REACTIVITY OF CHARCOAL-DERIVED WATER SOLUBLE

BIOMARKERS IN RIVER WATER

A Senior Scholars Thesis

by

MATTHEW NORWOOD

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

UNDERGRADUATE RESEARCH SCHOLAR

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Major: Marine Biology

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Approved by:

Research Advisor: Patrick Louchouarn
Director for Undergraduate Research: Sumana Datta

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ABSTRACT

Reactivity of Charcoal-derived Water Soluble Biomarkers in River Water. (April 2011)

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The purpose of this study is to evaluate the residence time of water-soluble levoglucosan and free lignin-derived phenols, from two different plant charcoals. Dissolved organic matter (DOM) was extracted from honey mesquite and cordgrass charcoal combusted at 250°C. The DOM was incubated with aliquots of filtered water collected from the Trinity River, (TX) for 37 days. We found that the reactivity of levoglucosan was similar to that of freely dissolved lignin-derived phenols in natural water. The results also indicate a shift in lignin aldehydes into acid moieties during the early stages of biodegradation (~2 days for cordgrass and ~6 days for honey mesquite). Based on the molecular nature of levoglucosan (carbohydrate derivative), it was anticipated that it would react quickly and be consumed faster by bacteria than the lignin phenols. The contradiction between the results of the experiment (similar reactivity of both biomarkers) and this initial prediction suggests that anhydrosugars are sufficiently altered to increase their residence time in natural waters to a range similar to more recalcitrant aromatic constituents of DOM. Furthermore, the near complete consumption

of these freely dissolved monomers within ~ 3 weeks suggests that combustion-derived

water-soluble organics can fuel significant bacterial respiration in natural waters.

NOMENCLATURE

CG_{con}	Cordgrass control
CG_{inc}	Cordgrass incubate
DOM	Dissolved organic matter
HM/CG_{con}	Both honey mesquite and cordgrass control groups
HM/CG_{inc}	Both honey mesquite and cordgrass incubated groups
HM_{con}	Honey mesquite control
HM_{inc}	Honey mesquite incubate
LP	Freely-dissolved lignin phenols
LvG	Levoglucosan
POM	Particulate organic matter
TOM	Terrigenous organic matter
$T_{1/2}$	Half life

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CHAPTER I

INTRODUCTION

Lignin is the second most abundant naturally occurring polymer after cellulose, and it is a complex and important structural component of vascular plants (Louchouarn et al., 2009; Kuo et al., 2008a; Sarkanen and Ludwig, 1971). Results from previous studies indicate that along with photochemical degradation, thermal alteration is an important abiotic degradation process for lignin (Kuo et al., 2008a). During the combustion of terrigenous organic matter (TOM), free lignin phenols (LP) and dehydro-monosaccharide derivatives such as levoglucosan are formed (Kuo et al., 2008b; 2011). The dehydro-monosaccharide levoglucosan (1,6-anhydro-β-D-glucopyranose) and its isomers (galactosan and mannosan) are derived from the combustion of cellulose and hemicelluloses, respectively (Elias et al., 2001; Kuo et al., 2008b; Louchouarn et al., 2009). Biomass combustion, from either wildfires and/or prescribed agricultural and forest burning, lead to large fluxes of thermally altered organic matter to soils (Preston and Schmidt, 2006; Alexis et al., 2007). Recent evidence suggests that charcoal and other forms of black carbon (BC) are susceptible to environmental processes of degradation releasing water-soluble derivatives into soils pore waters and eventually natural water systems (Hockaday et al., 2007).

This thesis follows the style of *Organic Geochemistry.*

However, although the flux of soil BC to streams bears important implications for the global carbon cycle, the dissolution, export, and fate of carbon-rich combustion residues is yet unmeasured. Because low temperature charcoals (150-300°C) are predominantly produced during biomass combustion (Alexis et al., 2007) and yield maximum quantities of soluble constituents (Kuo et al., 2008b, 2011), more work needs to address the potential influence of these BC species on aquatic metabolism.

In this study, we evaluated the fate of water-soluble biomarkers of biomass combustion by incubating charcoal dissolved organic matter (DOM) with natural river water collected in a coastal environment from Eastern Texas. Lignin biomarkers and levoglucosan have been successfully utilized as molecular tracers of combusted TOM in diverse terrestrial, aquatic, and atmospheric environments (Elias, et al., 2001; Simoneit, 2002; Hunsinger et al., 2008; Kuo et al., 2008a,b; Louchouarn et al., 2009). However, the residence time for levoglucosan and lignin phenols in natural water systems is unknown. Such information is critical to a) evaluate the potential of such molecular markers to act as viable tracers for the movement of combustion-derived DOM in aquatic environments at relevant time scales of transport (days to weeks), and b) to evaluate the rate(s) of reaction of different water-soluble constituents of BC. This study utilized two different charcoals derived from honey mesquite (*Prosopis glandulosa Torr.*) and cordgrass (*Spartina alterniflora*) to extract DOM. Each of the two terrestrial plants contains different concentrations of lignin and levoglucosan. Honey mesquite is enriched in lignin with respect to cellulose/hemicelluloses, whereas the opposite is true

for cordgrass (Kuo et al., 2008a). Although abiotic processes can lead to the degradation of lignin-derived phenols and anhydrosugars in the environment (Opsahl and Benner, 1998; Kuo et al., 2008a,b), we focused on the fate of these constituents under exclusive biological degradation in aquatic environments. The objective for this study was to test if carbohydrate by-products of combustions, such as levoglucosan, degrade substantially faster than lignin-derived phenols in natural waters. Due to the chemical nature of levoglucosan, a dehydro-monosaccharide derivative, we expected to observe a faster degradation rate and with a shorter residence time than those of the apparently more recalcitrant lignin phenols. To test this hypothesis, water-soluble charcoal-derived DOM from the two selected plant tissues was incubated over a period of 37 days. Each time series was sequentially extracted to isolate lignin and levoglucosan biomarkers, respectively. The extract series were then quantified using gas chromatography-mass spectrometry under full scan mode and using electronic ionization. Degradation was determined in comparison to concentrations in controls and at initial time of incubation, and modeled as first order exponential decay rates.

CHAPTER II

MATERIALS AND METHODS

Two different angiosperms, honey mesquite (*Prosopis glandulosa Torr.*) and cordgrass (*Spartina spartinae*), were selected for combustion. These samples were collected from Laguna Atascosa Natural Wildlife Refuge (Rio Hondo, TX) in July 2005. A detailed description of charcoal preparation is given in Kuo et al, 2008b. Briefly, the plant material was cut into small pieces and dried. Both the honey mesquite and cordgrass were combusted at 250°C for one hour in the absence of oxygen. Two grams of crushed and homogenized charcoal from each plant was mixed with precombusted sand and placed into an accelerated solvent extractor (ASE) at a pressure of 1500 psi for one hour. All of the glassware used in this study was soaked in microbial-90 and combusted at 450°C. The dissolved organic matter (DOM) was extracted into milli-Q water. After extracting the DOM the total volume of each vial after extraction was brought up to a volume of 250ml. The samples were partitioned into 20ml aliquots and placed into test tubes corresponding to controls (HM_{cont} and CG_{cont}, respectively) and incubated (HM_{inc} and CG_{inc}, respectively). The incubated extracts were spiked with 2ml (10%, v/v) river water taken from the Trinity River, TX on January 12, 2011. Each of the controls was treated with 1.5ml (15M) solution of sodium azide (NaN_3) in order to kill any native

bacteria. The vials were marked with the dates of extraction (0-37 days), which were extracted at predetermined time intervals. During the course of the trial the samples were continuously shaken in a dark room in-order to keep the solutions homogenous and protect against photochemical breakdown, respectively.

The water-soluble lignin phenols were determined according to methods developed in previous studies (Hedges and Ertel, 1982; Goni and Hedges, 1992; Louchouarn et al., 2000; 2009; 2010; Kuo et al., 2008b). Briefly, the surrogate standards trans-cinnamic acid (3-phenyl-2-propenoic acid) and d7-levoglucosan were spiked into each treatment (50µl) in order to calculate recovery rates for the extraction of lignin phenols and levoglucosan, respectively. The aqueous solution was first acidified to a pH of 0-2 using a 6M HCl solution. The LP were first extracted with ethyl acetate twice and treated with sodium sulfate (Na_2SO_4) to remove excess water. The organic extract was evaporated off using a LabConco™ solvent concentrator into a DOM paste. The residual water from the ethyl acetate extraction was then freeze-dried into a DOM paste. Both DOM pastes were diluted first into a small volume of pyridine (400µL). Following further dilution with pyridine (50:300µL), an aliquot (75 µL) was transferred to a 1.5 mL glass vial to which 75 µL of N,O-bis (trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS; Supelco, PA, USA) was added. The samples were then derivatized under normal atmosphere by heating at 75 °C for 1 h in a 20-wells block heater. After derivatization, each sample was transferred to a 250 µL glass autosampler vial insert.

Separation and quantification of trimethylsilyl (TMS) derivatives of CuO oxidation by-products were performed using gas chromatography–mass spectrometry (GC/MS) with a Varian triple quadrupole 480-300 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). Each sample was injected, under splitless mode, into a deactivated glass liner inserted into the GC injection port and using He as the carrier gas (~ 1.0 mL min^{-1}). The GC oven was programmed from 65 °C (with a 2 min initial delay) to 300 °C (held 10 min) using a 4 °C min^{-1} temperature ramp. The GC injector and GC/MS interface were maintained at 280 °C and 270 °C, respectively. The mass spectrometer was operated in the electron ionization mode (EI, 70 eV) using full scan mode (FS). Compound identification was performed using GC retention times and by comparing full mass spectra with those of commercially available standards. The instrument was operated in the mass range m/z 50–500, while 1–3 target ions (including the base ion) were selected for quantification.

Duplicate incubations were performed for five time periods (T0-T12) to evaluate the experimental variability for both biomarker series. The average coefficients of variability (CV) for lignin concentrations across replicate time periods was $2.6 \pm 3.2\%$ and $2.8 \pm 3.3\%$, respectively, for the control and incubation time series. The average CV for levoglucosan concentrations across replicate time periods was 2.8 ± 3.1 % and $3.9 \pm 4.2\%$, respectively, for the control and incubation time series. To estimate significant departures in biomarker concentrations in incubates from the natural experimental

variability of analysis, the expanded uncertainty (U) around the average of all controls

(n=14), was calculated as U = ks, where s is one standard deviation of the analyte mean,

and k is the coverage factor determined from the Student's *t*-distribution corresponding

to the associated degrees of freedom at the 95% confidence level (NIST, 2007). The

retention time and spectra of each standard were used for compound recognition. The

quantification was performed based upon relative response factors using absolute and

surrogate standard compounds.

CHAPTER III

RESULTS

We used sodium azide to kill the natural bacteria within the HM/CG$_{con}$. The bacteria occur from accidental inoculums from the milli-Q water used to extract the DOM. No significant decrease in the target molecular constituents was found within a 95% confidence window showing the efficient sterilization of the control solutions. In contrast, we found a significant decrease in lignin phenols (mg/L; Figs. 2-7) for HM/CG$_{inc}$, within the first four days, and with half-lives (t$_{1/2}$) of 4.1 and 4.7 days respectively. The *k*-value for HM/CG$_{inc}$ differed for the individual molecular constituents. The syringyls have slightly greater *k*-values than the vanillys in both plant charcoals, (Table 1 in appendix). Although the two lignin constituents have different *k*-values, both have very similar t$_{1/2}$ within the first 5 days of incubation. The exponential decay of both vanillin (aldehyde) and vanillic acid (acid) for HM/CG$_{inc}$ suggests a shift in molecular species from aldehyde into acids, (Fig. 10, 11, 12, 13). These figures illustrate the molecular transformation of aldehydes into acid moieties during the first days of incubation and also show a longer period of transformation for syringyls (~6 days) than for vanillyls (~2 days). Beyond six days, syringic and vanillic acids decrease substantially, which indicates that once the aldehydes are turned into acids, potentially via hydration reactions, resident bacteria consume the acids during metabolic processes. Although the individual lignin phenols degrade at different rates for the incubated

charcoals, there is no difference for similar compound groups across the different types of terrestrial plant materials (CG vs. HM).

Over the 37 days incubation period, the biodegradation of levoglucosan (LvG) was characterized by k-values of -0.09 and -0.15 d^{-1} for HM_{inc} and CG_{inc}, respectively. The $t_{1/2}$ values for HM/CG_{inc} are 7.7 and 4.7 days, respectively. These data indicate similar decay rates and residence times for levoglucosan and freely dissolved lignin phenols derived from low temperature charcoals.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The objectives for this study were first to measure the biodegradation rates (k) and half-lives ($t_{1/2}$) of different biomass combustion-derived biomarkers (lignin-derived phenols and levoglucosan) during incubatins with natural river bacterial communities. Secondly, we sought to investigate potential differences in degradation rates across molecular species. Contrary to expectations, lignin-derived phenols and cellulose-derived levoglucosan degrade at very comparable rates under biological degradation ($t_{1/2}$ of 4-5 *vs*. 4-7 days, respectively). Over the course of the 37 day incubation period, the charcoal-derived dissolved monomers were rapidly utilized pointing to the reactivity of water-soluble organic matter derived from biomass combustion. The half-lives of both compound groups (4-7 days), suggest that they will only be present in natural waters within time scales relevant to rapid mixing and transport (days to weeks).

The rapid utilization of vanillyl phenols in the first three days, prior to significant changes in syringyl phenols, contradicts earlier studies suggesting that they are more labile than vanillyls. Our results also show that the shifts in phenol concentrations and the transformations of aldehydes into acid moieties have a strong potential to affect lignin source and diagenetic ratios (S/V and acid/aldehyde ratios; Louchouarn et al., 2010).

In nature, the factors affecting the degradation of the studied biomarkers include bacteria, fungi, and abiotic process such as photochemical degradation. Due to the fact that we incubated the DOM in the absence of light we eliminated the photochemical processes that could contribute to the breakdown of lignin-derived molecular constituents in particular (Opsahl and Benner, 1998). We found that bacterial degradation is the cause for degradation within the 37 day incubation period. This is illustrated in the HM/CG controls in which there is no significant change (95% confidence interval) in the targeted concentrations treated with sodium azide. Sodium azide is a chemical that inhibits the glycolytic pathways of bacterial cellular respiration, resulting in cell death. The constant biomarker concentrations in the controls demonstrate the targeted inhibition in bacterial activity. The biomarker LvG can be used as a carbon source by bacteria, fungi, and yeast by converting it to glucose-6-phosphate by specific enzymes (Nakagawa et al. 1984; Kitamura et al. 1991). The transformation of anhydrosugars by bacteria can be an important method for the turnover of carbon in the environment. During fire events, black carbon (BC) is produced, releasing thermally altered methoxy phenols and anhydrosugars into sediments. Water run-off from rainfall, floods, and snow melt leaches these constituents out of sediments into solution. The biotic and abiotic processes that break down these carbon moieties form the link between carbon tied up in terrestrial plants and free carbon in the environment.

This study is important in putting together a clear picture of the importance BC to microbial communities within natural waters, such as coastal rivers, estuaries, and

coastal systems. The flux of BC constituents into these systems are not largely known so this work should be followed up by further studies in which the total carbon and polymeric lignin turnover rates are investigated by using dissolved organic carbon (DOC) analysis and the copper oxidation method (CuO), respectively.

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APPENDIX

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┌─────────────────────────────────────────────┐
│  Accelerated Solvent Extraction of Charcoal  │
│              Solvent: H₂O                     │
│            (DOM 250ml X2)                     │
└─────────────────────────────────────────────┘
                      ↓
┌─────────────────────────────────────────────────────┐
│ Partition aliquots of HM/CG controls and HM/CG       │
│ incubate (20 ml)                                     │
│ (Spike incubate with 10% River Water)                │
└─────────────────────────────────────────────────────┘
                      ↓
┌──────────────────────────────────────────────────────────┐
│ Partition samples into duplicates (5 ml) and add          │
│ surrogate standards                                       │
│ (50µl of 3-phenyl-2-propenoic acid and d7-Levoglucosan)   │
└──────────────────────────────────────────────────────────┘
                      ↓
┌──────────────────────────────────────────────────────────┐
│ Acidify to pH of 0-2 with 6M HCL and add 2.5 ml of        │
│ Ethyl Acetate (EA)                                        │
│ (Vortex for 1 minute and centrifuge for 5 minutes X2)     │
└──────────────────────────────────────────────────────────┘
          ↓                              ↓
┌────────────────────────┐   ┌────────────────────────────┐
│ LP extracted into EA    │   │ Anhydrosugars are in H₂O    │
│ and placed on cold      │   │ solution and freeze dried   │
│ centri-vap              │   │ (Anhydrosugar Paste)        │
│ (LP Paste)              │   │                             │
└────────────────────────┘   └────────────────────────────┘
          ↓                              ↓
┌────────────────────────┐   ┌────────────────────────────┐
│ Paste worked into       │   │ Paste worked into Pyridine  │
│ Pyridine                │   │ (diluted to 50:300µl)       │
│ (diluted to 50:300µl)   │   │                             │
└────────────────────────┘   └────────────────────────────┘
          ↓                              ↓
          └──────────────┬───────────────┘
                         ↓
        ⬡───────────────────────────────────⬡
        │      Quantitative analysis of       │
        │ Lignin phenols and Anhydrosugars    │
        │     Gas Chromatography Mass         │
        │ Spectrometer (GC/MS) Varian 480-300 │
        ⬡───────────────────────────────────⬡
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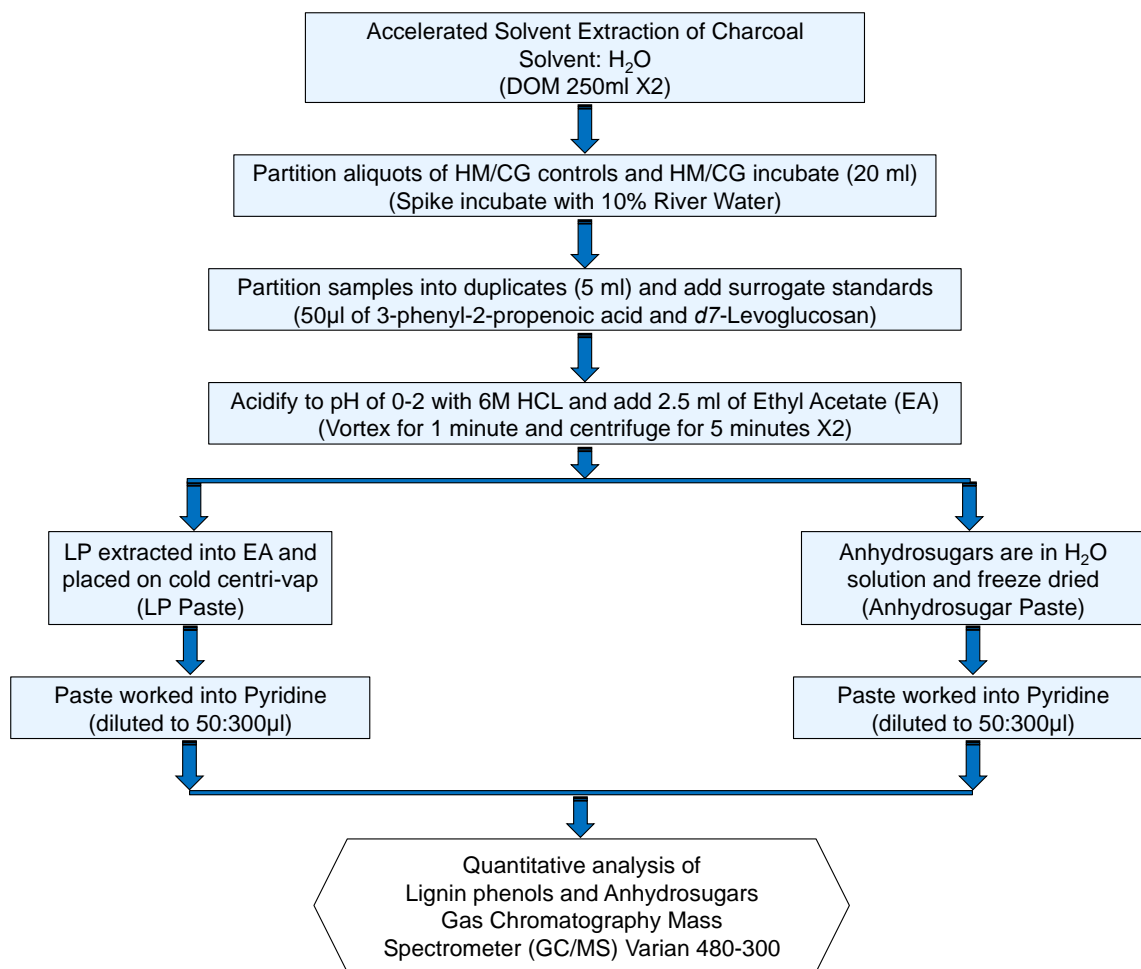
Figure 1. Flow chart of the sequential extraction protocol and analytical approaches to measure water-soluble lignin phenols and levoglucosan in charcoal dissolved organic matter.
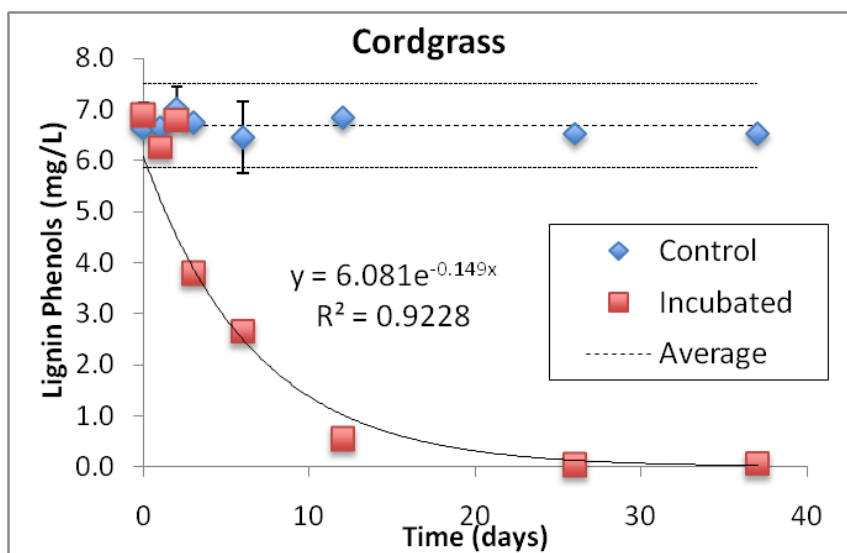
Figure 2. Exponential decrease of water-soluble lignin phenols derived from cordgrass charcoal over 37 days incubation (with 10% river water). The value of control represents the mean of all replicates (n=14).
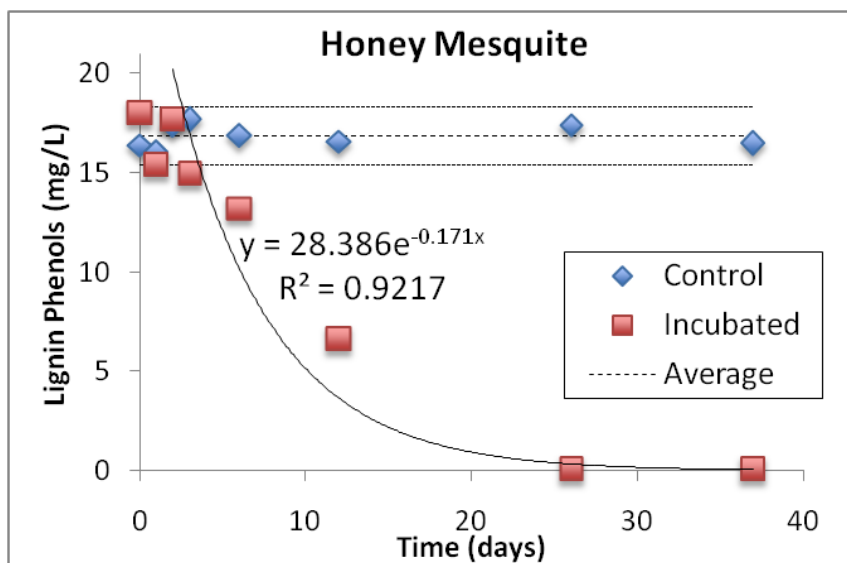
Figure 3. Exponential decrease of water-soluble lignin phenols derived from honey mesquite charcoal over 37 days incubation (with 10% river water). The value of control represents the mean of all replicates (n=14).
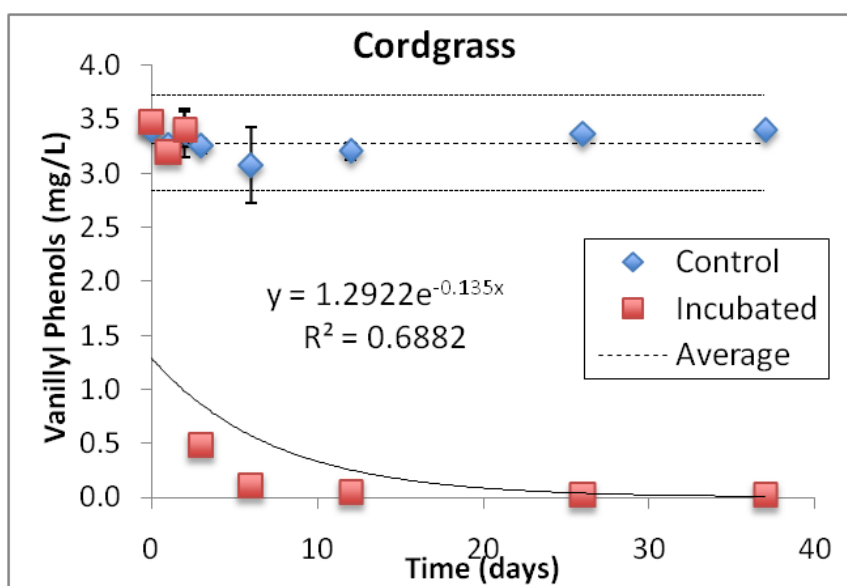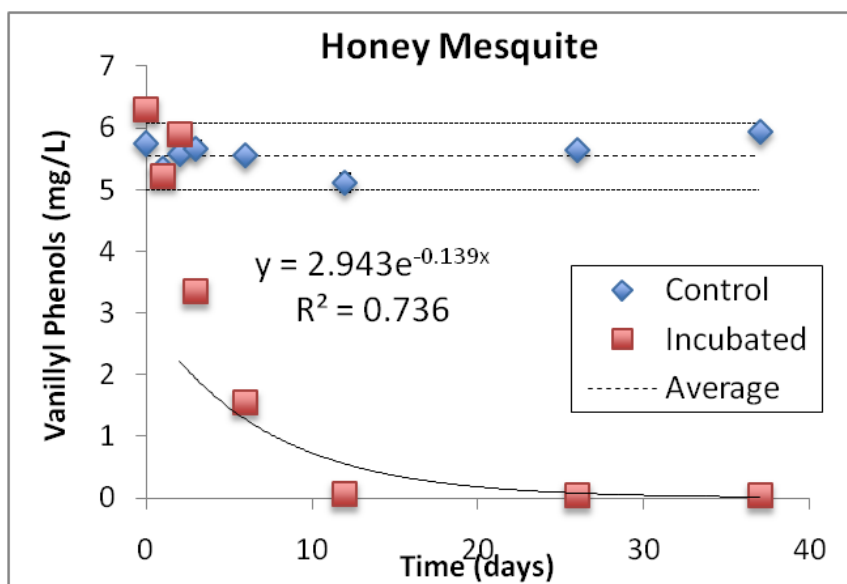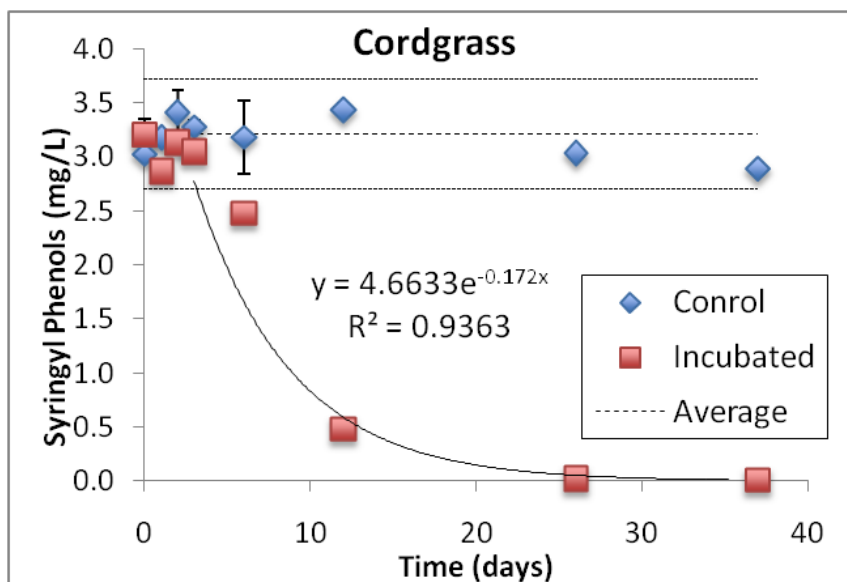
Figure 4. Exponential decrease of water-soluble vanillyl phenols derived from cordgrass charcoal over 37 days incubation (with 10% river water). The value of control represents the mean of all replicates (n=14).
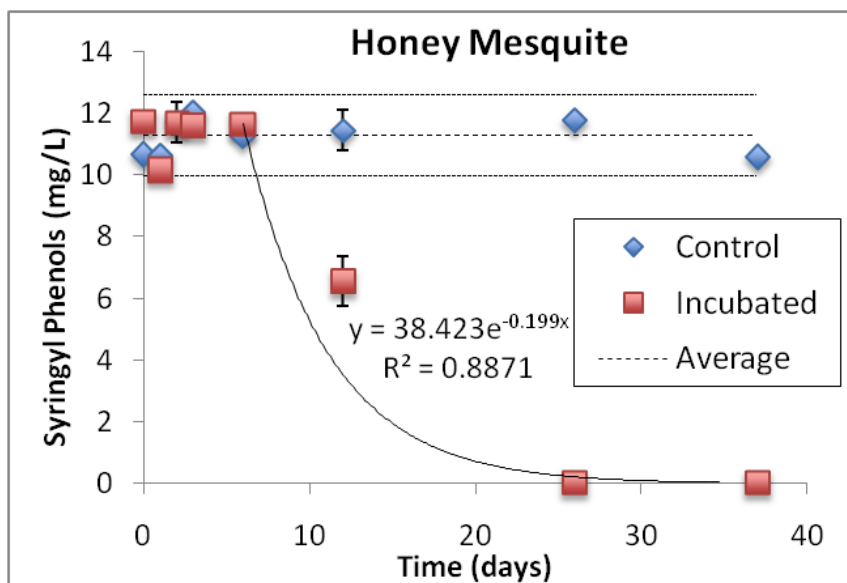

Figure 5. Exponential decrease of water-soluble vanillyl phenols derived from honey mesquite charcoal over 37 days incubation (with 10% river water). The value of control represents the mean of all replicates (n=14).
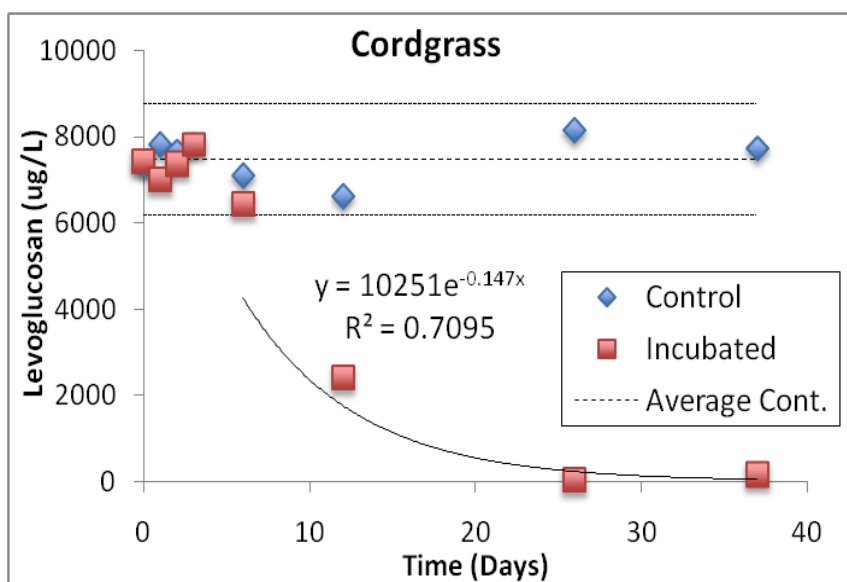
Figure 6. Exponential decrease of water-soluble syringyl phenols derived from cordgrass charcoal over 37 days incubation (with 10% river water). The value of control represents the mean of all replicates (n=14).

Figure 7. Exponential decrease of water-soluble syringyl phenols derived from honey mesquite charcoal over 37 days incubation (with 10% river water). The value of control represents the mean of all replicates (n=14).

Figure 8. Exponential decrease of water-soluble levoglucosan from cordgrass charcoal over 37 days incubation (with 10% river water). The value of control represents the mean of all replicates (n=14).
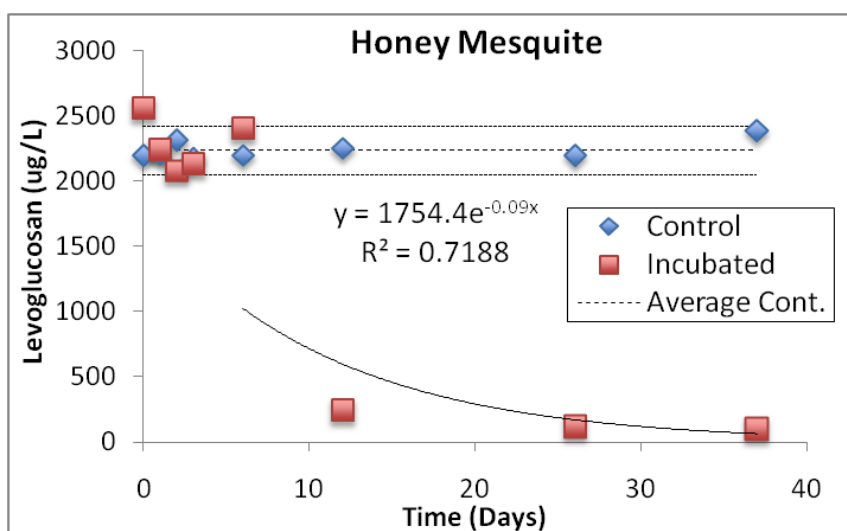

Figure 9. Exponential decrease of water-soluble levoglucosan from honey mesquite charcoal over 37 days incubation (with 10% river water). The value of control represents the mean of all replicates (n=14).
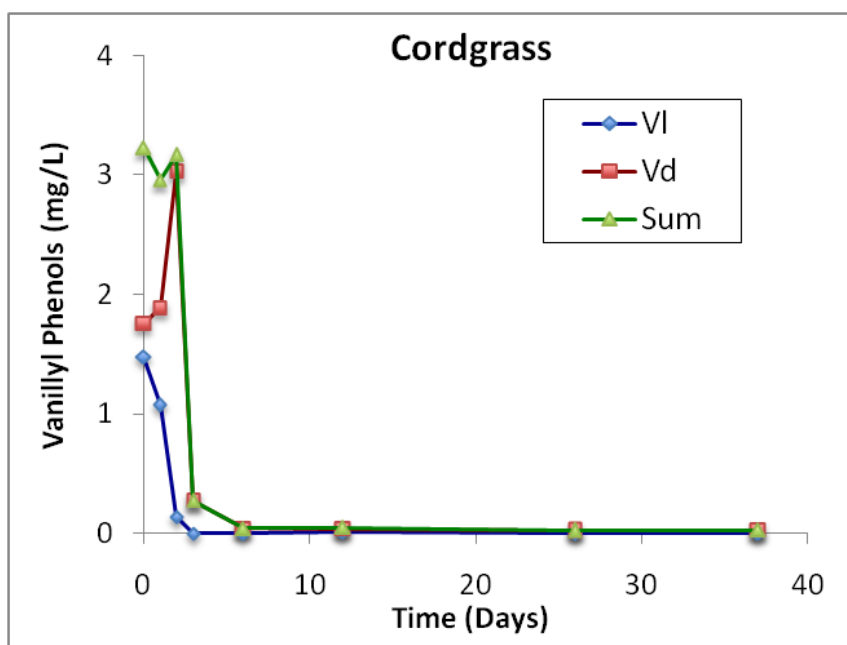
Figure 10. Changes in concentrations in vanillin (aldehyde) and vanillic acid (acid) in cordgrass charcoal DOM over the 37 days incubation period.
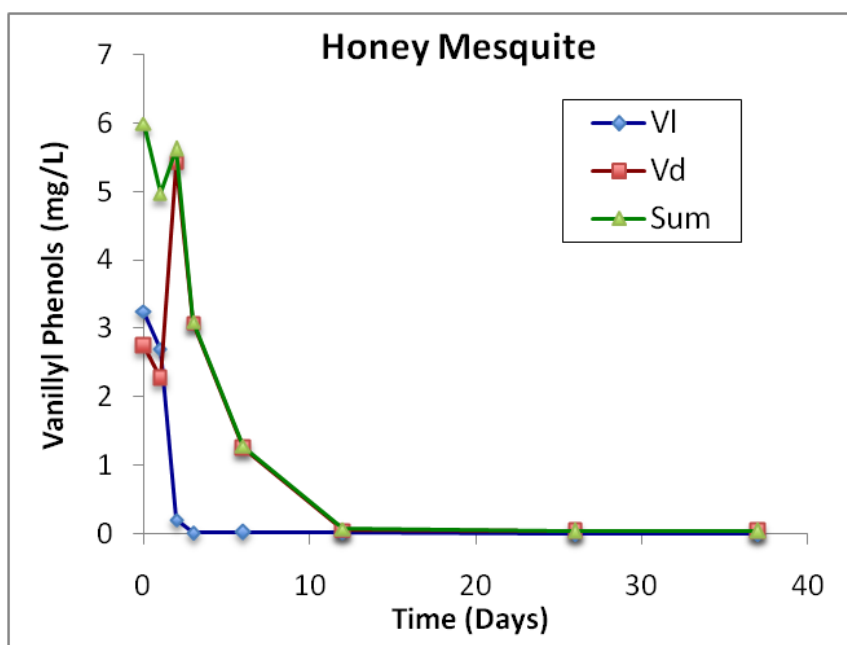

Figure 11. Changes in concentrations in vanillin (aldehyde) and vanillic acid (acid) in honey mesquite charcoal DOM over the 37 days incubation period.
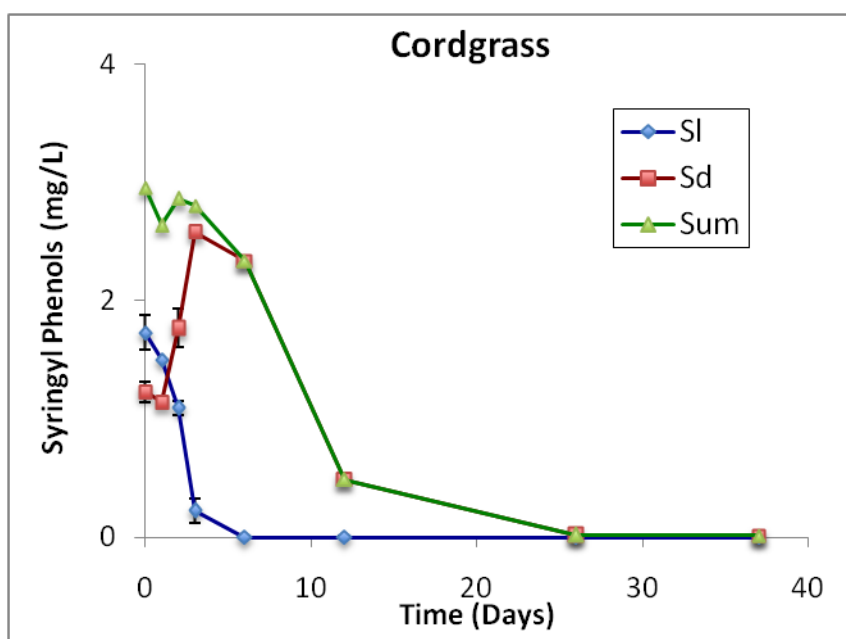
Figure 12. Changes in concentrations in syringe aldehyde (aldehyde) and syringic acid (acid) in cordgrass charcoal DOM over the 37 days incubation period.
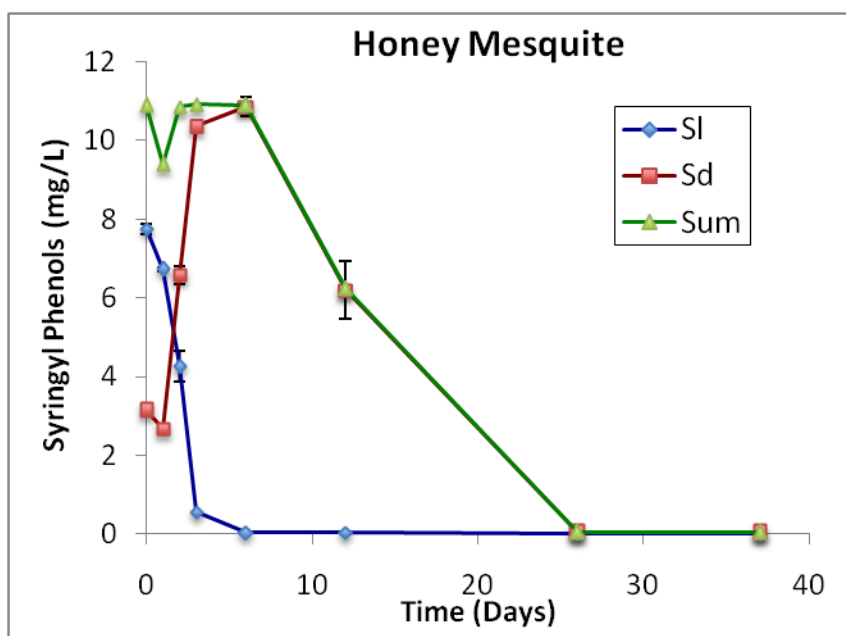

Figure 13. Changes in concentrations in syringe aldehyde (aldehyde) and syringic acid (acid) in honey mesquite charcoal DOM over the 37 days incubation period.

Table 1: The biodegradation rates and half-life values for HM/CG molecular constituents; lignin and levoglucosan. This data represents the 37 days incubation period.

Molecular Component	k (day^{-1})		$t_{1/2}$ (days)	
	HM	CG	HM	CG
Sigma 8 Lignin Phenols	-0.17	-0.15	4.1	4.7
Vanillyls	-0.14	-0.14	5.0	5.1
Syringyls	-0.20	-0.17	3.5	4.0
Levoglucosan	-0.09	-0.15	7.7	4.7

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