Figure 32). As end members, the means of the Russian rivers (Salinity = 0, DOC = 580 µM, THNS = 25 µM C) and the water of the Kara Sea (Salinity = 34.8, DOC = 54 µM,

THNS = 2.3 µM C) were used. The DOC concentrations of stations 3 and 6B were in good agreement to the expected values. At St. 6B, which is near the mouth of the rivers Ob and Yenisey, Anderson et al. (1999) found the maximum influence of terrestrial DOM, based on fluorescence measurements. The other DOC concentrations were more than two times higher than expected from the corresponding salinity. Explanations might be DOM release from POM, which was suggested for the Amazon estuary (Keil et al., 1997), and autochthonous DOC production. Amon and Spitzy (1999) assumed on the basis of DOC data from the southern Kara Sea a DOC surplus as a result of a large phytoplankton production.

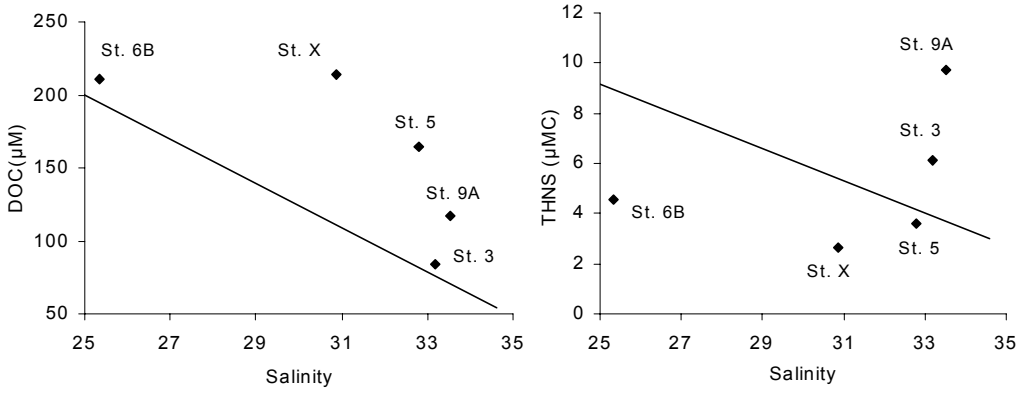


Figure 32: Salinity versus DOC and THNS at the Russian inshore stations; solid lines: dilution line extrapolated from estimates of Arctic surface seawater and river means.

Concentrations of total carbohydrates decreased by a similar factor as DOC, from 25 µM C at the river stations to 5.5 µM C on average at the inshore stations. A DOC surplus due to phytoplankton production should be reflected by a similar carbohydrate surplus. This was only found at stations 3 and 9A (Figure 32), at which the carbohydrate concentrations were higher than expected from conservative mixing. The low THNS concentrations at stations 6B and X suggest selective removal from DOM, e.g., preferential utilization of carbohydrates, since their DOC concentrations were higher than those expected of conservative mixing.

It can only be speculated about the reasons for this deviating behaviour. At the inshore stations with salinities higher than 25, physical desorption of DOM from POM is unlikely to occur, since these processes take place in estuaries where salinity increases rapidly. Adsorption of DOM on mineral surfaces may lead to lower concentrations than expected from conservative mixing, but this process decreases with increasing marine influence (Keil et al., 1997). This phenomenon suggests that the sources of carbohydrates are different in estuarine and marine environments and indicates that transformation processes take place in the transition zones.

The low carbohydrate contributions to DOC indicate that in most of the samples the major part of DOC is derived from the rivers, and phytoplankton production contributed only a minor fraction to DOC. However, at some inshore stations, especially at stations 5 and 6B, the pronounced increase in glucose proportion may be attributed to autochthonous production and release of fresh glucose-rich THNS by phytoplankton (Figure 33).

The terrestrial influence on the composition of DOC is well reflected by the THNS distribution (Figure 33). The THNS pattern of St. 3 is very similar to the nearby discharging Vaskina River. At this station the almost conservative mixing of DOC (Figure 32) indicates that DOM was dominated by riverine input. The THNS compositions of stations 5 and 6B were similar, but different to the vicinal rivers Moroyyakha and Yenisey. A composition similar to that of the Yenisey exhibited St. X, located northeast of the river mouth in the Yenisey plume.

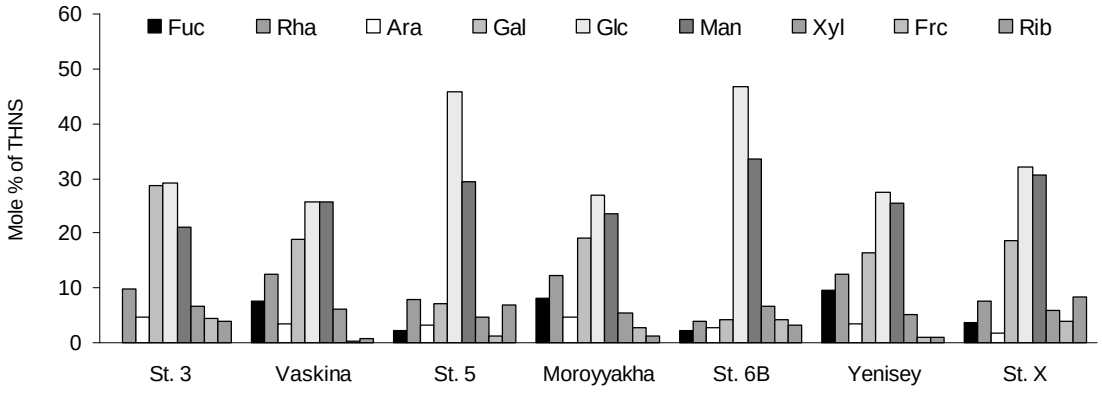


Figure 33: THNS distribution of the inshore stations and corresponding Russian rivers.

Compared to the rivers, the THNS composition varied much more at the inshore stations. The low fucose, rhamnose, and galactose concentrations of the inshore stations were most evident compared to the clearly higher concentrations in the rivers.

Ribose was measured only in traces (below 1% of THNS) in the river samples. According to Cowie and Hedges (1984a), it is a valuable indicator to distinguish between terrestrial and marine carbohydrate sources, since it is a component of RNA and many nucleotides which are present at greater levels in small, metabolically active organisms than in plant tissues, in which ribose is superimposed by structural polysaccharides. At the marine stations, ribose concentrations reached much higher values than in the rivers and ranged between 2.4 and 8.3% (4.1% on average). The highest values were measured at the stations 5 and X, which is a strong evidence for dissolved carbohydrates produced by phytoplankton on the shelves. While the high ribose proportions at St. 5 were in accordance with high glucose and THNS proportions and thus indicating marine phytoplankton production, DOM at St. X is partially derived from the river, since glucose and THNS proportions were low.

It is striking that the deoxysugars, fucose and rhamnose, contributed more to the THNS pool in the rivers than at the shelf stations. High proportions of these deoxysugars were observed by several authors in different organic materials (e.g., Cowie and Hedges, 1984b; Frimmel, 1998; Opsahl and Benner, 1999). An increase of these sugars in DOM was assigned to their abundance in algal cell walls and their subsequent release by bacterial destruction (e.g., Haug and Myklestad, 1976). The low proportions of the deoxysugars at the shelf stations indicated high phytoplankton activity and comparatively low bacterial modification. This implies net production of DOC. Confirming these results, Amon and Spitzy (1999) assumed on the basis of DOC data from the southern Kara Sea a DOC surplus as a result of phytoplankton production.

Arabinose, mannose, and xylose may also be indicative of different sources of terrestrial DOM (e.g., Cowie and Hedges, 1984a; Frimmel, 1998; Opsahl and Benner, 1999), but also of marine DOM (e.g., Ittekkot et al., 1981; Biersmith and Benner, 1998). The proportions of xylose were nearly constant in all samples, while arabinose proportions weakly decreased in West-East direction. Since the terrestrial influence was highest in the West, arabinose might therefore be used as a weak tracer for terrigenous DOM in the marine environment. Mannose proportions slightly increased from West to East at the shelf stations, which in turn exhibited higher mannose proportions than the river samples. Mannose might therefore be used as a weak tracer of phytoplankton-derived DOM.

At the inshore stations, contrasting to the river samples, no free monosaccharides were detected. This was in clear contrast to open ocean and coastal waters from temperate regions (Ittekkot et al., 1981; Pakulski and Benner, 1994; Børsheim et al., 1999), where the ratios between mono- and polysaccharides ranged from 0.1 to 6.3. The authors attributed these high ratios to a combination of cellular and in situ degradation as well as excretion from healthy cells. However, it is still unknown how these high amounts of monosaccharides are protected from utilization and degradation and why free monosaccharides were not detected in the Arctic Ocean.

5.1.3 The Open Arctic Ocean

In the Arctic Ocean, water masses of terrestrial, Atlantic, and Pacific Ocean origin are found, which are clearly distinguishable by temperature, salinity, and DOC concentrations (e.g., Aagaard and Carmack, 1994; Bussmann and Kattner, 2000). Carbohydrate variations may therefore be due to the distribution of water masses and the biogeochemical features of the different areas. In addition, the high irradiance during the polar summer may cause phytoplankton blooms, except in areas of permanent ice cover, which results in patchiness of surface DOC (e.g., Lobbes, 1998; Kattner et al., 1999; Bussmann and Kattner, 2000) and carbohydrate distribution.

The central Arctic Ocean exhibited DOC concentrations between 42 to 80 µM (Bussmann and Kattner, 2000), which are typical for open ocean regions (e.g., McCarthy et al., 1993; Sharp, 1997; Skoog and Benner, 1997), but much lower than in shelf areas. In contrast, carbohydrate concentrations in offshore areas were with 5.1 µM C on average similar to these in inshore areas, resulting in higher contributions of carbohydrates to DOC in the open Arctic Ocean.

Surface waters of the Amundsen Basin partly reached exceptional high carbohydrate concentrations (>7 µM C) in the Trans Polar Drift region, where waters from the Laptev Sea are transported across the Arctic Ocean. Bussmann and Kattner (2000) assigned high DOC values in this area to the freshwater influence from the Siberian rivers. Sea ice melting and subsequent release of organic material was insignificant, as water temperature was still below freezing point (Bussmann and Kattner, 2000).

In the Laptev Sea, surface carbohydrate concentrations also exceeded 7 µM C. Highest values were found in the area of warmer water and enhanced chlorophyll *a* and silicate concentrations in the eastern boundary of the Lena River plume (Kattner et al., 1999). These carbohydrate maxima may therefore be attributed to autochthonous primary production. Both ice melting and subsequent release of the sediment and organic matter load may enhance primary production. In the Lena River plume, however, concentrations were lower.

Surface concentrations of carbohydrates in the northern Kara Sea were lower than in the Laptev Sea (3.0 to 5.0 µM C). As in the Lena River plume, elevated carbohydrate concentrations were not characteristic for the plumes of the rivers Ob and Yenisey, which follow the shore to the Northeast towards Severnaya Zemlya and Vilkitsky Strait (Pavlov and Pfirman, 1995). A high carbohydrate concentration was found near Vilkitsky Strait on the Kara Sea shelf (St. 9A, 9.7 µM C), which was much higher than the adjacent surface values in the Arctic Ocean. However, the highest carbohydrate concentrations in the Kara Sea of up to 8 µM C (11% of DOC) were found in the deep water samples of the Voronin Trough. The Voronin Trough is separated from the adjacent Santa Anna Trough by a shallow bank in the West that prevents intrusion of deeper water from the Barents Sea into the trough. In the Santa Anna Trough, carbohydrate concentrations (2.3 to 3.5 µM C below 100 m depth, approx. 5% of DOC) were much lower than in the Voronin Trough.

The origin of the high carbohydrate concentration is unknown. Schauer et al. (subm.) found no indications of outflow of dense bottom waters from the Kara Sea. They assumed that water from the Santa Anna Trough follows the topography and entered the Voronin Trough from the North. This is in contrast to the carbohydrate distribution, which suggests inshore or surface sources.

These high carbohydrate concentrations might be the source for the high carbohydrate concentrations at the Laptev Sea slope. Here, in approx. 2000 m depth, a boundary current is found (Rudels et al., 2000). Its water derives from the Atlantic water of the Barents Sea shelf, which follows the continental slope of the northern Kara Sea towards the Laptev Sea. However, as pointed out before, the Barents Sea water is low in carbohydrates, but the boundary current may transport the higher amounts of carbohydrates, injected via Voronin Trough, towards the Laptev Sea.

The major basins of the central Arctic Ocean exhibited different organic matter composition. Due to the separation by the Lomonosov Ridge, there are differences in the oceanographic properties of both deep water masses, i.e., the Eurasian side is supplied with colder and fresher water of the shelves than the Makarov Basin (Swift et al., 1997). In the Nansen Basin, TCHO concentrations were low and rather homogenously distributed throughout the water column, except near the surface, where they reached up to 5 µM C. In the Amundsen Basin, deep water TCHO concentrations were even lower. In the deep Makarov Basin, at the eastern side of the Lomonosov Ridge, which is less influenced by riverine and shelf waters, the lowest TCHO values were measured. This distribution is reverse to the DOC distribution reported by Bussmann and Kattner (2000). They found an increase of the DOC concentrations from the Nansen to the Makarov Basin. This contrariness reflects the different origins of organic matter in these regimes. Olsson and Anderson (1997) suggested that most of the organic material of the central Arctic Ocean is derived from the rivers. Rich et al. (1997) measured very high turnover rates and rapid uptake of all DOM components along a transect through the central Arctic. Based on these results and due to the isolation of the deeper waters from the upper layer, Wheeler et al. (1997) found no evidence for export of DOC from shelf waters to the deep oceans. Therefore, the organic material should show increasing state of degradation with increasing distance from the Siberian shelves where most of the primary production takes place (Olsson and Anderson, 1997). This is probably also the reason for the decreasing carbohydrate proportion towards the inner part of the Arctic Ocean.

The most degraded material should exist in the Fram Strait region where water masses leave the Arctic Ocean. The very low THNS proportions of DOC in these water masses and especially the very low carbohydrate concentrations in the deep water support this assumption.

Following the diagenetic sequence of glucose for terrestrial and riverine organic matter, the contributions of all seven neutral sugars to THNS in DOM of the open Arctic Ocean were plotted in a similar way (Figure 34). The data of fructose and ribose were not included since only few data are available in the literature. Neutral sugar data from primary sources of the oceanic environment, such as phytoplankton and bacteria cultures (Cowie and Hedges, 1984a), have been included for comparison. Since only one data set of neutral sugars for the Arctic Ocean was available (Rich et al., 1997), data from other oceans were also included (Borch and Kirchman, 1997; Skoog and Benner, 1997).

The assumed diagenetic sequence of marine DOM deduced from neutral sugar proportions was confirmed by culture experiments of phytoplankton and bacteria (Biersmith and Benner, 1998; Cowie and Hedges, 1984a) and deep sea sediment pore water (Burdige et al., 2000). Although variations among THNS composition of phytoplankton and bacteria culture samples were large and strongly dependent on growth conditions, e.g., nutritional state, and large variations were even observed between the investigated species, however, high amounts of deoxysugars and arabinose were characteristic for deep sea bacteria and deep sea sediment pore water. These sugars may therefore be indicators for microbial degradation processes. Rather low proportions of galactose, glucose, mannose, and xylose were measured in bacteria cultures and the deep sea sediment pore water. These components may be, in contrast, indicators for algal activity.

The proportions of individual THNS in marine DOM are highly variable. None of the neutral sugars is of sole terrestrial or marine origin. All components occur in all environments and water masses. Generally, the hexoses, glucose and mannose, were the most abundant sugars and contributed up to 86% (Glc) and 41% (Man) to total THNS in surface waters of the Arctic Ocean. These values are even higher than in fresh plants. Lowest glucose proportions were found in deep waters of the Arctic Ocean, in Atlantic surface water and sediment pore water.

As discussed before, the glucose proportion of THNS seems to be a valuable indicator to determine the diagenetic state of marine DOM. However, its application has severe limitations. Thus, in deep waters of the equatorial Pacific Ocean, the glucose proportion was much higher than in surface waters (23 and 55%, Skoog and Benner, 1997). In contrast, decreasing glucose proportion with depth was observed in the northern Pacific Ocean (Borch and Kirchman, 1997), similar to the Arctic Ocean. In the North Atlantic, the proportion of glucose was also higher in surface than in deep water (Borch and Kirchman, 1997; Mopper et al., 1980). These contradictory results show the necessity to consider the hydrological and nutritional properties of the different areas. In contrast to the Arctic Ocean and North Atlantic, the surface waters studied in the equatorial Pacific are characterized by continuous nutrient surplus by upwelling, which makes this area to one of the most productive regions of the open ocean (Chavez and Barber, 1987). Here, the carbon fluxes, and in particular the neutral sugar fluxes, are much higher than in oligotrophic regions, where the carbohydrate concentration is low throughout the water column (Ittekkot et al., 1984).

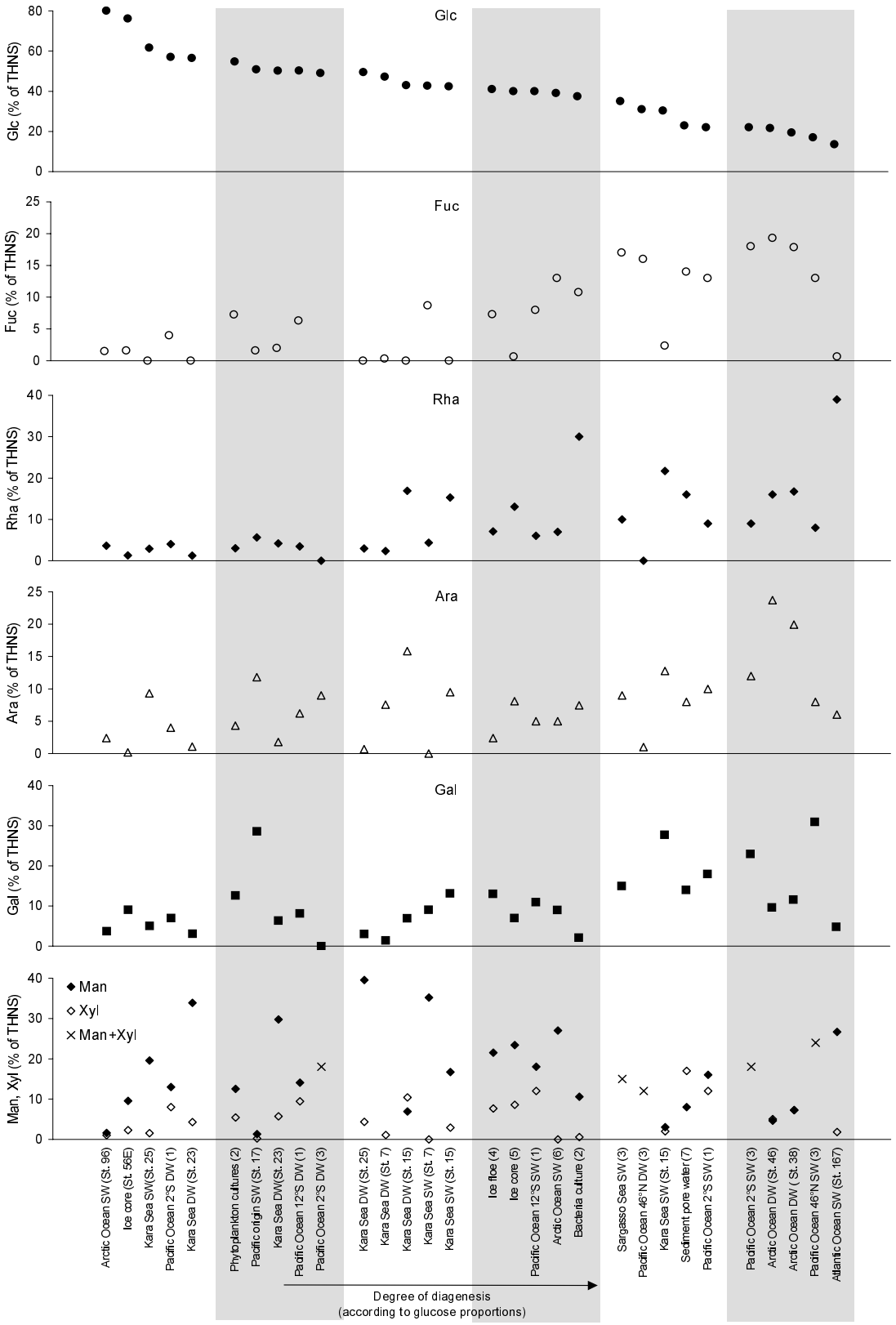


Figure 34: THNS composition of marine water samples; SW = surface water; DW = deep water;
[1]: Skoog and Benner (1997); [2]: Cowie and Hedges (1984a), [3]: Borch and Kirchman (1997), [4]: Amon et al. (2001), [5]: Engbrodt (unpublished data), [6]: Rich et al. (1997), [7]: Burdige et al. (2000).

Hernes et al. (1996) examined the modification of particulate carbohydrates and observed losses of glucose and enrichment of rhamnose and fucose with depth in the Pacific waters during the same period in the equatorial Pacific. Even higher values of up to 59% in 4000 m depth have been found by Skoog and Benner (1997). The higher productivity near the equator leads to higher export rates and hence to DOM of younger diagenetic state compared to the Arctic and North Atlantic.

Glucose is considered to be a suitable indicator for degradation processes, but other neutral sugars may also inhere potential as diagenetic tracers. The distribution of arabinose in the Arctic Ocean is striking. Its proportion to THNS in surface waters was low and similar to the riverine and inshore samples, while in deep waters its proportion increased to 25%. In addition, the proportions of fucose and rhamnose increased significantly with depth in the Arctic Ocean. Thus, the increasing proportions of these sugars with depth are additional indicators for degradation of DOM.

Surface water samples of the Atlantic and the strongly Atlantic influenced water masses of the Arctic Ocean had high amounts of rhamnose and arabinose, which suggests a high diagenetic degree compared to surface water samples of the Arctic Ocean. The high glucose proportions indicated, on the other hand, high contributions of fresh phytoplankton-derived DOM.

In the surface water of Pacific origin (St. 17, 50 m) sampled in the Fram Strait, the THNS composition partly showed indications of Pacific and Arctic surface waters. Low rhamnose and high arabinose and galactose proportions were similar to the surface samples of the Pacific Ocean (Skoog and Benner, 1997; Borch and Kirchman, 1997), whereas proportions of fucose, mannose, and xylose were similar to Arctic Ocean surface water.

In a similar way, the high freshness of the DOM of the Arctic ice floe (St. 56E) can be shown, compared, e.g., to DOM of an Antarctic ice core (Engbrodt, unpublished data), which is supposed to be also young. The glucose proportion to THNS in the ice floe sample was one of the highest overall and the proportions of the deoxysugars and arabinose were very low. Microbially degraded DOM of the same Arctic ice floe sample (Amon et al., 2001) had much higher deoxysugar and arabinose proportions, and the glucose proportion was correspondingly much lower. This further indicated that these proportions can be well used to attribute microbial diagenesis. The glucose proportion in the Antarctic ice core was similar to the degraded Arctic ice floe DOM, while rhamnose and arabinose proportions were even higher. For these Antarctic ice core samples, a high influence of microbial secondary production on the DOM composition was observed, which was, in contrast, low in the Arctic ice floe.

The proportions of galactose to THNS could not clearly be attributed to diagenetic alteration in all samples, just as the mannose and xylose proportions. Therefore, their suitability as diagenetic indicators is limited.

5.2 Composition of Refractory Carbohydrates

Detailed information on the molecular structure of carbohydrates within the DOM pool cannot be achieved with hydrolytic release of sugar monomers from bulk DOM. However, it is essential for our understanding of carbohydrate cycling in the ocean whether they are easily available as polysaccharides or bound in refractory humic substances. Extraction with XAD resins is a suitable tool to yield humic substances and to separate them from polysaccharides, which are not retained on the resin (Malcolm and MacCarthy, 1992). A separation cannot be obtained by ultrafiltration, since this method extracts a high molecular weight fraction of humic substances, together with polysaccharides (Amon and Benner, 1994; Benner, 1998).

Aged bulk material should become refractory due to losses of biodegradable compounds. Thus, a correlation between age and extraction efficiency with XAD can be assumed. The carbon extraction efficiency for DOC with XAD for surface water samples was significantly lower (45% on average) than for deep waters (58% on average), which indicates a higher amount of refractory organic matter in the deep sea. These efficiencies are similar to values reported by other authors who used similar procedures (e.g., Lara and Thomas, 1994; Frimmel, 1998). At the surface, extraction efficiencies with ultrafiltration are comparable to that of XAD (e.g., McCarthy et al., 1996; Skoog and Benner, 1997). In deep water, however, ultrafiltration is much less effective than XAD (e.g., McCarthy et al., 1996; Skoog and Benner, 1997). This confirms the selectivity of XAD for the humic fraction of DOM.

Generally, carbohydrate extraction efficiencies increased clearly with depth. For TCHO, the efficiencies were similar to the respective carbon extraction yields. For THNS, they were only one fourth of TCHO for surface samples (8%) and one half for deep water samples (24%). The much higher amount of XAD-extractable carbohydrates of deep sea samples suggest that a high proportion of carbohydrates is bound in refractory substances, which was also found, but to a lesser extent, by Hedges et al. (1992b).

Deviating results between the bulk determination (TCHO) and the chromatographic method (THNS) were already observed in seawater samples. Largest differences were found for glucose-rich and hence fresh DOM, where THNS values were up to two times higher than TCHO values. These differences were attributed to lower efficiencies of the TCHO hydrolysis for fresh polysaccharides (section 4.4). In contrast, for refractory carbohydrates, the TCHO values were generally higher than the THNS values, except for the XAD-fractionated ice floe sample. The discrepancies were larger in surface samples than in deep sea samples, which strongly indicate different chemical structures of refractory, fresh, and bulk DOM.

Additionally, the XAD-2:XAD-4 ratios of the fractionated carbohydrates in surface waters were much higher than in deep waters. With respect to the relatively low XAD-extraction efficiency, this finding indicates that large, labile polysaccharides are preferentially found in surface samples, which is congruent with findings from

ultrafiltration (e.g., Benner et al., 1992). With depth, the XAD-2:XAD-4 ratios decreased significantly for both methods. This finding is further evidence for structural differences not only between fresh and humic substances, but also between humic substances of surface and deep waters.

Lower THNS- than TCHO extraction efficiencies were attributed to the individual monosaccharide composition, since other than the determined neutral sugars were not included in the concentration of THNS. In consequence, high amounts of those carbohydrates lead to underestimates of the carbohydrate concentrations. Substituted carbohydrates, like aminosugars, uronic acids, and sugar alcohols cannot be detected with the chromatographic method, since charged carbohydrates are lost during sample preparation, but they are detected by bulk determination. On the abundance of charged carbohydrates in marine environments, contradictory results have been reported. Mopper (1977) and Gremm and Kaplan (1997) supposed that they are less abundant than neutral monosaccharides, while Sakugawa and Handa (1985) reported that uronic acids comprise 11% to 43% of polysaccharides from the North Pacific and Bering Sea. High amounts of sulphate esters or polysaccharides were found by Mopper et al. (1995). The different carbohydrate values determined by the TCHO and THNS methods indicated, that charged carbohydrates made up a considerable fraction of up to $^2/_3$ of refractory carbohydrates in surface waters, but only $^1/_3$ in deep waters. On the other hand, no evidence was found that these sugars contributed significantly to the total DOM, since TCHO did not yield more carbohydrates than THNS from bulk DOM.

The HbN:HbA ratio of fractionated THNS increased with depth, which reflects the hydrophobic character of refractory marine DOM in the deep sea (Figure 24). Furthermore, the deep water samples had lower HbN:HbA ratios for TCHO than for THNS. From the chemical nature, one would expect high amounts of substituted and charged carbohydrates to be found in the acidic fraction of XAD-extraction, which would lead to lower HbN:HbA ratios for TCHO than for THNS. This is in contrast to the fact that charged carbohydrates are predominantly found in humic substances of surface water. This indicates, that the adsorption behaviour is not determined by the carbohydrate part, but primarily by the overall chemical structure of the humic substances.

The composition of marine humic substances is determined by the formation processes. Generally, humic substances are produced by the transformation of different biomolecules into more refractory forms. Whereas terrestrial humus mainly contains degradation products of vascular plants and large amounts of aromatic structures, marine humic substances are characterized by the high aliphatic carbon backbone (e.g., Hedges et al., 1992b). In addition to carbohydrates, amino acids and lipids are major constituents of marine humic substances (e.g., Harvey et al., 1983). They provide a wide range of possible “secondary structures” which is far beyond chemical determination and even characterization.

The calculated HbN:HbA ratios may help to characterize the vicinity of carbohydrates in humic substances. The ratio was much lower for fractionated DOC than for fractionated carbohydrates (Table 6 to Table 8). This shows that carbohydrates (TCHO and THNS)

were preferentially eluted in the neutral and not in the acidic fraction. The occurrence in the neutral fraction reveals that the carbohydrates are bound or associated with a high portions of low-polar constituents to result in this neutral structure. Most structural properties of XAD-fractionated humic substances were in good agreement with fractionated matter obtained by ultrafiltration. However, one of the most striking differences is the depth-depending selectivity. XAD exhibited much higher carbohydrate extraction efficiencies in deep sea samples than in surface samples. Using ultrafiltration, this efficiency decreases with depth. For example, in surface waters of the Pacific Ocean, about 70% of the carbohydrates were isolated by ultrafiltration, but in deep waters, it decreased to only 15% (Skoog and Benner, 1997). This underlines that refractory carbohydrates are preferentially extracted by XAD-fractionation. Furthermore, the general lower XAD-2:XAD-4 ratios in deep than in surface waters indicated that carbohydrates are present in smaller molecules in old and refractory humic substances of the deep water than in surface samples. This is again in good agreement with the low ultrafiltration efficiencies of deep waters. The comparison between carbohydrates isolated by XAD and ultrafiltration reveals details about their biogeochemical characteristics.

Generally, neither the ultrafiltration fraction nor the XAD fractions have THNS compositions identical to the original seawater sample. They were most similar in the ultrafiltration fraction of a surface sample, also reflected by similar C/N ratios and carbohydrate concentration estimated on ^{13}C-NMR measurements (e.g., Benner et al., 1992; Hedges et al., 1992b) (Figure 35 and Figure 36).

The two methods were directly compared using a surface water sample. In surface waters, carbohydrates are primarily polysaccharides produced by phytoplankton (e.g., Benner et al., 1992). One fifth of these carbohydrates exhibited molecular weights of more than 1 kDa. From this ultrafiltration fraction, only one fourth of the carbohydrates (approx. 7% of the total) could be extracted with XAD. These carbohydrates are probably bound or associated to the humic substances. Additionally, the XAD-2:XAD-4 ratio of this XAD-fraction (7% of the total) is much higher than the XAD-2:XAD-4 ratio of the extracted DOC of this fraction (Figure 26). The carbohydrates of this fraction are thus of a higher molecular weight than the total DOM of this fraction.

From the small size fraction (<1 kDa), which comprises the vast majority (75%) of all carbohydrates, nearly no carbohydrates could be further extracted with XAD (Figure 27). Additionally, no free neutral sugars were found. The cut-off of ultrafiltration is equivalent to molecules of a comparable size of hexasaccharides, which are too polar to be XAD-extractable. Hence, the majority of carbohydrates is found in hydrophilic molecules with molecular masses between approx. 200 Da and 1000 Da. It can be assumed, that fragments of metabolites contribute to this fraction.

Of the total carbohydrates (THNS and TCHO), about 10% were extracted with the humic substances fraction. One half elutes in aqueous solution (HbA), the other half in methanol (HbN). “Pure” carbohydrates such as polysaccharides are highly hydrophilic and are not XAD-reactive. The occurrence of carbohydrates in the most unpolar fraction

(HbN) indicates again, that these carbohydrates have a completely different structure compared to polysaccharides.

In Figure 35, the selective fractionation for the individual sugars of XAD-extraction and ultrafiltration is shown. Rhamnose and galactose were preferentially extracted with XAD than arabinose, thus contributing a higher proportion to the humic substances. The other sugars were present in similar mole percentages compared to the original sample. These compositional changes are in accordance with transformations occurring during diagenesis (section 5.1.3) indicating a higher diagenetic state of the humic substances than bulk DOM.

A more different THNS composition compared to the original sample is obtained with ultrafiltration, although a common feature for both fractionation methods was the increase of rhamnose and decrease of arabinose. In contrast to XAD and the original sample, glucose strongly decreased, whereas xylose increased by a factor of about four in the ultrafiltration fraction.

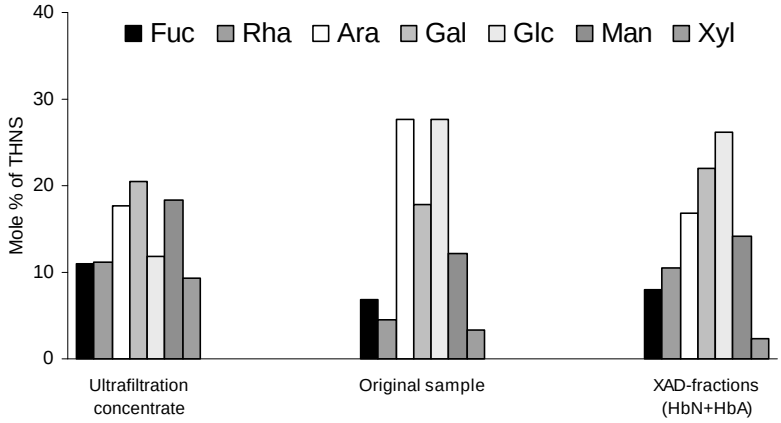


Figure 35: THNS composition of the ultrafiltration concentrate and XAD-fractions in comparison to the original sample of the intercomparison experiment.

In surface samples, fucose and rhamnose were mostly found in the humic substances indicated by the XAD-fractionation (Figure 25, Figure 36a), whereas in deep sea samples, these deoxysugars are probably not chemically bound to humic substances. Due to the high diagenetic degree of DOM in the deep sea samples, the glucose proportion was lower in the original sample but increased during XAD-fractionation. The proportions of mannose and xylose increased similar to glucose. The proportions of the deoxysugars and arabinose were very low in the XAD-fraction compared to the original sample. The THNS composition of ultrafiltrated surface and deep sea waters of the Pacific and Atlantic Oceans (Sargasso Sea) were similar, despite the regional differences in the oceanic regime (Figure 36b, c) (Skoog and Benner, 1997; McCarthy et al., 1996). Most evident in deep waters, the proportion of glucose decreased by ultrafiltration while it increased by XAD. The uniformity of their carbohydrate composition led the authors to the assumption that carbohydrate composition is similar in all size classes, which is in contrast to this study. The significant differences in THNS composition of total and XAD-

extractable DOM are evidence that carbohydrates are bound to different macromolecules of different size and polarity and not homogenously distributed. For example, deoxysugars abundant in DOM are considered to be released from algal cell wall components by bacterial destruction (e.g., Haug and Myklestad, 1976). Their nearly exclusive abundance in surface waters and in the ice sample may suggest that cell wall material contributes to the DOM fractions. With ongoing degradation, the deoxysugars were removed from the humic substances, which seems to be similar for arabinose, while mannose exhibited an opposite behaviour.

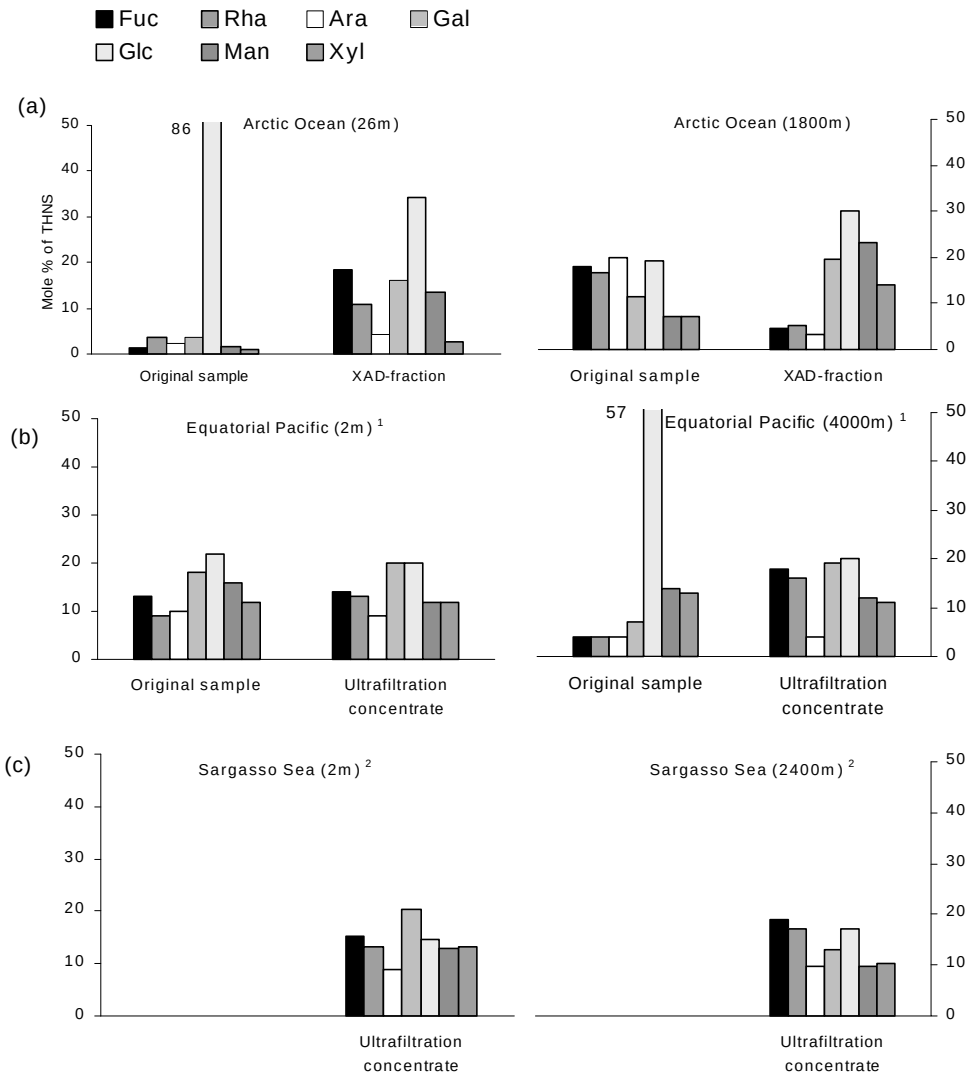


Figure 36: Comparison of changes in THNS composition of surface and deep water samples and fractionation by XAD and ultrafiltration;
[1]: Skoog and Benner (1997), [2]: McCarthy et al. (1996).

Surface water of Pacific origin, sampled at St. 17, showed a deviating THNS composition compared to the other surface waters (Figure 25). XAD-extraction efficiencies for DOC and carbohydrates were much higher and similar to deep waters,

which is probably due to the history of this water mass. High amounts of non-refractory galactose were detected, which may derive from the Bering Sea (Sakugawa and Handa, 1985).

The differences in the THNS composition between surface and deep sea samples and the changes in refractory carbohydrates composition were attributed to microbial modification processes. With respect to the radiocarbon age of up to 6000 years of deep sea DOM (Williams and Druffel, 1987), even slow chemical processes may account for the THNS composition. For example, Mopper et al. (1980) assumed that fructose might be an epimerization product of glucose. This may explain high amounts of fructose in the samples of Arctic deep waters and water of Pacific origin.

In this study, microbial degradation processes were not studied. Bussmann (1999) showed that the humic substances, which were isolated in this study, were accessible for microbial utilization, although the surface humic substances were of significantly better bacterial food quality than the deep sea humic substances. It was also found, that the bioavailability of surface humics showed nearly the same quality as a bacterial carbon source than unfractionated seawater DOM. Amon et al. (2001) conducted a microbial respiration experiment with material of the Arctic ice floe (St. 56E) and monitored the changes in THNS composition. After one week of microbial respiration, the THNS composition of DOM extracted with XAD from the Arctic ice floe (St. 56E) was very similar to that of the original sample (Figure 37) (Amon et al., 2001). Compared to the original, fresh sample, the proportions of deoxysugars and arabinose increased, whereas glucose decreased. This is further evidence for the selectivity of XAD for refractory compounds and showed that the generation of humic substances may directly be coupled with microbial activity. However, the mannose and xylose proportions of fractionated THNS were still similar to the total THNS composition, whereas during degradation these proportions increased by a factor of two. Again, the decrease of glucose indicates a higher diagenetic degree, which further supports the glucose-based diagenetic sequence of DOM.

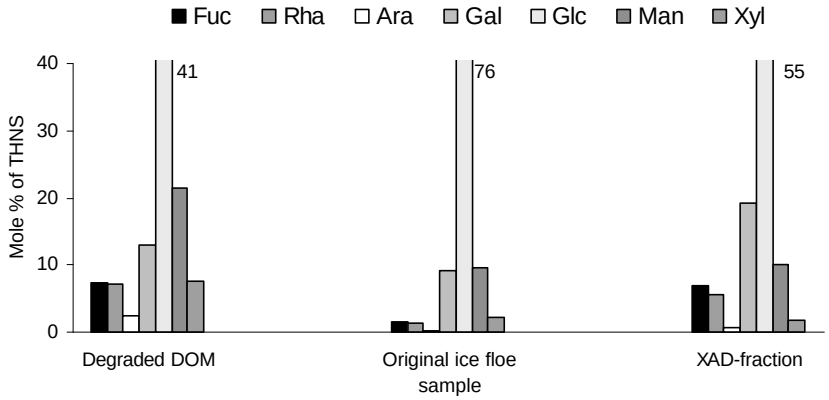


Figure 37: Comparison of changes in THNS composition of ice floe DOM during microbial degradation (Amon et al., 2001) and XAD-fractionation.

Since the proportions of the two deoxysugars, fucose and rhamnose, of THNS increased with depth in the total sample, their abundance may be used as an indicator of microbial activity. On the other hand, their abundance in fractionated matter decreased significantly with depth. Furthermore, the proportions of arabinose and xylose of fractionated DOM increased with depth. For quantitative assessment of the diagenetic degree of marine humic substances, so far, the extraction efficiencies of total carbon and carbohydrates can be used, as they lead to higher values with greater depth. Additionally, based on this study, proportions of individual neutral sugars can be used for the same purpose. The ratio between the proportions of fucose plus rhamnose and the sum of arabinose plus xylose (Fuc+Rha):(Ara+Xyl) in the XAD-fractionated matter was calculated. In Figure 38a, the (Fuc+Rha):(Ara+Xyl) ratios of the extracted carbohydrates are plotted versus the DOC extraction efficiencies. Samples with high extraction efficiencies, e.g., the deep water samples, exhibited low (Fuc+Rha):(Ara+Xyl) ratios, while the ice floe and surface water samples with the lowest extraction efficiencies exhibited the highest ratios. As shown, the THNS composition of the sample of St. 17, the water of Pacific origin, partly resembles the composition of deep water samples. This is also reflected by its (Fuc+Rha):(Ara+Xyl) ratio of the extracted THNS, which had the lowest value of all surface samples. Hence, the humic substances of the Arctic Ocean can be well characterized by their degree of degradation by using the (Fuc+Rha):-(Ara+Xyl) ratio.

The X-axis intercept of the line fit is between 60 and 70% of extraction efficiency, which is in good agreement with extraction efficiencies of riverine samples (e.g., Aiken et al., 1979). Additionally, in humic substances of rivers, the (Fuc+Rha):(Ara+Xyl) ratio is mostly below one (e.g., Watt et al., 1996; Frimmel, 1998). The composition of THNS of deep sea humic substances seems thus to be comparable to the composition of terrestrial humic substances. In addition, the ratio of the proportions of mannose plus xylose to arabinose plus xylose has been used for the characterization of terrestrial humic substances (Guggenberger and Zech, 1984; Watt et al., 1996; Frimmel, 1998). For instance, freshly produced humic substances of an effluent of a sewage treatment plant, isolated with XAD-extraction, could be distinguished from humic substances of groundwater. The latter had low values of both neutral sugar ratios, and the humic substances of the sewage treatment effluent much higher ratios (Figure 38b).

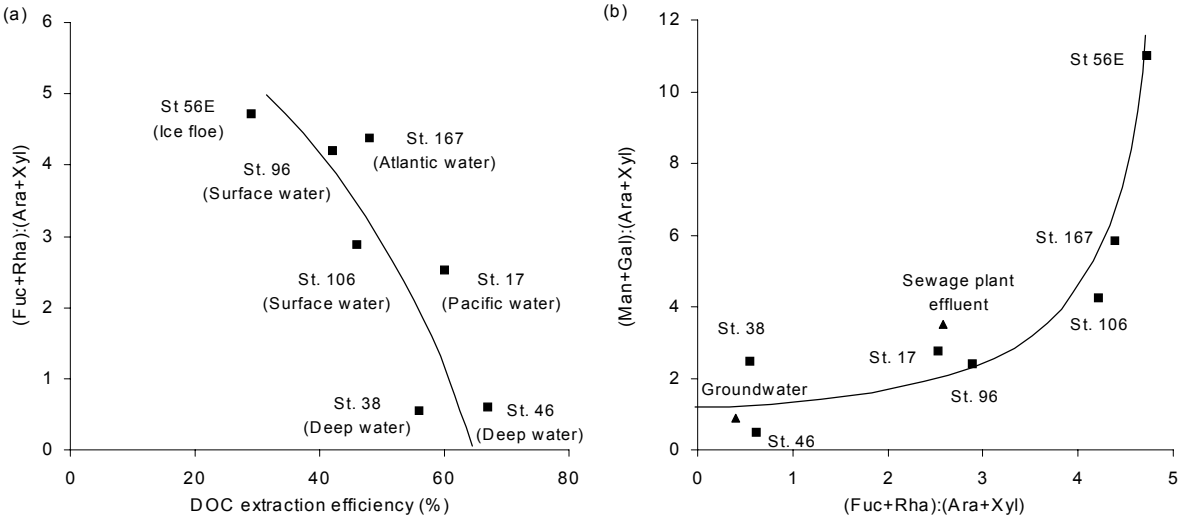


Figure 38: (a) DOC extraction efficiency versus (Fuc+Rha):(Ara+Xyl) ratio and (b) (Fuc+Rha):(Ara+Xyl) versus (Man+Gal):(Ara+Xyl) ratios of the XAD-fractionated DOM; data of the sewage treatment plant and groundwater from Frimmel (1998).

Using these neutral sugar ratios, also the humic substances of the Arctic Ocean can be characterized. The deep sea samples low (Fuc+Rha):(Ara+Xyl) ratios, and in surface samples, both ratios increased. The ratios were highest in the XAD-extracted DOM of the ice floe sample. This suggests that the freshest humic matter was found here.

The (Fuc+Rha):(Ara+Xyl) ratio of the marine samples was deduced from changes of neutral sugar proportions to THNS with depth due to bacterial degradation. The (Man+Gal):(Ara+Xyl) ratio was introduced in limnic studies to distinguish between woody and non-woody plants. It is obvious, that this differentiation cannot be used for marine humic substances, since Hedges et al. (1992b) found that terrestrial humic matter is rapidly removed from seawater. In marine environments, mannose and galactose have been observed to derive preferentially from phytoplankton, since they are components of their cell walls and extracellular polymers. However, it was not possible to attribute them clearly to phytoplankton. These neutral sugar ratios were only a suitable tool for the characterization of marine humic substances. If calculated for seawater samples, these ratios were widely variable without clearly visible trends, whereas they are very similar for ultrafiltration fractions.

The composition of neutral sugars in marine samples is a valuable tool for the characterization of DOM. The diagenetic degree of THNS was determined by the proportions of glucose, fucose, rhamnose, and mannose. Although labile polysaccharides account for the majority of carbohydrates, considerable amounts, about 10% of the total carbohydrates of freshly produced DOM, were found as carbohydrates in the humic matter fraction. Most of these carbohydrates exhibited properties clearly different from polysaccharides. Decreasing proportions of individual sugars with increasing degradation, e.g., fucose, rhamnose, and arabinose, may be attributed to preferential utilization, while

other sugars, e.g., mannose and xylose, are retained. Thus, to a certain amount, selective preservation of individual sugars is likely.

On the basis of the minimum concentrations of each neutral sugar in all samples, a "background level" of THNS for seawater can be determined, accounting for about 550 nM C in total (Figure 39a). From this minimum concentrations, 64 nM C (12% of the total) are XAD-extractable (Figure 39b). The composition of the individual carbohydrates reflects well the diagenetic sequence of glucose, and shows also the increase in fucose and arabinose proportions in XAD-fractionated matter. However, both background levels differed from the composition of individual seawater samples. Even deep water samples and their fractionated organic matter exhibited different neutral sugar compositions. Only the concentrations of fucose and xylose of deep waters were identical to the background level.

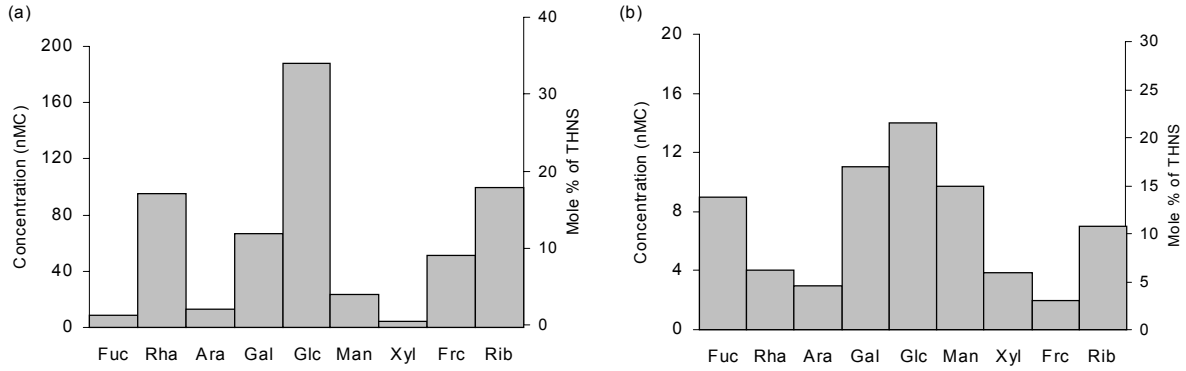


Figure 39: "Background levels" of neutral sugars, i.e., minimum concentration of each component, and their composition
(a) total THNS and
(b) XAD-fractionated THNS.

In summary, carbohydrates play a central role in biogeochemical cycles, due to their ubiquitous abundance in marine environments. In the investigated samples, their contribution to DOC ranged from 1%, measured in deep water, to 20% in the Kara Sea. These results confirmed that carbohydrates are the largest pool of DOM, which can be chemically identified. Their proportions were much higher than those of amino acids in the same polar regions, which ranged from 1.4 to 2.6% of DOC (Fitznar, 1999). Therefore, individual sugars are an important and valuable tool for the characterization of DOM. With the analytical techniques established in this study, a direct and sensitive determination of fucose, rhamnose, arabinose, galactose, glucose, mannose, xylose, fructose, and ribose in freshwater and seawater samples has been achieved.

With the comprehensive and detailed determination of the concentrations of total and individual carbohydrates, samples from terrestrial sources in rivers of northern Russia, inshore and offshore regions of the Arctic Ocean, and deep waters were characterized. During the transition from the Russian estuaries to the inshore stations the carbohydrate concentrations rapidly decreased and the compositions changed. A still considerable

terrestrial influence on DOM was observed, similar to other studies (e.g., Olsson and Anderson, 1997). The contribution of terrestrial DOM might be the reason for differences of DOM in the deep waters, where with increasing distance from the Eurasian shelves, the carbohydrate concentrations and proportions to DOC decreased continuously from the Nansen to the Makarov Basin. On the contrary, the freshwater mixing behaviour of DOC and THNS was non-conservative. On basis of a DOC surplus, phytoplankton production can be assumed. For carbohydrates, formation and depletion has to be considered, so that the exact mechanisms are still unknown. This is in contrast to results from other studies, e.g., Kattner et al. (1999), who observed conservative mixing in the vicinal Laptev Sea.

In Figure 40, a summary of mean concentrations and proportions of individual sugars in the different environments is presented. The highest overall concentrations were found in the rivers, in which the rather uniform neutral sugar composition was characteristic.

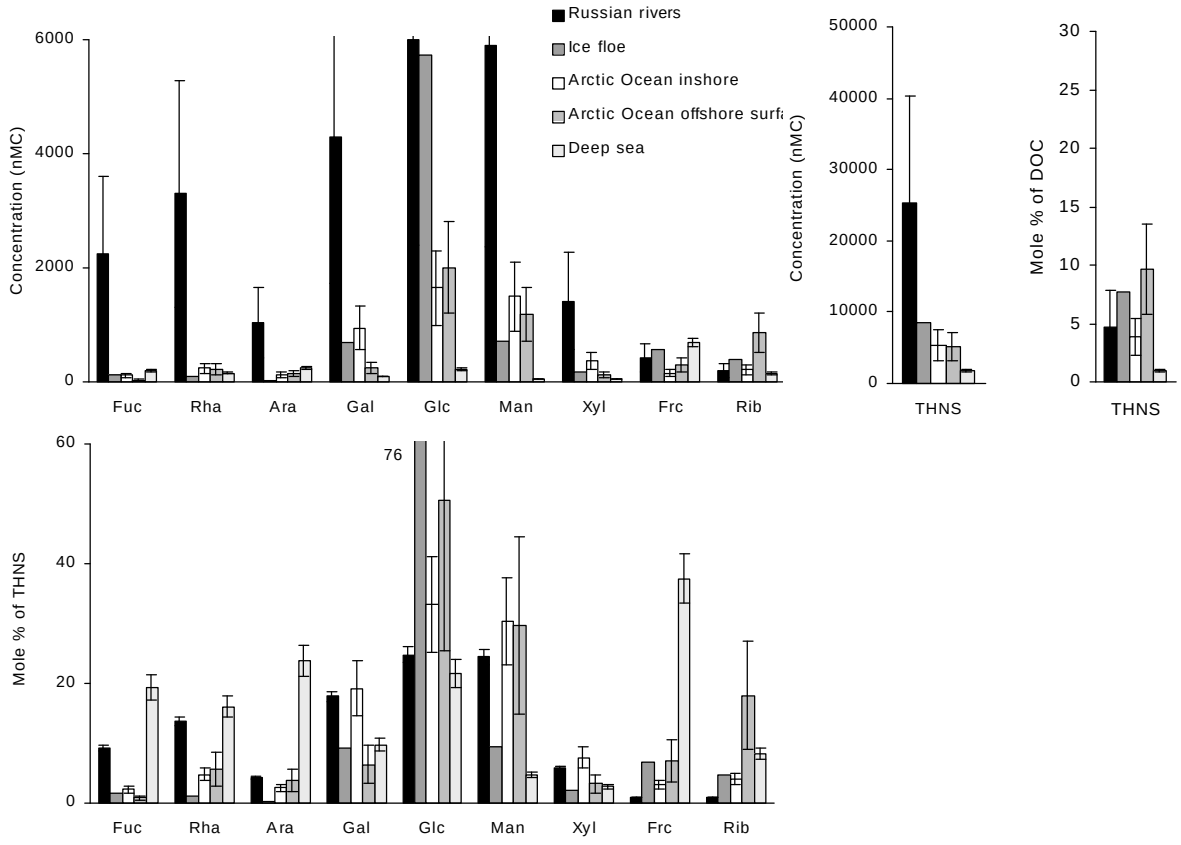


Figure 40: Summary of concentrations of individual neutral sugars, total carbohydrates, and their proportion of DOC of the various polar regions.

Their sugar composition was different from the marine environment, which was clearly attributed to terrestrial and marine phytoplankton sources. For DOM of Arctic ice and surface waters, the high THNS proportion of DOC and the also high ribose proportion of THNS were striking. In contrast, aged and degraded DOM exhibited lowest THNS

concentrations and proportions to DOC, while the proportions of the deoxysugars, arabinose, and fructose to THNS were enhanced.

The carbohydrate composition of refractory DOM, the marine humic substances, allowed conclusions about the chemical association of the neutral sugars with humic substances. More than half of these sugars in the XAD-fractions, which made up to 60% of total carbohydrates in deep waters, were incorporated into neutral molecules of a size of less than 1000 Da. These sugars exhibited chemical properties and compositions clearly different from polysaccharides produced by phytoplankton. This structural composition, the ”secondary structure”, in which the sugars are integrated, demands a more detailed study of the chemical nature, which was not further possible in this thesis. For this purpose, a molecular size class separation may be suitable, e.g., the method described by Huber and Frimmel (1994), which separates the multiplicity of organic compounds in both fractionated and total seawater samples by their hydrodynamic size. Subsequent non-destructive determination of low-molecular weight compounds could be done by mass-spectrometric analysis techniques (e.g., Boon et al., 1998; Kazmaier et al., 1998) in order to obtain the molecular composition. Due to the generally low amount of DOM in seawater, these techniques are a great challenge.

6 Literature

Aagaard, K. and Carmack, E.C., 1989. The Role of Sea Ice and Other Fresh Water in the Arctic Circulation. *J. Geophysical Res.* **94**, 14485-14498.

Aagaard, K. and Carmack, E.C., 1994. The Arctic Ocean and Climate: A Perspective. In: *Geophysial Monograph 85*, AGU, Washington, 5 pp.

Aagaard, K., Swift, J.H., and Carmack, E.C., 1995. Thermohaline circulation in the Arctic Mediterranean Seas. *J. Geophys. Res.* **90**.

Aiken, G.R., Thurman, E.M., Malcolm, R.L., and Walton, H.F., 1979. Comparison of XAD Macroporous Resins for the Concentration of Fulvic Acid from Aqueous Solution. *Anal. Chem.* **51**, 1799-1803.

Amon, R.M.W. and Benner, R., 1994. Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. *Nature* **369**, 549-552.

Amon, R.M.W., Fitznar, H.P., and Benner, R., 2001. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. *Limnol. Oceangr.* **46**, 287-297.

Amon, R.M.W. and Spitzy, A., 1999. Distribution of dissolved organic carbon during estuarine mixing in the southern Kara Sea. *Ber. Polarforschung* **300**, 102-109.

Anderson, L.G. et al., 1999. The Effect of the Siberian Tundra on the Environment of the Shelf Seas and the Arctic Ocean. *Ambio* **28(3)**, 270-280.

Barber, R.T., 1968. Dissolved organic matter from deep sea waters resists microbial oxidation. *Nature* **220**, 274-275.

Barker, S.A. and Somers, P.J., 1970. Bacterial and Fungal Polysaccharides. In: *The Carbohydrates: Chemistry and Biochemistry*, Academic Press, New York.

Bauch, D., 1995. Die Verteilung von delta 18-O im Arktischen Ozean: Implikationen für die Süßwasserbilanz der Halokline und die Quellen des Tiefen- und Bodenwassers. *Ber. Polarforschung* **159**.

Bauer, J.E., Williams, P.M., and Druffel, E.R.M., 1992. 14-C activity of dissolved organic carbon fractions in the north-central Pacific and Sargasso Sea. *Nature* **357**, 667-670.

Benner, R., Biddanda, B., Black, B., and McCarthy, M., 1997. Abundance, size distribution, and stable carbon and nitrogen isotopic compositions of marine organic matter isolated by tangential-flow ultrafiltration. *Mar. Chem.* **57**, 243-263.

Benner, R., Pakulski, D., McCarthy, M., Hedges, J.I., and Hatcher, P.G., 1992. Bulk Chemical Characteristics of Dissolved Organic Matter in the Ocean. *Science* **255**, 1561-1564.

Benner, R. and Strom, M., 1993. A critical evaluation of the analytical blank associated with DOC measurements by high-temperature catalytic oxidation. *Mar. Chem.* **41**, 153-160.

Benner, R.H., 1998. Cycling of Dissolved Organic Matter in the Ocean. In: H. Tranvik (Editor), Aquatic Humic Substances. Springer Verlag, Berlin, Heidelberg, 317-331.

Berner, E.K. and Berner, R.A., 1987. The Global Water Cycle, Prentice-Hall, Englewood Cliffs, NJ.

Biersmith, A. and Benner, R., 1998. Carbohydrates in phytoplankton and freshly produced dissolved organic matter. *Mar. Chem.* **63**, 131-144.

Blindheim, J., 1989. Cascading of Barents Sea bottom water into the Norwegian Sea. *Rapp. P.-V. Reun. Cons. Int. Explor. Mer.* **188**, 49-58.

Boon, J.J., Klap, V.A., and Eglinton, T.I., 1998. Molecular characterization of microgram amounts of oceanic colloidal organic matter by direct temperature-resolved ammonia chemical ionization mass spectrometry. *Org. Geochem.* **29**, 1051-1061.

Borch, N.H. and Kirchman, D.L., 1997. Concentration and composition of dissolved combined neutral sugars (polysaccharides) in seawater determined by HPLC-PAD. *Mar. Chem.* **57**, 85-95.

Børsheim, K.Y., Myklestad, S.M., and Sneli, J.-A., 1999. Monthly profiles of DOC, mono- and polysaccharides at two locations in the Tronheimsfjord (Norway) during two years. *Mar. Chem.* **63**, 255-272.

Burdige, D.J., Skoog, A., and Gardner, K., 2000. Dissolved and particulate carbohydrates in contrasting marine sediments. *Geochim. Cosmochim. Acta* **64**, 1029-1041.

Bussmann, I., 1999. Bacterial utilization of humic substances from the Arctic Ocean. *Aquat. Microb. Ecol.* **19**, 37-45.

Bussmann, I. and Kattner, G., 2000. Distribution of dissolved organic carbon in the central Arctic Ocean: the influence of physical and biological properties. *J. Mar. Syst.* **27**, 209-219.

Cauwet, 1978. Organic chemistry of sea water particulates concepts and developments. *Oceanol. Acta* **1**, 99-105.

Cauwet, G. and Sidorov, I., 1996. The biogeochemistry of Lena River: organic carbon and nutrient distribution. *Mar. Chem.* **53**, 211-227.

Cavalieri, D.J. and Martin, S., 1994. The contribution of Alaskan, Siberian, and Canadian coastal polynyas to the cold halocline layer of the Arctic Ocean. *J. Geophys. Res.* **99**, 18343-18362.

Chavez, F.P. and Barber, R.T., 1987. An estimate of new production in the equatorial Pacific. *Deep-Sea Res.* **37**, 1733-1752.

Cowie, G.L. and Hedges, J.I., 1984a. Carbohydrate sources in a coastal marine environment. *Geochim. Cosmochim. Acta* **48**, 2075-2087.

Cowie, G.L. and Hedges, J.I., 1984b. Determination of Neutral Sugars in Plankton, Sediments, and Wood by Capillary Gas Chromatography of Equilibrated Isomeric Mixtures. *Anal. Chem.* **56**, 497-504.

Cowie, G.L. and Hedges, J.I., 1994. Biochemical indicators of diagenetic alteration in natural organic matter mixtures. *Nature* **369**, 304-307.

Decho, A.W. and Lopez, G.R., 1993. Exopolymer microenvironments of microbial flora: Multiple and interactive effect on trophic relationships. *Limnol. Oceanogr.* **38**, 1633-1645.

DIONEX, TN20. Analysis of Carbohydrates by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection, DIONEX Corp., Sunnyvale, CA.

DIONEX, TN21. Optimal Settings for Pulsed Amperometric Detection of Carbohydrates using DIONEX Pulsed Electrochemical and Amperometric Detectors, DIONEX Corp., Sunnyvale, CA.

Dixon, R.K., Brown, S., Houghton, R.A., Solomon, A.M., Trexler, M.C., and Wisniewski, J., 1994. Carbon pools and flux of global forest ecosystems. *Science* **263**, 185-190.

Druffel, E.R.M., Williams, P.M., Bauer, J.E., and Ertel, J.R., 1992. Cycling of dissolved and particulate organic matter in the open ocean. *J. Geophys. Res.* **97**, 15639-15659.

Eberlein, K. and Hammer, K.D., 1980. Automatic Determination of Total Carbohydrates in Sea Water. *Fresenius Z. Anal. Chem.* **301**, 17-19.

Eberlein, K., Leal, M.T., Hammer, K.D., and Hickel, W., 1985. Dissolved organic substances during a Phaeocystis pouchetii bloom in the German Bight (North Sea). *Mar. Biol.* **89**, 311-316.

Eberlein, K. and Schütt, M., 1986. Automatic methods for the determination of total dissolved and particulate carbohydrates in the marine environment. *Fresenius Z. Anal. Chem.* **323**, 47-49.

Engbrodt, R., 1997. Kohlenhydrate in polaren Meeresgebieten: Über ihre Verteilung in Wasser, Eis und Huminstoffen. Diploma Thesis, RWTH Aachen, 64 pp.

Ernst, W., 1983. Organische Spurenstoffe im Meer. *Nachr. Chem. Tech. Lab* **31**, 880-888.

Fitznar, H.P., 1999. D-Aminosäuren als Tracer für biogeochemische Prozesse im Fluß-Schelf-Ozean-System der Arktis. *Ber. Polarforschung* **334**.

Frimmel, F.H., 1998. Characterization of natural organic matter as major constituents in aquatic systems. *J. Contaminant Hydrol.* **35**, 201-216.

Fuhrmann, J.A. and Suttle, C.A., 1993. Viruses in marine planktonic systems. *Oceannography* **6**, 51-63.

Gordeev, V.V., Martin, J.M., Sidorov, I.S., and Sidorova, M.V., 1996. A reassesment of the Eurasian river input of water, sediment, major elements, and nutrients to the Arctic Ocean. *Amer. J. Sci.* **296**, 664-691.

Gremm, T.J. and Kaplan, L.A., 1997. Dissolved carbohydrates in streamwater determined by HPLC and pulsed amperometric detection. *Limnol. Oceanogr.* **42**, 385-393.

Guggenberger, G. and Zech, W., 1984. Composition of dissolved carbohydrates and lignin-degradation products in two coniferous forests. *Soil Biol. Biochem.* **26**, 19-27.

Harvey, G.R., Boran, D.A., Chesal, L.A., and Tokar, J.M., 1983. The structure of marine fulvic and humic acids. *Mar. Chem.* **12**, 119-132.

Haug, A. and Myklestad, S., 1976. Polysaccharides of marine diatoms with special references to Chaetoceros Species. *Mar. Biol.* **34**, 217-222.

Hedges, J.I., 1992a. Global biogeochemical cycles: progress and problems. *Mar. Chem.* **39**.

Hedges, J.I., Cowie, G.L., Richey, J.E., Quay, P.D., Benner, R., Strom, M., and Forsberg, B.R., 1994. Origins and processing of organic matter in the Amazon River as indicated by carbohydrates and amino acids. *Limnol. Oceanogr.* **39(4)**, 743-761.

Hedges, J.I., Hatcher, P.G., Ertel, J.R., and Meyers-Schulte, K.J., 1992b. A comparison of dissolved humic substances from seawater with Amazon River counterparts by 13-C-NMR spectrometry. *Geochim. Cosmochim. Acta* **56**, 1753-1757.

Hellebust, J.A., 1965. Excretion of some organic compounds by marine phytoplankton. *Limnol. Oceanogr.* **10**, 192-206.

Hernes, P.J., Hedges, J.I., Peterson, M.L., Wakeham, S.G., and Lee, C., 1996. Neutral carbohydrate geochemistry of particulate material in the central equatorial Pacific. *Deep-Sea Res. II* **43(4-6)**, 1181-1204.

Hirajama, H., 1974. Fluorimetric Determination of Carbohydrates in Sea Water. *Anal. Chim. Acta* **70**, 141-148.

Huber, S.A. and Frimmel, F.H., 1994. Direct Gel Chromatographic Charaterization and Quantification of Marine Dissolved Organic Carbon Using High-Sensitivity DOC detection. *Environ. Sci. Technol.* **28**, 1194-1197.

Ittekkot, V. and Arain, R., 1986. Nature of particulate organic matter in the river Indus, Pakistan. *Geochim. Cosmochim. Acta* **50**, 1643-1653.

Ittekkot, V., Brockmann, U., Michaelis, W., and Degens, E.T., 1981. Dissolved Free and Combined Carbohydrates During a Phytoplankton Bloom in the Northern North Sea. *Mar. Ecol. Prog. Ser.* **4**, 299-305.

Ittekkot, V., Degens, E.T., and Brockmann, U., 1982. Monosaccharide composition of acid-hydrolyzable carbohydrates in particulate matter during a plankton bloom. *Limnol. Oceanogr.* **27**, 770-776.

Ittekkot, V., Deuser, W.G., and Degens, E.T., 1984. Seasonality of the fluxes of sugars, amino acids, and amino sugars to the deep ocean: Sargasso Sea. *Deep-Sea Res.* **31**, 1057-1069.

Johnson, D.C. and LaCourse, W.R., 1990. Liquid Chromatography with Pulsed Electrochemical Detection at Gold and Platinum Electrodes. *Anal. Chem.* **62**, 589A-597A.

Johnson, K.M. and Sieburth, J.M., 1977. Dissolved carbohydrates in seawater. *Mar. Chem.* **5**, 1-13.

Jones, A.E.P., Anderson, L.G., and Swift, J.H., 1998. Distribution of Atlantic and Pacific waters in the upper Arctic Ocean: Implications for circulation. *Geophysical Research Letters* **25(6)**, 765-768.

Jones, E.P., Rudels, B., and Andersson, L.G., 1995. Deep waters of the Arctic Ocean: origins and circulation. *Deep-Sea Res. I* **42**, 737-760.

Josefsson, B., Uppström, L., and Östling, G., 1972. Automatic spectrophotometric procedures for the determination of the total amount of dissolved carbohydrates in sea water. *Deep-Sea Res.* **19**, 385-395.

Kaiser, K. and Benner, R., 2000. Determination of Amino Sugars in Environmental Samples with High Salt Content by High-Performance Anion-Exchmage Chromatography and Pulsed Amperometric Detection. *Anal. Chem.* **72**, 2566-2572.

Kattner, G., 1999. Storage of dissolved inorganic nutrients in seawater: poisoning with mercuric chloride. *Mar. Chem.* **67**, 61-66.

Kattner, G., Lobbes, J.M., Fitznar, H.P., Engbrodt, R., Nöthig, E.-M., and Lara, R.J., 1999. Tracing dissolved organic substances and nutrients from the Lena River through Laptev Sea (Arctic). *Mar. Chem.* **65**, 25-39.

Kazmaier, T., Roth, S., Zapp, J., Harding, M., and Kuhn, R., 1998. Quantitative analysis of malto-oligosaccharides by MALDI-TOF mass spectrometry, capillary electrophoresis and anion exchange chromatography. *Fresenius Z. Anal. Chem.* **361**, 473-478.

Keil, R.J., Mayer, L.M., Quay, P.D., Richey, J.E., and Hedges, J.I., 1997. Loss of organic matter from riverine particles in deltas. *Geochim. Cosmochim. Acta* **61**, 1507-1511.

Lara, R.J., Rachold, V., Kattner, G., Hubberten, H.W., Guggenberger, G., Skoog, A., and Thomas, D.N., 1998. Dissolved organic matter and nutrients in the Lena River, Siberian Arctic: Characteristics and distribution. *Mar. Chem.* **59**, 301-305.

Lara, R.J. and Thomas, D.N., 1994. Isolation of Marine Dissolved Organic Matter: Evaluation of Sequential Combinations of XAD Resins 2,4, and 7. *Anal. Chem.* **66**, 2417-2419.

Ledl, F. and Schleicher, E., 1990. Die Maillard-Reaktion im menschlichen Körper - neue Ergebnisse zu Chemie, Biochemie und Medizin. *Angew. Chem.* **102**, 597-626.

Lobbes, J., 1998. Biogeochemische Tracer im Arktischen Ozean. PhD Thesis, Universität Bremen, 63 pp.

Lobbes, J.M., Fitznar, H.P., and Kattner, G., 2000. Biogeochemical characteristics of dissolved and particulate organic matter in Russian rivers entering the Arctic Ocean. *Geochim. Cosmochim. Acta* **64**, 2973-2983.

Malcolm, R.L., 1989. Factors to be considered in the isolation and characterization of aquatic humic substances. In: B. Allard, H. Borén and A. Grimvall (Editors), Humic substances in the aquatic and terrestrial environment. Springer Verlag, Berlin, 9-36.

Malcolm, R.L., 1990. The uniqueness of humic substances in each of soil, stream, and marine environments. *Anal. Chim. Acta* **232**, 19-30.

Malcolm, R.L. and MacCarthy, P., 1992. Quantitative evaluation of XAD-8 and XAD-4 resins used in tandem for removing organic solutes from water. *Environment International* **18**, 597-607.

McCarthy, M., Hedges, J.I., and Benner, R., 1996. Major biochemical composition of dissolved high molecular weight organic matter in seawater. *Mar. Chem.* **55**, 281-297.

McCarthy, M.D., Hedges, J.I., and Benner, R., 1993. The chemical composition of dissolved organic matter in seawater. *Chem. Geol.* **197**, 503-507.

Mopper, K., 1977. Sugars and uronic acids in sediment and water from the Black Sea and North Sea with emphasis on analytical techniques. *Mar. Chem.* **5**, 585-603.

Mopper, K., 1978a. Improved Chromatographic Separations on Anion-Exchange Resins I. Partition Chromatography of Sugars in Ethanol. *Anal. Biochem.* **85**, 528-532.

Mopper, K., 1978b. Improved Chromatographic Separations on Anion-Exchange Resins III. Sugars in Borate Medium. *Anal. Biochem.* **87**, 162-168.

Mopper, K., Dawson, R., Liebezeit, G., and Ittekkot, V., 1980. The monosaccharide spectra of natural waters. *Mar. Chem.* **10**, 55-66.

Mopper, K., Schultz, C.A., Chevolot, L., Germain, C., Revuelta, R., and Dawson, R., 1992. Determination of Sugars in Unconcentrated Seawater and Other Natural Waters by Liquid Chromatography and Pulsed Amperometric Detection. *Environ. Sci. Technol.* **26**, 133-138.

Mopper, K., Zhou, J., Ramana, K.S., Passow, U., Dam, H.G., and Drapeau, D.T., 1995. The role of surface-active carbohydrates in the flocculation of a diatom bloom in a mesocosm. *Deep-Sea Res. II* **42**, 47-73.

Myklestad, S., Haug, A., and Larsen, B., 1972. Production of carbohydrates by the marine diatom Chaetoceros affinis var. willei (gran) Husted. II. Preliminary investigation of the extracellular polysaccharide. *J. exp. mar. Biol. Ecol.* **9**, 137-144.

Olsson, K. and Anderson, L.G., 1997. Input and biogeochemival transformation of dissolved carbon in the Siberian shelf seas. *Cont. Shelf Res.* **17**, 819-833.

Opsahl, S. and Benner, R., 1997. Distribution and cycling of terrigenous dissolved organic matter in the ocean. *Nature* **386**, 480-482.

Opsahl, S. and Benner, R., 1999. Characterization of carbohydrates during early diagenesis of five vascular plant tissues. *Org. Geochem.* **30**, 83-94.

Pakulski, J.D. and Benner, R., 1992. An inproved method for the hydrolysis and MBTH analysis of dissolved and particulate carbohydrates in seawater. *Mar. Chem.* **40**, 143-160.

Pakulski, J.D. and Benner, R., 1994. Abundance and distribution of carbohydrates in the ocean. *Limnol. Oceanogr.* **39(4)**, 930-940.

Pavlov, V.K. and Pfirman, S.L., 1995. Hydrodynamic structure and variability of the Kara Sea: implications for pollutant distribution. *Deep-Sea Res. II* **42**, 1369-1390.

Percival, E., 1979. Algal carbohydrates. In: W. Pigman and D. Horton (Editors), The Carbohydrates: Chemistry and Biochemistry. Academic Press, New York.

Pütter, A., 1909. Die Ernährung der Wassertiere und der Stoffhaushalt der Gewässer, Fischer, Jena.

Rich, J., Gosselin, M., Sherr, E., Sherr, B., and Kirchman, D.L., 1997. High bacterial production, uptake and concentrations of dissolved organic matter in the Central Arctic Ocean. *Deep-Sea Res. II* **44**, 1645-1663.

Romankevich, E.A., 1984. Geochemistry of organic matter in the ocean, Springer, Berlin, Heidelberg, New York.

Rudels, B., 1989. The formation of Polar Surface Water, the ice export and the exchange through the Fram Strait. *Prog. Oceanogr.* **22**, 205-249.

Rudels, B., Jones, E.P., Anderson, L.G., and Kattner, G., 1994. On the intermediate depth waters of the Arctic Ocean. In: O.M. Johannessen, R.D. Muench and J.E. Overland (Editors), The Role of the Polar Oceans in Shaping the Global Climate. *Geophysical Monographs 85*. AGU, Washington, 33-46.

Rudels, B., Muench, R.D., Gunn, J., Schauer, U., and Friedrich, H.J., 2000. Evolution of the Arctic Ocean boundary current north of the Siberian shelves. *J. Mar. Syst.* **25**, 77-99.

Sakugawa, H. and Handa, N., 1985. Chemical studies on dissolved carbohydrates in the water samples collected from the North Pacific and Bering Sea. *Oceanol. Acta* **8**, 185-195.

Schauer, U., Rudels, B., Jones, E.P., Anderson, L.G., Muench, R.D., Björk, G., Swift, J.H., Ivanov, V., and Larsson, A.M., submitted. Confluence and redistribution of Atlantic water in the Nansen, Amundsen, and Makarov Basins. *Annales Geophys.*

Schlosser, P., Swift, J.H., Lewis, D., and Pfirman, S.L., 1995. The role of the large-scale Arctic Ocean circulation in the transport of contaminants. *Deep-Sea Res. II* **42(6)**, 1341-1367.

Senior, W. and Chevolot, L., 1991. Studies of dissolved carbohydrates (or carbohydrate-like substances) in an estuarine environment. *Mar. Chem.* **32**, 19-35.

Sharp, J.H., 1997. Marine dissolved organic carbon: Are the older values correct? *Mar. Chem.* **56**, 265-277.

Siegenthaler, U. and Sarmiento, J.L., 1993. Atmospheric carbon dioxide and the ocean. *Nature* **365**.

Skoog, A. and Benner, R., 1997. Aldoses in various size fractions of marine organic matter: Implications for carbon cycling. *Limnol. Oceanogr.* **42**, 1803-1813.

Skoog, A., Thomas, D.N., R.J., L., and Richter, K.U., 1997. Methodological investigations on DOC determinations by the HTCO method. *Mar. Chem.* **56**, 39-44.

Snyder, L.R., Glaich, J.L., and Kirkland, J.J., 1988. High performance liquid chromatography - Methodology. In: *Practical HPLC method development*, Wiley-Interscience, New York, 17 pp.

Snyder, L.R. and Kirkland, J.J., 1979. An Introduction to Modern Liquid Chromatography, Wiley-Interscience, New York, Chapt. 2 pp.

Strasburger, E., 1983. Lehrbuch der Botanik, Gustav Fischer Verlag, Stuttgart, 1161 pp.

Strom, S.L., Benner, R., Ziegler, S., Dagg, M.J., 1997. Planktonic grazers are potentially important source of marine dissolved organic matter. *Limnol. Oceanogr.* **42(6)**, 1364-1374.

Sweet, M.S. and Perdue, E.M., 1982. Concentration and speciation of dissolved sugars in river water. *Environ. Sci. Technol.* **16**, 692-698.

Swift, J.H., Jones, E.P., Aagard, K., Carmack, E.C., Hingston, M., Macdonald, R.W., McLaughlin, F.A., and Perkin, R.G., 1997. Water of the Makarov and Canada Basins. *Deep-Sea Res. II* **44**, 1503-1529.

Telang, S.A., Pocklington, R., Naidu, A.S., Romankevich, E.A., Gitelson, I.I., and Gladyshev, M.I., 1991. Carbon and mineral transport in major north American, Russian Arctic, and Siberian Rivers: the Lawrence, the MacKenzie, the Yukon, the Arctic Alaskan Rivers, the Arctic Basin Rivers in the Soviet Union, and the Yenisei. In: E.T. Degens, S. Kempe and L.E. Richey (Editors), Biogeochemistry of major world rivers. *SCOPE Report 42*. John Wiley and Sons Ltd, Chichester, UK, 75-104.

Thurman, E.M. and Malcolm, R.L., 1981. Preparative Isolation of Aquatic Humic Substances. *Environ. Sci. Technol.* **15**, 463-466.

Times Atlas, 1997. *The Times Atlas of the World*, Harper Collins, London, UK.

Tranvik, L.J. (Editor), 1992. Rapid microbial production and degradation of humic-like substances in lake waters. In: *Microbial Ecology of Pelagic Environment*. Schweitzerbat'sche Verlagsbuchhandlung, Stuttgart, 43 - 50 pp.

Volk, C.J., Volk, C.B., and Kaplan, L.A., 1997. Chemical Composition of biodegradable dissolved organic matter in stream water. *Limnol. Oceanogr.* **42(1)**, 39-44.

Wangersky, P.J., 1972. The cycle of organic carbon in seawater. *Chimica* **26**, 559-564.

Watt, B.E., Malcolm, R.L., Hayes, M.H.B., Clark, N.W.E., and Chipman, J.K., 1996. Chemistry and potential mutagenicity of humic substances in waters from different watersheds in Britain and Ireland. *Wat. Res.* **30 (6)**, 1502-1516.

Wheeler, P.A., Watkins, J.M., and Hansing, R.L., 1997. Nutrients, organic carbon and organic nitrogen in the upper water column of the Arctic Ocean: implications for the sources of dissolved organic carbon. *Deep-Sea Res. II* **44**, 1571-1593.

Williams, P.M. and Druffel, E.R.M., 1987. Radiocarbon in dissolved organic matter in the central North Pacific Ocean. *Nature* **330**, 246-248.

7 Appendix

A1: SWEDARCTIC Tundra Expedition 1994 Russian Rivers

A2: SWEDARCTIC Tundra Expedition 1994 Russian Inshore Stations

A3: ARK XI/1 1995 Laptev Sea Stations Transect C

A4: ARK XII 1996 Central Arctic Ocean Transect 1

A5: ARK XII 1996 Central Arctic Ocean Transect 2

A6: ARK XIII/3 1997 Fram Strait and Greenland Sea Stations

A7: ANT XIV/3 Weddell Sea Ice Core

A8: Chromatographic Conditions and Detector Settings for the HPAEC-PAD Method

A1: SWEDARCTIC Tundra Expedition 1994
Russian Rivers:

St.		Vizjas	Vaskina	Velikaja	Moroyyakha	Yenisey	Olenek	Indigirka	Kolyma
Ident.		B	B	B	B	D-2B	116306 B	116357F1	116368-1C
Lat.°N		66.43	68.46	69.23	70.20	73.37	73.00	71.20	68.80
Long. °E		045.54	048.46	049.37	067.47	080.26	119.80	150.50	155.00
Tundra	(%)	50	100	100	100	5	50	50	5
Silicate	(µM)	5.20	1.01	6.58	1.15	12.58	23.35	0.48	
DOC	(µM)	912	550	404	409	711	850	404	387
C/N		62.9	39.4	43.6	57.2	69.1	47.5	33.4	
TDAA	(nM)	3855		4678	3699	3266	3546	2212	1822
Free neutral sugars									
Fuc	(nMC)	0	0	0	0	0	0	0	0
Rha	(nMC)	18	198	0	0	0	30	0	0
Ara	(nMC)	0	0	0	0	0	0	0	0
Gal	(nMC)	0	0	0	0	24	0	0	0
Glc	(nMC)	30	456	0	0	36	144	0	0
Man	(nMC)	0	0	0	0	66	0	0	0
Xyl	(nMC)	0	0	0	0	0	0	0	0
Frc	(nMC)	18	480	0	0	0	90	0	0
Rib	(nMC)	0	160	0	0	0	60	95	75
TFNS	(nMC)	66	1294	0	0	126	324	95	75
Combined neutral sugars									
Fuc	(nMC)	2610	2502	1962	3120	2712	1842	2406	810
Rha	(nMC)	5004	4068	2922	4806	3546	2388	2040	1632
Ara	(nMC)	1585	1140	780	1865	1005	845	648	414
Gal	(nMC)	5634	6090	3990	7506	4704	2874	1974	1620
Glc	(nMC)	8172	8346	6210	10506	7848	3906	3636	2538
Man	(nMC)	7794	8364	5076	9228	7296	3474	3666	2298
Xyl	(nMC)	1910	1960	1370	2060	1470	1310	650	594
Frc	(nMC)	1092	120	156	1050	288	330	30	210
Rib	(nMC)	285	225	145	510	285	50	45	60
THNS	(nMC)	34086	32815	22611	40651	29154	17019	15095	10176
THNS	(%DOC)	3.7	6.0	5.6	9.9	4.1	2.0	3.7	2.6
Fuc	(%THNS)	8	8	9	8	9	11	16	8
Rha	(%THNS)	15	13	13	12	12	14	14	16
Ara	(%THNS)	5	4	3	5	4	5	4	4
Gal	(%THNS)	17	19	18	19	16	17	13	16
Glc	(%THNS)	25	26	28	27	27	23	24	26
Man	(%THNS)	24	26	23	24	26	21	24	23
Xyl	(%THNS)	6	6	6	5	5	8	4	6

A2: SWEDARCTIC Tundra Expedition 1994
Russian Inshore Stations:

St.		3 N Kolgujev	5 W Jamal	6B SE Belyj	X E Arktitjeskogo Instituta	9A SW Mys Tjeljuskina
Ident.		3-2B	5-2A	6B-2B	X-2B	9A-B
Depth	(m)	10	10	20	20	50
Lat.°N		68.59	70.14	73.10	75.06	77.20
Long. °E		050.17	066.17	072.25	085.17	100.44
Salinity	(PSU)	33.18	32.80	25.34	30.86	33.52
Silicate	(µM)	0.32	1.60	16.54	6.07	3.58
DOC	(µM)	84	164	211	214	117
C/N		16.8	33.0	21.0	21.4	9.7
TDAA	(nM)	554	496	498	881	332
Combined neutral sugars						
Fuc	(nMC)	6	72	96	84	312
Rha	(nMC)	546	258	162	174	48
Ara	(nMC)	255	105	110	40	115
Gal	(nMC)	1602	234	180	432	2292
Glc	(nMC)	1626	1518	1968	744	2358
Man	(nMC)	1182	972	1410	708	3228
Xyl	(nMC)	370	150	280	135	955
Frc	(nMC)	270	42	186	102	192
Rib	(nMC)	245	245	145	220	235
THNS	(nMC)	6102	3596	4537	2639	9735
THNS	(%DOC)	3.7	1.7	2.3	2.3	9.4
Fuc	(%THNS)	0	2	2	4	3
Rha	(%THNS)	10	8	4	8	1
Ara	(%THNS)	5	3	3	2	1
Gal	(%THNS)	29	7	4	19	25
Glc	(%THNS)	29	46	47	32	25
Man	(%THNS)	21	29	34	31	35
Xyl	(%THNS)	7	5	7	6	10

A3: ARK XI/1 1995
Laptev Sea Stations Transect C:

St.	Depth (m)	Lat. °N	Long. °E	Silicate (µM)	DOC (µM)	C/N	TDAA (nM)	TCHO (µMC)	Chl *a* (µg/l)	Temp (°C)	Salinity (PSU)	Ice (%)
16	5	76.00	129.97	6.19	151	25.6	541	10.8	0.07	2.648	28.647	0
	10			6.26	160	25.4	635	10.7	0.10	2.583	28.694	
17	5	76.89	129.99	5.48	152	22.0		7.4	0.05	2.428	28.717	0
	9			5.22	158	23.0	557	7.1	0.08	0.952	29.222	
	16			7.22	156	20.8		8.2	0.10	-1.364	31.303	
	21			7.54	143	20.5	511	6.4	1.12	-1.472	32.590	
	30			7.08	99	16.9		4.1	0.49	-1.520	33.127	
	38			6.60	91	16.3	425	3.8	0.32	-1.546	33.550	
	47			8.56	89	18.6		3.7	0.09	-1.411	33.850	
	58			9.97	87	18.5	444	4.1	0.07	-0.831	34.160	
18	5	77.60	130.01	4.73	148	21.5		8.5	0.23	1,561	28.915	0
	15			6.46	150	20.2		6.9	1.03	-1.552	31.959	
	20			6.80	130	19.0	485	6.0	0.70	-1.595	32.851	
	30			6.34	87	14.1		8.9	0.78	-1.613	33.738	
	50			4.93	86	16.6		2.6	0.05	-1.565	33.877	
	60			5.40			343	2.9	0.03			
	75			6.05				3.0	0.05	-0.926	34.199	
19	4	77.62	130.06		138			7.4	0.21	0.190	29.485	0
	10				140		560	6.9	0.48	-1.018	30.143	
	19				118		517	6.4	0.91	-1.506	31.327	
	30				77		469	2.7	0.50	-1.589	33.283	
	39				85		379	1.3	0.07	-1.619	33.723	
	50				82		378	1.0	0.04	-1.498	33.959	
	74				84		615	1.3	0.02	-0.986	34.203	
	174				81		345	0.7	0.04	-0.833	34.349	
	200				79		263	0.6	0.02	-0.840	34.351	
	232				86		196	0.9	0.02	-0.825	34.354	
20	10	77.70	130.05		131	18.4	425	5.9	0.27	-0.444	30.184	
	30			6.24	86	14.4		3.9	0.32	-1.598	32.324	
	40			5.35	72	13.3		3.5		-1.615	33.453	
	50			5.13	82		224	2.9	0.09	-1.604	33.802	
	75			5.28	230	46.1		2.5	0.05	-1.533	34.015	
	125			4.93				1.9	0.01	-1.252	34.276	
23	10	78.20	129.98	5.22	160	24.8	871	5.3	0.30	-0.234	29.404	0
	20			6.44	150	23.8	500	4.0	0.14	-1.369	30.678	
	30			5.05	134	23.7		2.8	0.05	-1.660	31.996	
	59			3.14	101	20.5	306	4.3	0.01	-1.833	33.671	
	74			3.27	94	19.7		3.7	0.01	-1.839	33.744	
	121			3.99	88	21.6	232	4.3	0.01	-1.181	34.274	
	400			5.61	50	14.5	238	3.2		1.239	34.891	
	499			5.99	80	24.5	489	3.6		0.792	34.886	
	798			6.27	53	14.0	248	3.4		-0.036	34.873	
	1000			6.78	82	21.1	322	3.9		-0.202	34.886	
	1250			7.80	77	23.9	289	4.0		-0.293	34.898	
	2325			11.53	72	23.9	227	4.4		-0.794	34.927	

A3 (cont.):

St.	Depth (m)	Lat. °N	Long. °E	Silicate (µM)	DOC (µM)	C/N	TDAA (nM)	TCHO (µMC)	Chl *a* (µg/l)	Temp (°C)	Salinity (PSU)	Ice (%)
24	11	79.04	131.41	6.89	131	18.0	489	6.5	0.25	-1.392	30.195	80
	50			3.60	84	16.0	307	3.6	0.01	-1.772	33.353	
	73			4.05	85	16.5		3.7	0.00	-1.767	33.723	
	99			4.17	65	14.8	258	2.3	0.00	-1.490	34.153	
	201			5.46	65		232	2.8	0.00	1.689	34.834	
	349			5.70	62		202	3.3		1.502	34.898	
	501			6.12	55		206	4.1		0.760	34.882	
	1005			7.39	60		214	3.5		-0.280	34.890	
47	11	80.88	131.06	4.20	105	21.8	454	6.4	0.41	-1.631	31.206	100
	21			4.21	111	24.6	434	5.0		-1.667	31.256	
	30			5.13	97	23.0		4.0	0.12	-1.729	32.384	
	51			3.26	78	21.1	330	4.2	0.02	-1.804	33.586	
	73			3.41	60	17.3		3.9	0.01	-1.730	33.889	
	101			3.95	61	17.3	314	3.7	0.01	-1.480	34.151	
	150			4.93	57	17.2		0.7		0.620	34.594	
	301			5.69	63	22.4	385	0.8	0.00	1.620	34.880	
	1000			8.03	88	28.9	235	1.3		-0.190	34.898	
	3001			11.70	49	18.9	227	1.1		-0.780	34.930	
	3914			11.67	52		305	1.4		-0.690	34.937	

A4: ARK XII 1996
Central Arctic Ocean
Transect 1:

St.	Lat.°N	Long.°E	Ident.	Depth (m)	Chl *a* (µg/l)	DOC (µM)	TCHO (µMC)	Temp. (°C)	Salinity (PSU)	Silicate (µM)
3	81.38	064.87	165323	4	3.48	74	4.8	-1.280	33.579	2.43
			165321	15	3.03	66	4.9	-1.286	33.658	2.51
			165319	25	0.88	71	4.1	-1.119	34.024	3.36
			165317	35	0.45	62	4.1	-1.395	34.245	3.93
			165315	45	0.30	57	3.8	-1.524	34.372	4.51
			165312	86	0.03	71	4.0	-1.550	34.488	5.28
5	81.46	066.86	165372	20	0.34	66	3.0	-1.763	33.965	2.83
			165370	30	0.06	54	2.6	-1.746	34.033	2.99
			165361	99	0.01	57	2.4	2.774	34.858	6.16
			165351	299	0.00	52	2.4	2.526	34.954	6.44
			165349	399	0.00	50	2.3	1.726	34.919	6.95
			165345	523	0.00	153	2.3	1.134	34.905	7.25
			165343	543	0.00		2.8	0.962	34.911	7.55
7	81.22	070.06	165427	19		64	4.9	-1.766	33.847	2.74
			165424	49		57	4.1	-1.774	34.141	3.63
			165421	100		51	3.5	-0.917	34.471	3.99
			165419	150		46	3.0	0.793	34.737	5.11
			165415	199			3.4	1.403	34.838	5.72
			165411	300			2.7	1.481	34.898	6.37
			165409	400			2.6	0.140	34.835	6.07
			165405	500		57	2.6	-0.350	34.875	7.32
			165401	582		79	2.6	-0.307	34.910	9.31
8	81.26	070.95	165447	50		63	4.6	-1.800	34.063	4.05
			165444	100		58	3.8	-1.169	34.374	4.25
			165441	200		48	3.0	1.379	34.818	5.62
			165435	500		50	3.2	-0.763	34.813	5.75
			165433	583		49	3.1	-0.300	34.906	8.46
			165431	602			3.1	-0.305	34.905	8.58
9	81.29	072.02	165485	10		93	2.9	-1.792	33.732	3.50
			165477	50		67	2.9	-1.802	34.115	3.86
			165472	100			2.7	-0.936	34.386	3.73
			165467	200		50	2.6	1.680	34.847	5.42
			165463	300		63	2.8	1.425	34.879	5.80
			165461	401			3.2	0.861	34.879	6.36
			165459	500		51	3.1	-0.343	34.845	6.44
			165455	596		54	3.1	-0.297	34.892	8.84
12	81.43	073.79	165572	20	0.41	94	4.5	-1.797	33.714	3.55
			165567	49	0.05	93	4.9	-1.800	33.896	3.47
			165561	100	0.01	101	3.1	0.134	34.616	4.67
			165556	200	0.00	74	3.1	-0.611	34.739	5.27
			165550	400	0.00		2.9			
			165540	512	0.00	73	2.7	-0.425		6.79
15	81.42	074.56	165658	20	0.37	74	4.2	-1.727	33.887	3.33
			165656	30	0.02	68	4.3	-1.718	34.124	4.59
			165647	100	0.15	55	3.0	-1.314	34.645	4.80
			165642	200	0.01	61	3.5	-1.308	34.739	5.13
			165639	264	0.01	57	3.2	-1.279	34.746	5.22
			165626	284	0.01	54	3.2	-1.288	34.746	5.26
18	81.46	077.46	165711	20	0.28	59	4.1	-1.656	34.263	4.16
			165707	40	0.16	53	0.0	-1.540	34.382	4.42
			165703	60	0.03	67	2.6	-0.235	34.718	5.24
			165699	100	0.01	59	2.6	0.039	34.765	5.36
			165697	125	0.01	60	2.4	0.055	34.770	5.39

A4 (cont.):

A4 (cont.):

St.	Lat.°N	Long.°E	Ident.	Depth (m)	Chl *a* (µg/l)	DOC (µM)	TCHO (µMC)	Temp. (°C)	Salinity (PSU)	Silicate (µM)
23	81.54	082.71	165802	20	0.38	61	4.4	-1.795	33.920	3.84
			165796	50	0.02	44	3.1	-1.535	34.441	4.66
			165784	200	0.00	42	3.2	-0.416	34.764	5.47
			165774	287	0.00	44	4.9	-1.102	34.766	5.69
25	81.96	083.90	165868	20	0.38	94	4.5	-1.776	33.999	3.57
			165865	50	0.20	74	4.5	-1.809	34.105	3.76
			165862	100	0.01	67	4.3	-1.230	34.488	4.36
			165857	200	0.00	59	3.4	0.350	34.794	5.46
			165855	300	0.00	60	3.9	-0.673	34.799	5.79
			165840	396	0.00	178	3.7	-0.643	34.827	6.31
28	81.81	089.31	165962	20		86	4.6	-1.629	33.977	3.78
			165959	50		106	4.2	-1.572	34.415	3.55
			165956	100		111	2.3	-0.021	34.718	4.73
			165941	182			2.7	0.061	34.776	5.44

St.		7.50	7-500	15-20	15-100	15-284	23-200	23-287	25-50	25-200	25-300
Ident.		165424	165405	165658	165647	165626	165784	165774	165865	165857	165855
Depth	(m)	50	500	20	100	284	200	287	50	200	300
Combined neutral sugars											
Fuc	(nMC)	132	12	56	0	0	0	114	0	0	0
Rha	(nMC)	66	90	516	407	264	90	240	504	90	234
Ara	(nMC)	0	240	253	210	206	65	85	295	240	45
Gal	(nMC)	138	54	660	350	109	228	366	<10	156	240
Glc	(nMC)	648	1800	720	1127	669	4116	2880	4932	1908	3900
Man	(nMC)	534	1530	72	444	108	2472	1710	<10	606	3120
Xyl	(nMC)	<10	35	40	65	135	260	275	<10	40	285
Frc	(nMC)	420	30	804	203	276	120	612	<10	<10	270
Rib	(nMC)	2200	600	2710	478	265	800	25	<10	255	225
THNS	(nMC)	4138	4391	5831	3284	2031	8130	6282	5731	3301	8319
Fuc	(%THNS)	8.7	0.3	2.4	0.0	0.0	0.0	2.0	0.0	0.0	0.0
Rha	(%THNS)	4.3	2.4	22.3	15.6	17.7	1.2	4.2	8.8	3.0	3.0
Ara	(%THNS)	0.0	6.4	10.9	8.1	13.8	0.9	1.5	5.1	7.9	0.6
Gal	(%THNS)	9.1	1.4	28.5	13.5	7.3	3.2	6.5	0.0	5.1	3.1
Glc	(%THNS)	42.7	47.9	31.1	43.3	44.9	56.9	50.8	86.1	62.8	49.8
Man	(%THNS)	35.2	40.7	3.1	17.1	7.2	34.2	30.2	0.0	19.9	39.9
Xyl	(%THNS)	0.0	0.9	1.7	2.5	9.1	3.6	4.9	0.0	1.3	3.6
THNS	(%DOC)	7.3	7.7	7.9	6.0	3.7	19.8	14.3		5.6	13.8

A5: ARK XII 1996 Central Arctic Ocean Transect 2:

St.	Lat.°N	Long.°E	Ident.	Depth (m)	Chl *a* (µg/l)	DOC (µM)	TCHO (µMC)	Temp. (°C)	Salinity (PSU)	Silicate (µM)
34	82.09	091.50	166084	20		67	4.1	-1.784	33.910	3.09
			166081	50		225	3.5	-1.783	33.944	3.26
			166073	300		49	3.3	1.784	34.876	5.83
			166071	500		49	3.9	0.118	34.847	6.44
			166069	700		51	3.8	-0.337	34.867	7.45
			166064	1200		52	3.7	-0.250	34.903	8.33
			166059	1506		44	3.4	-0.582	34.911	10.47
43	84.20	100.53	166456	10		65	5.7	-1.796	34.232	3.16
			166447	60		65	5.4	-1.819	34.303	3.18
			166444	100		62	4.9	-1.564	34.390	4.18
			166441	235		54	4.2	2.127	34.921	5.96
			166434	301		54	4.0	1.631	34.890	5.95
			166427	500		49	4.1	1.021	34.912	6.42
			166418	1001		52	3.5	-0.215	34.906	7.90
			166416	1400		45	3.8	-0.546	34.917	9.65
			166408	2000		140	3.7	-0.749	34.924	10.92
			166400	2600		49	3.5	-0.794	34.931	11.30
			166398	3000		47	3.6	-0.780	34.937	11.50
			166394	3727		36	3.6	-0.706	34.942	11.74
			166393	3779		55	3.6	-0.700	34.942	11.75
51	85.28	111.59	166769	20	0.16	79	5.7	-1.769	34.176	2.87
			166767	40	0.12	86	5.5	-1.826	34.268	2.99
			166764	99	0.01	75	4.4	-1.795	34.320	3.31
			166761	232	0.00	65	3.5	1.553	34.879	6.04
			166758	500	0.00	74	3.3	0.625	34.889	6.59
			166754	1000	0.00	56	3.7	-0.348	34.908	8.03
			166751	1600	0.00	61	2.7	-0.523	34.930	10.56
			166749	2000	0.00	78	2.1	-0.540	34.936	11.02
			166744	2999	0.00	72	2.8	-0.759	34.930	11.12
			166739	4000	0.00	61	2.6	-0.690	34.944	11.72
			166736	4365	0.00	66	2.4	-0.652	34.943	11.58
			166735	4416	0.00	69	2.6	-0.646	34.943	11.59
58	86.39	134.19	167050	10	0.66	101	7.4	-1.286	32.816	3.03
			167046	60	0.04	81	5.1	-1.701	33.912	3.88
			167044	100	0.00	69	3.4	-1.219	34.246	4.41
			167042	210	0.00	53	3.5	1.544	34.853	5.55
			167034	1001	0.00	62	3.2	-0.270	34.894	7.35
			167031	1601	0.00	51	2.9	-0.622	34.918	9.96
			167029	2001	0.00	56	2.4	-0.736	34.916	10.37
			167026	2601	0.00	53	2.3	-0.782	34.928	11.81
			167024	3000	0.00	49	1.9	-0.770	34.933	11.66
			167018	3969	0.00	55	2.5	-0.692	34.939	11.66
			167017	4020	0.00	51	2.9	-0.687	34.944	11.58
62	86.45	141.10	167184	10	0.29	100	5.3	-1.663	33.404	2.44
			167176	60	0.32	81	4.2	-1.638	34.065	3.83
			167167	235	0.00	74	4.2	1.764	34.875	5.56
			167160	500	0.00	69	4.0	0.644	34.870	6.07
			167154	1000	0.00	67	3.6	-0.172	34.884	7.07
			167153	1055	0.00	69	3.5	-0.237	34.886	7.07
			167152	1107	0.00	68	3.5	-0.266	34.890	7.18
			167151	1154	0.00	63	3.6	-0.294	34.892	7.32

A5 (cont.):

St.	Lat.°N	Long.°E	Ident.	Depth (m)	Chl *a* (µg/l)	DOC (µM)	TCHO (µMC)	Temp. (°C)	Salinity (PSU)	Silicate (µM)
68	86.24	153.02	167342	20	0.22	81	5.9	-1.707	33.464	2.45
			167339	60	0.08	81	5.3	-1.788	33.909	3.60
			167337	100	0.00	69	4.1	-1.253	34.239	5.74
			167333	250	0.00	58	3.9	1.119	34.848	6.21
			167330	500	0.00	58	4.0	0.491	34.868	6.94
68	86.24	153.02	167327	800		54	3.5	-0.003	34.882	7.30
			167322	1421		42	3.5	-0.411	34.932	10.86
			167321	1470		40	3.4	-0.426	34.940	
70	85.99	159.97	167413	9		74	6.3	-1.573	33.205	2.64
			167405	60		43	5.0	-1.830	33.854	3.37
			167401	100		54	4.6	-1.530	34.132	5.63
			167396	251		71	3.1	1.481	34.866	5.82
			167391	500		51	3.1	0.463	34.868	6.76
			167387	1001		45	3.2	-0.209	34.898	7.60
			167384	1601		34	3.2	-0.437	34.935	11.21
			167382	2065		33	2.9	-0.427	34.944	12.47
			167381	2117		29	2.0	-0.415	34.947	12.60
			167380	2167		32	2.0	-0.410	34.945	12.81
71	85.97	160.51	167445	21	0.26	79	5.4	-1.623	33.485	2.98
			167442	60	0.03	70	4.6	-1.836	33.980	3.75
			167440	100	0.01	63	4.9	-1.812	34.045	3.84
			167433	501	0.00	64	3.4	0.458	34.868	7.29
			167429	1001	0.00	63	3.2	-0.178	34.899	7.82
			167424	2000	0.00	71	2.7	-0.431	34.943	12.16
			167418	2790	0.00	60	2.1	-0.367	34.951	12.78
			167417	2840	0.00	58	2.4	-0.364	34.951	12.76
			167416	2891	0.00	56	1.9	-0.359	34.951	12.66
72	85.83	161.68	167474	20	0.60	79	5.8	-1.676	33.405	
			167463	100	0.01	67	3.6	-1.491	34.134	
			167454	259	0.01	51	3.7	0.965	34.839	
			167511	500	0.00	53	3.3	0.483	34.871	
			167505	1000	0.00	54	3.3	-0.151	34.890	
			167496	2000	0.00		2.9	-0.406	34.947	
			167489	3000	0.00	60	2.2	-0.358	34.954	
			167484	3801	0.00	49	1.8	-0.294	34.952	
			167483	3850	0.00	48	1.9	-0.289	34.953	
			167479	3900	0.00	44	1.6	-0.284	34.953	

A6: ARK XIII/3 1997
Fram Strait and Greenland Sea Stations:

St.		14	17	20	38	46	56E	56W	60	62	96	106	133	167
Depth	(m)	2900	50	20	1800	4526	ice floe	1	5650	BWS	26	45	1000	20
Lat.°N		81.07	81.06	81.04	79.67	79.67	80.89	80.89	79.17	76.79	77.92	75.21	75.00	75.00
Long.°E		-4.99	-7.01	-7.78	-3.00	3.30	-2.57	-2.57	2.66	6.98	-5.30	-15.01	-0.29	17.99
Temp.	(°C)	-0.924	-1.610	-1.641	-0.642	-0.956	n.d.	-1.500	-0.977	3.900	-1.379	-1.673	-0.898	2.034
Salinity	(PSU)	34.934	32.998	31.611	34.924	34.925	n.d.	31.470	34.922	34.630	32.210	33.338	34.884	34.122
DOC	(µM)	50	81	83	39	45	110	91	50	108	86	85	54	63
TCHO	(µMC)	1.0	4.2	4.3	1.0	0.6	4.8	n.d.	0.7	n.d.	3.8	2.0	2.1	2.1
THNS	(µMC)		5.56		1.44	1.83	8.50				4.62			1.84
Fractionated DOC														
2-A	(µM)	3	15	11	3	8	6	n.d.	6	n.d.	11	12	9	6
2-N	(µM)	8	18	15	9	9	16	n.d.	10	n.d.	15	19	15	9
4-A	(µM)	8	5	4	3	3	2	n.d.	4	n.d.	3	2	3	2
4-N	(µM)	12	11	7	7	10	8	n.d.	12	n.d.	7	6	0.5	13
Hb	(µM)	31	49	37	22	30	32	43	32	30	36	39	27.5	30
Hb	(%DOC)	62	60	45	56	67	29	47	64	28	42	46	51	48
2:4		0.6	2.1	2.4	1.2	1.3	2.2	n.d.	1.0	n.d.	2.6	3.9	6.9	1.0
N:A		1.8	1.5	1.5	2.7	1.7	3.0	1.2	2.2	1.7	1.6	1.8	1.3	2.8
Fractionated TCHO														
2-A	(µMC)	0.01	0.70	0.39	0.20	0.19	0.29	n.d.	0.02	0.45[1]	0.50	0.39	0.53	0.41
2-N	(µMC)	0.08	1.95	1.35	0.07	0.16	0.59	n.d.	0.27	0.50[2]	0.96	0.45	0.29	0.47
4-A	(µMC)	0.16	0.08	0.10	0.05	0.00	0.31	n.d.	0.06	n.d.	0.06	0.00	0.11	0.04
4-N	(µMC)	0.35	0.59	0.10	0.19	0.07	0.20	n.d.	0.10	n.d.	0.06	0.09	0.14	0.11
Hb	(µMC)	0.59	3.33	1.94	0.50	0.42	1.4	n.d.	0.45	0.95	1.58	0.94	1.05	1.03
Hb	(%DOC)	1.2	4.1	2.3	1.3	0.9	1.3	n.d.	0.9	0.9	1.8	1.1	2.0	1.6
Hb	(%TCHO)	59	79	45	50	70	11	n.d.	64	n.d.	47	47	50	49
2:4		0.2	3.9	8.4	1.2	5.3	1.7	n.d.	1.8	n.d.	12.6	8.7	3.4	5.9
N:A		2.6	3.3	3.0	1.0	1.2	1.3	n.d.	4.8	1.1	1.8	1.4	0.7	1.3
Fractionated THNS														
Hb	(µMC)		0.50		0.30	0.50	2.14				0.50			0.10
Hb	(%DOC)		0.62		0.81	1.00	1.94				0.58			0.16
Hb	(%THNS)		9		21	27	25				11			6
2:4			2.1		1.9	1.0	2.6				1.8			1.6
N:A			1.9		4.5	n.d.	1.3				0.8			1.7

[1]: 2+4-A
[2]: 2+4-N

St.	Fuc (%THNS)	Rha (%THNS)	Ara (%THNS)	Gal (%THNS)	Glc (%THNS)	Man (%THNS)	Xyl (%THNS)
THNS							
17	1.6	5.6	11.8	28.6	50.8	1.3	0.2
38	17.8	16.7	19.9	11.6	19.4	7.3	7.3
46	19.3	16.0	23.7	9.7	21.6	4.7	5.0
56E	1.6	1.3	0.2	9.1	76.1	9.5	2.3
96	1.5	3.7	2.4	3.8	87.3	1.1	0.2
167	0.7	49.3	6.4	0.8	14.4	28.4	0.0
Fractionated THNS							
17Hb	12.0	8.4	6.5	12.3	49.4	9.9	1.6
38Hb	4.4	5.1	3.4	19.6	30.2	23.3	14.0
46Hb	14.5	2.3	27.4	7.3	42.3	6.2	0.0
56E Hb	7.0	5.6	0.8	19.3	55.4	10.0	1.9
96 Hb	18.3	10.9	4.2	16.0	34.2	13.6	2.7
167 Hb	16.5	12.3	6.1	20.3	26.3	18.0	0.5

A6 (cont.):

St.	Fraction	Fuc (nMC)	Rha (nMC)	Ara (nMC)	Gal (nMC)	Glc (nMC)	Man (nMC)	Xyl (nMC)	Frc (nMC)	Rib (nMC)	THNS (nMC)
Fractionated THNS											
17	Original	28	97	205	496	882	23	4	566	262	2563
	HI	0	78	190	468	768	0	0	340	233	2077
	Hb	28	19	15	28	114	23	4	226	29	486
	2-A	6.8	6.5	4.7	7.3	14.8	6.5	1.0	5.6	26.7	79.8
	2-N	16.1	9.2	8.1	18.0	87.4	13.6	2.1	95.5	0.0	250.1
	4-A	2.2	1.2	0.7	1.2	4.7	0.8	0.2	76.6	0.0	87.6
	4-N	2.6	2.4	1.4	1.8	7.1	2.0	0.4	48.5	2.2	68.4
38	Original	175	164	195	114	190	71	71	303	154	1436
	HI	168	156	190	84	144	36	50	288	150	1266
	Hb	7	8	5	30	46	35	21	15	4	295
	2-A	1.9	1.3	0.5	3.7	2.9	5.9	0.0	2.0	0.0	18.2
	2-N	4.8	6.4	2.5	16.9	26.9	14.8	21.1	0.0	0.0	93.4
	4-A	0.0	0.0	1.8	0.0	1.9	4.4	0.0	2.2	2.6	12.9
	4-N	0.0	0.0	0.4	8.9	13.8	10.1	0.0	11.0	1.3	45.5
46	Original	193	160	237	96	216	47	50	687	150	1835
	HI	168	156	190	84	144	36	50	288	150	1266
	Hb	25	4	47	12	72	11	0	399	0	569
	2-A+N	24.6	4.0	46.5	12.4	60.9	10.5	0.0	128.8	0.0	288
	4-A+N	0.0	0.0	0.0	0.0	11.0	0.0	0.0	270.7	0.0	282
56E	Original	119	95	13	682	5722	717	172	578	396	8493
	HI	0	0	0	354	4782	546	140	186	350	6358
	Hb	119	95	13	328	940	171	32	392	46	2135
	2-A	89.1	60.9	0.0	178.6	204.4	98.6	22.4	145.6	20.6	820.2
	2-N	17.0	16.8	0.0	130.6	407.1	42.5	1.3	111.6	0.0	726.9
	4-A	6.4	5.3	4.3	1.3	34.3	8.6	2.1	46.2	9.5	118.0
	4-N	6.3	12.0	8.9	17.2	294.3	20.9	6.3	88.4	15.9	470.2
96	Original	61	151	99	156	3571	67	42	51	427	4625
	HI	0	114	85	102	3456	21	33	49	305	4165
	Hb	61	37	14	54	115	46	9	2	122	460
	2-A	18.9	12.0	5.0	22.9	55.6	16.7	2.2	0.0	16.6	149.9
	2-N	29.9	16.6	5.8	19.4	21.2	17.2	3.6	2.0	29.0	144.7
	4-A	4.1	2.9	1.3	4.3	24.9	6.1	1.9	0.0	66.2	111.7
	4-N	8.6	5.3	2.0	7.2	13.0	5.5	1.4	0.0	10.4	53.4
106	Original	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	HI	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Hb	22	23	12	22	65	16	4	69	58	291
	2-A	6.4	7.2	5.0	7.0	18.1	5.8	1.0	3.7	19.0	73.2
	2-N	11.2	11.7	4.8	11.1	28.1	8.1	2.2	65.7	39.1	182.0
	4-A	1.2	1.2	0.5	1.0	11.7	0.0	0.0	0.0	0.0	15.7
	4-N	2.9	3.3	1.5	2.5	6.9	2.3	0.6	0.0	0.0	20.1
167	Original	9	643	83	67	188	370	25	179	279	1842
	HI	0	636	80	56	174	360	25	178	236	1745
	Hb	9	7	3	11	14	10	0	1	43	97
	2-A	1.6	0.7	0.4	1.7	1.9	0.9	0.0	0.0	11.0	18.2
	2-N	3.5	2.5	1.7	5.1	5.7	3.8	0.0	0.5	18.2	41.0
	4-A	0.6	1.3	0.0	0.5	0.4	1.7	0.3	0.0	13.4	18.2
	4-N	3.3	2.1	1.2	3.7	6.2	3.4	0.0	0.0	0.0	19.9

A6 (cont.):

St.	Fraction	Fuc (nMC)	Rha (nMC)	Ara (nMC)	Gal (nMC)	Glc (nMC)	Man (nMC)	Xyl (nMC)	THNS (nMC)	THNS (%initial conc.)
Comparison between XAD and ultrafiltration										
62	Original	85	55	340	220	340	150	40	1230	100.0
	2+4-A	8	9	14	20	22	11	3	86	7.0
	2+4-N	3	5	8	9	12	8	1	45	3.6
	Hb	10	14	22	29	34	19	3	131	10.6
	HI	75	41	318	191	306	131	37	1099	89.4
Concentrate of ultrafiltration										
	Original	42	42	67	78	45	70	36	381	30.9
	Hb	7	13	13	15	7	15	10	80	6.5
	HI	35	30	54	63	38	55	26	301	24.5
Permeate of ultrafiltration										
	Original	10	10	268	10	293	10	10	610	49.6
	Hb	5	5	24	5	24	5	5	73	5.9
	HI	5	5	244	5	268	5	5	537	43.6

A7: ANT XIV/3
Weddell Sea Ice Core:

N140 1803
72.87°S 19.21°W

		A14	A13	A12	A11	A9	A7	A1
Depth	(cm)	100	110	120	130	150	170	220
Salinity	(PSU)	5.1	4.7	4.5		3.6	4.1	8
Chl a	(µg/l)	1.2	0.4	0.5	0.5	0.5	1.1	312.6
Bacteria	(tot/l)	n.d.	2E+08	1E+08	9E+07	1E+08	2E+08	2E+09
DOC	(µM)	172	27	16	96	67	39	202
Silicate	(µM)	4.07	3.30	2.33	1.12	0.58	1.03	44.20
Nitrate	(µM)	1.27	0.44	0.29	0.46	0.56	0.15	9.47
Free neutral sugars								
Fuc	(nMC)	0	0	0	0	0	0	0
Rha	(nMC)	890	198	336	222	174	222	252
Ara	(nMC)	325	35	60	70	60	155	65
Gal	(nMC)	0	0	0	0	0	0	0
Glc	(nMC)	1668	1020	852	1662	3186	2978	3078
Man	(nMC)	0	0	0	0	0	0	0
Xyl	(nMC)	0	0	0	0	0	0	0
Frc	(nMC)	5118	948	1338	618	1098	888	1752
Rib	(nMC)	595	905	870	605	2230	1595	360
TFNS	(nMC)	8596	3106	3456	3177	6748	5838	5507
TFNS	(%DOC)	5.0	11.7	21.7	3.3	10.1	15.0	2.6
Combined neutral sugars								
Fuc	(nMC)		0		0	0	71	1014
Rha	(nMC)		578		647	1058	1495	5814
Ara	(nMC)		202		203	197	567	9255
Gal	(nMC)		526		169	532	1445	3204
Glc	(nMC)		1372		1293	3014	2280	4398
Man	(nMC)		1681		1665	3016	2210	8548
Xyl	(nMC)		760		550	799	1296	2177
Frc	(nMC)		852		462	3290	2882	15822
Rib	(nMC)		965		1246	1042	607	7045
THNS	(nMC)		6936		6235	12948	12853	57277
THNS	(%DOC)		26.1		6.5	19.5	33.0	26.7
Fuc	(%THNS)		0.0		0.0	0.0	0.8	2.9
Rha	(%THNS)		11.3		14.3	12.3	16.0	16.9
Ara	(%THNS)		3.9		4.5	2.3	6.1	26.9
Gal	(%THNS)		10.3		3.7	6.2	15.4	9.3
Glc	(%THNS)		26.8		28.6	35.0	24.3	12.8
Man	(%THNS)		32.8		36.8	35.0	23.6	24.8
Xyl	(%THNS)		14.8		12.1	9.3	13.8	6.3
Total neutral sugars (TCHO=THFS+THNS)								
TCHO	(nMC)		3132		3183	6767	5871	5534
TCHO	(%DOC)		38		10	30	48	29

A8: Chromatographic Conditions and Detector Settings:

GP-40

Time events:

Time	Event	Injection port	Output
Init	Equilibration	Load	TTL1 off
0.00	Start autosampler	Load	TTL1 on
1.60	Injection	Inject	TTL1 off
21.60	End of run	Load	TTL1 off

General settings:

Pressure max:	3000 PSI
Pressure min:	0 PSI

ED-40

Collect	Offset
Begin	x

Mode: Integrated amperometry

Waveform: Carbohydrates (Ag/AgCl reference)

Time (s)	Potential (V)	Integration
0.00	+0.05	
0.20	+0.05	Begin
0.40	+0.05	End
0.41	+0.75	
0.60	+0.75	
0.61	-0.15	
1.00	-0.15	

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