APPLICATION OF CARBOHYDRATES AS BIOMARKERS TO STUDY
DISSOLVED ORGANIC MATTER RESERVOIRS IN ARCTIC RIVERS.

An Undergraduate Research Scholars Thesis

By

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ABSTRACT

Application Of Carbohydrates And Phenols As Biomarkers To Study Dissolved Organic Matter Reservoirs In Arctic Rivers. (May 2014)

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Arctic rivers are the dominant pathways for the transport of terrestrial dissolved organic carbon to the Arctic Ocean, but knowledge of sources, transformations and transfer of organic carbon and nitrogen in Arctic river watersheds is extremely limited. Here we use chemical analysis of carbohydrates to investigate the bioavailability of dissolved organic matter in five major Arctic river watersheds. In addition, neutral sugar analyses were integrated with existing measured chemical parameters from the PARTNERS data set to identify DOM sources. The results show the bioavailability of DOM in Arctic rivers is strongly correlated with seasons, vegetation topography and water residence time in the watersheds. Pulses of bioavailable DOM are observed in the Siberian rivers during the Spring flood, whereas the Mackenzie River shows extensively degraded DOM throughout all stages of the hydrograph. $\Delta^{14}$C-DOC was not correlated to higher carbohydrate yields possibly indicating export of recently mobilized ancient permafrost organic carbon.
ACKNOWLEDGEMENTS

Thank you to Dr. Karl Kaiser for being a constant source of information and skill. The samples were numerous and the instruments sensitive your guidance made this thesis and research possible.

The information and data collected through Dr. Rainer Amon and the PARTNERS group helped to build the story that is the Arctic Ocean and the base for further research to be built upon.
CHAPTER I

INTRODUCTION

Artic watersheds store approximately 50% of the global soil organic carbon, much of which is held in shallow permafrost (Tarnocai et al. 2009). In addition, these watersheds hold a significant portion of the global biomass in the form of terrestrial biomass (McGuire et al. 2009, 2010). The Arctic Ocean receives about 10% of the global runoff, while having only 1% of the total ocean volume. (Aagaard and Carmack 1989, Menard and Smith 1966).

The concentration of dissolved organic carbon (DOC) in the Arctic Ocean is heavily influenced by river runoff (Anderson 2000). The Artic rivers Lena, ‘Ob, and Yenisey are all classified in the same class size as the Mississippi river meaning the rivers are large and contribute to DOM in the Arctic Ocean.

The scientific community still has a very limited understanding of what sources and transformation processes of Arctic riverine DOM predominate at different stages of the hydrograph and within the different enormous watersheds understanding the sources of DOM in these watersheds weather it be vegetation, soil or peat it is crucial in predicting the effect of warmer climate will have on the massive carbon and nitrogen sinks from these watersheds to the Artic Ocean.
Table 1. Geographical, climatic and geochemical characteristics of river/watershed systems investigated in this project (from Amon et al. 2012).

<table>
<thead>
<tr>
<th>River/Watershed characteristics</th>
<th>Mackenzie</th>
<th>Ob</th>
<th>Yenisey</th>
<th>Lena</th>
<th>Kolyma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discharge (km$^3$ yr$^{-1}$)</td>
<td>298</td>
<td>427</td>
<td>636</td>
<td>581</td>
<td>111</td>
</tr>
<tr>
<td>Length (km)</td>
<td>3679</td>
<td>3977</td>
<td>4803</td>
<td>4387</td>
<td>2091</td>
</tr>
<tr>
<td>Catchment ($10^6$ km$^2$)</td>
<td>1.78</td>
<td>2.99</td>
<td>2.54</td>
<td>2.46</td>
<td>0.65</td>
</tr>
<tr>
<td>MAAT (°C)</td>
<td>0.7</td>
<td>1.4</td>
<td>-1.0</td>
<td>-6.5</td>
<td>-10.1</td>
</tr>
<tr>
<td>Mean slope (m km$^{-1}$)</td>
<td>2.23</td>
<td>1.28</td>
<td>1.94</td>
<td>1.83</td>
<td>2.16</td>
</tr>
<tr>
<td>Sediment flux ($10^6$ t/y)</td>
<td>124</td>
<td>15.5</td>
<td>4.7</td>
<td>20.7</td>
<td>10.1</td>
</tr>
<tr>
<td>Southernmost Lat. (°N)</td>
<td>52.2</td>
<td>45.3</td>
<td>45.7</td>
<td>52.2</td>
<td>60.6</td>
</tr>
<tr>
<td>Cont. permafrost (%)</td>
<td>13</td>
<td>1</td>
<td>31</td>
<td>77</td>
<td>99</td>
</tr>
<tr>
<td>Deciduous BL forest (%)</td>
<td>1.4</td>
<td>10.2</td>
<td>3.4</td>
<td>1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Evergreen NL forest (%)</td>
<td>23.7</td>
<td>14.9</td>
<td>20.6</td>
<td>7.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Deciduous NL forest (%)</td>
<td>0</td>
<td>1.5</td>
<td>32.7</td>
<td>58.8</td>
<td>49.1</td>
</tr>
<tr>
<td>Mixed forest (%)</td>
<td>9.2</td>
<td>12.0</td>
<td>10.6</td>
<td>4.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Total forest (%)</td>
<td>34.4</td>
<td>38.6</td>
<td>67.3</td>
<td>72.1</td>
<td>49.9</td>
</tr>
<tr>
<td>Forest – MODIS (%)</td>
<td>35</td>
<td>25</td>
<td>35</td>
<td>32</td>
<td>10</td>
</tr>
<tr>
<td>Shrubland (%)</td>
<td>10.5</td>
<td>2.6</td>
<td>9.0</td>
<td>12.5</td>
<td>32.1</td>
</tr>
<tr>
<td>Grassland (%)</td>
<td>30.0</td>
<td>15.9</td>
<td>7.2</td>
<td>0.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Cropland (%)</td>
<td>2.4</td>
<td>22.9</td>
<td>6.2</td>
<td>0.6</td>
<td>0</td>
</tr>
<tr>
<td>Wetlands (%)</td>
<td>0.1</td>
<td>8.5</td>
<td>2.6</td>
<td>3.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Water bodies (%)</td>
<td>10.3</td>
<td>2.4</td>
<td>2.1</td>
<td>1.7</td>
<td>1.6</td>
</tr>
</tbody>
</table>

MAAT = mean annual air temperature, BL = broad leaf, NL = needle leaf.

The numerous rivers that enter the Arctic Ocean drain enormous areas with very different vegetation and soil conditions as seen in Table 1. The Ob has the least permafrost of the five rivers experiencing the most varied of weather conditions (Yang et al. 2002). The Yenisey is the longest of the rivers and shows the largest discharge in the largest watershed of the five rivers (Holmes et al. 2012). The Mackenzie River has the highest flux of sediments of all Arctic rivers but a rather low discharge level.

About 76% of the total watersheds area is located within Eurasia (Zhulidov et al. 1997). The latitudinal vegetation gradient transitions from boreal forests in the more southern latitudes to tundra populated by lichens, mosses and angiosperms in the north. Above 50-60°N the
watersheds are dominated by permafrost both discontinuous and continuous that lead to large peat lands especially in the Siberian river watersheds (McGuire et al 2009,2010).

The Artic has displayed a larger increase in temperatures than the rest of the global mean annual air temperature (MAAT) over the last decades (IPPC 2007) and temperatures are expected to increase another 4-7° C by 2100 (Artic Climate Impact Assessment 2005). Temperature and moisture are key to governing the fate of organic matter in these watersheds, recent estimates for carbon fluxes in this region indicated that the large significant net sinks for CO₂ (200-400 Tg/yr McGuire et al. 2009), net sources for CH₄, and deliver between 25 and 36 Tg C/yr in the form of dissolved organic carbon (DOC) to the artic ocean (Raymond et al 2007, Holmes et al. 2012).

Most studies agree that the carbon cycle in the Arctic will intensify with the increase in mean temperatures; estimates of future terrestrial carbon transport in these rivers are variable.

Arctic biomes have been an important terrestrial carbon sinks during the current Holocene. Ongoing climatic change could result in mobilization of the immense soil carbon reservoirs stored in these regions with global ramifications. Arctic rivers are the dominant pathways for the transport of terrestrial dissolved organic matter (DOM) to the Arctic Ocean. Discharge has significantly increased over the last century and appears to be strongly linked to Arctic Oscillation (Peterson et al. 2004, Fichot et al. 2013).

The bioavailability of Arctic riverine DOM appears to be closely linked to its chemical composition and sources. High bioavailability of spring freshet DOM reflects the recent origin of the DOM leached from the fresh litter and surface soil horizons of these major watersheds, and little previous decomposition during cold seasons (Holmes et al. 2008, Mann et al. 2012,
Wickland et al. 2012). As fresh litter is released in the spring floods it is followed by a significant increase in the rates of microbial decomposition in the summer. Thus, summer DOM is likely more decomposed and more resistant to further decomposition in the river channel.

The primary goal of the following research is to investigate the bioavailability of Arctic river DOM further. Water samples collected in the six largest Arctic rivers will be analyzed for carbohydrates and together with lignin phenol will serve as indicators of DOM bioavailability (Cowie and Hedges 1994). It is predicted that seasonal changes in the bioavailability of DOM reflect variable DOM sources in Arctic river watersheds and chemical compositions. This research will improve our understanding of the fate and removal of riverine DOM at a pan-Arctic scale.

The study utilizes 75 water samples collected during the Pan-Arctic River Transport of Nutrients, Organic Matter, and Suspended Sediments (PARTNERS) project between 2003 and 2007. Fig 1. Shows the collection sites and watershed areas for these samples. C-normalized concentrations of neutral sugars and phenols serve as molecular indicators for the bioavailability of riverine DOM (Cowie and Hedges 1994). Carbohydrates are analyzed by high-performance liquid chromatography and electrochemical detection (Skoog and Benner 1998, Kaiser and Benner 2000). The phenol data already available through published resources (Amon et. al. 2012) coupled with the carbohydrate and amine data is utilized together to analyze riverine DOM.
Fig. 1: Map of major Arctic river watersheds and rivers investigated by the Pan-Arctic River Transport of Nutrients, Organic Matter, and Suspended Sediments (PARTNERS) project project. Red dots indicate sampling stations. The red line indicates the area contributing freshwater to the Arctic Ocean.
CHAPTER II

METHODS

Samples:
A total of 75 samples are available for 5 major Arctic rivers sampled by the PARTNERS project between 2003-2007 (Table 1). Raymond et al. (2007), McClelland et al. (2008), and Holmes et al. (2012) described detailed sampling programs and procedures. The PARTNERS collection protocol was designed to capture base flow (under ice), spring melt, and late summer conditions. The collection device was a torpedo shaped, Teflon coated, 60 kg depth-integrated sampler (US D-96). The rivers were sampled at five different locations along a cross-channel transect and combined into one homogeneous sample using a Teflon churn. With the exception of winter samples, which were collected by drilling a hole in the ice, each water sample is representative not only of surface to bottom, but cross-channel chemistry. Water from the Teflon churn was then filtered (0.45 µm Pall Aquaprep 600 capsule filters) into acid washed 1 L polycarbonate bottles and frozen.

The full PARTNERS data set includes over 50 measured parameters, which are accessible online through the Cooperative Arctic Data and Information Service (CADIS) of the Arctic Observing Network (AON) and the Arctic Great Rivers Observatory website (http://www.greatarcticrivers.org). Monitoring and sampling at these rivers continues as part of the Arctic Great Rivers Observatory project.

Yields of neutral sugars and phenols will serve as molecular indicators for bioavailability of riverine DOC (Cowie and Hedges 1994). For example, decreases in neutral sugar and phenol yield indicate a decrease in the bioavailability of bulk DOM. These indicators track the
decomposition of different organic sources in riverine DOM. Carbohydrate compositions are sensitive to their source in polysaccharides of all plants. Also available to this study is a rather large data set of collect data collected by the PARTNERS group that analyzed the same 50 samples used in the study. This will allow for a detailed comparison of different components including total dissolved organic carbon (DOC), lignin data, and river watershed characteristics.

Carbohydrates are analyzed in a three step process which includes filtration, neutralization and solid phase extraction, and high pressure liquid chromatography (HPLC) analysis (Fig. 2). Seven neutral sugars (fucose, rhamanose, arabinose, galactose, glucose, manose, xylose) are measured according to Skoog and Benner (1997) with modifications (Kaiser and Benner 2000).

Samples are first filtered from the thawed sample set through GFF filters into 20 ml vial containers. Then, samples are hydrolyzed in 1.2 molar sulfuric acid for 5 hours. Neutralization is performed by passing the hydrolyzed samples through about 5 ml of self-absorbed ion retardation resin (AG11A8, Biorad, Kaiser and Benner, 2000).

After neutralization the samples are desalted using a mixture of cation and anion exchange resins. Shortly after cleaning the samples are analyzed for neutral sugars by ion chromatography with 25 mM NaOH on a PA 1 column in a Dionex 500 system with pulsed amperiometric detection (PAD). **Fig.2** shows a more detailed description of the experimental setup. The detector settings were analogous to Skoog and Benner (1997). Calibration was performed with a set of internal and external standards.
Fig 2. A systematic approach to the analysis of samples including filtration, hydrolyzation, neutralization, purification, and HPLC.
A time and discharge weighted mean of the parameters was calculated based on the time window and discharge of each sample (Table 2). The time window for each sample was assigned based on the time from of 2003-2006 over about 1500 days. Then they were weighed based on their discharge values. The resulting parameters include carbon normalized neutral sugar concentrations in the unit of nmol/mg C. The neutral sugar yield (NS % yield) describes the amount of carbon contained in the analyzed neutral sugars in percent. Neutral sugar yields serve as a relative measure for the bioavailability of DOM, with higher yields indicating more bioavailable DOM. The mol percentage (mol %) of glucose was the measure of relative glucose concentrations compared to total neutral sugar concentrations.

DOC concentrations increased from low to high flow conditions in all rivers. The Lena exhibited highest concentration, whereas lowest concentrations were observed in the Mackenzie. The Lena and Yenisey showed the highest DOC discharge of all rivers (5.08-6.47 Tg DOC yr$^{-1}$) accounting for 70% of total DOC discharge of the combined discharge. For comparison, the Eurasian rivers account for ~84% of total DOC discharge to the Arctic Ocean with the difference discharged by the Yukon, the Mackenzie and other small North American Rivers (Amon et al. 2012).

Lignin volume and C-normalized concentrations based on concentrations of vanillyl and syringyl phenols changed during different stages of the hydrography (Fig. 2, Table 2). Highest lignin C-normalized concentrations were observed in the Lena and Yenisey. Lowest concentrations were observed in the Mackenzie that did not change with discharge volume.
C-normalized concentrations of neutral sugars were highest in the Lena and a factor of 10 higher than in the Kolyma and Mackenzie indicating extensive decomposition in the latter. Mol% of glucose were fairly similar among all five rivers and ranged from 22-26%.

Table 1. Mean and discharge-weighted average values for DOC, lignin phenols and neutral sugars in the five rivers.

<table>
<thead>
<tr>
<th>Type</th>
<th>Kolyma</th>
<th>Lena</th>
<th>Mackenzie</th>
<th>Ob</th>
<th>Yenisey</th>
<th>Annual Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC (mg/L)</td>
<td>6.56</td>
<td>11.37</td>
<td>4.35</td>
<td>10.48</td>
<td>8.80</td>
<td></td>
</tr>
<tr>
<td>DOC(Tg/yr)</td>
<td>0.71</td>
<td>6.47</td>
<td>1.20</td>
<td>3.04</td>
<td>5.08</td>
<td>16.5</td>
</tr>
<tr>
<td>% total DOC</td>
<td>4.30</td>
<td>39.21</td>
<td>7.27</td>
<td>18.42</td>
<td>30.79</td>
<td>100</td>
</tr>
<tr>
<td>Lignin (ug/l)</td>
<td>0.60</td>
<td>0.93</td>
<td>0.27</td>
<td>0.56</td>
<td>0.97</td>
<td>3.33</td>
</tr>
<tr>
<td>NS (nmol/mg C)</td>
<td>27.01</td>
<td>331.87</td>
<td>8.93</td>
<td>238.87</td>
<td>159.31</td>
<td>765.98</td>
</tr>
<tr>
<td>NS % yield (% DOC)</td>
<td>1.08</td>
<td>2.33</td>
<td>0.42</td>
<td>1.66</td>
<td>1.78</td>
<td>-</td>
</tr>
<tr>
<td>Mol % glucose (% NS)</td>
<td>25.16</td>
<td>22.39</td>
<td>22.36</td>
<td>26.42</td>
<td>24.86</td>
<td>-</td>
</tr>
</tbody>
</table>

1 Amon et al. 2012

Throughout all five rivers there is a consistent trend of peak discharge and peak concentrations of neutral sugars occurring each spring flow. Fig. 3 shows the Kolyma and Mackenzie have the lowest discharge values as well as the lowest concentrations in each sample. The Lena River has the highest concentrations of neutral sugars of all rivers. In previous studies DOM components like lignin phenols correlated strongly with the highest discharge levels during each spring flood (Amon et al. 2012); the neutral sugar yields correlate with discharge in only some of the rivers like the Yenisey and to a lesser extent in the Ob, while the other rivers have an offset from the highest river discharges to the highest neutral sugar yields. This is most easily seen in the Lena and Kolyma Rivers, while the Mackenzie River shows no significant peak value. It is unclear why some rivers show highest neutral sugar concentrations during low flow conditions, and this needs to be investigated in more detail.
The data was divided based on a categorical system of flow ranges starting with base flow the lowest flow period, occurring each winter, mid flow that occurred shortly before and after the peak flow regimes in late winter and early fall, and then peak flow during the spring and summer when it’s the warmest and there is the highest ice melt.

In **Fig. 3** each river is plotted based on their NS yield and during each flow regime out of the total DOC measured from the PARTNERS dataset. The Siberian rivers show elevated carbon-normalized yields of carbohydrates during peak flow indicating export of bioavailable DOM. This is consistent with previous studies (Holmes et al. 2008, Amon et al. 2012). The Mackenzie

**Fig. 3**: Neutral sugar concentrations compared to water discharge for four major Siberian rivers and the Mackenzie River.
River shows consistently low yields indicating exported DOM is highly degraded throughout the year. The low yields in the Mackenzie river can be explained by a longer water residence time compared to the other rivers and extensive decomposition occurs already in the river channel. Fig. 4 displays the standard deviation of the collected samples through the aid of error bars added to the average neutral sugar yield. For the Kolyma, Lena, and Yenisey the standard deviation values are very large compared to the Ob and Mackenzie. Flow regime groupings integrate data from 3 years and this causes some of the large standard deviations in the data as NS yields varied between years for the same flow period. Also, many of the samples were collected during the summer season in which the spring flood had already occurred and the availability of samples for base and peak flow is more limited than the midflow. Nonetheless, the data clearly show pulses of bioavailable DOM released during spring discharge and relatively decomposed during mid and base flow.
The carbohydrate data collected can be compared to a large set of parameters analyzed for these river samples. Information already obtained includes measurements like $\Delta^{14}\text{C-DOC}$ and C-normalized concentrations of lignin yields. In Fig. 5 the Ob and Kolyma show weak correlations between carbohydrate and lignin yields, whereas the other rivers show no clear correlation. This suggests that the carbohydrates and lignin do not leach into the water column at the same timing, as their sources can be very different or there could be significant production of carbohydrates in the river. Lignin is a component of woody plant material while carbohydrates are more common in all plant sources as well as a product of phytoplankton growth. This also suggests that other factors besides discharge and production control the NS yield.

**Fig. 4:** Neutral sugar yields for base, mid and peak flow conditions from 2004-2006. Error bars describe the standard deviation for each set of samples grouped within a flow regime.
$\Delta^{14}C$-DOC was not correlated to higher carbohydrate yields indicating that younger DOC was not always associated with more bioavailable DOM (Fig. 5). It is possible that older DOC with higher carbohydrate yields reflects input of permafrost organic matter. Permafrost organic matter would have a much older age compared to the regular materials that are released into these rivers. The $\Delta^{14}C$-DOC is indicative of a very young flow of matter in these rivers suggesting that most of the matter flowing through these rivers is still relatively new in age and time within the river watersheds.

**Yenisey River**

![Graph for Yenisey River](image)

**Ob River**

![Graph for Ob River](image)
The compositional data suggests that each river has a unique pattern of individual neutral sugars in each phase of flow. In Fig. 6 glucose in both the Ob river and Lena River does not follow the regular pattern of increased contributions during higher discharge levels but instead follows a decreasing pattern as the discharge moves towards the peak flow. The other three rivers follow the more traditional conjecture. This indicates different carbohydrate sources during the different flow regimes. With the high base flow of glucose it is possible that glucose is not dependent on discharge levels of the river but rather consistently available in these rivers thus with the increase in flow the amounts are diluted during peak flow creating a decreasing trend.

**Fig. 5:** % DOC plotted against the lignin yields and C\textsuperscript{14} measurements show no direct correlation or significant trend.
Fig. 6: The Kolyma river is lacking some significant data current that would supply information for peak flow but currently the mid and base flow are shown. Each river is very unique flowing traditional conjectures but also having new features as well like high glucose levels at base flow.
Compositionally each river shows a unique signal. The Ob has increasing mol % of galactose manose and xylose from base to peak flow while the fucose and arabinose mol % stay consistent. In the Yenisey more of the sugars decrease with the increase in flow from base to peak than in the Ob but glucose increases from base to peak flow. The Kolyma River on the other hand shows very little change between base and peak flows. These widely varying compositions can be attributed to both differences in sources and degradation processes for each river. High lignin phenol yields during spring peak flow suggest fresh vascular plant leachates for peak flow (Amon et al. 2012). But neutral sugars can stem from a more varied source including lichen, and mosses that contain no lignin and vascular plants and phytoplankton growth, making it harder to pin point their source. With yields as low as 1% of the total DOC of these rivers the source information is heavily overshadowed by the degradation signal of bacteria and other microorganism that preferentially consume neutral sugars at part of bioreactive organic matter. As the climate is warming potentially more material can released throughout the whole year than in the recent and could lead to mobilization of ancient permafrost soils. So far the gathering of C\textsuperscript{14} isotopic measurements conclude that the DOC is still relatively young and organic matter derived from permafrost thawing is not an important source of DOM.
CHAPTER IV
CONCLUSION

In this study, neutral sugars were analyzed to investigate the bioavailability of Arctic river DOM at the different stages of the hydrograph. In summary, the data show a very consistent low yield of neutral sugars in all five rivers indicative of extensive decomposition of DOM. Spring discharge released pulses of bioavailable DOM in all Siberian rivers, whereas DOM in the Mackenzie was highly degraded throughout the whole year. Water residence time appears to be important in controlling the amount of bioavailable DOM that is exported to the Arctic Ocean. Peaks in neutral sugar and lignin phenol yields were observed in all rivers suggesting different mechanisms for release and decomposition of these biochemical. High yields of neutral sugars were not well correlated with radiocarbon ages indicating that permafrost soils could be contributing to riverine DOM. The sources of analyzed neutral sugars are less understood as decomposition overshadows the source signal erasing characteristic compositions of hemicelluloses or phytoplankton derived sources. Neutral sugars account for a very small portion of the overall DOC but are very useful tracer of DOM bioavailability. Climate driven changes in Arctic ecosystem could result in substantial release of organic matter stored in permafrost soils. The amount of bioavailable DOM derived from this source and delivered to the Arctic Ocean appears to be strongly controlled by water residence time. As such the potential for export of bioavailable DOM is largest in the Siberian rivers.
REFERENCES


