

**BIOMASS COMBUSTION AS A SOURCE OF TERRIGENOUS
ORGANIC MATTER TO THE COASTAL OCEAN**

A Senior Scholars Thesis

by

KAYCE PEIRCE

Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

HONORS UNDERGRADUATE RESEARCH FELLOW

May 2012

Major: Marine Biology

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ABSTRACT

Biomass Combustion as a Source of Terrigenous Organic Matter to the Coastal Ocean.
(May 2012)

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Natural and anthropogenic combustion processes are major sources of organic carbon into the environment. Biomarkers of biomass combustion can be used to monitor the impact of combustion on carbon cycling at multiple scales, particularly in natural systems. Biomarkers of biomass combustion (lignin-oxidized phenols and anhydrosugars) were selectively extracted from standard solutions and natural solutions using both a liquid-phase extraction (LPE) and a solid-phase extraction (SPE). The LPE was found to be both highly accurate and highly reproducible with an average recovery from standard solutions of $100\pm 9\%$ and $91\pm 7\%$ for lignin phenols and anhydrosugars, respectively. The quantities of lignin phenols and anhydrosugars extracted from dissolved organic matter derived from a honey mesquite charcoal were similar to values previously produced in the lab demonstrating the reproducibility of this method over time. The SPE extracted measurable but variable quantities of lignin phenols but demonstrated no recovery of anhydrosugars, showing that the SPE disks selected were not efficient for extracting the compounds of interest from natural waters. Further tests

will be required to identify a method of extraction for such highly polar molecules from aqueous solutions. We applied the LPE protocol to extract free lignin phenols from a series of natural waters along a seasonal gradient in an Arctic river and compared these to previously measured polymeric lignin phenol concentrations. The temporal trend in free phenol concentrations matches that of the polymeric lignin with a maximum during the spring freshet peak flow event. The proportion of total lignin phenols that occur as free phenol ranges 2-9% with a maximum prior to the spring freshet peak flow event and a minimum during the spring freshet. Molecular signatures of the free phenols show that the dissolved lignin during the spring freshet contains high proportions of acid groups, which suggests a more degraded state of the dissolved terrigenous organic matter.

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NOMENCLATURE

ASE	Accelerated Solvent Extractor
BC	Black carbon
DOM	Dissolved organic matter
GC-MS	Gas chromatogram-mass spectrometer
GFF	Glass-fiber filter
LOP	Lignin-oxidized phenols
LPE	Liquid-phase extraction
OM	Organic matter
PAHs	Polycyclic aromatic hydrocarbons
PM	Particulate matter
SPE	Solid-phase extraction

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CHAPTER I

INTRODUCTION

Combustion processes, both natural (wildfires) and anthropogenic (industrial, urban, and agricultural) are major sources of particulate matter (PM), black carbon (BC), and volatile organic carbon into the atmosphere. Over the 20th century, the increased emissions of combustion-derived PM and BC to the atmosphere have led to environmental impacts at local, regional, and global scales. At the local level, BC, PM, and associated contaminants (i.e. polycyclic aromatic hydrocarbons (PAHs) (Fine et al. 2004; Fraser et al. 2003; Kumata et al. 2006; Molina and Molina 2004) are associated with a range of negative health effects, including increased mortality (Rabl and Spadaro 2000; Smith 1993), heightened asthma and respiratory conditions, and increased risks of cardiovascular disease (Lighty et al. 2000; Radomski et al. 2005). At the regional level, these aerosols have been implicated in the spiraling-down of regional hydrological cycling (Ramanathan and Carmichael 2008; Ramanathan et al. 2001); and at the global level these pollutants have been demonstrated to significantly impact the earth's radiative budget (Hadley et al. 2007; Ramanathan and Carmichael 2008; Ramanathan et al. 2001).

The state of our knowledge is still at the exploratory level when it comes to understanding the influence of combustion processes on climate and the feedback loops

This thesis follows the style of *Limnology and Oceanography: Methods*.

that eventually effect natural and human systems. For example, large uncertainties still exist in our efforts to reconstruct past BC emissions. Theoretical models using fuel consumption data are susceptible to large ranges in BC emission factors (Elmqvist et al. 2007; Streets et al. 2001). The difference in fuel consumption and reported fuel consumption data (fuel consumption is intrinsically heterogeneous at the regional level, whereas consumption data is usually reported at the national level) only heightens these ambiguities (Novakov et al. 2003; Streets et al. 2001). This uncertainty was highlighted in a recent study by Louchouart et al. (2007) which demonstrated a discrepancy between modeled estimates of BC emissions for the early 20th century in the U.S. vs. actual reconstruction from historical sedimentary archives in the northeast. Similar work in other areas (L. Michigan, L. Washington, Puget Sound) confirms the maximum in BC concentrations during the mid-20th century but suggests regional differences with respect to both BC emissions and fuel consumption (Elmqvist et al. 2007; Griffin and Goldberg 1983; Kuo et al. 2011a; Wakeham et al. 2005). Such historical records are paramount to the efforts of climate modelers since they provide data against which the wide-scale distribution of BC emissions can be gauged. A recent paper by a group of leading climate scientists (Hansen et al. 2005) specifically points to the need for such historical reconstruction using ice core and sediment core analyses at many global sites. The authors further suggest that “high resolution analyses of many cores in one region have the potential to offer a useful BC history at the decadal, centennial, and millennial scales”.

Both historical and regional records of BC-emissions are now being published with increased frequency showing that biomass combustion from both natural and anthropogenic activities contributes to the majority of atmospheric light-absorbing carbonaceous aerosols (Gustafsson et al. 2009; Hegg et al. 2009; McConnell et al. 2007; Tollefson 2009). According to some models, up to 40% of global BC emissions may originate from wildfires and prescribed agricultural burning (Tollefson 2009), whereas actual apportionment studies show that this proportion can represent from 50-90% of BC emissions at the regional level (Gustafsson et al. 2009); Hegg et al. 2009).

Despite of these findings, biomarkers of biomass combustion are still only regularly monitored in atmospheric particles, with a handful of studies tracing these markers in sediments (Elias et al. 2001; Hunsinger et al. 2008; Kuo et al. 2011a) and even fewer in dissolved OM (Hockaday et al. 2007; Dittmar 2008; Ziolkowski et al. 2010). In fact, little is known about the role that wildfires and other biomass combustion processes may play in the transfer of terrigenous organic matter (OM) to aquatic systems. Since thermal alteration of plant material generates heterogeneous structures with a variable proportion of soluble functional groups (Hammes et al. 2006; Knicker et al. 2008; Kuo et al. 2008a-b), we can anticipate that certain forms of BC will be important contributors to the dissolved OM pool exported by aquatic systems in watersheds heavily impacted by wildfires (Hockaday et al. 2007). Similarly, the pervasive presence of biomass combustion by-products in atmospheric particles could potentially lead to a large transfer of thermally altered terrigenous OM to the surface of aquatic systems through both dry and wet deposition. For example, in the large scale coastal system for Puget Sound,

Washington, the average total flux of anhydrosugars, a suite of exclusive biomass combustion markers, at the sea-air interface ($300 \mu\text{g}/\text{m}^2/\text{d}$; Brandenberger et al. 2010) is 4 orders of magnitude higher than calculated fluxes at the water-sediment interface ($\sim 0.25 \mu\text{g}/\text{m}^2/\text{d}$; Kuo et al. 2011a). This difference suggests that by-products of biomass combustion compounds are highly reactive in aquatic systems and/or that they are highly soluble. Considering such large atmospheric depositional fluxes as well as the substantial post-fire incorporation of charred plant material in surface soils (Alexis et al. 2007; Hockaday et al. 2007; Kuo et al. 2008b; Preston and Schmidt 2006), we can expect an important contribution of biomass combustion byproducts to the particulate and dissolved pools of OM transported by rivers to the coastal ocean. Preliminary work in Dr. Louchouart's lab has shown that, in addition to anhydrosugars, lignin-derived monomers are released in substantial quantities from remains of charred vascular plant materials (Kuo et al. 2008b; Norwood et al. 2011). Hence, major wildfire events in watersheds may act as significant sources of terrigenous OM to aquatic systems, further pointing to the impact of climate change and episodic events on the transfer of terrigenous OM to aquatic systems. The monitoring of hydrophilic and hydrophobic markers of biomass combustion (such as anhydrosugars and lignin monomers; Kuo et al. 2008a-b; Norwood et al. 2011) in dissolved OM will thus further help characterize the impact of fire on carbon cycling at the watershed scale.

CHAPTER II

METHODS

Preparation of samples

Two approaches were used to evaluate the respective recoveries of lignin phenols and anhydrosugars under the liquid-phase (LPE) and solid-phase (SPE) extractions: natural solutions prepared from charcoal extracts and standard solutions prepared from pure standards spiked into MilliQ water. Solutions of natural water-soluble organic matter were extracted from honey mesquite (HM) wood combusted at 250°C for 1 hour.

Approximately 1 g of charcoal was run through an Accelerated Solvent Extractor (ASE) using MilliQ water as the solvent. The extract was brought to 500 ml with MilliQ water and then divided into ten equal parts for extraction. Standard solutions were prepared by spiking 500 ml of MilliQ water with the calibrations standards (lignin phenols and anhydrosugars) at an average concentration of 100-200 ng/ml.

Liquid-phase extraction

Liquid-phase extractions were performed on both natural and standard solutions.

Approximately 10 ml of solution was spiked with 50 µl each of surrogate standards for each compound group (*d7*-cinnamic acid for lignin phenols and *d7*-levoglucosan for anhydrosugars). The samples were then acidified to pH<2 with approximately 0.5 ml 6M HCl. Following acidification, the samples were extracted three times with ethyl acetate (EA), separating the organic EA layer from the aqueous layer after each extraction. The

organic layer was dried with Na_2SO_4 , and then extracted into a clean centrifuge tube. The EA was evaporated from the sample using a LabconcoTM CentriVap set at 45°C. Once the EA was completely evaporated, the compounds were taken back into solution with two 150 μl volumes of pyridine and transferred into 2 ml amber vials. The aqueous layer remaining after EA extraction was freeze-dried in a LabconcoTM freeze-drier to recover water-soluble compounds not extract by the EA. The dry extracts were then taken back into solution with two 150 μl volumes of pyridine and transferred into 2 ml amber vials.

Solid-phase extraction

Both the natural and standard solutions underwent SPE in order to compare recoveries between the SPE and LPE. Approximately 50 ml of solution was spiked with 50 μl each of surrogate standards for each compound group. The samples were then acidified to $\text{pH} < 2$ with approximately 0.5 ml 6M HCl. Following acidification, the samples were run through an SPE cartridge consisting of an Empore C18 disk and an Empore SDB-RPS disk layered between glass-fiber filters (GFF) at a rate of approximately 10 ml/minute. Before extraction, the SPE cartridges were primed with 10 ml EA followed by 10 ml dichloromethane-methanol (DCM-MeOH; 9:1), and 10 ml of acidified MilliQ water. After extraction, the wet disks were rinsed with 20 ml of acidified MilliQ water and then freeze dried. After drying, the C18 and SDB-RPS disks were run together through the ASE with a solution of DCM:MeOH (9:1). Following extraction, the extracts for each suite of compounds were concentrated to approximately 1.5 ml using a LabconcoTM

RapidVap, and then to complete dryness using a Labconco™ CentriVap. Once the solvent was completely evaporated, the compounds were taken back into solution with two 250µl volumes of pyridine and transferred into 2 ml amber vials.

Extraction of natural solutions

The LPE protocol was applied to duplicates of 7 samples collected from the Lena River at various times throughout the year of 2004 in order to collect a data set representing the seasonal hydrology. Samples were collected in an integrated sampler and then filtered through 0.45 µm Pall Aquaprep 600 capsule filters into acid-washed 1 liter polycarbonate bottles before freezing in the field for preservation. The samples were then frozen and kept in the dark during shipment and distribution.

Quantification and analysis

After extraction, all samples were quantified and analyzed using gas chromatography and mass spectrometry. Samples were prepared for gas chromatographic analysis by combining 50 µl BSTFA (Supelco, PA, USA), 50 µl sample, and 50 µL internal standard (d7-Anisic acid) in microvials. Prior to injection, samples were heated on a 20-well block WWR Digital Heatblock at 74.5°C for 15 minutes to trimethylsilylate any exchangeable hydrogens present in the extract. Separation and quantification of target analytes was performed using a Varian GC-MS with a triple-quadrupole 450-300 system fitted with a fused silica column (J&W DB-5MS, 30 m x 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies, CA). Each sample was injected under splitless mode

into a deactivated glass liner inserted into the GC injection port using HE as the carrier gas (1.0 ml/min). The GC oven was programmed from 65°C (with a 2-minute initial delay) to 240°C using a 6°C/min temperature ramp, at which point it was raised to 300°C (held for 10 minutes) at a rate of 10°C/min. The GC injector and GC-MS interface were maintained at 280°C and 270°C, respectively. The MS was operated in the electron ionization mode (70 eV) using full scan mode (FS). Compound identification was performed using GC retention times and comparisons of full mass spectra to commercially available standards.

CHAPTER III

RESULTS

Preparation of standard solutions

Standard solutions for extraction were prepared using MilliQ water and spiking standards in methanol for both lignin phenols and anhydrosugars. The concentration of the spiking solutions was verified by creating 5-points calibration curves for each standard and using deuterated compounds as surrogates (*d7*-cinnamic acid for lignin phenols and *d7*-levoglucosan for anhydrosugars). The calibration solutions was prepared in pyridine and ranged 0.2, 0.5, 1.0, 2.0, and 4.4 ng/ul for lignin phenols and 0.3, 0.6, 1.4, 1.6, and 2.8 ng/ul for anhydrosugars. The resulting calibration solutions and all other samples were then analyzed by GC-MS. Calibrations were linear over the range tested with average $r^2 \geq 0.996$ (Figures 1 and 2).

Liquid-phase extraction

The extraction of standard solutions using the LPE method yielded an average recovery of $100 \pm 9\%$ for the lignin-oxidized phenols (LOP; Table 1), indicating both a high internal reproducibility and a high accuracy for the LPE method. For natural solutions using extracts from honey mesquite combusted at 250°C (HM250), the LPE method produced an average value of 2.95 ± 0.15 ug per gram ($n=3$; Table 2), which shows again a very low variability (5%) in the extraction-quantification procedure. Back calculations using the aqueous concentrations of lignin phenols from the LPE of natural solutions

produced a value of 1.72 ± 0.09 mg of lignin constituents per gram of charcoal extracted. This value is comparable to the value of 2.10 ± 0.08 mg of lignin constituents per gram of charcoal found by Norwood et al. in 2011. The calculated value of lignin constituents per gram of charcoal for the LPE extractions was found to be $82 \pm 15\%$ of previously published values, demonstrating that the LPE method is also reproducible across time.

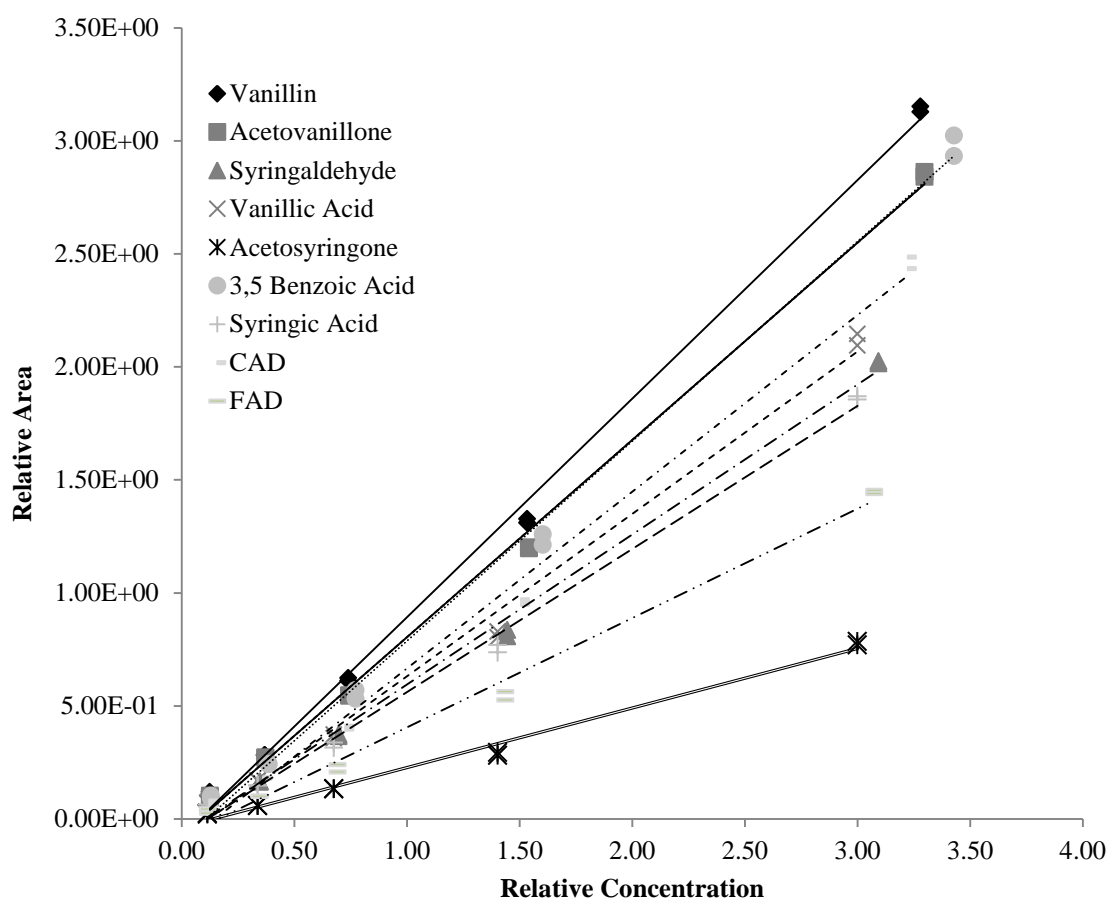


Figure 1: 5-point calibration curves for spiking solutions of lignin phenols. Each sample was injected twice. Concentrations are given in ng/ul.

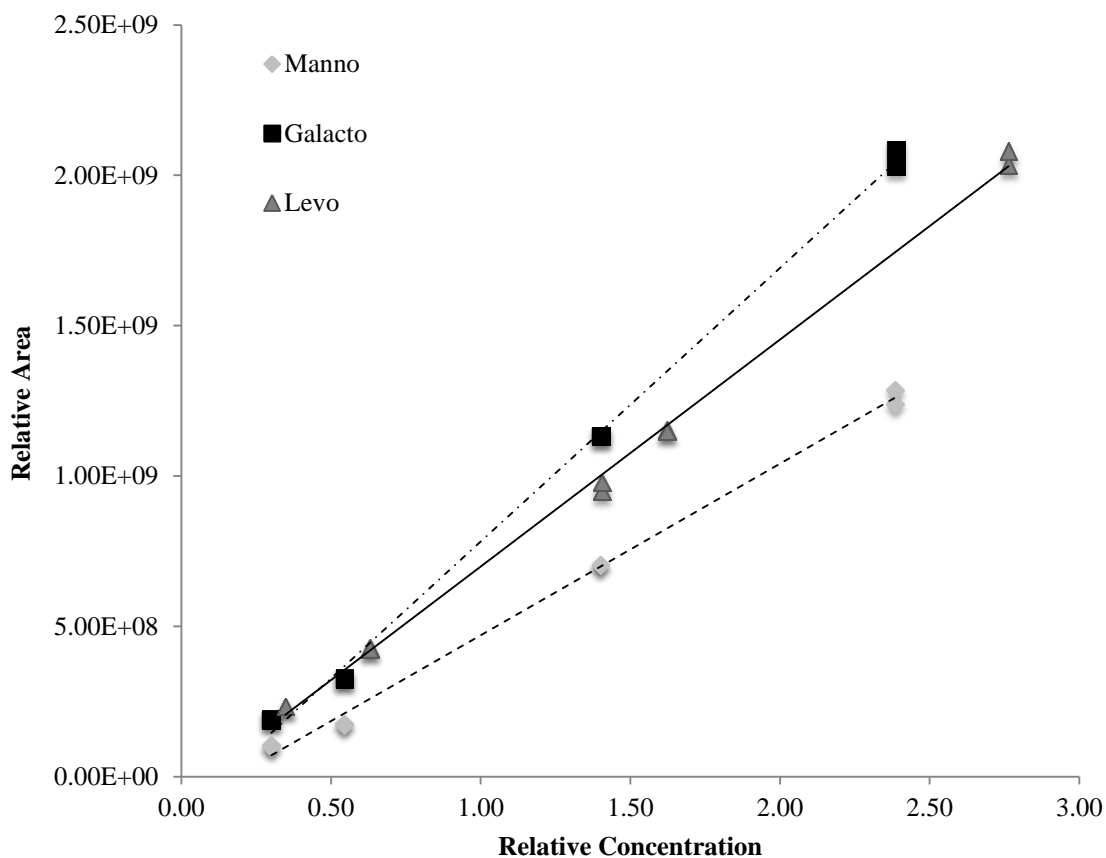


Figure 2: 5-point calibration curves for spiking solutions of anhydrosugars. Each sample was injected twice. Concentrations are given in ng/ul.

Table 1: Concentrations of lignin phenols in standard solutions from LPE. Concentrations are given in ng/ul.

Compound	Average Concentration	Standard Deviation	Percent Recovery
Vanillin	18.0	1.5	106.8
Acetovanillone	16.7	0.8	96.6
Syringaldehyde	18.3	1.3	106.3
Vanillic Ac	20.9	5.3	113.8
Acetosyringone	18.4	4.1	102.5
3,5 diOH Benzoic Ac	18.6	3.7	91.3
Syringic Ac	15.5	3.2	86.5
p-Coumaric Ac	18.4	4.5	94.0
Ferulic Ac	17.4	3.5	95.9
Average	18.0	3.3	100±9

Table 2: Concentrations of lignin phenols in LPE extraction of honey mesquite. Concentrations are given in ng/ul.

Compound	Average Concentration	Standard Deviation
Vanillin	0.5	0.0
Acetovanillone	0.0	0.0
Syringaldehyde	1.1	0.1
Vanillic Ac	0.4	0.0
Acetosyringone	0.1	0.0
3,5 diOH Benzoic Ac	0.0	0.0
Syringic Ac	0.5	0.0
p-Coumaric Ac	0.0	0.0
Ferulic Ac	0.0	0.0

Analysis of standard solutions spiked with anhydrosugars that underwent LPE demonstrated a variable recovery of sugars. The average recoveries of galactosan and levoglucosan were $86\pm 3\%$ and $111\pm 13\%$ respectively, while the average recovery for mannosan was $141\pm 6\%$. However, running standard solutions spiked with anhydrosugars that had not undergone LPE produced an average recovery of $91\pm 7\%$ (Table 3). For natural solutions using extracts from HM, LPE produced an average value of 327.7 ± 10.8 ug per gram of charcoal (Table 4; n=3), which is indistinguishable from previously published values for anhydrosugar content in charcoal (Table 5). In addition, the LPE extraction of anhydrosugars from natural solutions has a lower variability (4%) than previously produced data (5-20%). This indicates that the LPE method is both internally reproducible and highly accurate for compounds with a high anhydrosugar composition.

Solid-phase extraction

As of the current date, experiments regarding the solid-phase extractions have yielded no results for anhydrosugars. Analysis of standard solutions that underwent SPE demonstrated no recovery of anhydrosugars and variable quantities of lignin phenols. The extraction of LOP by the C18 material was actually much lower than the LPE, demonstrating that the specific filters used provide a much lower extraction performance than other C18 materials (syringes) used in prior publications (Louchouart et al. 2000). The SDB material, however, does not extract the more polar sugars from solution, which means that further tests will be required to identify the appropriate sorbent to extract such highly polar molecules from aqueous solutions.

Table 3: Concentrations of anhydrosugars in standard solutions without LPE. Concentrations are given in ng/ul.

Compound	Average Concentration	Standard Deviation	Percent Recovery
Galactosan	8.5	0.4	84.7
Mannosan	8.9	0.6	89.2
Levogluconan	9.9	0.4	98.9
Average	9.1	0.5	90.9

Table 4: Concentrations of anhydrosugars in LPE extraction of honey mesquite. Concentrations are given in ug/g.

Compound	Average Concentration	Standard Deviation
Galactosan	417.9	10.8
Mannosan	152.7	3.3
Levogluconan	655.4	21.6

Table 5: Average concentrations of anhydrosugars in honey mesquite charcoal from different methods of extraction (Norwood et al. 2011).

Method	Average concentration (ug/g)	(+/-)
H ₂ O Extraction	297.7	67.3
H ₂ O Extraction	310.2	61.4
MeOH Extraction	320.3	24.2
MeOH Extraction	325.5	17.5
LPE Extraction	327.7	10.8

Extraction of natural solutions

Analysis of extractions from LPE of natural solutions taken from the Lena River demonstrated a relationship between the concentrations of free phenols and polymeric lignin (Amon et al. 2011) present in the samples. Both the free phenols and polymeric lignin demonstrated a temporal trend along a seasonal gradient with a peak in concentration during the spring freshet peak flow event (Figure 3; Table 6), which points to a high input of lignin products during the spring flow. The ratio of free phenols to polymeric lignin varied from 2-9% with a maximum occurring prior to the spring freshet event and a minimum occurring during the spring freshet event (Table 6).

The acid to aldehyde ratios of both the free phenols and LOP varied temporally with the free phenols showing a much larger range (1.4-14.1; Table 6) than the LOP (1.0-1.6; Table 6). The peak of the acid-aldehyde ratio for free phenols corresponded with that of the maximum concentration of free phenols, both of which occurred during the spring freshet event.

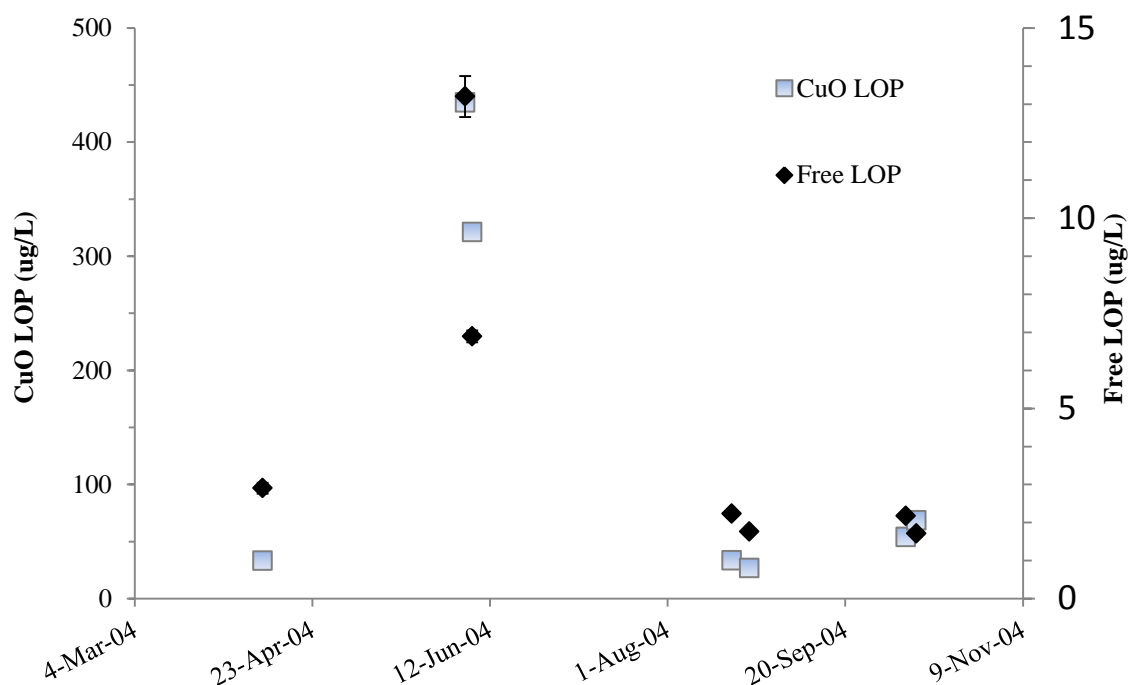


Figure 3: Concentrations of free phenols and polymeric lignin taken from Lena River samples collected in 2004. Free phenol concentrations were obtained by analysis of LPE extraction. Polymeric lignin concentrations were obtained by analysis of copper oxide (CuO) oxidation. Error bars denote standard deviation.

Table 6: Concentrations of free phenols and polymeric lignin extracted from samples taken from the Lena River. Free phenol concentrations were obtained by analysis of LPE. Polymeric lignin concentrations were obtained by analysis of copper-oxide (CuO) oxidation extraction (Amon et al. 2011). Sigma8 value represents ratio of free phenols to polymeric lignin. (Ad:Al) value represents ratio of acids to aldehydes in free phenol concentrations.

Sample Date	Free Phenol Concentration (ug/L)	Polymeric Lignin Concentration (ug/L)	Sigma8	(Ad:Al)
9-Apr-04	2.9	33.3	9%	2.81
5-Jun-04	13.2	434.9	3%	14.14
7-Jun-04	6.9	321.1	2%	8.58
19-Aug-04	2.2	33.4	7%	2.89
24-Aug-04	1.8	26.8	7%	2.94
7-Oct-04	2.2	53.9	4%	1.45
10-Oct-04	1.7	68.6	2%	4.40

CHAPTER IV

SUMMARY AND CONCLUSIONS

The analysis of the results from the liquid-phase extraction demonstrates the effectiveness of using this method to extract lignin phenols from both natural and standard solutions. The average recovery of lignin-oxidized phenols (LOP) from standard solutions was $100\pm 9\%$, indicating that the LPE procedure is both highly accurate and internally reproducible with low variability. The LPE of natural extracts from honey mesquite (HM) combusted at 250°C produced average values comparable to previously published concentrations of lignin constituents in honey mesquite charcoal. LPE of the HM extracts yielded a concentration of 1.72 ± 0.09 mg of lignin constituents per gram of charcoal, which is comparable to the concentration of 2.10 ± 0.08 mg of lignin constituents per gram produced during prior extractions of HM extracts (Norwood et al. 2011). The match between these two values signifies that the LPE method is both internally reproducible and reproducible across time.

Though the LPE of standard solutions spiked with anhydrosugars demonstrated a variable recovery of sugars, LPE was still an effective method of extraction for compounds with a high anhydrosugar composition. The average recoveries of the anhydrosugars galactosan, levoglucosan, and mannosan ($86\pm 3\%$, $111\pm 13\%$, and $141\pm 6\%$, respectively) from LPE demonstrate a high internal reproducibility as well as a low variability. Although the accuracy was acceptable for two of the anhydrosugars

(galactosan and levoglucosan), mannosan was overestimated by 40%, suggesting that there are possibly different efficiencies of extraction for different sugars. Interestingly, analysis of standard solutions that did not undergo preliminary LPE also demonstrated high recovery and low variability. These samples, which were spiked, freeze-dried, re-taken into pyridine, and analyzed by GC-MS, produced an average recovery of $91 \pm 7\%$, indicating that extraction of anhydrosugars may not be necessary for analysis of concentration. Using the natural solutions from the extraction of HM, LPE produced an average concentration of 327.7 ± 10.8 ug per gram of charcoal, a value indistinguishable from the previously published data derived from high-temperature, high-pressure solvent extractions for HM250 charcoal (320.3 ± 24.2 ug/g; Kuo et al., 2011). This type of reproducibility across methods further reinforces the effectiveness of the LPE as a method of extraction of polar compounds from both natural and standard solutions.

The SPE of both standard and natural solutions demonstrated measurable quantities of LOP and no recovery of anhydrosugars. The extraction of LOP by the C18 material, however, was highly variable and much lower than the LPE, demonstrating that the specific filters used provide a much lower extraction performance than other C18 materials (syringes) used in prior publications (Louchouart et al. 2000). The SBD-RPS material yielded no recovery of anhydrosugars from spiked or natural solutions, indicating that this particular material is not an appropriate sorbent to extract these highly polar molecules. Further testing is required to find a sorbent that will efficiently and accurately extract such molecules from aqueous solutions.

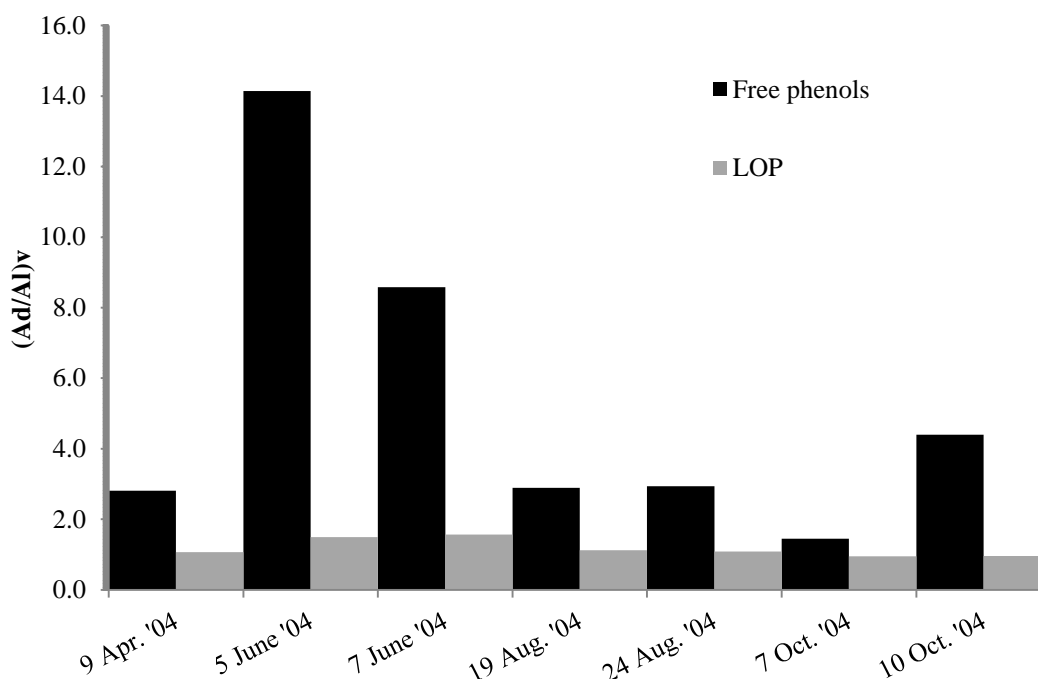


Figure 4: Acid to aldehyde (Ad/Al) ratios for free phenols and polymeric lignin in samples taken from the Lena River. Free phenol ratios were obtained by analysis of LPE extraction. Polymeric lignin ratios were obtained by analysis of copper oxide (CuO) oxidation.

After LPE was demonstrated to be an efficient method of extracting free lignin-phenols from natural solutions, we used this method to extract lignin phenols across a seasonal gradient of samples taken from the Lena River in 2004. The concentrations of free phenols demonstrated a temporal trend with a peak during the spring freshet peak flow event (Figure 3) that occurs during late May and early June (Amon et al. 2011). The polymeric lignin oxidation products (LOP) concentrations peaked during the spring freshet event with a minimum concentration occurring during the winter base flow (Figure 3). The free phenol concentrations matched those of the LOP, increasing by a full order of magnitude between winter base flow months and the spring freshet. The ratio of free phenols to polymeric lignin also exhibited a temporal trend with free

phenols making up the lowest proportion of polymeric lignin during winter months and the spring freshet (2%) and the highest proportion during the early spring and late summer (7-9%). In addition, the ratio of acid to aldehydes of free lignin phenols also demonstrated a seasonal trend similar to that observed in LOP but with a much wider range (1.4-14.1 vs. 1.0-1.6, respectively). The acid to aldehyde ratio of vanillyls [(Ad/Al)_v] for the free phenols was clearly affected by the river flow, increasing during the spring freshet and exhibiting a minimum during winter base flow (Figure 4).

Lignin monomers are often used as indicators of terrestrial organic matter in coastal systems. Amon et al. (2011) found a strong relationship between lignin constituents and the age of DOM in Arctic river systems. The concentration of lignin phenols decreases with increased age of DOM (determined by ¹⁴C signatures), meaning that younger DOM has higher concentrations of monomeric and polymeric lignin. Ad/Al ratios, which increase with increased degradation of organic matter, also demonstrate a strong relationship to the relative age of DOM, increasing with decreasing age of DOM. Combined with the lignin phenol concentrations found by this study and Amon et al. in 2011, this provides strong evidence that recently released vascular plant material is a major source of much of the DOM transported by Arctic rivers during the spring freshet. Although all lignin phenols are exclusive indicators of vascular plant material, they tend to predominantly exist in the environment in polymeric form, rather than monomeric form. In fact, to our knowledge, this is the first study that quantifies free lignin phenols in natural waters and compares their yield to polymeric lignin. The release of free lignin monomers in the environment can be mediated by microbial degradation as well as

abiotic processes such as thermal degradation (e.g. combustion). However, only combustion releases the anhydrosugar components that we were trying to identify in our study (Kuo et al. 2008). Without the analysis of the anhydrosugar concentrations, it is impossible to differentiate between combustion-derived LOP and humic-derived LOP. This further emphasizes the importance of developing a method for extracting these highly polar molecules from aqueous solutions. Several studies have pointed out the importance of combustion as a source of organic carbon into the environment (DeLuca and Applet 2008; Kuhlbusch and Crutzen 1995; Kuo et al. 2008; Skjemstad et al. 2002). With a changing global climate, combustion may prove to be an important source in the transfer of organic carbon to the coastal ocean. Increased fire events and changing hydrology may result in a faster turnover of DOM between terrestrial and aquatic systems, making the identification of DOM sources an important priority in studying the transfer of organic material in natural systems.

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