Characterizing Lumber Leachate by Tree Species

A Major Qualifying Project

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Abstract

This study provides characterizations and comparisons of leachates produced from 29 tree species in order to investigate the environmental impacts of leachates from different species and layers of trees. Analysis included COD, TDN, DOC, UV-visible and fluorescence spectroscopy, UV irradiation, and polyphenolic content. As confirmed by this research, bark produced leachate with higher concentrations of organic carbon, nitrogen, polyphenols, and condensed tannins. Hardwood leachate produced more humic-like material while softwood leachate produced more tryptophan-like material. Species found posing higher environmental risks included the Ash, Olive, Poplar, and Chestnut trees. Further investigation is recommended to reduce the impact of lumber leachate on aquatic ecosystems.

Acknowledgements

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Executive Summary

Many sites of wood storage use water sprinkling as a technique for conserving wood. Lumber processing also commonly involves techniques which expose wood samples to water. During these processes dissolved organic matter (DOM) is leached from wood into water, creating lumber leachate. The specific materials which leach into the water vary widely depending on the tree species, the length of contact time, and various other environmental conditions. Some constituents in lumber leachate, produced by water runoff from wood storage and lumber processing sites, present a serious concern for their impact on aquatic ecosystems. Research on the qualities and characteristics of lumber leachates has only been conducted on a limited number of tree species. This study provides characterizations and comparisons of leachates produced from 29 tree species in order to investigate the environmental impact of leachates from different species and layers of trees.

Each wood sample from the tree species selected was first divided into the layers of bark, sapwood, and heartwood. Wood samples were then chopped into small pieces of approximately 1 cm^2, measured into quantities of approximately 5 g, and exposed to 150 mL of ultra-pure water for 48 hours at 25°C in an orbital shaker operating at 150 rpm. The leachate produced by this method was then filtered and stored in a refrigeration unit. Leachate samples were then tested for dissolved organic carbon, total dissolved nitrogen, chemical oxygen demand, UV-visible and fluorescence spectroscopy, UV irradiation, total polyphenolic content, and condensed tannin content via both the vanillin assay and acidic-butanol assay in order to characterize and compare the environmental impact of leachate samples based on tree species, layers, and categories (hardwood vs softwood).

The constituents examined in leachate samples have varying impacts on aquatic ecosystems according to previous studies. Both organic carbon and nitrogen can cause hypoxic conditions in aquatic ecosystems, which lead to a loss of biodiversity. Both humic and fulvic-like materials are hydrophobic DOM which increase oxygen demand and microbial regrowth in water, and can form dangerous carcinogenic disinfection byproducts (DBP). Tryptophan-like material, which is hydrophilic DOM, is a precursor for DBP and it is difficult to remove through water treatment. Condensed tannins, which are a subset of polyphenols, are toxic to many fish, invertebrates, and amphibians at high concentrations. Other types of polyphenols can also affect reproductive and developmental health of aquatic species.

Generally, the bark layer of tree species produced leachate with higher concentrations of DOM than the other layers examined. Dissolved organic carbon testing (DOC) showed that bark sometimes produced leachate containing up to 15 times more carbon than that of the leachate produced by its core. Similarly total dissolved nitrogen testing (TDN) showed that tree bark from some species was able to produce leachate containing up to 6 times more nitrogen than that of the leachate produced by its core. Figure 1 shows that both the average DOC and TDN for bark leachate samples exceeded the average DOC and TDN for all leachate samples examined.

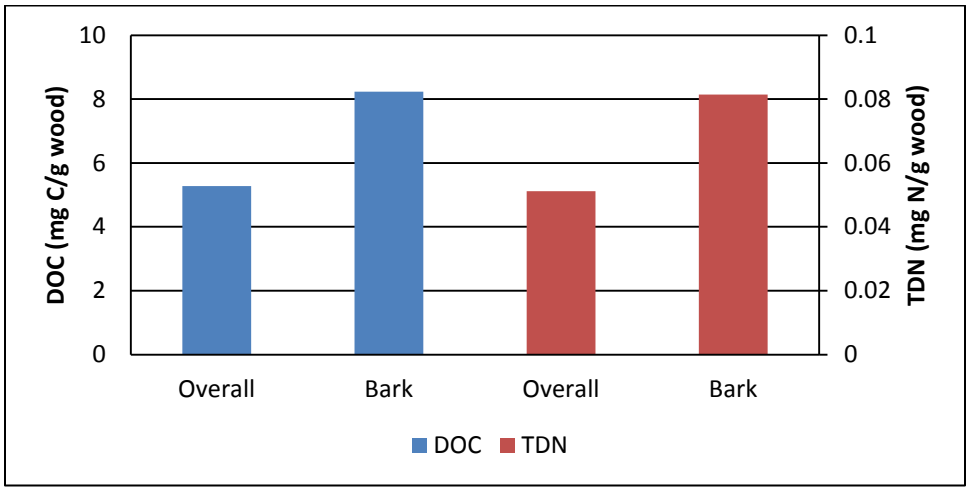

Figure 1: Average carbon and nitrogen content

Figure 2 shows that the average total polyphenolic content for bark leachate samples exceeded the average total polyphenolic content for all leachate samples examined. Similarly, Figure 2 shows condensed tannins content, as tested by both the vanillin assay and acidic butanol assay, for bark leachate samples exceeded the average condensed tannins content for all leachate samples examined.

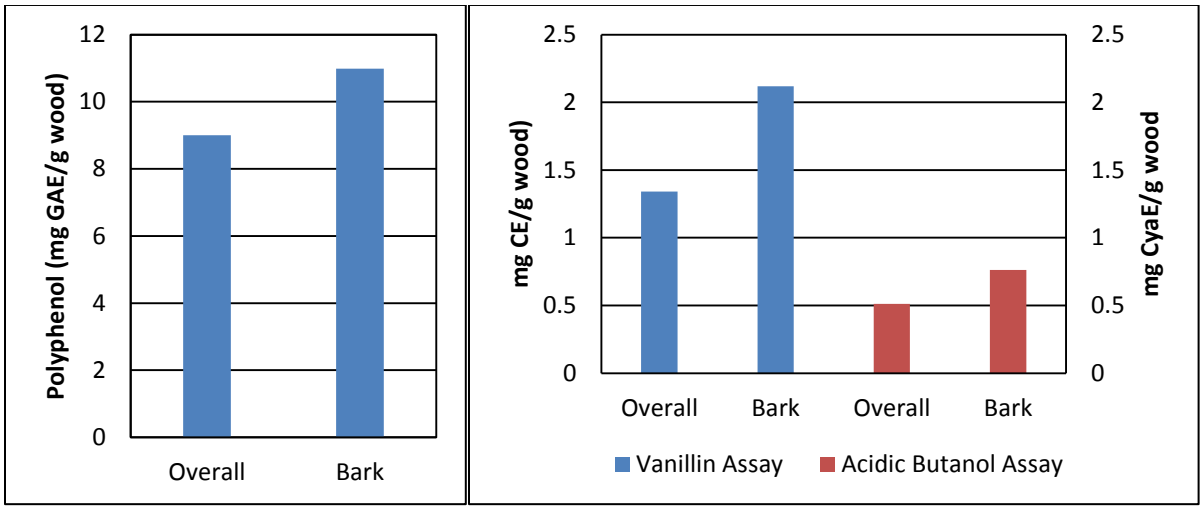

Figure 2: Average polyphenol content (left) and average condensed tannins content (right)

This indicates that bark has higher mass transfer of organic carbon, nitrogen, polyphenols, and condensed tannins into water than inner layers. UV-visible spectroscopy also indicated that bark leachate contains a high concentration of humic-like material.

The interior portions of wood also have unique leachate characteristics. Heartwood, the innermost layer of the tree, usually leached more organic carbon, fulvic-like material, humic-like material, and polyphenols than sapwood, but still less than the bark. However, given that bark is the primary layer exposed to environmental conditions in wood storage sites and lumber yards, the leaching ability of bark samples should be the greatest concern and may be the most useful indicator of the environmental impact of leachate produced by different tree species during wood storage or lumber processing.

When comparing leachate samples according to species, the Ash tree consistently leached many of the constituents more readily than other species. Ash tree bark leachate tested with one of the highest concentrations for tryptophan-like material, humic-like material, fulvic-like materials, polyphenols, and condensed tannins. Thus, the bark of Ash tree appears to be a dangerous source of leaching in wood storage and lumber processing sites.

Other species which exhibited more leaching ability include Olive, Poplar, and Chestnut. Olive tree leachate yielded the highest concentration of both organic carbon and nitrogen. Poplar bark leachate was notable as a source of organic carbon, tryptophan-like material, fulvic-like material, and polyphenols. Chestnut was significant in that its core sample produced the highest concentration of polyphenolic material in any leachate tested.

In comparisons of hardwood vs softwoods, hardwood tree species appeared to transfer fulvic-like and humic-like material to water more readily than softwood species. Gauss identification tests revealed that leachates produced by softwoods generally had higher B1 values, indicative of tryptophan-like material. Though softwood leachates made up only 20% of the leachate samples tested, their B1 values made up 28.5% of the sum total of B1 values for all leachate samples. Leachates produced by hardwoods generally had higher B2 and B3 values, indicative of fulvic-like and humic-like material, respectively. The softwood leachates made up only 8.27% and 9.57% of the total B2 and B3 values, respectively. UV-visible spectroscopy also confirmed that hardwoods leached humic-like material more readily than softwoods as the SUVA 280 and 340 values for hardwoods were generally higher than those of softwoods.

Another significant difference found between softwoods and hardwoods was the impact of UV irradiation testing, which mimics the effect of sunlight on leachate samples by delivering a dose of UV light at 254 nm to samples for 24 hours. The effect of UV irradiation on hardwoods

was clearly visible, while the effect on softwoods was minimal. Figure 3 shows three examples of the effect of UV irradiation testing. The blue line indicates the UV-visible spectroscopy performed before UV light exposure, while the red line indicates the UV-visible spectroscopy performed after 24 hours of exposure. As shown in Figure 3, UV irradiation had little to no effect on the peak at 280 nm, which indicates the presence of tryptophan-like materials. In some cases, the peak even increased, which can likely be attributed to polymerization of some tryptophan-like material. However, the peaks from 300-340 nm, which indicate the presence of humic and fulvic-like materials, experienced significant reductions in some leachate samples after UV light exposure.

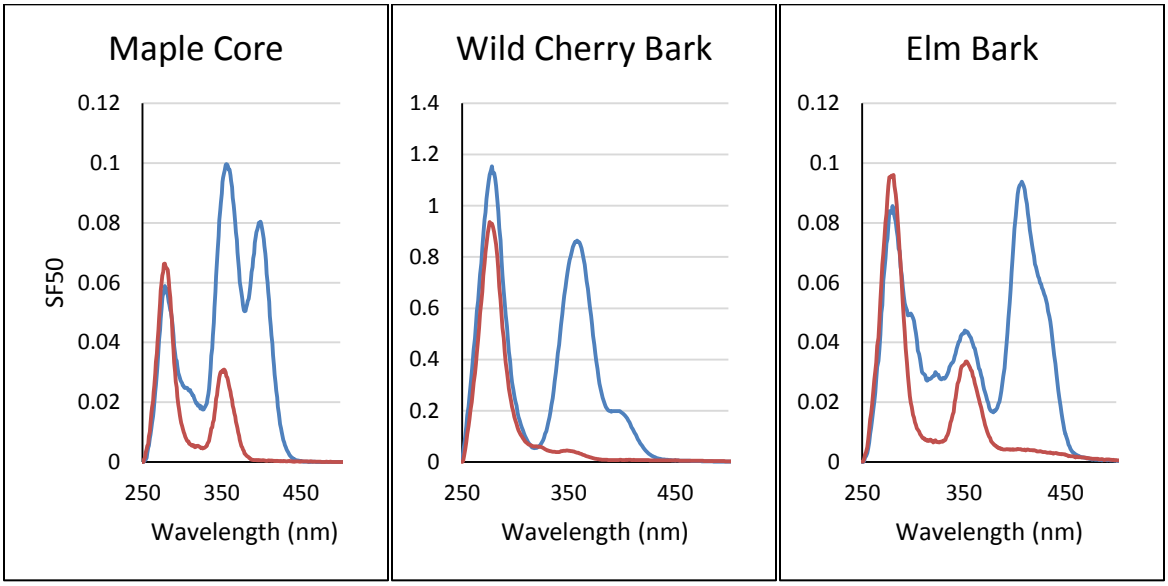

Figure 3: UV irradiation effect

On average, hardwoods experienced a global decrease of 28.74% after UV irradiation testing, while softwoods experienced only 1.75% global decrease. Hardwood bark and core experienced a global decrease of 31.62% and 25.85% on average, respectively, while softwood bark and core experienced a global decrease of -2.04% and 5.53% on average, respectively. The

negative global decrease of softwood barks actually indicates an increase, which is likely due to a UV light induced polymerization effect on tryptophan-like material.

Although UV irradiation offered one method of examining the effect of sunlight on leachate samples, there are many possibilities to study the effect of other environmental conditions on leachate. A UV irradiation test could be developed to examine the effect of sunlight during the leaching process. Various methods of leachate production such as using different wood to water ratios, exposing the wood to water through water sprinkling or still water, and adjusting the exposure time could also be used to imitate different natural and industrial wood processing environments. It is recommended that additional tests employing new leachate production methods be used to investigate the effect of various environmental conditions on leachate formation.

Introduction

In France, following three major winter storms in December 1999, the runoff from wood storage sites for damaged wood put water quality in aquatic environments at risk. This series of storms damaged an estimated 150-180 million m^3 of forest wood (Schelhaas, Nabuurs, & Schuck, 2003). Afterwards damaged trees were stacked and treated with water sprinkling to conserve the wood. These trees leached an unknown quantity of undetermined organic substances into sprinkling water, which then drained into local waterways, polluting aquatic environments and potentially harming the growth and development of aquatic species. This incident increased concern about the impact of lumber leachate, which is formed through water sprinkling as well as other lumber processing techniques, on aquatic ecosystems.

In the past decade more research has been conducted on wood samples to understand the qualities and characteristics of lumber leachates and the impact of these leachates on aquatic environments. While it is known that exposure to wood samples results in water with a greater concentration of dissolved organic matter, the identity of constituents transferred to the water from trees has only been researched for a small number of species, under a limited number of conditions. It is well proven that materials like organic carbon and nitrogen, polyphenols, and tryptophan, humic, and fulvic-like materials diminish water quality and even harm some aquatic species. However, the ability of various tree species to leach these materials into water is largely unknown.

This research tested 29 species of trees to characterize and compare leachate samples formed from various tree species. The wood samples collected were divided into appropriate layers of bark, sapwood, and heartwood according to each tree's anatomy before preparing leachate samples. Spectroscopy, polyphenolic content testing, and dissolved organic carbon

testing were among the analytical methods employed to characterize leachate samples. The data collected was organized and compared on the basis of tree species, layers, and categories (hardwood vs softwood) to investigate trends in leaching capabilities among various tree species.

Background

1999 Storm and Logging Industry

In December 1999, three large winter storms produced extreme winds over Europe, which severely impacted France, Southern Germany and Switzerland (Ulbrich, Fink, Klawa, & Pinto, 2001). During these storms, high extreme wind speeds devastated forest areas, damaging an estimated 150-180 million m^3 of wood (Schelhaas, Nabuurs, & Schuck, 2003; Costa & Ibanez, 2005). In France, most of the trees felled during the 1999 windstorm were dealt with by stacking the logs and using water sprinkling to conserve the wood. Water sprinkling, a form of wet wood storage, prevents the wood from rotting, growing mushrooms, and becoming infested with insects, all of which reduce the value of the wood. In water sprinkling, the water is usually drained from the soil and recycled. However, some amount of water will manage to escape the drainage process and enter local waterways. The main concern with this water is that the wood and bark of the trees have contaminated it with organic substances that may be toxic to aquatic ecosystems (Hedmark & Scholz, 2008).

Although the December 1999 storms in Europe are considered to be a rarity, because multiple high magnitude storms occurred within a few days, the occurrence of forest disturbances due to wind are increasingly more common as a result of climate change (Schelhaas et al., 2003; Usbeck et al., 2011). Furthermore, the combined method of log stacking and water sprinkling, which creates contaminated runoff water, is common within the logging industry (Hedmark & Scholz, 2008). Because of the short term use of many wood storage sites, runoff from water sprinkling is often untreated before release into aquatic environments (Hedmark & Scholz, 2008). In addition to water sprinkling, water from natural precipitation, log transportation, and equipment cleaning can produce additional sources of runoff (Zenaitis,

Sandhu, & Duff, 2002). With France's annual 35 to 40 million m^3 of wood harvested, peaking at 45 million as a result of the 1999 storm, and three to six percent volume of processed wood in log sort yards being lost as woody debris, there are ample sources for runoff which has been in contact with wood (Zenaitis et al., 2002; Elyakime & Cabanettes, 2009). Thus, it is important to understand how lumber leachate affects water quality and impacts local aquatic environments.

Biology of Trees

As living organisms, the composition of trees varies species to species as well as between individual trees within a species. One important aspect of the analysis of the lumber industry is the attributes from each type of wood. To do this a multitude of aspects of trees and wood need to be understood. Among individual trees, characteristics such as moisture content and composition can vary widely (Samis, Liu, Wernick, & Nassichuk, 1999). However, there are overall trends in species that allow for comparisons to be made between hardwood and softwood species as well as between three main sections of tree: heartwood, sapwood, and bark.

Hardwood vs Softwood

The defining difference between hardwood and softwood is that hardwoods, or angiosperms, are flowering trees, while softwoods, or gymnosperms, are conifers bearing. These two distinct categories of tree species are further divided by biological and physical differences between the two (Hon & Shiraishi, 2000). Hardwoods have a higher carbohydrate content, higher cellulose, and higher fatty acids (Samis et al., 1999). They generally have a higher density than softwoods, making hardwood ideal for construction, pallets, and high-quality furniture (Haynes, 2003). Softwoods have higher phenolic content, lignin content, and higher proportion of bark by volume (Samis et al., 1999). Softwoods are used for paper, residential upkeep, low-budget

construction, and more (Haynes, 2003). For each tree species, whether hardwood or softwood, specific portions of the trees' anatomy have distinct characteristics and uses.

Tree Anatomy

Trees consist of several sections including the bark, cambium, xylem, sapwood, heartwood, and pith (Hon & Shiraishi, 2000). Figure 4 shows an example cross section of a tree with each layer identified.

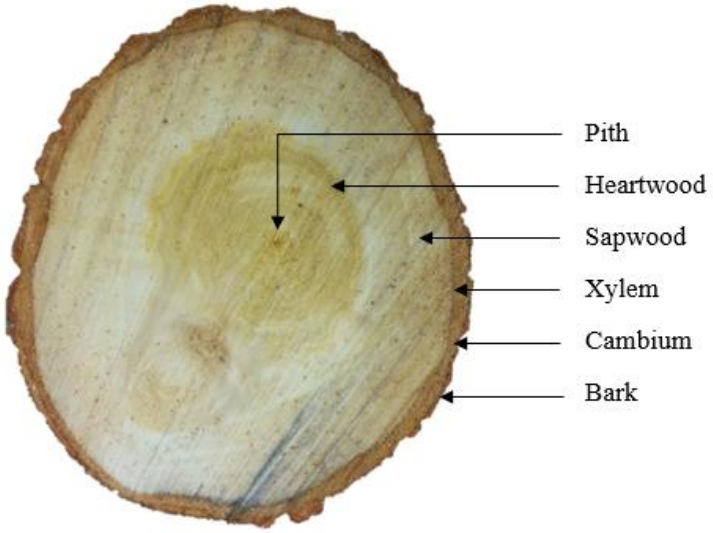

Figure 4: Willow sample with labeled anatomy

The three main sections of a tree are the heartwood, sapwood, and bark. Heartwood, which is found surrounding the pith at the center of a tree, is made up of dead cells, while sapwood, the layer surrounding the heartwood, is made up of living cells (Hon & Shiraishi, 2000). Thus, the sapwood has a higher moisture content, making it more susceptible to decay during the logging process (Hon & Shiraishi, 2000). Additionally, the heartwood is usually darker than the sapwood due to a higher concentration of lignans stored in the heartwood (Samis et al., 1999; Lee, 2007).

Bark, the outermost layer of a tree, functions as the protective boundary for the heartwood and sapwood, as well as all other parts of the tree. As it is generally the only exposed layer, bark can vary widely in response to different environments. This layer has more water insoluble compounds, which makes trees more resistant to biodegradation and insect infestation (Samis et al., 1999). While bark contains less carbohydrates than other portions of the tree, those carbohydrates have a higher pectin content, which provides structural stability (Samis et al., 1999). Similarly, bark generally contains the same inorganic components, proteins, and phenolic materials (such as tannins) as other layers, but at higher concentrations (Samis et al., 1999). For example, in oak trees, tannins are produced in the cambium level, which immediately precedes the bark, but are stored in the bark (Hathway, 1958). The impact of the composition of different tree species, as well as the composition of each layer within a species, are important considerations to make when lumber is introduced to water during wood processing and storage, and subsequently leached to the environment.

Lumber Leachate

The length of time over which leaching occurs depends on several factors including the initial concentration of a given constituent in the wood, the volume of water in contact with the wood, and the contact time between the wood and water sample. In natural environments, each of these factors varies widely, making it difficult to estimate an average leaching time. In one study conducted in British Columbia, leaching was posited to last for more than three years at many wood residue sites under average regional climatic conditions (Samis et al., 1999). Under natural conditions of variable water volume, wetness and dryness, and water purity the leaching process may endure much longer. The materials found in lumber leachate can have drastic effects on ecosystems, depending on concentration and length of exposure. Dissolved organic

matter (DOM) may impact soil development and increase microbial growth in water systems (Qualls & Haines, 1991). DOM creates a higher demand for oxygen in a waterway as the DOM is degraded (Hedmark & Scholz, 2008). High concentrations of DOM in wood leachate has been attributed as a main source of oxygen depletion, affecting the water quality and the health of plant and animal species that service the water source (Svensson, Svensson, Hogland, & Marques, 2012). Hypoxia, the condition of insufficient oxygen in an aquatic ecosystem, is a leading cause of death for aquatic species and loss of biodiversity (Vaquer-Sunyer & Duarte, 2008, Riedel et al., 2014).

Characteristics of Lumber Leachate

Oxygen demand, which indicates the health of an ecosystem, may be characterized several different ways including Chemical Oxygen Demand (COD). COD is used to quantify the organic matter in water when the concentration of organic matter exceeds 1.0 mg/L (Chandrappa & Das, 2014). COD values correspond to the amount of oxygen that is necessary to oxidize all of the organic matter in a water sample into carbon dioxide and water. COD levels generally correlate to the concentration of DOM in a leachate sample.

The content of DOM in leachate may also be characterized by measuring Dissolved Organic Carbon (DOC), simply the concentration of organic carbon in a water sample. While this measurement does not reveal the specific compounds which make up the DOM, the test indicates the presence of organic contaminants (Hedmark & Scholz, 2008). Paired with other tests like UV-Visible Spectroscopy and Fluorescence Spectroscopy, the specific makeup of DOM may be investigated to indicate the presence of toxic organic components.

Classifying DOM is important to understand the quality of the water and the necessary treatment steps to purify it. Firstly, DOM can be broken down between hydrophobic and

hydrophilic. Hydrophobic DOM is generally naturally forming from plant degradation and is often classified as humic and fulvic acids (Bieroza, Bridgeman, & Baker, 2010). These are rich in aromatic carbon and phenol compounds (Hua & Reckhow, 2007). These materials generally have higher molecular weight and are preferential for typical water treatment methods (Bieroza et al., 2010). Hydrophobic DOM presence increased oxygen demand, microbial regrowth, and can lead to the formation of dangerous carcinogenic disinfection byproducts (DBP) (Bieroza et al., 2010). However, due to their hydrophobic nature and relatively large molecular weight, hydrophobic DOM can be removed fairly easily with traditional water treatment methods.

Hydrophilic DOM, on the other hand, have lower molecular weights and are biodegradable (Bieroza et al., 2010). Often classified as tryptophan like, these often microbial derived organic materials are harder to remove from water systems during treatment (Bieroza et al., 2010). Furthermore, though it is commonly thought that hydrophobic DOM is the precursor for DBP, hydrophilic DOM can also increase the formation of DBP in low humic content water systems (Leenheer & Croué, 2003). As a result, the classification of leachate can indicate the water treatment necessary based on the species of trees at the site.

Toxicity of Lumber Leachate

The toxicity of some materials in lumber leachate such as resin acids and phenolic materials can affect fish development and behavior, potentially resulting in death (Samis et al., 1999). Many phenolic compounds are established endocrine disrupters and carcinogens for both humans and aquatic life. Additionally, high concentrations of phenol in leachate has been linked to elevated pH values (Kurata, Ono, & Ono, 2008). The damaging effects of high alkalinity on aquatic ecosystems is well documented with side effects on fish including gill failure, loss of eye

sight, and reproductive failure (Erickson, McKim, Lien, Hoffman, & Batterman, 2006; Yao, Lai, Zhou, Rizalita, & Wang, 2010; Wood et al., 2012).

Tannins, a subset of polyphenols found in various plant species, have varied effects on aquatic species. In lower concentrations, tannins provide antioxidant effects while in higher concentrations, tannins are known toxins to fish, invertebrates, and amphibians at higher concentrations (Earl & Semlitsch, 2015). The derivation of tannins also appears to have an effect on toxicity as tannins may have varying oxidative and protein binding abilities (Salminen & Karonen, 2011). In plants, the two main types of tannins are hydrolysable and condensed tannins which are distinguished by their structure and response to acid hydrolysis (Meyers, Swiecki, & Mitchell, 2006). Condensed tannins, also called proanthocyanidins are polymers of flavan-3-ol molecules that are broken down into flavan-3-ol monomers known as anthocyanidins during acid hydrolysis. Proanthocyanidins are commonly found in many tree species (Kawakami, Aketa, Nakanami, Iizuka, & Hirayama, 2010).

One study found that the polyphenolic compounds which cause brown or black water coloration are generally not significant in terms of toxicity and are not biodegradeable (Paixao, 1999). However, tannins are both highly toxic and biodegradable, creating a greater oxygen demand on the water (Paixao, 1999). Given that various plant species are currently known to synthesize over 4,000 different phenols, many of which have not been fully investigated, the effect of leachate from many tree species in completely unknown (Svensson et al., 2012).

Lumber leachate may also contain a higher concentration of dissolved nitrogen than natural waterways. Excess nitrogen can adversely affect waterways by creating hypoxic or acidic conditions. Nitrogen may also spur algal blooms, which lowers biodiversity by creating hypoxic

conditions (Baron et al., 2013). The nitrogen content of lumber leachate may be analyzed by measuring the Total Dissolved Nitrogen (TDN) of a sample.

Case Studies on Tree Effects

Previous studies have begun to investigate and compare the effects of different tree parts and tree species on leachate composition and toxicity. In 2012, a study compared samples of bark and sawdust from five different tree samples: oak, pine, larch, spruce, and beech. pH tests, total inorganic carbon (TIC), total organic carbon (TOC), and liquid chromatography tests, as well as acute toxicity, were run on two specific species. In the end, all samples were found to produce high levels of toxic leachate due to the presence of phenolic and acid components. Oak generally leached the highest amount of phenols and oak and pine produced high dissolved organic carbon (DOC). However, in all samples, bark produced worse conditions than sawdust, indicating the distinct hazards of bark (Svensson et al., 2012).

In a 2013 study, leachate samples from woodchip and sawdust of oak, maple, pine and beech were compared over time for pH, conductivity, color and biological oxygen demand (BOD). As in the previous study, oak leachate had higher phenol and DOC levels. While pine did not have high phenol levels, its leachate had the second highest DOC. Generally, hardwood samples were predicted to have more DOC based on the assumption that the large pore size of hardwood would facilitate more mass transfer. However, the softwood pine leachate had higher DOC than both of the hardwoods, maple and beech. This study, removing the variable of bark, also found that sawdust released more DOC than woodchip samples, indicating that size has a large effect on DOC (Svensson, Marques, Kaczala, & Hogland, 2013). While both of these studies have initiated a discussion on the impact that organic matter from lumber leachate may

have on aquatic ecosystems, this research will develop the ability to characterize, understand, and compare organic matter in different lumber leachates.

Characterizing Lumber Leachate

Dissolved organic matter (DOM) consists of various soluble organic compounds in a water based mixture, including carbon, nitrogen, and phosphorus. Although DOM consists of a range of compounds, the qualifying characteristic is that the solutes must pass through a filter less than 0.7 micrometers in pore size (Michalzik, Kalbitz, Park, Solinger, & Matzner, 2001). DOM, which is derived from both microorganisms and terrestrial sources such as trees, is important for the functioning of aquatic ecosystems because of its effect on COD, BOD, pH, the carbon cycle, and the storage of carbon, nitrogen, and phosphorus (Qu et al., 2013). DOM may consist of many different substances and its composition also varies widely between locations. As such, there are many options for characterizing the properties of a DOM sample, such as testing carbon, nitrogen, and/or phosphorus content, or using electromagnetic spectroscopy to identify constituents (Qu et al., 2013). The tests used in this research, DOC, TDN, UV-visible spectroscopy, fluorescence spectroscopy, COD, total polyphenolic content, condensed tannins content, and proanthocyanidin content were selected based on the tests performed in previous case studies.

Dissolved Organic Carbon Testing

The DOC of samples was measured at Laboratoire Réactions et Génie des Procédés (LRGP) using a Shimadzu TOC-VCSH (Total Organic Carbon analyzer) with an ASI-V injection syringe autosampler. This machine oxidizes carbon with high temperature combustion by heating samples to 680°C. The Shimadzu TOC-VCSH model has five main pieces of equipment: the autosampler injection syringe, combustion cell, dehumidifier, halogen scrubber,

and NDIR gas analyzer. The ASI-V holds up to 68 x 40 mL vials at once and loads, sparges, and injects each sample automatically, washing the injector in between each use. The autosampler also autodilutes the samples if necessary. Carrier gas, flowing at 150 mL/min, brings the samples into the combustion cell which is where oxidation occurs at 680°C. The oxidation process yields carbon dioxide, which exits the combustion cell in the carrier gas stream. Next, the electronic dehumidifier cools and removes water from the gas stream carrying carbon dioxide. The halogen scrubber removes chlorine and other halogens from the gas stream before analysis in the NDIR gas analyzer.

The NDIR (non-dispersive infrared) sensor detects components in a gas by passing infrared energy through the gas stream and measuring the absorbed wavelengths against a reference gas such as nitrogen. The data output by the NDIR is in the form of an analog signal with a peak, which is proportional to the total carbon concentration in the sample. To determine the organic carbon concentration, the inorganic carbon must be removed from the total carbon concentration. This is accomplished by acidifying the sample to a pH less than three and sparging gas through the sample, which removes the inorganic carbon. The resulting concentration, the DOC, is measured in the units of mg/L (Shimadzu Corporation International Marketing Division, 2011).

Total Dissolved Nitrogen Testing

The TDN of the samples was tested using the Shimadzu TNM-1 (Total Nitrogen Measuring unit) accessory with the TOC-VCSH and ASI-V autosampler. The TNM-1 functioned simultaneously with the TOV-VCSH to provide DOC and TDN reading in the same run. The TNM-1 runs by flowing the carrier gas stream through a thermal decomposition catalyst at 720°C which produces nitrogen monoxide. The carrier gas then passes through a dehumidifier to

remove water from the gas and nitrogen monoxide stream. The nitrogen monoxide is sensed and measured as the stream then passes through a chemiluminescence detector which applies an ozone activation reaction to produce nitrogen dioxide and oxygen. The concentration of nitrogen dioxide, which is directly proportional to the amount of nitrogen, is measured and recorded as an analog signal forming a peak. Using a calibrated curve, the peak area can be used to calculate the total nitrogen (TDN) present in terms of mg/L (Shimadzu Corporation International Marketing Division, 2011).

Chemical Oxygen Demand

The COD of the samples was tested by using a DigiPREP CUBE digestion system from SCP Science and a DR/2400 Portable Spectrophotometer by Hach. The DigiPREP CUBE is a digestion block which holds up to 25 samples in 16 mm tubes. The heating block is composed of Teflon-coated graphite and operates on a predefined program for COD testing which heated samples to 150°C for two hours. Before starting the digestion period, an acid solution and a digestion solution are added to each leachate sample. During digestion, the acid acts as a catalyst to oxidize hexavalent dichromate ions ($Cr_2O_7^{2-}$) to give up oxygen which reacts with organic carbon, forming carbon dioxide. This thermally-driven oxidation reaction transforms hexavalent dichromate ions into chromium ions (Cr^{3+}) which absorb visible light at 420 nm and 600-620 nm, respectively (SCP Science, n.d.). After digestion, the absorbance of samples is measured at 620 nm in the DR/2400 Portable Spectrophotometer to give the value for COD. The DR/2400 operates with a low-voltage Tungsten bulb and LED to read the absorbency of the samples. At 620 nm the chromium ion is visible, but the dichromate ion does not absorb any light. A calibration curve with a slope of 2884 mg O_2/L•% absorbance allows the absorbance of samples

at this wavelength to be correlated to the amount of oxygen that reacted with organic carbon, referred to as the chemical oxygen demand (HACH Company, 2003).

UV-Visible Spectroscopy

Ultraviolet-visible spectroscopy (UV-visible spectroscopy) is an analysis method that uses electromagnetic spectroscopy, specifically within the UV-visible spectrum, to identify the components of a solution. In UV-visible spectroscopy, a solution is loaded into a cuvette through which a beam of light in the 200 to 800 nm wavelength is projected. The energy from this beam of light is absorbed by some molecules when excited electrons move to higher energy orbitals. The remaining light, which remains unabsorbed, passes through the sample and is read by a probe which reads the results of the spectroscopy, showing which UV-visible wavelengths were absorbed and which were not. From this information, components of a solution may be identified (Reusch, 2014).

At LRGP, the samples for this study were analyzed in a Secomam Anthelie UV/Visible Light Advanced Spectrophotometer. This machine uses a pre-adjusted deuterium lamp to produce ultraviolet light and a pre-adjusted halogen lamp to produce visible light in the range of 190 to 900 nm. The components of each sample are detected by a silicium diode, which records the absorption spectra passing through a sample cuvette. Before testing samples, a cuvette filled with only ultra-pure water is measured. The absorption intensity of this "blank" is used to calibrate all following samples. For the standard UV-visible spectrometry, the cuvettes used to measure samples were composed of one cm^2 quartz (Secomam, n.d.). For the UV-irradiation tests, PMMA cuvettes were used to measure samples.

Fluorescence Spectroscopy

Fluorescence spectroscopy is a form of electromagnetic spectroscopy which is used to analyze a solution based on its fluorescent properties. Fluorescence occurs when a substance, having absorbed light or electromagnetic radiation, emits light. In fluorescence spectroscopy, a solution is loaded into a sample cuvette which is excited with a beam of light of 180 to 800 nm (Birdwell & Engel, 2010). The light that is emitted by the sample at a right angle to the excitation light is measured and this measurement corresponds to fluorescence. The fluorescent properties of the sample are used to identify substances within the sample.

Two types of fluorescence spectroscopy are commonly used to characterize samples, total luminescence spectroscopy (TLS) and synchronous fluorescence spectroscopy. TLS uses a range of excitation and emission wavelengths to produce an emission-excitation data matrix whereas synchronous fluorescence spectroscopy maintains a constant difference between the excitation and emission wavelength throughout testing to produce a graph of absorbance intensity vs wavelength (Sikorska et al., 2004). In this research, synchronous fluorescence spectroscopy with a constant wavelength difference of 50 nm was used.

Fluorescence emission intensities, which are measured in Raman Units, usually need to be corrected because of a phenomenon known as the inner filter effect (IFE). When IFE occurs, the substance being examined absorbs the exciting light as well as some of the emitted fluorescent light. IFE causes fluorescence emission intensities to be measured as lower than they actually are. The correction for IFE is displayed in the following equation:

$$F_{corr} = F_{obs} \times 10^{(\frac{A_{exc}+A_{em}}{2})}$$

where F_{corr} is the corrected fluorescence intensity, F_{obs} is the uncorrected fluorescence intensity, A_{exc} is the absorbance value at the current excitation wavelength, and A_{em} is the absorbance

value at the current emission wavelength (Larsson, Wedborg, & Turner, 2007). Using this correction, fluorescence emission intensities of a set of samples can be accurately compared between themselves.

At LRGP, fluorescence spectroscopy was performed using a Hitachi Digilab F-2500 Fluorescence Spectrophotometer. This machine uses a 150 W Xenon Lamp to produce fluorescent light in the wavelength range of 220 to 730 nm at a rate of 12000 nm/min (Hitachi High-Technologies Corporation, 2001). The machine uses a monochromatic light filter to detect the adsorption spectra passing through a sample cuvette by measuring the light of excitation against the light emitted at a right angle to the excitation light. Before testing samples, the Raman peak of water is tested using the Raman spectroscopy method on a "blank" cuvette filled with only deionized water. This data is used as a standard to transform the units of the direct intensity readings from the fluorescence spectrophotometer into Raman units (Hitachi High Technologies America, 2009). All cuvettes used to measure samples were disposable one cm^2 PMMA cuvettes.

Gauss Identification

In order to characterize the results from the fluorescence spectra, there are various tests than can be run, two of the most common being Principal Component Analysis (PCA) and decomposition by Gauss function. Both of these methods allow for the comparison of peaks in the spectra to characterize the material and identify fluorophore groups. While PCA removed assumptions on the number of fluorophore groups present in the sample, the Gauss decomposition is faster and easier to interpret (Assaad, Pontvianne, Corriou, & Pons, 2015). For Gauss decomposition, the synchronous fluorescence spectrum of each fluorophore is represented by a Gauss function and the spectra decomposes into a specific number of Gauss functions that

indicate fluorophores (Assaad et al., 2015). Each substance should have a Gauss shape that is determined by its height, position, and width. Software through Fortran code uses sequential quadratic programming to identify these peaks (Assaad et al., 2015).

UV Irradiation

To simulate some environmental effects, such as sunlight, and continue to characterize the leachate samples, UV irradiation can be performed on the samples. Humic, fulvic, and tryptophan-like materials are affected differently by UV irradiation. Humic and fulvic-like substances readily react with water-dissolved molecules upon absorption of radiation (Bianco et al., 2014). For example, humic-like aromatic structures are very susceptible to irradiation due to their free radical generation (Rodríguez-Zúñiga et al., 2008). After encountering irradiation, tryptophan-like materials are posited to transform into larger materials as a result of photochemical polymerization (Bianco et al., 2014). These effects can be analyzed by comparing synchronous fluorescence spectroscopy from before and after the application of UV irradiation.

Total Polyphenolic Content

One way to investigate specific characteristics of a sample is investigating the total phenolic content. One method of accomplishing this is using a Folin-Ciocalteu (F-C) assay. Phenolic compounds act as oxygen radical scavengers because they have a lower electron reduction potential than that of oxygen radicals (Ainsworth & Gillespie, 2007). Quantification of total phenolic content is possible through a reaction with a colorimetric reagent which can be quantified with visible light (Ainsworth & Gillespie, 2007). The F-C assay reaction is largely unknown, but it relies on the transfer of electrons from phenolic compounds to acid complexes. It is believed that sequences of one or two electron reductions create a blue species, detectable at 760 nm (Ainsworth & Gillespie, 2007). Gallic acid can be used as a standard and the absorbance

to concentration calibration can be created (Ainsworth & Gillespie, 2007). F-C method does not result in absolute measurements, but offers a value for chemical reducing capacity relative to an equivalent reducing capacity of gallic acid (Frankel, Waterhouse, & Teissedrespt, 1995).

Condensed Tannins Content

The content of condensed tannins, also known as proanthocyanidins, can be determined through various analytical methods including the vanillin assay and the acidic butanol assay.

Vanillin Assay

The content of condensed tannins can be determined by use of a vanillin assay reaction and UV absorbance. When vanillin is in an acid solution, it is protonated, thereby acting as a weak electrophilic radical (Sarkar & Howarth, 1976). The vanillin assay reacts with flavonoid rings, or condensed tannins, to form a red compound that absorbs at 500 nm (Broadhurst & Jones, 2006; Sarkar & Howarth, 1976). The vanillin assay is specific to flavanols in which aromatic aldehyde condenses with certain flavonoids and their oligomers (Beta, Rooney, Marovatsanga, & Taylor, 1999). This enables vanillin to be used as a test to distinguish condensed tannins from total polyphenol content. Catechin, a monomeric flavanol, is used to create a calibration curve and quantify the condensed tannins content (Beta et al., 1999). While this test is specific, it lacks reliability and reproducibility (Broadhurst & Jones, 2006).

Acidic Butanol Assay

The condensed tannin content of a sample may also be determined with the use of an acidic butanol assay in which a solution of iron dissolved in butanol and hydrochloric acid is mixed with each sample, heated, and then tested at 530 nm (Abdalla et al., 2014). In this test, the acid acts as a catalyst while the butanol depolymerizes proanthocyanidins into red

anthocyanidins via oxidation (Makkar, Gamble, & Becker, 1999; Schofield, Mbugua, & Pell, 2001). The iron, which acts as a transition metal ion, catalyzes the red color formation during the acidic butanol assay (Schofield et al., 2001). The concentration of proanthocyanidin in a sample is quantified in terms of cyanidine equivalents (Abdalla et al., 2014). Despite the longtime use of the acidic butanol assay as a method for measuring condensed tannin content, there is still a lack of knowledge about the interference of other polyphenol groups with the condensed tannin reading, which may make the test less reliable (Makkar et al., 1999; Schofield et al., 2001).

Materials & Methodology

Wood Samples

Aleppo Pine and Eucalyptus bark samples, as well as Olive tree branches and Date Palm debris were provided by Dr Hajjaji and Dr Khila (Univ. Gabès, Tunisia). The Douglas Fir sample was provided by A. Dufour (LRGP). Boysenberry branches were collected in a private garden. Maritime Pine bark was obtained from a local garden center. The remaining 22 samples of wood were collected by the Forestry Lab of INRA (LERFOB, Champenoux, France).

Names, species, and some attributes about the species and samples are listed in Table 1.

Table 1: Wood samples with characteristics

English Name	French Name	Scientific	Samples (B:Bark, S:Sapwood, H:Heartwood)	Hard or Soft	Native (not naturalized)
Alder	Aulne	Alnus	B, S	Hard	Europe, Northern Hemisphere
Aleppo Pine	Pin d'Alep	Pinus halepensis	B	Soft	Mediterranean
Ash	Frêne	Fraxinus	B, S	Hard	Europe, Asia, North America
Aspen	Tremble	Populus tremula	B, S	Hard	Asia, Europe, North America
Birch	Bouleau	Betule	B, S	Hard	Northern Hemisphere
Boysenberry	Mûre de Boysen	Rubus ursinus	Branches	Hard	Europe, North America
Checker	Alisier	Sorbus torminalis	B, S, H	Hard	Europe, Africa, Asia
Chestnut	Châtaignier	Castanea sativa	B, S	Hard	Europe, Asia Minor
Common Beech	Hêtre	Fagus grandifolia	B, S	Hard	North America
Common Walnut	Noyer	Juglans regia	B, S, H	Hard	Europe, Asia
Date Palm	Palmier dattier	Phoenix dactylifera	Debris	Hard	Tropical and subtropical regions
Douglas Fir	Sapin de Douglas	Pseudotsuga menziesii	B, S	Soft	North America
Elm	Orme	Ulmus	B, S, H	Hard	Asia
Eucalyptus	Eucalyptus	Eucalyptus obliqua	B	Hard	Americas, Europe, Africa, Mediterranean, Asia
European larch	Mélèze	Larix decidua	B, S	Soft	Europe
Fir	Sapin	Abies	B, S	Soft	North America, Europe, Asia, Africa
Hornbeam	Charme	Carpinus spp.	B, S	Hard	Asia, Europe, North America

Lime	Tilleul	Tilia	B, S	Hard	Europe, North America, Asia
Locust	Robinier	Robinia	B, S, H	Hard	North America
Maple	Érable	Acer	B, S	Hard	Asia, Europe, Africa, North America
Maritime Pine	Pin Maritime	Pinus pinaster	B	Soft	Mediterranean
Norway Spruce	Épicéa	Picea abies	B, S	Soft	Europe
Oak	Chêne	Quercus	B, S, H	Hard	Northern Hemisphere
Olive	Olivier	Olea europaea	Branches	Hard	Africa, Mediterranean, Asia
Pine	Pin	Pinus	B, S	Soft	Northern Hemisphere
Poplar	Peuplier	Populus	B, S, H	Hard	North America, Europe, Asia, Africa
Service	Cormier	Sorbus domestica	B, S	Hard	Europe, Africa, Asia
Wild Cherry	Merisier	Prunus avium	B, S, H	Hard	Europe, Western Asia
Willow	Saule	Salix	B, S, H	Hard	Northern Hemisphere

Sample Preparation

For the 23 large pieces of wood, each was cut into a slice approximately 2 cm thick. Then, the sample was divided into up to three sections by the use of a chisel: bark, sapwood, and heartwood. When separating the bark from the wood, the cambium and exterior xylem was included in the bark sample.

Figure 5: Separating the bark

The sapwood and heartwood are generally distinguishable by color. However, as heartwood only appears as a tree ages, the interior samples were only separated when there was a

distinct difference. Otherwise, the entire core of the wood was considered to be one homogeneous sample. The pieces were cut to be approximately 1 x 0.5 x 0.5 cm in size. Though many of the pieces had abnormal dimensions, the goal was to keep the overall size as consistent as possible.

Figure 6: Separating the sapwood and heartwood

The extra slices were stored with air access while the woodchips were labeled and stored in sealed containers until the tests.

For smaller and irregular wood samples that came in the form of branches, mulch, or pre-cut bark samples, the wood was split into small pieces approximately 1 x 0.5 x 0.5 cm in size and kept as one single sample.

Batch Experiments

In order to compare the water quality between different trees and samples, a consistent leachate process had to be determined. Using equivalently size woodchips, five grams of each sample and 150 mL of ultra-pure water were placed into a 250 mL glass bottle. The glass bottle was sealed and the samples were placed in an orbital shaker incubator. The orbital shaker was set at 150 rpm and 25°C and the samples were left for 48 hours.

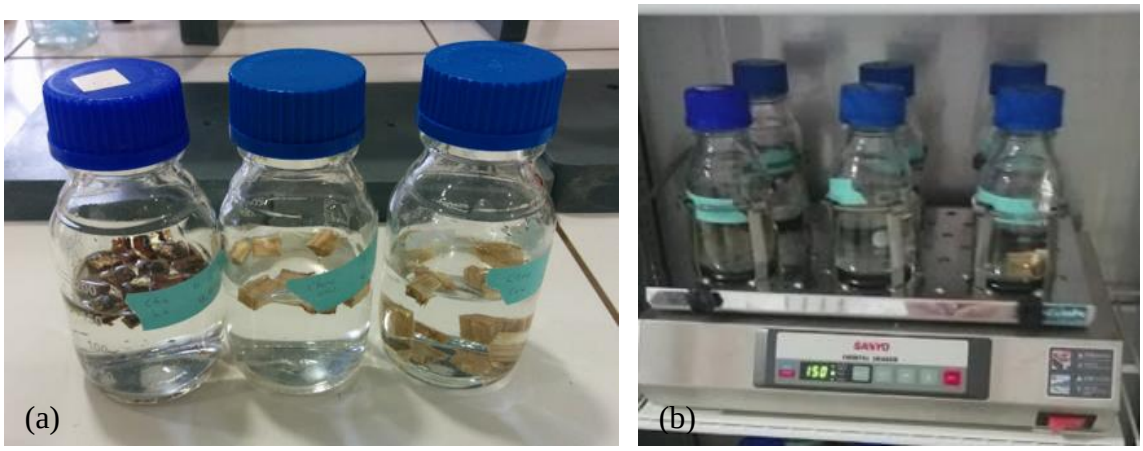

Figure 7: (a) Samples in bottles and (b) bottles in orbital shaker incubator

Filtration

After 48 hours, the samples were removed and double filtered. First, the samples entire contents were poured into a paper filter (~20 μm) in a funnel and filtered into a clean glass jar. Then the contents were filtered through glass microfiber filters (1.0 μm) using a syringe.

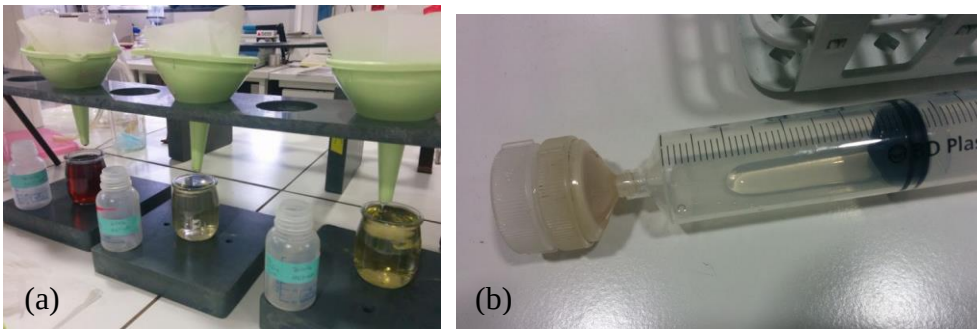

Figure 8: (a) Filtration with paper filter and (b) filtration with glass microfiber filter

Approximately 40 mL of each solution was set aside in a 40 mL glass vial for DOC/N tests while the remainder of the sample was put in a plastic bottle. When possible, the UV-visible Spectroscopy and Fluorescence Spectroscopy were run within five hours after filtration. Otherwise, the sample was stored in the fridge for no more than 60 days.

Tests

Dissolved Organic Carbon/Total Dissolved Nitrogen

When at least 10 samples had been collected, the Shimadzu TOC-VCSH with ASI-V and the Shimadzu TNM-1 were used to determine total organic carbon and total dissolved nitrogen.

Chemical Oxygen Demand

Of all the samples, 24 of the darkest leachates (#10, 17, 18, 19, 21, 22, 24, 25, 27, 29, 31, 34, 37, 39, 40, 46, 51, 53, 55, 57, 60, 61, 62, and 63) were randomly selected to be tested for COD. Each sample was prepared in 16 mm glass vials by adding 0.5 mL of leachate sample and then adding a prescribed acid solution to each vial. These 24 samples were inserted to the DigiPREP CUBE digestion block and heated for two hours at 150°C. After the digestion period, the samples were removed and allowed to cool for 30 minutes. Each sample was then tested in the DR/2400 Portable Spectrophotometer at a wavelength of 620 nm. Three absorbency tests were performed on each vial and the absorbency readings of the three tests were averaged. This value was then multiplied by the value 2884 to yield the COD in mg O_2/L.

UV-visible Spectroscopy

All samples underwent UV-visible spectroscopy in the Secomam Anthelie UV/Visible Light Advanced Spectrophotometer. The samples were run in a 10 mm quartz cuvette after the system was calibrated with ultra-pure water. All samples were exposed to a range of UV-visible light from 200 to 600 nm. Between each sample, the cuvette was rinsed with the subsequent sample. Due to the limitations of the spectrophotometer, many of the samples had to be diluted in order to observe the entire spectrum. Samples were first diluted by 10 by micropipetting 1 mL of the sample and adding it to 9 mL of ultra-pure water. If this dilution was not sufficient, the

sample was diluted by 20 by micropipetting 0.5 mL of the sample and adding it to 9.5 mL of ultra-pure water.

Fluorescence Spectroscopy

All samples underwent fluorescence spectroscopy in the fluorescence spectrophotometer, the Hitachi Digilab F-2500. The system was calibrated with a blank PMMA cuvette filled with ultra-pure water to determine the conversion for Raman Units. Then a cuvette with ultra-pure water was run to create a baseline. Finally, each sample was tested in the fluorescence spectrophotometer with light in the range of 230 to 600 nm. For each sample, a new PMMA cuvette was used. Samples that had been diluted during UV-visible spectroscopy were diluted to the same degree for fluorescence spectroscopy.

Gauss Identification

After testing, the fluorescence spectrometry and UV-visible data were corrected for dilutions and Raman units and combined to create synchronous fluorescence spectroscopy curves with a constant $\Delta\lambda$=50 nm. These synchronous fluorescence spectroscopy, or SF50 curves, underwent Gauss decomposition using Fortran software as used in *Spectrophotometric characterization of dissolved organic matter in a rural watershed* (Assaad et al., 2015). The peaks that were identified from this software were grouped to be analyzed as fluorophores.

UV Irradiation

All samples underwent UV irradiation testing in addition to UV-visible spectroscopy. In UV irradiation testing, each sample was tested with both UV-visible and fluorescence spectroscopy before and after exposure to UV radiation to observe the effects of UV radiation on the materials. Each sample was loaded into a PMMA cuvette which was then tested with the

fluorescence spectroscopy procedure described above. Each sample was also tested with the UV-visible spectroscopy procedure described above with one modification: the UV-visible spectrophotometer was set to test at a wavelength range of 250 to 600 nm. The blank cuvette filled with ultra-pure water used to calibrate the machines was also tested with UV-visible and fluorescence spectroscopy.

Next, each sample was placed in an irradiation chamber for 24 hours. The blank cuvette was also placed in the irradiation chamber to test for UV irradiation effects on the PMMA cuvette. After 24 hours all samples were removed from the UV irradiation chamber. A new blank PMMA cuvette filled with ultra-pure water was loaded and used to calibrate the UV-visible spectrophotometer (250 to 600 nm) and the fluorescence spectrophotometer. Then each sample, including the blank cuvette exposed to UV radiation, was tested with both UV-visible spectroscopy and fluorescence spectroscopy.

Total Polyphenolic Content

The Folin-Ciocalteu method was run on each sample to determine the total phenolic content. In individual glass vials, 0.5 mL of each sample was mixed with 2.5 mL of Folin reagent and 2 mL of calcium carbonate (75 g/L). Each sample was placed in a hot water bath at 50°C for 5 minutes. This process can be detailed in the article *Characterisation of maritime pine bark tannins extracted under different conditions by spectroscopic methods* (Chupin, Motillon, Charrier-El Bouhtoury, Pizzi, & Charrier, 2013). These samples underwent UV-visible spectroscopy in the Secomam Anthelie UV/Visible Light Advanced Spectrophotometer. The samples were tested in PMMA cuvettes after the system was calibrated with ultra-pure water. All samples were exposed to a range of UV-visible light from 700 to 800 nm in order to determine the absorbance at 760 nm. The same process was run with gallic acid at various dilutions to

create a calibration curve. The absorbances from the samples were then converted to gallic acid equivalent (GAE) and reported in mg GAE per g of dry wood.

Condensed Tannins Content

The content of condensed tannins, also known as proanthocyanidins, was determined by two methods, the vanillin assay and acidic butanol assay. The results from both methods were analyzed and compared.

Vanillin Assay

The vanillin method was run on each sample to determine the condensed tannins content. 1 mL of the sample was mixed with 2 mL of the vanillin assay. The vanillin assay was formed with approximately 1 g of vanillin and 100 mL of 70% sulfuric acid. Each sample was mixed and placed in a hot water bath at 35°C for 15 minutes. This process is detailed in the article *MALDI-TOF Analysis of Aleppo Pine Bark Tannin* (Abdalla et al., 2014). These samples underwent UV-visible spectroscopy in the Secomam Anthelie UV/Visible Light Advanced Spectrophotometer. These samples were tested in PMMA cuvettes after the system was calibrated with ultra-pure water. All samples were exposed to a range of UV-visible light from 450 to 550 nm in order to determine the absorbance at 500 nm. The same process was run with catechin at various dilutions to create a calibration curve. The absorbance's from the samples were then converted to catechin equivalent (CE) and reported in mg CE per g of dry wood.

Acidic Butanol Assay

An acidic butanol assay was performed on all samples to determine the condensed tannins content of each sample. First 500 mL of Butanol-HCl in a 2:3 volumetric ratio were prepared. 0.0779 g of iron sulfate ($FeSO_4 \bullet nH_2O$) was measured and added to the 500 mL of

Butanol-HCl to create an iron solution. In individual glass vials, 0.5 mL of each leachate sample was mixed with 5 mL of the prepared iron solution. Each of the samples was then placed in a water bath at 95°C for 15 minutes. The samples were then removed and allowed to cool in a cold water bath for approximately 15 minutes before being loaded into cm^3 PMMA cuvettes. The samples then underwent UV-visible spectroscopy in the Secomam Anthelie UV/Visible Light Advanced Spectrophotometer after the machine was calibrated with ultra-pure water. The samples were tested at a wavelength range of 480 to 580 nm in order to determine the absorbance at 530 nm. The absorbance value for each sample was expressed as measure of mg of cyanidin per g of dry bark (mg Cya/g bark).

$$\frac{mg\ CyaE}{g\ bark} = \frac{A \times V \times D \times M \times 1000}{\varepsilon \times l \times m}$$

where A is the absorbance reading at 530 nm, V is the volume of the reaction (5.5 mL), D is the dilution factor (1), M is the cyanidin molar mass (287 g/mol), ε is the molar extinction coefficient (34,700 L/mol•cm), l is the path length (1 cm) and m is the mass of dry bark for the given sample. This equation is fully described in the article *Characterisation of maritime pine (Pinus pinaster) bark tannins extracted under different conditions by spectroscopic methods, FTIR and HPLC* (Chupin et al., 2013).

Results and Discussion

Each of the eight tests was analyzed for all 60 unique samples. Trends among bark, core, sapwood, and heartwood; softwood and hardwood, and individual species were investigated. Due to the large number of samples, samples on each graph were labeled as their sample number. Table 2 correlates the sample number to the species and wood portion. The graphs were color coded to allow for easier visual analysis. For the graphs in this section, softwood is indicated with a red outline. When the general comparison is made, the bark is green, the core is blue, the branches are yellow, and the debris is purple. When a comparison is made between tree samples that have heartwood and sapwood, the bark is green, the sapwood is yellow and the heartwood is red.

Table 2: Sample numbers and characteristics

Sample #	Species	Hard or Soft	Portion
7	Checker	Hard	Bark
8	Checker	Hard	Sapwood
9	Checker	Hard	Heartwood
10	Oak	Hard	Bark
11	Oak	Hard	Sapwood
12	Oak	Hard	Heartwood
13	Common Beech	Hard	Bark
14	Common Beech	Hard	Core
15	Maple	Hard	Bark
16	Maple	Hard	Core
17	Chestnut	Hard	Bark
18	Chestnut	Hard	Core
19	Locust	Hard	Bark
20	Locust	Hard	Sapwood
21	Locust	Hard	Heartwood
22	Wild Cherry	Hard	Bark
23	Wild Cherry	Hard	Sapwood
24	Wild Cherry	Hard	Heartwood
25	Norway Spruce	Soft	Bark
26	Norway Spruce	Soft	Core
27	Service	Hard	Bark

28	Service	Hard	Core
29	European Larch	Soft	Bark
30	European Larch	Soft	Core
31	Common Walnut	Hard	Bark
32	Common Walnut	Hard	Sapwood
33	Common Walnut	Hard	Heartwood
34	Poplar	Hard	Bark
35	Poplar	Hard	Sapwood
36	Poplar	Hard	Heartwood
37	Ash	Hard	Bark
38	Ash	Hard	Core
39	Pine	Soft	Bark
40	Pine	Soft	Core
41	Lime	Hard	Bark
42	Lime	Hard	Core
43	Elm	Hard	Bark
44	Elm	Hard	Sapwood
45	Elm	Hard	Heartwood
46	Willow	Hard	Bark
47	Willow	Hard	Sapwood
48	Willow	Hard	Heartwood
49	Alder	Hard	Bark
50	Alder	Hard	Core
51	Fir	Soft	Bark
52	Fir	Soft	Core
53	Aspen	Hard	Bark
54	Aspen	Hard	Core
55	Hornbeam	Hard	Bark
56	Hornbeam	Hard	Core
57	Birch	Hard	Bark
58	Birch	Hard	Core
59	Maritime Pine	Soft	Bark
60	Aleppo Pine	Soft	Bark
61	Eucalyptus	Hard	Bark
62	Olive	Hard	Branches
63	Date Palm	Hard	Debris
64	Douglas Fir	Soft	Bark
65	Douglas Fir	Soft	Core
66	Boysenberry	Hard	Branches

Dissolved Organic Carbon

Bark vs Core

Overall bark leached the greatest amount of dissolved organic carbon into water. All leachate samples with 6 mg C/g wood or higher were bark samples, with the exception of the Chestnut core sample and Olive branches. The 17 samples that had leachate greater than 6 mg C/g wood represent the top 27.0% of samples tested and account for 65.7% of the total DOC leached in all samples combined. Within each tree species, the bark produced leachate with a higher concentration of DOC than the inner layers 95.7% of the time. Checker was the only tree sample in which an inner layer produced a higher concentration leachate than the bark.

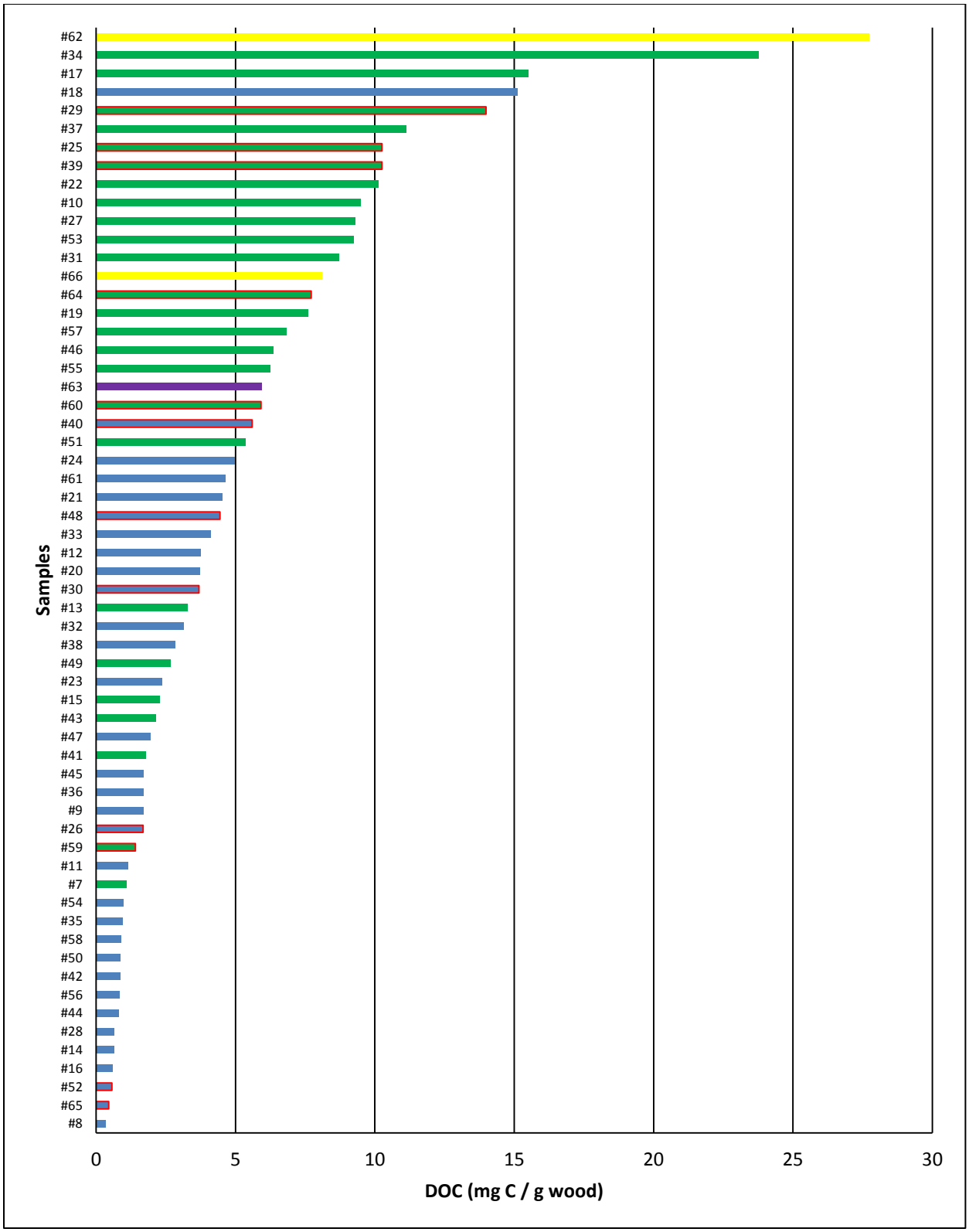

Figure 9: Overview of DOC

Bark, Sapwood, and Heartwood

In all tree species with three layers, including the Checker tree, the heartwood always produced leachate with a higher concentration of DOC than the sapwood. All leachate samples with 1 mg C/g wood or lower were core or sapwood samples. The 13 samples that produced leachate less than 1 mg C/g wood represent the bottom 21.7% of samples tested and account for just 3.0% of the total DOC leached in all samples combined. Core and sapwood were generally significantly less toxic than the bark and heartwood layers of the trees. The average DOC concentration from bark leachates was the highest, measuring 8.67 mg C/g wood. The average DOC concentration from sapwood leachates was the lowest at 1.80 mg C/g wood. The average TDN concentration from heartwood leachates was 3.37 mg C/g wood. The standard deviation for the bark, sapwood, and heartwood leachate DOC concentration averages was 6.94, 1.20, and 1.42, respectively. While leachate samples from all layers varied widely among species, the heartwood leachate samples experienced the least variation between species. Heartwood leachate samples from all species always fell within the range of 1.5-5 mg C/g wood, making heartwood the most consistent tree layer in terms of predicting DOC leaching.

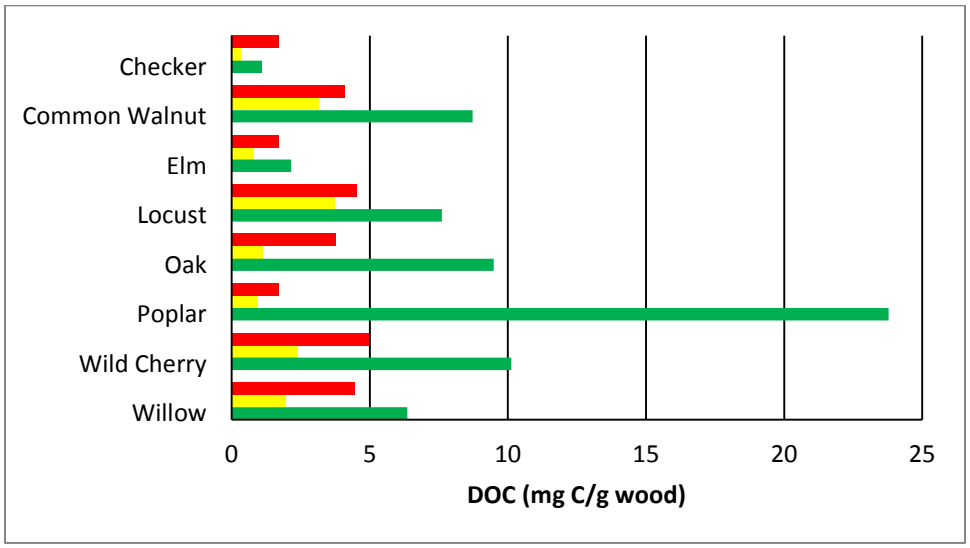

Figure 10: DOC of multi-layer tree species

Hardwood vs Softwood

When comparing just the exterior samples including bark, branches, and debris from each species, softwoods made up 24.1% of the samples tested and accounted for 23.0% of the sum total DOC from all bark samples tested. 42.9% of the softwood bark samples tested also produced leachates in the top quartile of DOC concentrations. The average DOC concentration for softwood bark samples was 7.84 mg C/g bark which is only slightly less than 8.36 mg C/g bark, the average DOC concentration for hard wood bark samples.

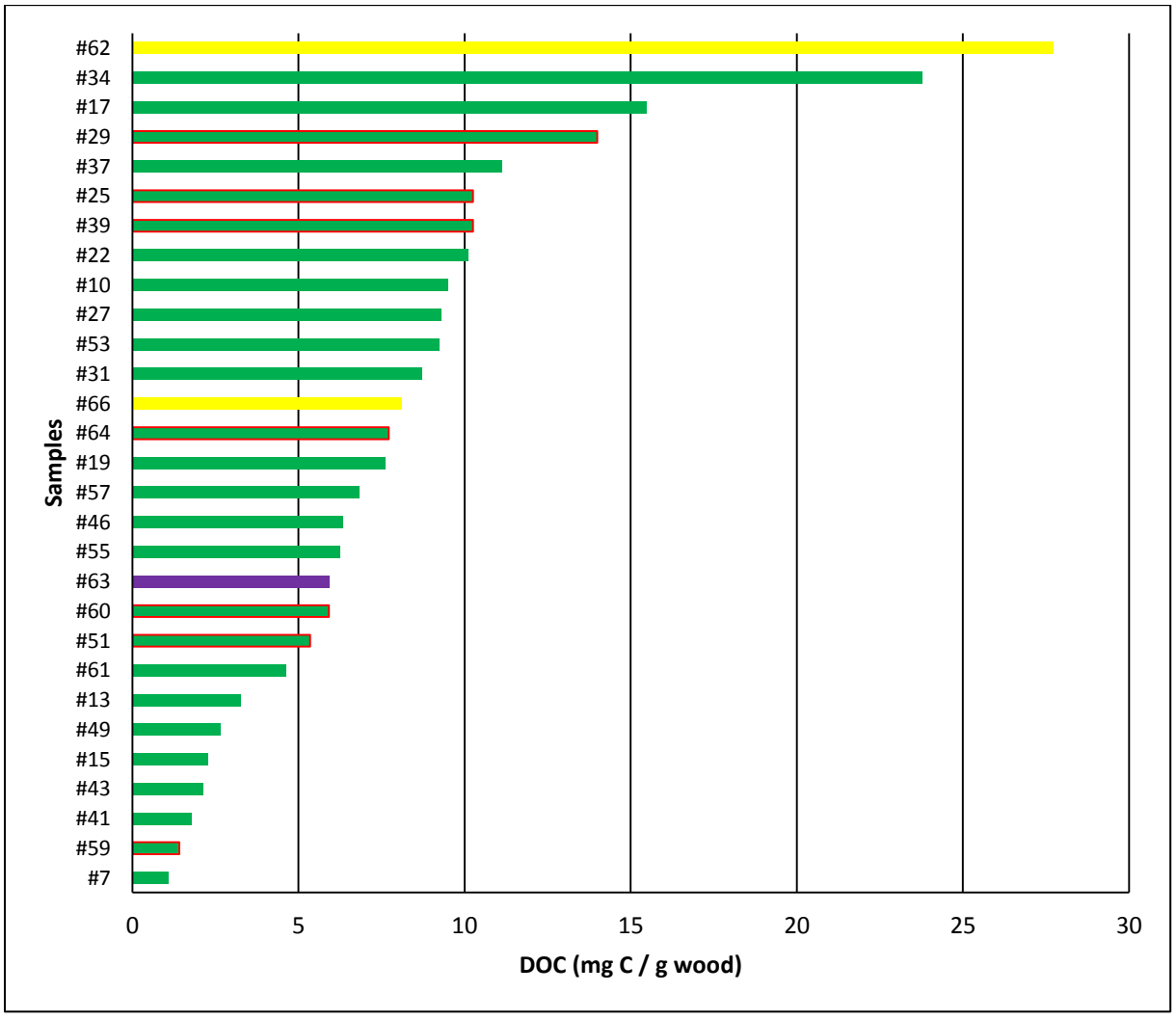

Figure 11: DOC of exterior/bark samples

When comparing samples from all tree layers, softwoods generally produced higher DOC concentration leachate samples as 58.3% of the softwood leachate samples fell in the upper half of leachate concentrations.

Species

Species exhibiting the highest values of DOC (mg C/g wood) in leachate samples included Olive, Poplar, Chestnut, European Larch, Ash, and Norway Spruce. Species exhibiting the lowest values of DOC (mg C/g wood) in leachate samples were Checker, Fir, Maple, Common Beech, Service, and Elm. When comparing bark and equivalent exterior wood (branches, debris), Table 3 provides a ranking of DOC leachate concentrations in order of highest to lowest.

Table 3: DOC of exterior/bark samples

Species	DOC (mg C/g bark)
Olive	27.7
Poplar	23.8
Chestnut	15.5
European Larch	14.0
Ash	11.1
Norway Spruce	10.3
Pine	10.3
Wild Cherry	10.1
Oak	9.49
Service	9.28
Aspen	9.24
Common Walnut	8.72
Boysenberry	8.11
Locust	7.61
Birch	6.83
Willow	6.36
Hornbeam	6.26
Date Palm	5.94
Aleppo Pine	5.92
Fir	5.35
Eucalyptus	4.64

Common Beech	3.27
Alder	2.66
Maple	2.27
Elm	2.15
Lime	1.79
Maritime Pine	1.41
Checker	1.10

Figure 12 compares the DOC concentrations in the bark and core samples for each species in which two layers were tested. The values for bark to core DOC ratios are graphed in blue with values for the sum total DOC in the bark and core samples from each species in pink to show the comparative toxicity of each species. The values graphed for softwood species are outlined in red. The tree exhibiting the highest DOC concentration ratio for bark to core was the Service tree with a ratio over 30. The bark of the Service tree produces leachate with a DOC concentration over 30 times greater than that of its core. In comparison, both the Aleppo Pine and Chestnut trees produce leachate samples of nearly equal DOC concentrations for the bark and core samples. Chestnut, which has nearly equally harmful bark and core in terms of DOC leaching, has the greatest total DOC out of the samples. There was no apparent trend for hardwood vs softwood in terms of bark to core ratio.

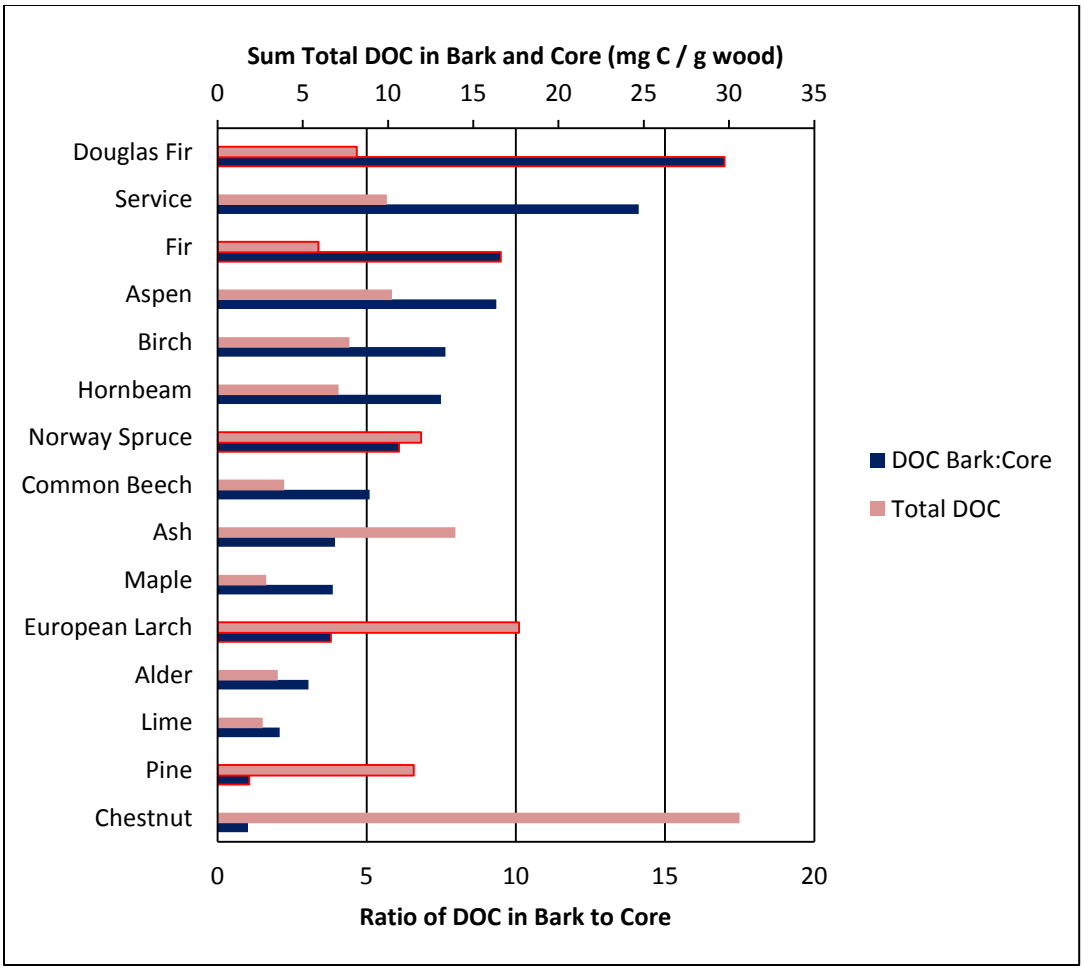

Figure 12: DOC ratios (bark:core) and sum total

For trees with high bark to core ratios, removing the bark before storage could reduce DOC leaching into runoff. For trees with low bark to core ratios, bark removal will make little difference in DOC leaching into runoff. However, as bark plays an important role in protecting the wood, its removal would make the wood structure vulnerable to the environment.

Discussion

Dissolved organic carbon testing indicates the presence of organic contaminants in a water sample (Hedmark & Scholz, 2008). A comparison of the DOC concentrations in leachates from various tree species and layers provides a basic understanding of the potential of these

species and layers to conduct mass transfer of organic compounds into water. After testing a wider range of tree species, this study confirms the result that bark leaches more DOC than inner tree layers as previously proved (Svensson et al., 2013, 2012). Additionally, it reveals that they facilitate mass transfer of organic compounds into water without a significant difference between these two categories of trees.

Total Dissolved Nitrogen

Bark vs Core

On average, bark and equivalent outer tree portions leach more TDN into water than inner tree layers. The average TDN leached from each sample was 0.0512 mg N/g wood. Only 9.7% of the inner tree layer samples produced leachate of equal or greater concentration to this value, despite the fact that inner tree layers accounted for half of the samples tested. The average leachate TDN concentration from bark and equivalent outer tree portions was 0.0814 mg N/g wood, approximately 3.6 times greater than the average inner layer leachate concentration of 0.0229 mg N/g wood. Within each tree species, the bark produced leachate with a higher TDN concentration than that of the inner layers 96.8% of the time. Common Walnut was the only tree sample in which an inner layer produced a higher concentration leachate than the bark. When examining tree species with only two layers, the bark produced leachate with a higher TDN concentration than that of the core 100% of the time.

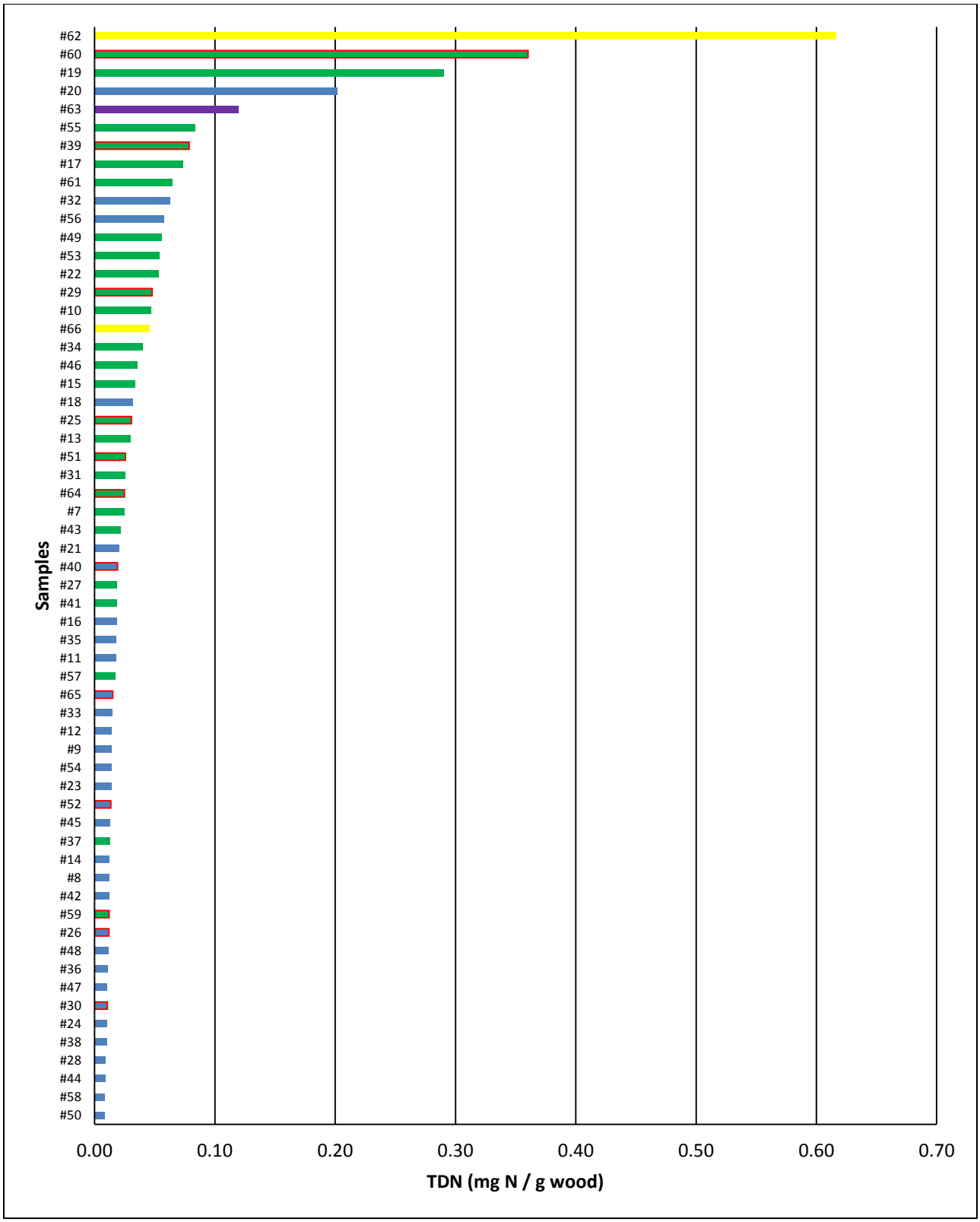

Figure 13: Overview of TDN

Bark, Sapwood, and Heartwood

When examining tree species with three layers, the sapwood produced a leachate with higher TDN concentration than that of the heartwood 62.5% of the time. The bark layer produced a leachate with a higher TDN concentration than that of the inner layers 87.5% of the time. The average TDN concentration from bark leachates was the highest, measuring 0.537 mg N/g wood. The average TDN concentration from sapwood leachates was 0.346 mg N/g wood. The average TDN concentration from heartwood leachates was the lowest at 0.110 mg N/g wood. The standard deviation for the bark, sapwood, and heartwood leachate TDN concentration averages was 0.0909, 0.0664, and 0.0030, respectively. Both bark and sapwood leachate samples varied widely among species, but the heartwood leachate samples experienced little variation between species. All heartwood leachate samples fell in the range of 0.0100-0.0210 mg N/g wood, making heartwood the most predictable tree layer in terms of TDN leaching.

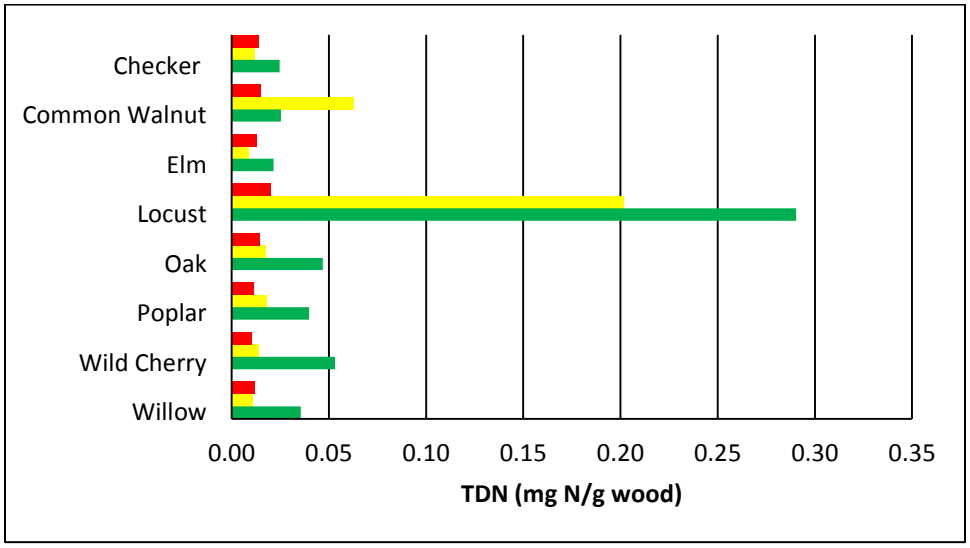

Figure 14: TDN of multi-layer tree species

Hardwood vs Softwood

When comparing just the exterior samples including bark, branches, and debris from each species, softwoods make up 24.1% of the samples tested and account for 24.7% of the sum total

TDN from all bark samples tested. There does not appear to be a significant trend in hardwood vs softwood bark samples. Both the lowest and second highest TDN concentrations measured were softwood bark samples, showing that both categories of trees are highly variable in terms of TDN leaching. When comparing samples from all tree layers, there also did not appear to be a significant trend in TDN leaching from hardwoods vs softwoods.

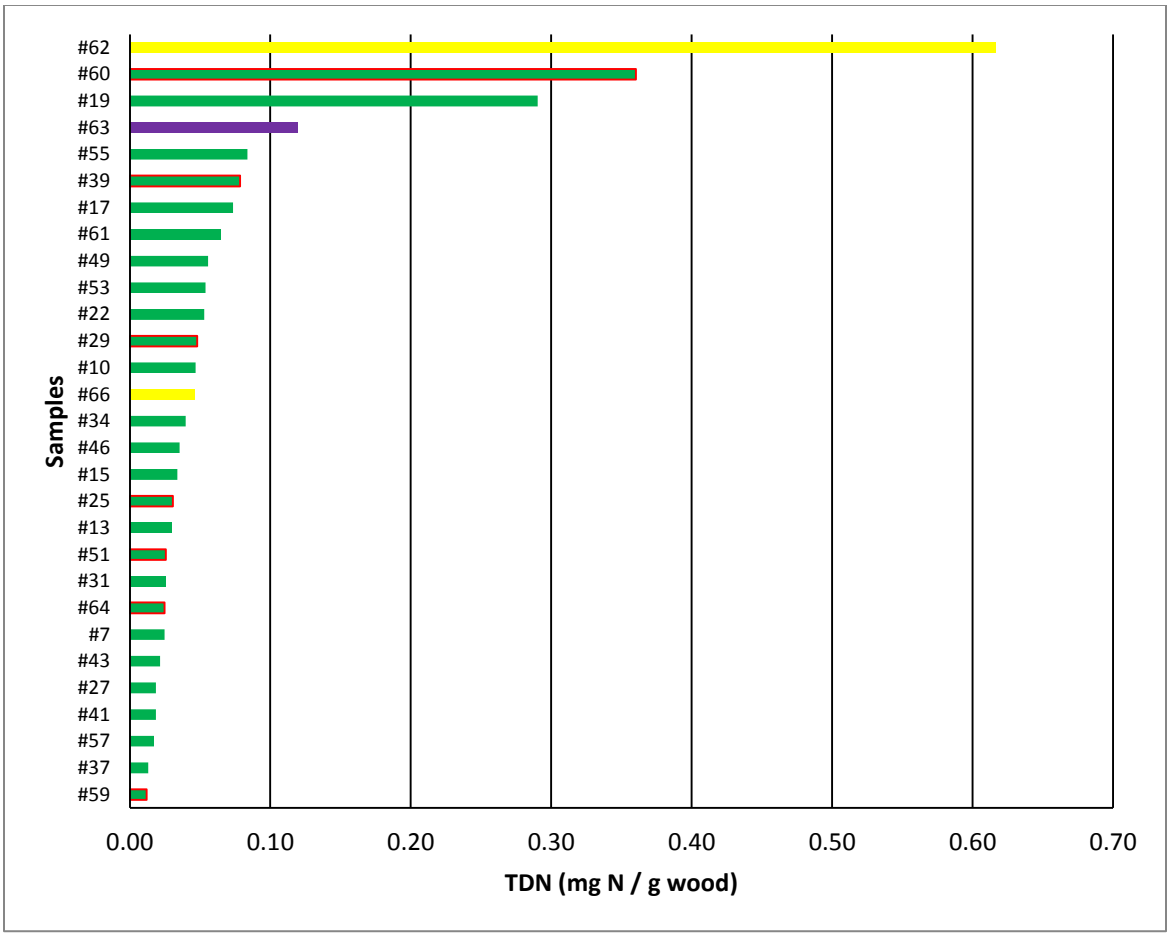

Figure 15: TDN of exterior/bark samples

Species

Species exhibiting the highest values of TDN (mg N/g wood) in leachate samples included Olive, Aleppo Pine, Locust, Date Palm, Hornbeam, and Pine. Species exhibiting the lowest values of TDN (mg N/g wood) in leachate samples were Alder, Birch, Elm, Service, Ash,

and Wild Cherry. When comparing bark and equivalent exterior wood (branches, debris), Table 4 provides a ranking of TDN leachate concentrations in order of highest to lowest.

Table 4: TDN of exterior/bark samples

Species	TDN (mg N/g bark)
Olive	0.617
Aleppo Pine	0.360
Locust	0.290
Date Palm	0.120
Hornbeam	0.0838
Pine	0.0785
Chestnut	0.0734
Eucalyptus	0.0647
Alder	0.0558
Aspen	0.0539
Wild Cherry	0.0531
European Larch	0.0480
Oak	0.0468
Boysenberry	0.0458
Poplar	0.0399
Willow	0.0355
Maple	0.0338
Norway Spruce	0.0307
Common Beech	0.0299
Fir	0.0256
Common Walnut	0.0253
Douglas Fir	0.0247
Checker	0.0247
Elm	0.0216
Service	0.0186
Lime	0.0185
Birch	0.0171
Ash	0.0131
Maritime Pine	0.0121

Figure 16 compares the TDN concentrations in the bark and core samples for each species in which two layers were tested. The values for bark to core TDN ratios are graphed in blue with values for the sum total TDN in the bark and core samples from each species in pink to

show the comparative toxicity of each species. The values graphed for softwood species are outlined in red. The tree exhibiting the highest TDN concentration ratio for bark to core was the Alder tree with a ratio of approximately 7. The bark of the Alder tree produces leachate with a TDN concentration nearly 7 times greater than that of its core. In comparison, the Ash tree produces leachate samples with nearly equal TDN concentrations for the bark and core samples. Hornbeam, which has the second lowest TDN bark to core ratio has the greatest total TDN out of the species examined. Softwoods generally had greater bark to core ratios, with two of the five softwood samples producing the second and third highest TDN bark to core ratios.

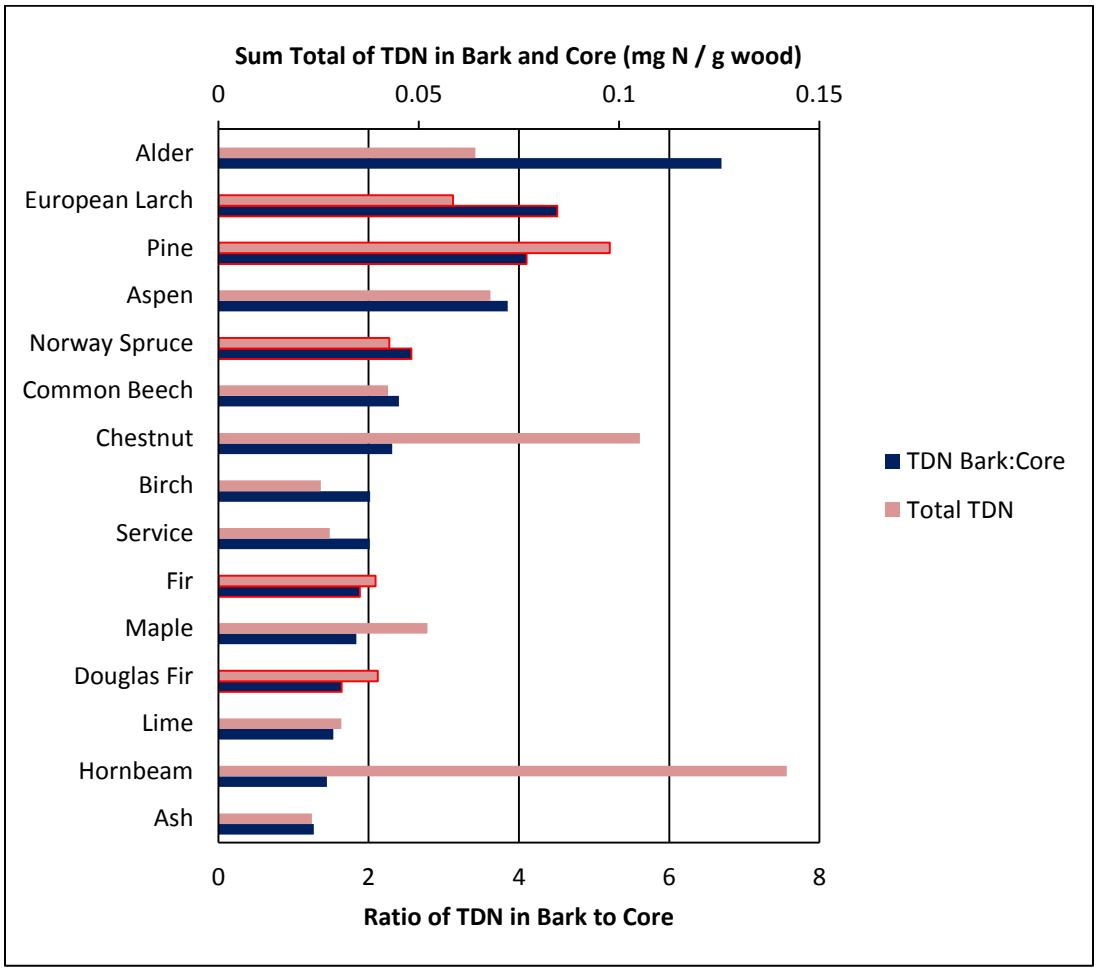

Figure 16: TDN ratios (bark:core) and sum total

For trees with high bark to core ratios, removing the bark before storage is a potential way to reduce TDN leaching into runoff. For trees with low bark to core ratios, bark removal will make little difference in TDN leaching into runoff. However, as previously discussed, removing bark industrially could have adverse effects on the wood structure making this procedure ill-advised.

Discussion

TDN testing is an important way to analyze lumber leachate since excess nitrogen in waterways can create hypoxic or acidic conditions which affect the health of organisms living in those waterways (Baron et al., 2013). A comparison of the TDN concentrations in leachates from various tree species and layers provides a basic understanding of the potential of these species and layers to conduct mass transfer of dissolved nitrogen into water. After testing a wider range of tree species, this study confirms the result that bark leaches more TDN than inner tree layers as previously proved (Svensson et al., 2013, 2012). However, bark from different tree species varies widely in terms in of TDN leaching abilities. The sapwood and especially the heartwood are much easier to predict in terms of TDN leaching ability.

Although the main purpose of this study is to examine leaching in the context of water pollution, it is interesting to note that nitrogen leaching may be a useful technique for preparing fuelwood. Trees that are commonly used as fuelwoods such as Oak, Maple, and Birch may be purposefully leached to remove nitrogen from the wood, which pollutes the air with nitrogen oxide when burned. Intentional use of nitrogen leaching would need to be examined further in research before determining the feasibility of this technique.

Chemical Oxygen Demand

Trend with DOC

As most of the materials in Chemical Oxygen Demand tests of water were organic, it would follow that COD and DOC should have a direct relationship. With this assumption and due to limited time and resources, this test was run on 24 samples where trends could be initially investigated. Of those samples, 23 were tested, normalized for bark weight, and plotted as shown in Figure 17. Sample #37's absorbance maxed out the DR/2400 Portable Spectrophotometer and was considered to be an inaccurate source of data.

Of the samples tests, 82.6% were exterior samples such as bark, branches, and debris, while only 17.4% were interior samples such as core and heartwood. Bark and exterior samples are indicated with green and interior samples are indicated with blue. It should be noted that most of the interior samples have smaller DOC's and are therefore condensed on the bottom left corner of the graph. 73.9% of the samples tested were hardwood while 26.1% of the samples were softwood. The softwood samples are identified with a red outline.

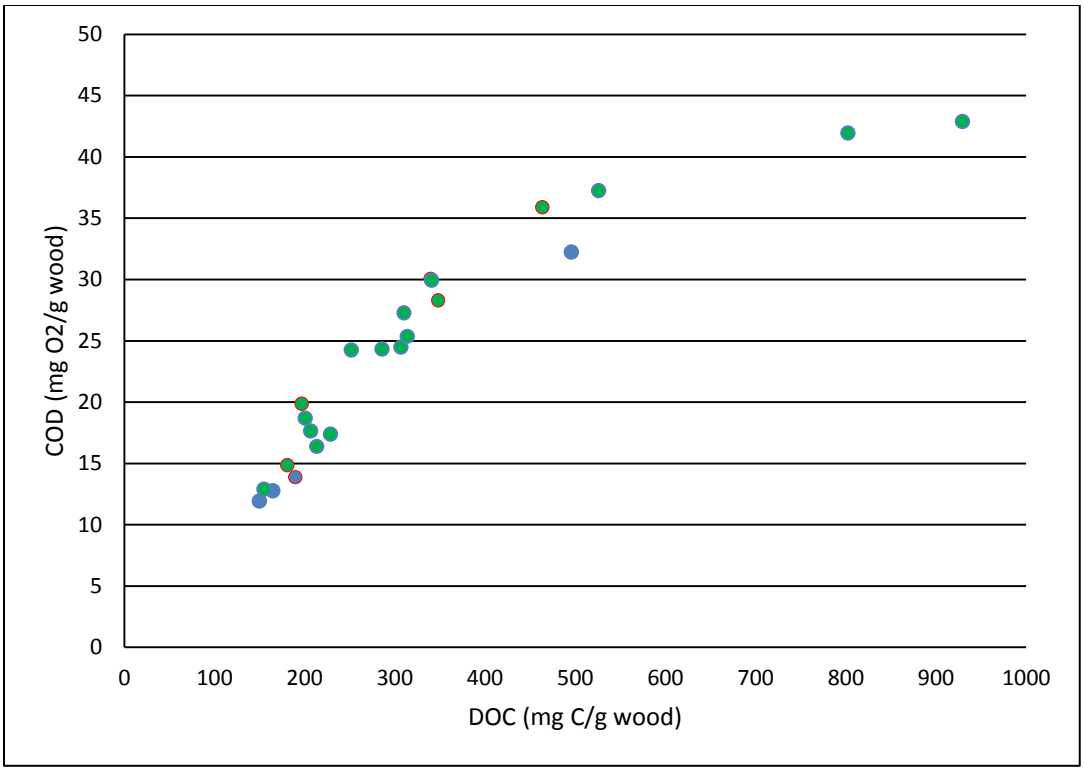

Figure 17: COD and DOC analysis

When the data was plotted, two trend lines were used to track the trend. Both linear and logarithmic trends had reasonable accuracy and without further data, a decision could not be made. Without more pieces of data, the decision was to investigate those datum points that landed below (shown in purple) and above (shown in red) both trend lines.

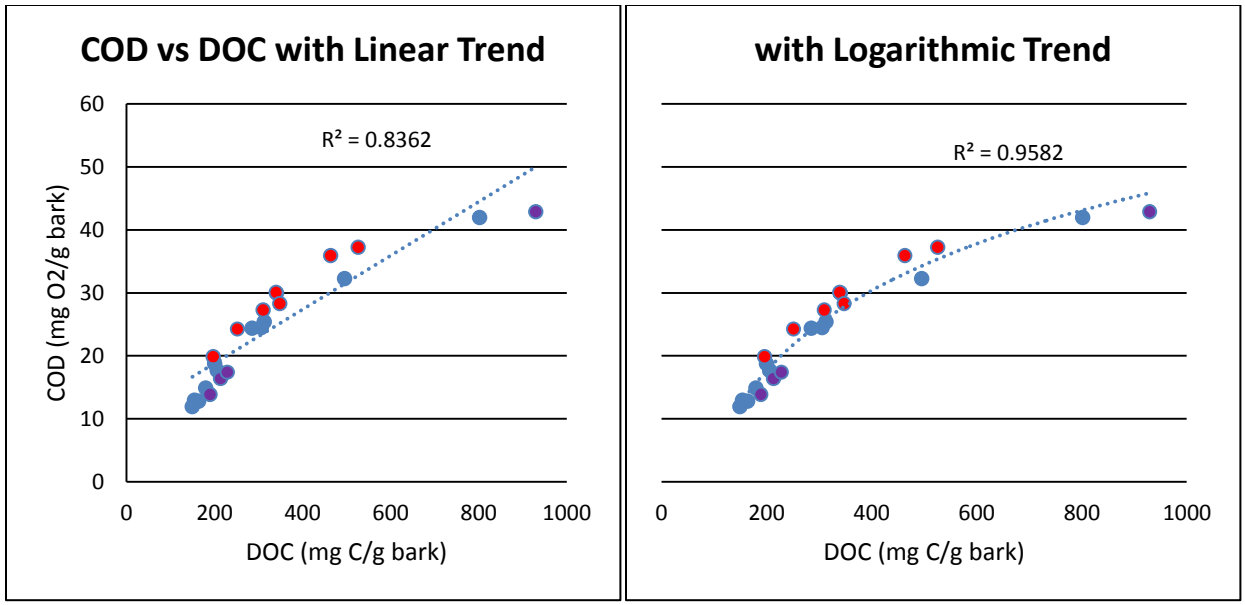

Figure 18: COD vs DOC with linear trend and with logarithmic trend

Below and Above the Trend

Of those samples that fell below that trend, 75% were exterior samples. As there were only four samples and the total data set was made up of 82.6% exterior samples, this is not a significant different. Similarly, though the total data set had 73.9% hardwood samples, the samples from below the trend were made up of 75% hardwood samples.

Of the eight samples that fell above the trend, 100% were bark samples. Furthermore, 50% of the samples that landed above both trends were softwood.

Discussion

With the samples that were tested, it is fair to say that COD had a direct correlation with DOC. Without more investigation it is hard to determine the type of correlation. It is also possible that the type of wood sample may change the relationship above or below the overall trend. For example, it appears that there might be a trend in which softwood bark leachate has more COD than would be predicted from its DOC. This could indicate dissolved organic

material that required more oxygen for breakdown or a presence of inorganic material. This test displays the need to analyze and understand the actual composition and makeup of the DOC.

UV-Visible Spectroscopy

The most visible peaks appeared at around 280 nm and 340 nm. A table of the SUVA values can be found in Appendix II.

Bark vs Core

Bark has overall higher values for SUVA 280 and 340 when comparing all species. This is largely due to the very large values of the highest samples. Within each species, bark has a higher SUVA 280 value only 51.5% of the time and a higher SUVA 340 value only 54.5% of the time.

When comparing wood samples with two portions (bark and core), bark has a higher SUVA 280 value 56.3% of the time which bark has a higher SUVA 340 value 50% of the time. This change is due to the varying range of the heartwood and sapwood.

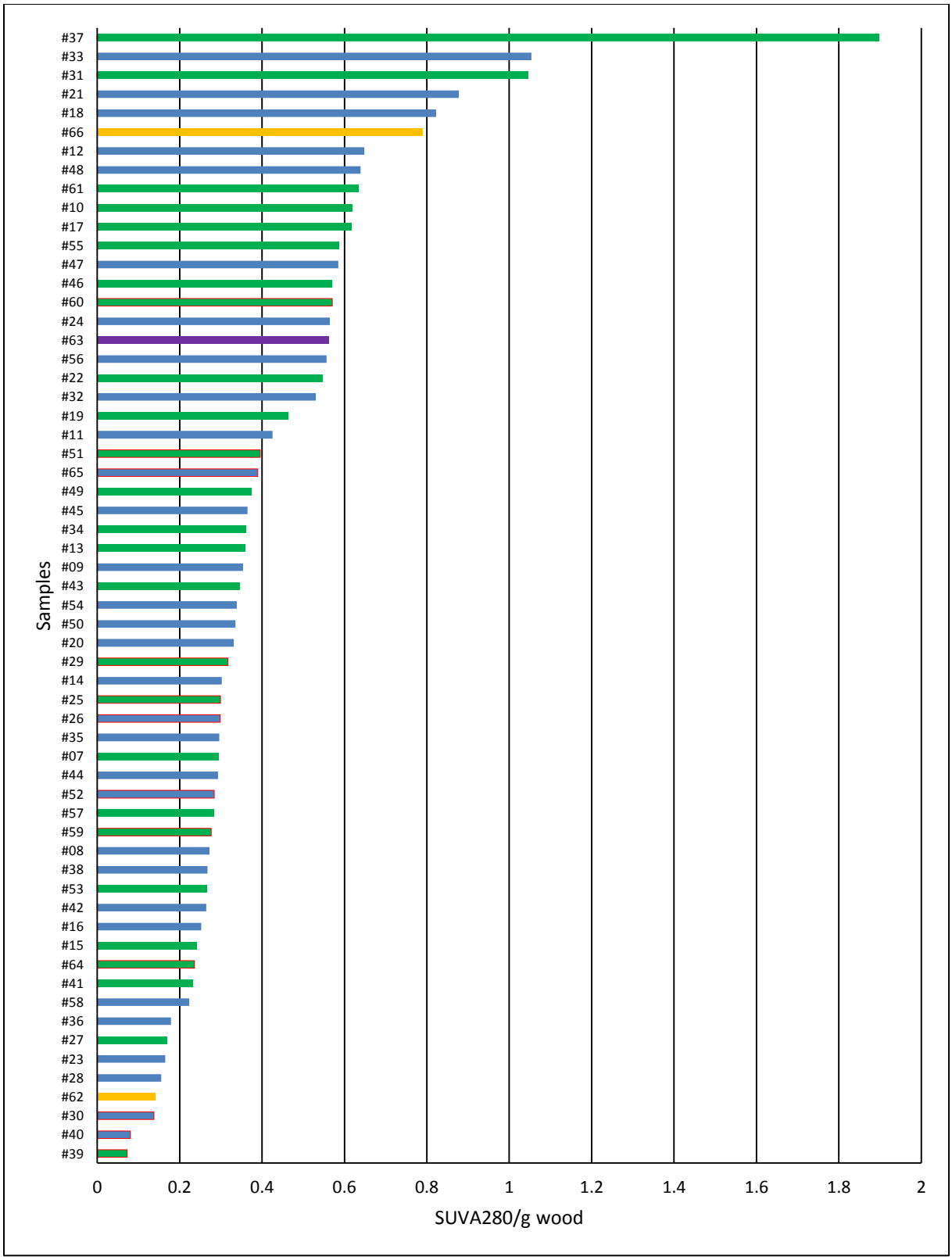

Figure 19: UV analysis: specific ultraviolet absorption at 280 nm

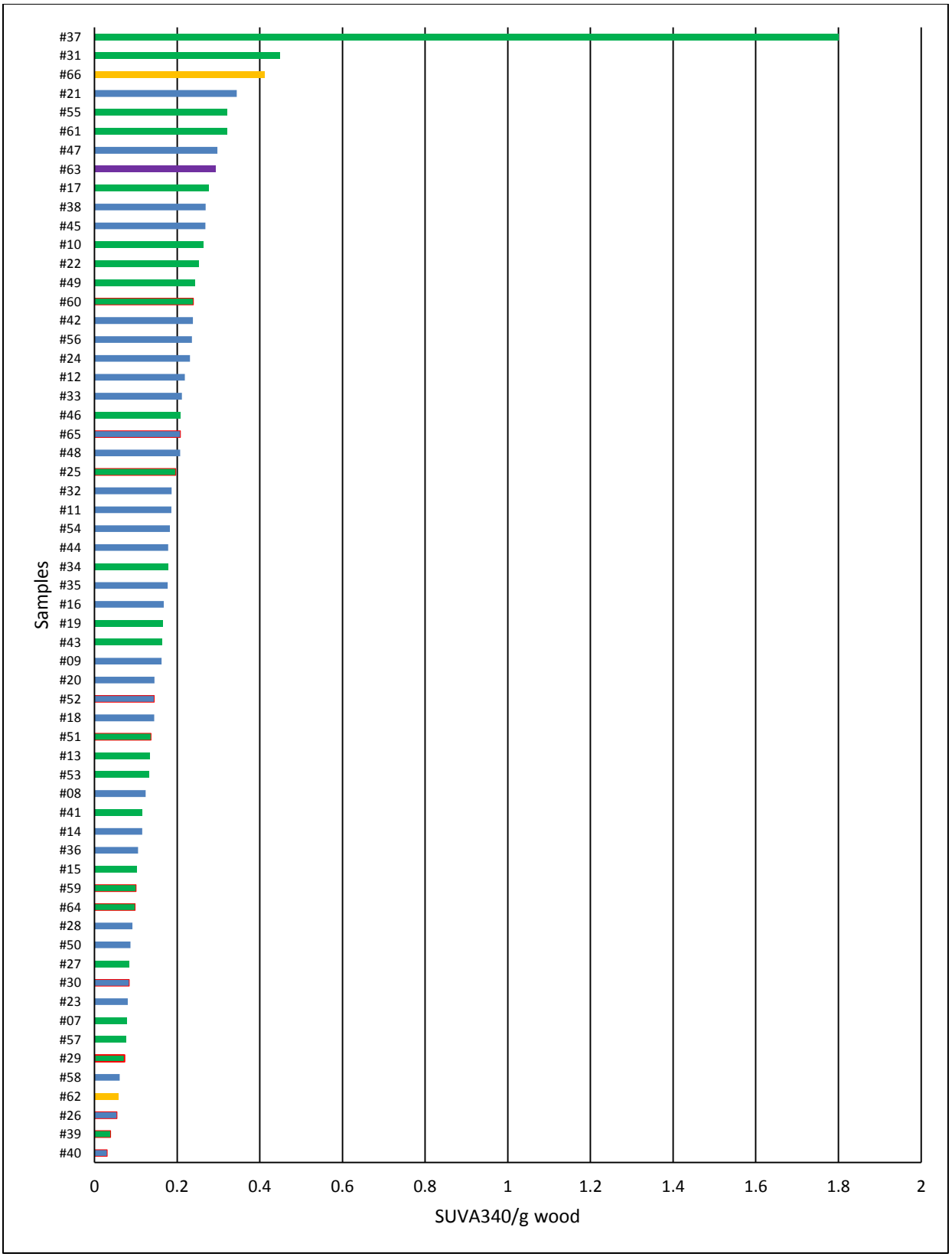

Figure 20: UV analysis: specific ultraviolet absorption at 340 nm

Bark, Sapwood, and Heartwood

For SUVA 280 values, heartwood is higher than bark 87.5% of the time which is higher than sapwood 87.5% of the time. For SUVA 340 values, bark is higher than heartwood 62.5% of the time which is higher than sapwood 75% of the time.

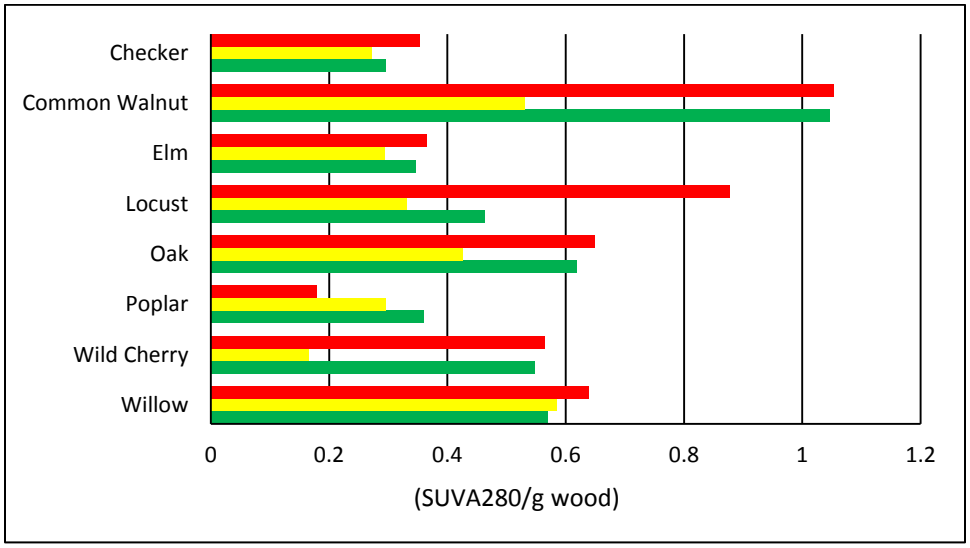

Figure 21: SUVA280 of multi-layer tree species

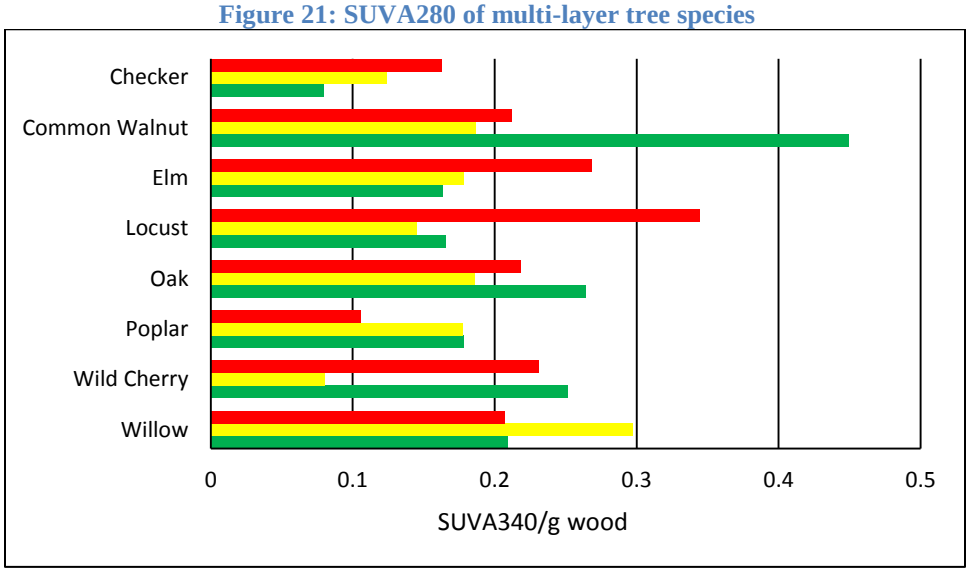

Figure 22: SUVA340 of multi-layer tree species

Hardwood vs Softwood

Firstly, hardwoods SUVA 280 and 340 values are generally higher than softwoods. When a comparison of softwood and hardwood samples that had a bark and a core, softwood bark

SUVA 280 values were higher than core values 60% of the time. Softwood bark SUVA 340 values were, however, only higher than core samples 40% of the time. In comparison to all samples when bark SUVA 280 and SUVA 340 values were higher 56.3% and 50% of the time, respectively, the isolation of softwood against hardwood reveals that softwoods bark and core differences may be more defined than those of hardwood.

Species

Species with the highest SUVA 280 values include Ash, Common Walnut, Locust, Chestnut, Boysenberry, and Oak with Ash being almost twice the next highest values. Lowest SUVA 280 values include Pine, European Larch, Olive, Service, Wild Cherry, and Poplar. Samples with highest SUVA 340 values include Ash, Common Walnut, Boysenberry, Locust, Hornbeam, and Eucalyptus with Ash being approximately four time the next highest values. Lowest include Pine, Norway Spruce, Olive, Birch, European Larch, and Checker.

When comparing bark and other exterior portions of wood, most often in contact with water, these are the values from highest to lowest.

Table 5: SUVA280 and SUVA340 of exterior/bark samples

Species	SUVA280/g bark	Species	SUVA340/g bark
Ash	1.90	Ash	1.80
Common Walnut	1.05	Common Walnut	0.449
Boysenberry	0.791	Boysenberry	0.412
Eucalyptus	0.635	Hornbeam	0.321
Oak	0.618	Eucalyptus	0.321
Chestnut	0.617	Date Palm	0.293
Hornbeam	0.588	Chestnut	0.276
Willow	0.570	Oak	0.264
Aleppo Pine	0.570	Wild Cherry	0.252
Date Palm	0.562	Alder	0.244
Wild Cherry	0.547	Aleppo Pine	0.239
Locust	0.463	Willow	0.209
Fir	0.396	Norway Spruce	0.197

Alder	0.374	Poplar	0.178
Poplar	0.360	Locust	0.165
Common Beech	0.359	Elm	0.163
Elm	0.346	Fir	0.137
European Larch	0.317	Common Beech	0.133
Norway Spruce	0.299	Aspen	0.132
Checker	0.295	Lime	0.116
Birch	0.284	Maple	0.102
Aleppo Pine	0.277	Aleppo Pine	0.101
Aspen	0.266	Douglas Fir	0.0984
Maple	0.241	Service	0.0843
Douglas Fir	0.236	Checker	0.0794
Lime	0.233	Birch	0.0771
Service	0.169	European Larch	0.0725
Olive	0.141	Olive	0.0572
Pine	0.0726	Pine	0.0392

Discussion

While several UV-visible absorbances and ratios have been used to characterize organic matter in soil, aquatic research has encountered more limited absorbance analysis (Leenheer & Croué, 2003). Scientists have largely attributed absorption from UV-visible light to be an indicator of aromatic, humic-like material (Leenheer & Croué, 2003). According to SUVA 280 and SUVA 340 values, hardwood appears to have a higher concentration of aromatic material. Furthermore, heartwood and bark had highest concentration of humic-like material. However, since this test was based off of UV-visible absorbance, there are some potential limitations in the data analysis. UV-visible absorbing compounds in water such as turbidity or inorganic substances can interfere with the characterization (Bieroza et al., 2010).

Gauss Identification

The three peaks found during the Gauss Identification were 280 nm (B1), 300 nm (B2), and 340 nm (B3). The table can be found in Appendix II.

Bark vs Core

For comparing all species, core samples had higher B1 values 61.3% of the time in comparison to bark. Neither the bark nor the core values were more often higher than the other for B2 values. However, bark has higher B3 values 54.8% of the time. When samples with only two portions were compares, the trends change. Core samples have higher B1, B2, and B3 values 68.8%, 68.8%, and 56.3% of the time, respectively. This change in trend should indicate a discrepancy between comparing heartwood and sapwood with general core wood.

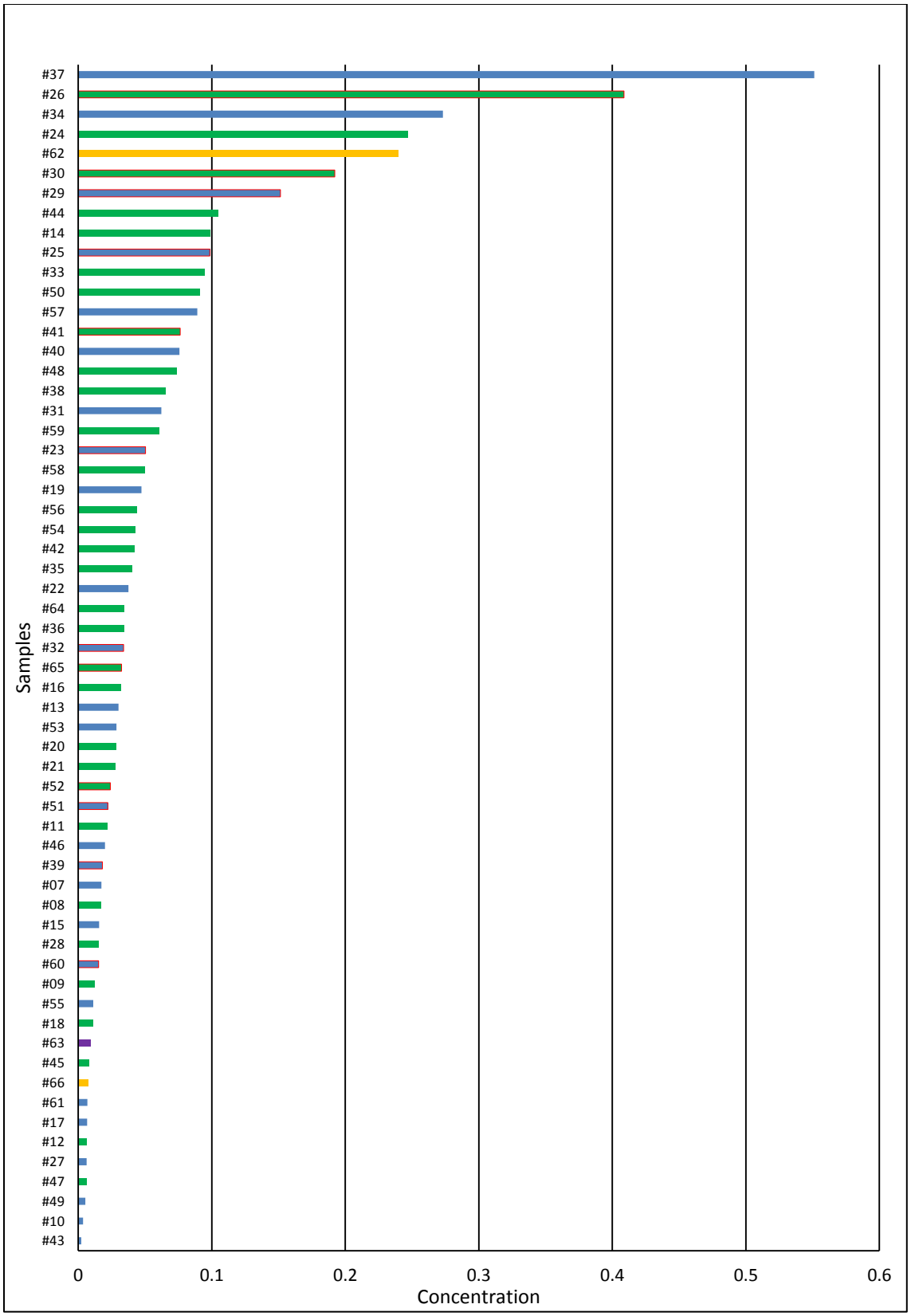

Figure 23: B1 values from Gauss identification

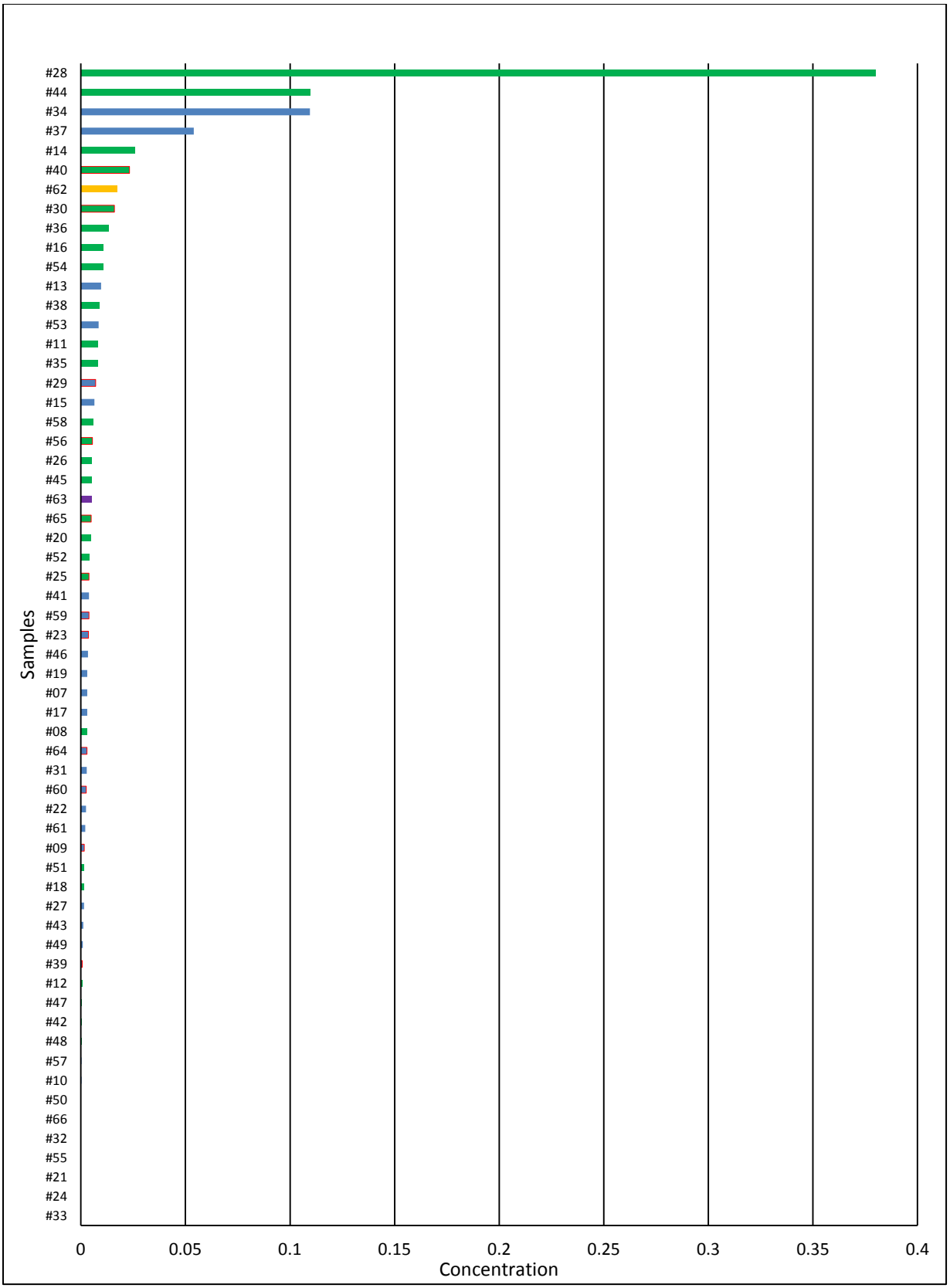

Figure 24: B2 values from Gauss identification

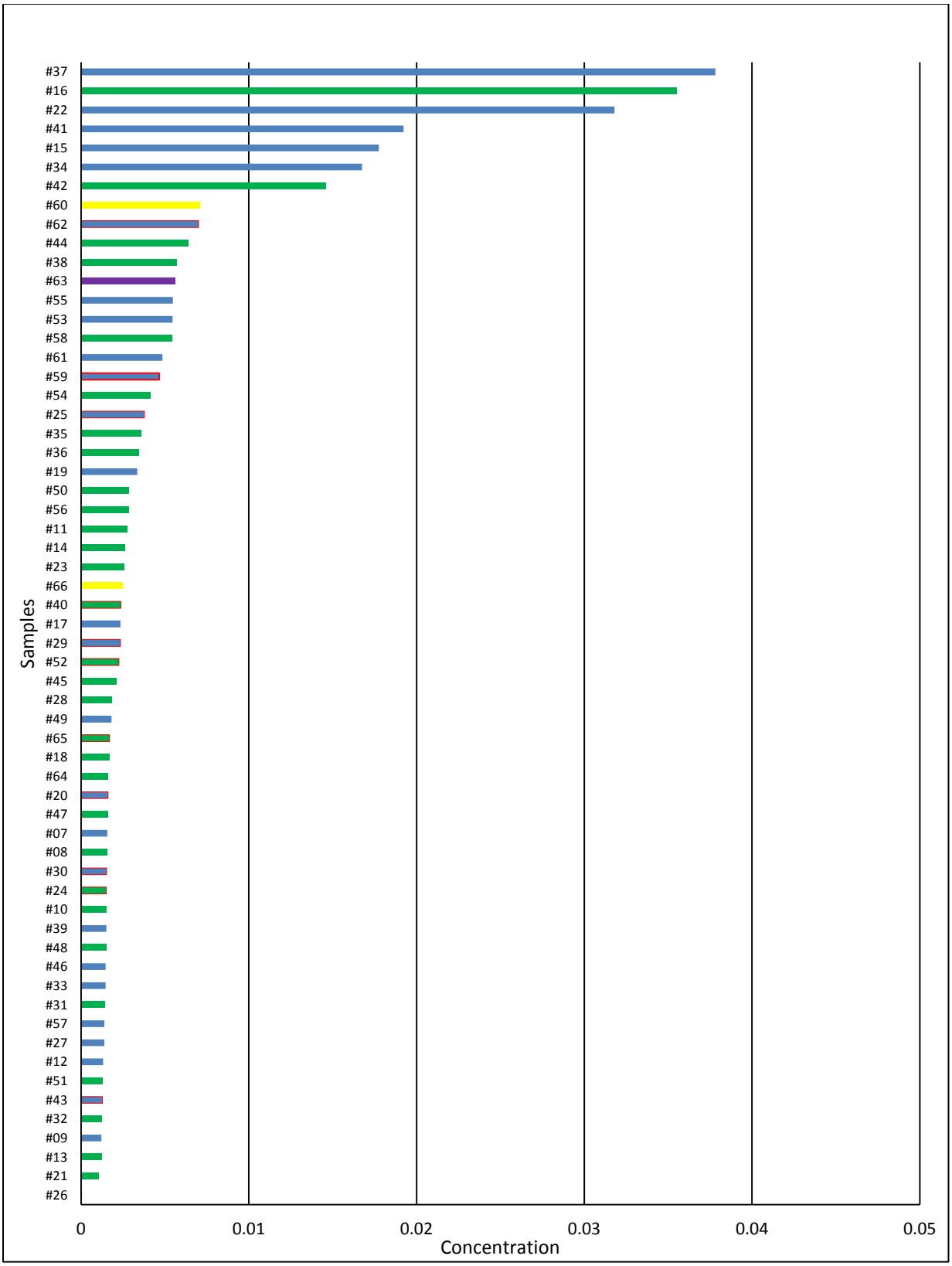

Figure 25: B3 values from Gauss identification

Bark, Sapwood, and Heartwood

When the samples had three portions, the trends varied. For B1 values, there was essentially no trend. The bark, sapwood, and heartwood were each the highest at some point during the analysis. For the B2 values, the bark and the sapwood were about the same. 50% of the time the B2 values in the sapwood were higher than that of the bark. The heartwood contained the lowest values for B2. 75% of the time the bark B2 values were greater than the heartwood values while 87.5% of the time the sapwood values were greater than the heartwood values. For B3, 62.5% of the time the bark has higher values than sapwood and 87.5% of the time the sapwood has higher values than the heartwood.

Since the heartwood samples are always the lowest, they skewed the original overall trends making it appear that the bark is higher than the core when more often than not if there is a trend, the core is higher.

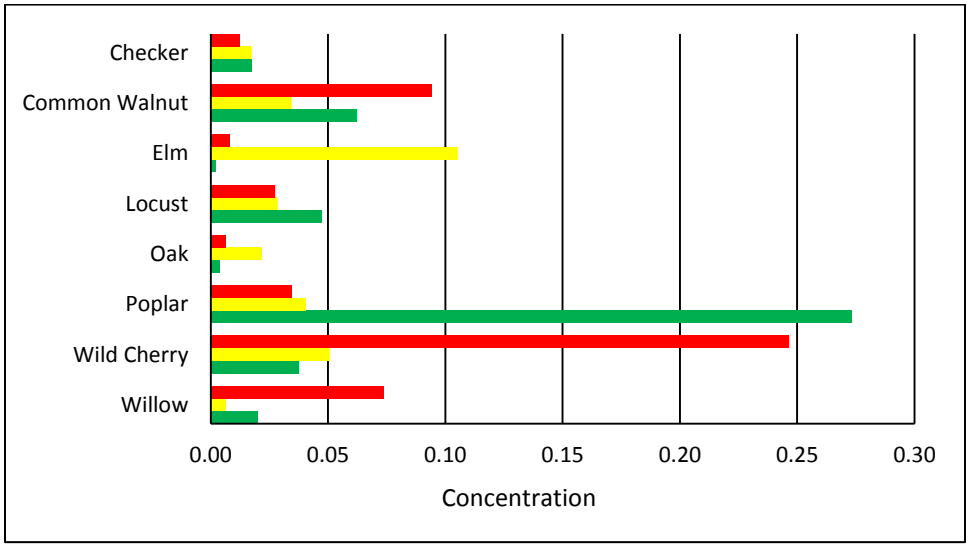

Figure 26: B1 of multi-layer tree species

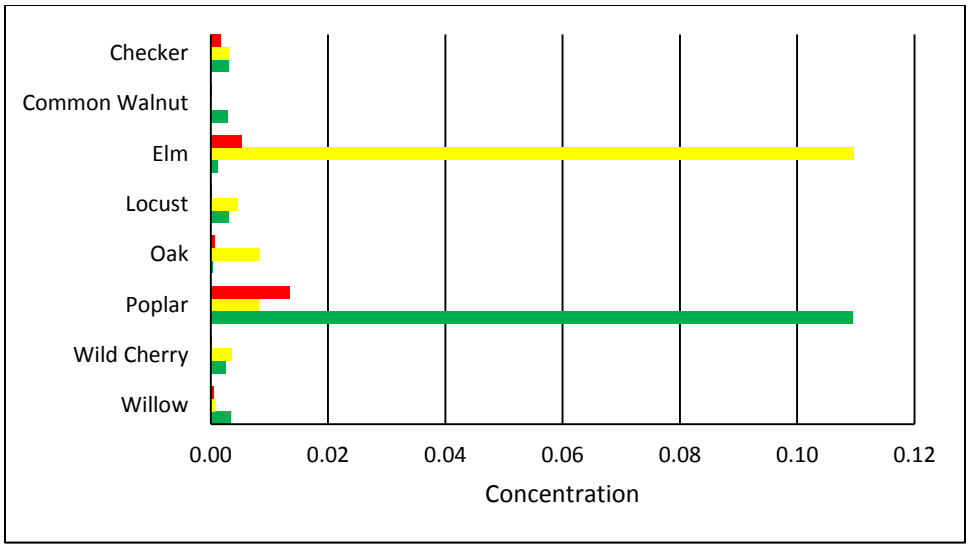

Figure 27: B2 of multi-layer tree species

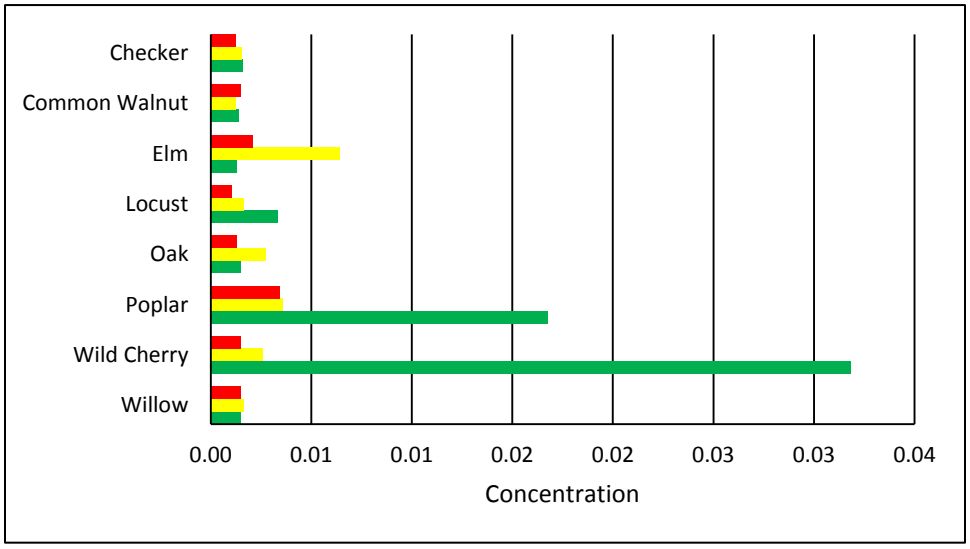

Figure 28: B3 of multi-layer tree species

Hardwood and Softwood

Overall, of the woods that were tested, softwood had higher B1 values and lower B2 and B3 values than those of hardwood. For example, through softwoods make up 20% of the samples, their B1 values make up 28.5% of the total. On the other hand, the softwood B2 and B3 values make up 8.27% and 9.57% of the total values, respectively. When comparing bark and

core samples between hard and softwood, softwoods have stronger trends. The B1, B2, and B3 values are higher for core samples 80%, 100%, and 60% of the time respectively. On the other hand, for hardwood the B1, B2, and B3 values are higher for core samples 63.6%, 54.5%, and 54.5% of the time respectively. Since the original averages were 68.8%, 68.8%, and 56.3%, it is apparent that the trend for softwoods is more distinct.

Species

Species with the highest B1 peaks include Ash, Norway Spruce, Poplar, Wild Cherry, Olive, and European Larch. Species with the lowest B1 peaks include Elm, Oak, Alder, Willow, Service, and Chestnut. Species with the highest B2 peaks include Service, Elm, Poplar, Ash, Common Beech, and Pine. Species with the lowest B2 peaks include Common Walnut, Wild Cherry, Locust, Hornbeam, Boysenberry, and Alder. Species with the highest B3 peaks include Ash, Maple, Wild Cherry, Lime, Poplar and Aleppo Pine. Species with the lowest B3 peaks include Norway Spruce, Locust, Common Beech, Checker, Common Walnut, and Elm.

When comparing bark and other exterior portions of wood, most often in contact with water, these are the values from highest to lowest.

Table 6: B1, B2, and B3 values of exterior/bark samples

Species	B1 (conc./g wood)	Species	B2 (conc./g wood)	Species	B3 (conc./g wood)
Ash	0.551	Poplar	0.110	Ash	0.0378
Poplar	0.273	Ash	0.0540	Wild Cherry	0.0318
Olive	0.240	Olive	0.0176	Lime	0.0192
European Larch	0.151	Common Beech	0.00968	Maple	0.0178
Norway Spruce	0.0985	Aspen	0.00849	Poplar	0.0168
Birch	0.0892	European Larch	0.00705	Aleppo Pine	0.00706
Lime	0.0764	Maple	0.00642	Olive	0.00700
Common Walnut	0.0621	Date Palm	0.00508	Date Palm	0.00559
Maritime Pine	0.0607	Norway Spruce	0.00386	Hornbeam	0.00547
Locust	0.0473	Lime	0.00384	Aspen	0.00545
Wild Cherry	0.0375	Maritime Pine	0.00384	Eucalyptus	0.00485

Douglas Fir	0.0344	Willow	0.00344	Maritime Pine	0.00466
Common Beech	0.0302	Locust	0.00306	Norway Spruce	0.00377
Aspen	0.0285	Checker	0.00304	Locust	0.00334
Fir	0.0220	Chestnut	0.00301	Boysenberry	0.00248
Willow	0.0200	Douglas Fir	0.00291	Chestnut	0.00235
Pine	0.0180	Common Walnut	0.00278	European Larch	0.00235
Checker	0.0174	Aleppo Pine	0.00258	Alder	0.00182
Maple	0.0156	Wild Cherry	0.00247	Douglas Fir	0.00162
Aleppo Pine	0.0152	Eucalyptus	0.00208	Checker	0.00156
Hornbeam	0.0113	Fir	0.00164	Oak	0.00151
Date Palm	0.00892	Service	0.00155	Pine	0.00151
Boysenberry	0.00759	Elm	0.00119	Willow	0.00146
Eucalyptus	0.00677	Alder	0.000889	Common Walnut	0.00141
Chestnut	0.00660	Pine	0.000734	Birch	0.00139
Service	0.00620	Birch	0.000358	Service	0.00139
Alder	0.00529	Oak	0.000326	Fir	0.00129
Oak	0.00354	Boysenberry	0.000157	Elm	0.00127
Elm	0.00213	Hornbeam	4.08E-05	Common Beech	0.00120

Discussion

The overall conclusions are that the core often had the highest concentration of B1, B2, and B3, specifically the sapwood. Softwood had generally higher B1 while hardwood had generally higher B2 and B3. From this analysis, softwoods appears to have had higher concentrations of tryptophan-like material, indicated from peak B1, while hardwoods had more fulvic and humic-like material, indicated from peak B2 and B3. This indicates that water produced around hardwood could be cleaned with traditional water treatment techniques while water around softwood may need more advanced water treatment. Furthermore, core material actually produced more of these substances than the bark which could indicate that depending on the logging and de-barking process, different water treatment techniques may be necessary. Also, some species had significantly higher concentrations of material which indicates the need to differentiate between tree species for environmental concerns.

UV Irradiation

Comparison to water

UV irradiation was tested on various samples as well as on ultra-pure water. To compare the effectiveness, the total values of the UV-visible spectrum were summed and a percent decrease was calculated. For water, the average decrease was 9.7%. This decrease can be due to degradation of plastic cuvettes or further purification from initial contaminants. For the samples, the average decrease was about 20.2%. Since this decrease was larger than the water average, it was valid to assume that the UV-irradiation has an effect on the samples.

Size of sample

Firstly, as the synchronous fluorescence spectrum varied in range, a trend was run to compare sample size and global decrease. Overall, larger sample spectrum has larger decreases. Though this trend is largely driven by very large and very small values and there is much variation from other factors, it's a possible confounding factor.

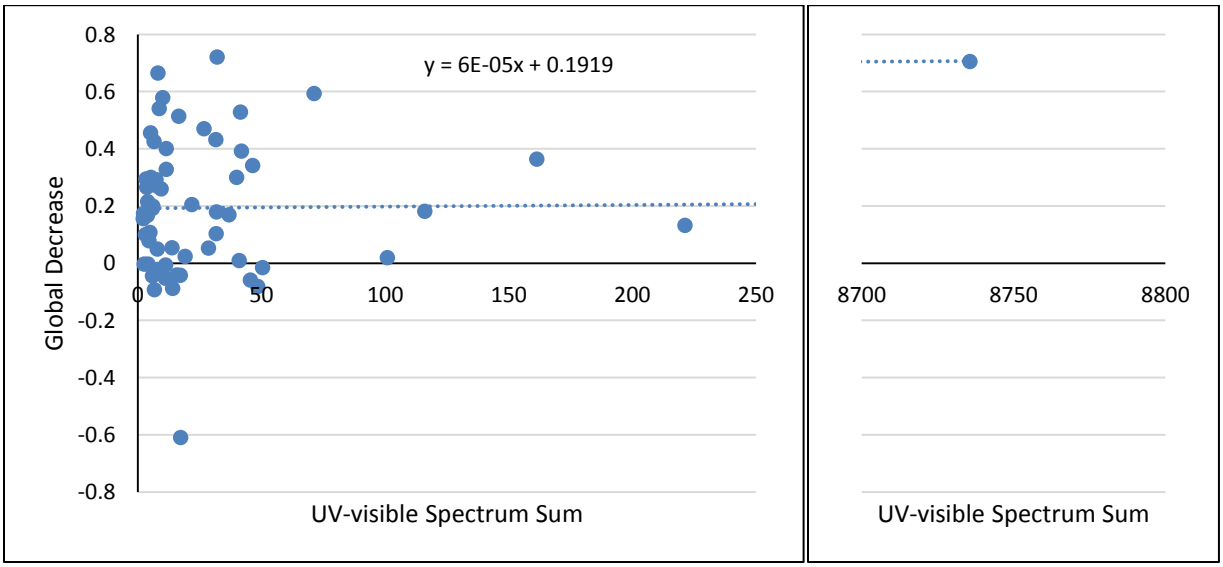

Figure 29: Global decrease from UV irradiation

Table 7: Global decrease from UV irradiation

Part	Total	Hard	Soft	Bark	All Cores	Core Only	Heartwood	Sapwood
Decrease	20.2%	23.4%	7.4%	23.8%	16.6%	19.1%	5.8%	22.6%
Mean Sum	171.94	209.3	22.5	369.86	16.00	9.71	31.82	11.99

From this, it could be predicted that bark would have the largest decrease and Core Only would have the smallest decrease. However, since that is not the case it can be assumed that trends other than size of the spectrum sum have an effect on predicting the global decrease.

Bark vs Core

When comparing all samples, the average decrease for bark was 23.8% while the average decrease for all core samples was 16.6%. When only looking at samples that had two portions, the average decrease of bark was 20.4% and the average decrease for core was 19.1%. Since many of the two portion samples are softwood, it is also possible that softwood samples have less global decrease for bark and more for core samples.

Bark, Sapwood, and Heartwood

For samples with three portions, the average decrease in bark, sapwood, and heartwood is 23.8%, 22.6% and 5.8%, respectively. The trend that bark has a larger decrease is still visible in these samples. Furthermore, the heartwood has nearly no decrease as a result of the UV irradiation.

Hardwood vs Softwood

Table 8: Global decrease from UV irradiation for hardwood vs softwood

	Total Average	Bark Average	Core
Total	19.74%	20.40%	19.08%
Softwood	1.75%	-2.04%	5.53%
Hardwood	28.74%	31.62%	25.85%

There is a significant different in the effect of UV irradiation between softwood and hardwood. With the hardwood, the same trends between bark and core are visible and the decreases are expected. With the softwood, the core and the bark do not have significant decreases, in fact the bark samples often increase. As these values are less than the global decrease average of water values, it can be assumed that any increases are negligible, potentially due to evaporation, and that UV irradiation simply has no effect on softwood samples.

Species

The species that were most susceptible to UV irradiation here Hornbeam, Ash, Maple, Wild Cherry, Elm, and Locust. The species that were the least susceptible to UV irradiation were Locust, Oak, Pine, Common Walnut, Birch and Norway Spruce. It is interesting to note that the Locust tree appears on both lists due to its significant bark decrease and its unaffected heartwood sample.

Table 9 is a comparison of the bark of each species listed from greatest to smallest global decrease.

Table 9: Global decrease from UV irradiation of exterior/bark samples

Species	Global Decrease
Hornbeam	0.720
Ash	0.705
Wild Cherry	0.593
Maple	0.578
Elm	0.541
Eucalyptus	0.514
Date Palm	0.470
Aleppo Pine	0.432
Lime	0.425
Locust	0.391
Olive	0.364
Alder	0.292
Maritime Pine	0.281
Checker	0.192

Common Beech	0.179
Chestnut	0.169
Poplar	0.132
Aspen	0.103
Willow	0.0539
Service	0.0497
Douglas Fir	0.0239
European Larch	0.0192
Common Walnut	0.00882
Oak	-0.00627
Norway Spruce	-0.0153
Boysenberry	-0.0409
Fir	-0.0420
Birch	-0.0590
Pine	-0.0878

Discussion

When investigating the specific effect of the UV irradiation, the spectra were reviewed. Trends became apparent among the samples. Peaks at 280 experienced very small decreases while the peaks from 300-400nm experienced large decreases. In Figure 30, the blue line represents the spectra before UV irradiation and the red indicates the spectra after UV exposure.

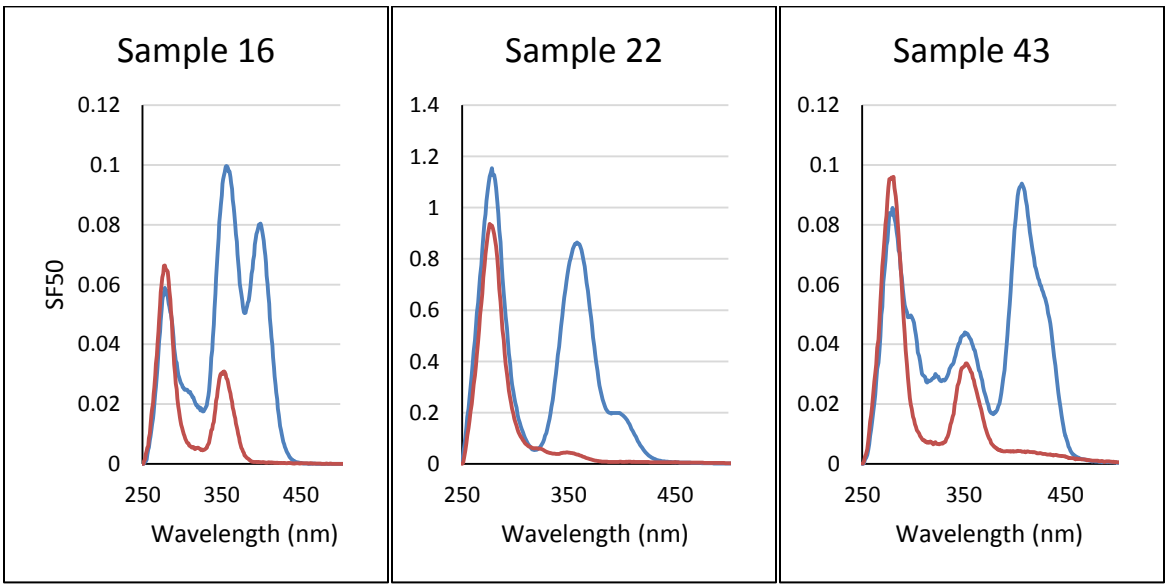

Figure 30: UV irradiation trends for samples 16, 22, and 43

When comparing to the literature, peaks in the 280nm range would indicate tryptophan-like materials while peaks from 300-400nm would indicate humic and fulvic-like materials. These spectra confirm that humic and fulvic-like are affected significantly by irradiation while tryptophan-like materials only experience marginal decreases or even increases.

As softwoods are not affected by UV irradiation as stongly as hardwood, these tests indicate that hardwoods have more humic and fulvic-like material while softwoods have more tryptophan-line material. In addition, as heartwood showed very little global decrease, it can also be assumed that heartwood is low in humic and fulvic-like material.

Total Polyphenol Content

The gallic acid calibration curve shown in Figure 31 was created to normalize the polyphenol content tests by. The linear best fit line yielded the equation of $y = 0.0072*x+0.0433$, with a resistance (R^2) value of 0.997, indicating an accurate fit. This equation, in which the y value represents absorbance at 760 nm and the x value represents gallic acid concentration in μg/mL, was used to calculate gallic acid concentrations from the absorbance value measure for each sample. Normalizing by the sample masses, gallic acid equivalents, in terms of mg GAE/g wood, were calculated for each sample to give a comparative value for phenolic content.

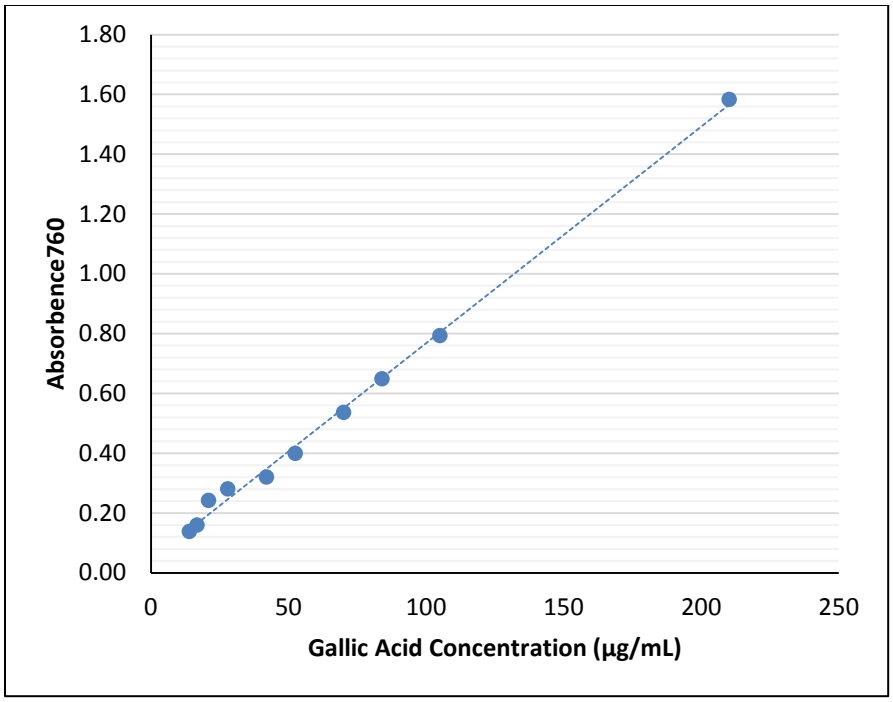

Figure 31: Gallic acid calibration curve

The polyphenolic content (mg GAE/g wood) of each sample is shown in Figure 32.

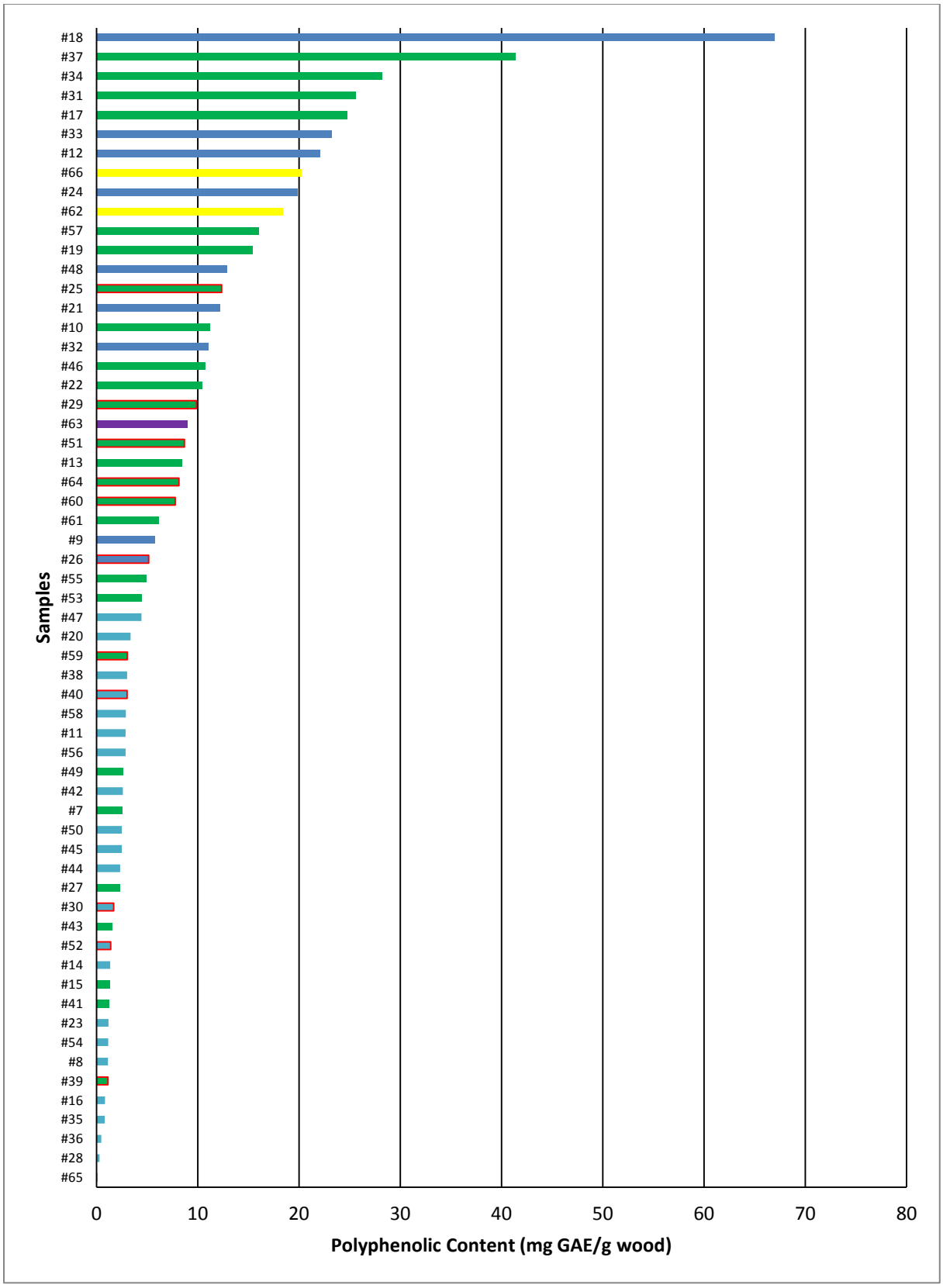

Figure 32: Overview of total polyphenolic content

Bark vs Core

When comparing the polyphenolic content of bark to core samples, bark produced a leachate with higher polyphenolic content than that of its core (including heartwood and sapwood) 71.0% of the time. When examining species with only two layers the bark produced a leachate with higher polyphenolic content than that of its core 80.0% of the time. Despite the general trend of bark leachate containing greater concentrations of polyphenolic material, the highest polyphenolic content was measured in the Chestnut core sample. The Chestnut core produced a leachate sample with polyphenolic content more than 7 times greater than the average polyphenolic content of the samples tested.

The average polyphenolic content for all samples was 9.00 mg GAE/g wood. Bark and equivalent outer wood samples produced leachate of equal or greater polyphenolic content to the average concentration 48.3% of the time. In comparison, core and inner layers produced leachate of equal or greater polyphenolic content to the average concentration only 22.6% of the time.

Bark, Sapwood, and Heartwood

When examining tree species with three layers, the heartwood produced leachate with higher polyphenolic content than that of its surrounding sapwood 87.5% of the time. On average, the polyphenolic content of heartwood leachate was about 5.0 times greater than that of its respective sapwood. The heartwood produced leachate with higher polyphenolic content than that of its respective bark 62.5% of the time. On average, the polyphenolic content of heartwood leachate was about 1.3 times greater than that of its bark.

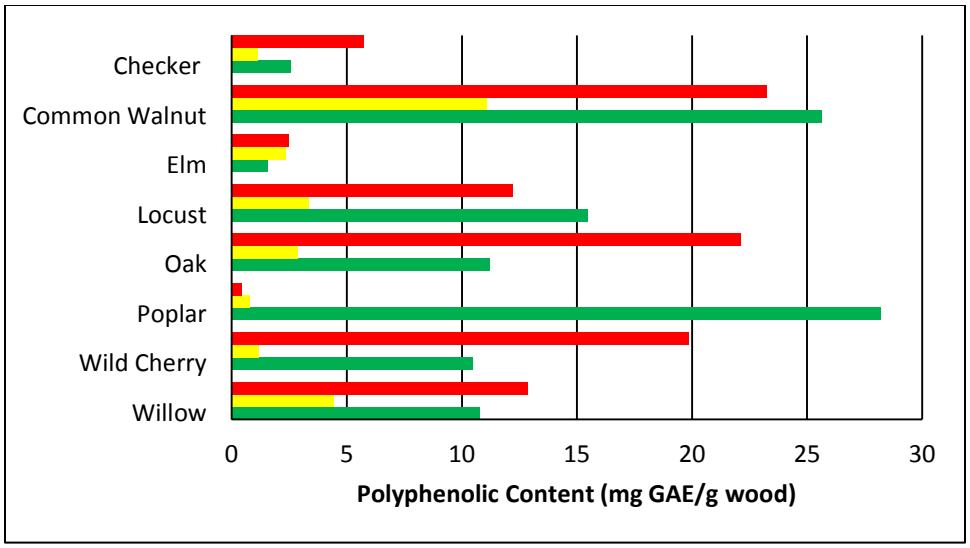

Figure 33: Total polyphenolic content of multi-layer tree species

Hardwood vs Softwood

The polyphenolic content of hard and softwood bark leachates were compared to the average polyphenolic content of all bark/exterior leachate samples (11.0 mg GAE/g wood). Softwood bark samples produced leachate with lower polyphenolic content than the average bark leachate sample 85.7% of the time. Hardwood samples generally produced leachate with higher polyphenolic content. Hardwood bark leachates contained a higher concentration of polyphenols than the average bark leachate 40.9% of the time. The average polyphenolic content of hardwood bark/exterior leachate samples was 12.2 mg GAE/g wood, significantly higher than 7.3 mg GAE/g wood, the average polyphenolic content of softwood bark samples. Additionally, the 13 samples with the highest polyphenol content, representing the top 21.7% of samples, included no softwood species.

Species

The tree species which produced the highest content of polyphenols in leachate included Chestnut, Ash, Poplar, Common Walnut, Oak, and Boysenberry. The tree species which

produced the lowest content of polyphenols in leachate included Douglas Fir, Service, Poplar, Maple, Pine, and Checker. When comparing bark and exterior wood, Table 10 provides a ranking of total polyphenolic content in leachate samples in order of highest to lowest.

Table 10: Total polyphenolic content of exterior/bark samples

Species	Total Polyphenolic Content (mg GAE/ g bark)
Ash	41.4
Poplar	28.2
Common Walnut	25.6
Chestnut	24.8
Boysenberry	20.3
Olive	18.4
Birch	16.1
Locust	15.5
Norway Spruce	12.3
Oak	11.2
Willow	10.8
Wild Cherry	10.5
European Larch	9.87
Date Palm	9.02
Fir	8.68
Common Beech	8.48
Douglas Fir	8.12
Aleppo Pine	7.77
Eucalyptus	6.18
Hornbeam	4.94
Aspen	4.49
Maritime Pine	3.04
Alder	2.68
Checker	2.58
Service	2.31
Elm	1.57
Maple	1.31
Lime	1.24
Pine	1.12

Discussion

This study revealed that hardwood samples generally leached more polyphenolic material than softwoods. In regards to layers, bark and heartwood generally leached more polyphenolic material. Previous studies of tree anatomy have proved that the polyphenols are generally stored in the bark layer of trees, perhaps accounting for the higher ability of bark samples to leach polyphenols (Samis et al., 1999). Given that the average polyphenolic content of a leachate sample from the heartwood of a multi-layer tree species was on average 1.3 times that of the bark from the same species, the heartwood and bark are considered to be comparable indicators of the toxicity of a multi-layer tree species. In order to extend this analysis to trees having just two layers, bark sample concentrations were used to assess relative tree toxicities. Although the list of polyphenolic content in bark sample leachates generally provides an accurate indication of relative species toxicities, the Chestnut tree is a strong exception, because of the large amount of polyphenolic material leached from its core sample. The Chestnut core sample produced a leachate more than 7 times greater than the average polyphenolic content of all samples tested, making it significantly toxic as a source of polyphenolic material.

Polyphenolic materials are a major concern in aquatic environments, because of the effect polyphenols have on the behavior and development of aquatic species (Samis et al., 1999; Yao et al., 2010, Wood et al., 2012). Thus, runoff from the species which leach the highest amount of polyphenolic material should be treated before release into the environment. Since bark is generally more harmful the core and sapwood tree layers, removing the bark from wood samples is also a potential way to minimize polyphenol leaching. However, the bark protects lumber from environmental sources of degradation, making this an unfavorable option in terms of lumber preservation.

Condensed Tannins Content

Vanillin Assay

Catechin Calibration

To create a calibration curve, 30 ppm catechin was run at 8 different dilutions. As the range of absorbance's only fully encompassed approximately 46.7% of the data values, extrapolation was needed. A linear relationship with the total set of data was unsuccessful as it predicted many of the concentrations as negative. As a result, two linear equations were used, one for when the absorbance was below 0.187 and one for above 0.187. These values were chosen as the catechin dilutions had two absorbances between 0.186 and 0.189, making it a safe start and end for the ranges. The blank was also used during this process to ensure that the linear equation took into account a sample with a concentration of zero. For comparison, these values were normalized and converted to mg Catechin Equivalent (CE) / g wood.

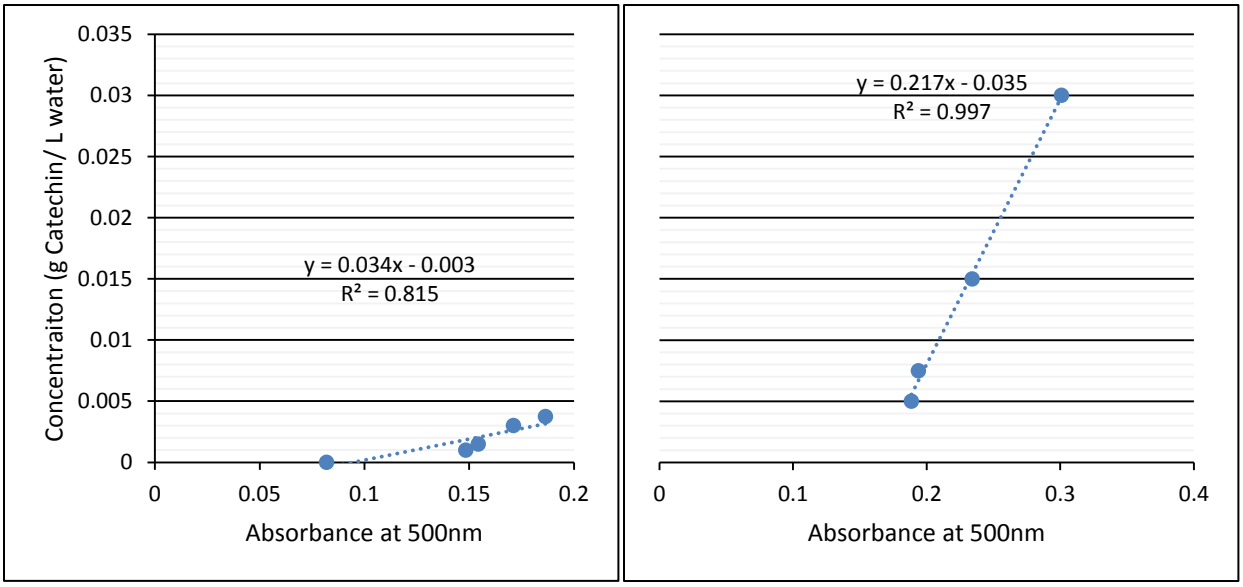

Figure 34: Catechin calibration below 0.187 (left) and above 0.187 (right)

Bark vs Core

Condensed tannins content displaying a very distinct trend with bark and core samples. 93.5% of the time, bark samples were higher than their core or sapwood/heartwood samples. In fact, although bark and exterior samples only make up 48.3% of the total samples, they make up 73.2% of the sum of mg CE/g wood. Furthermore, for samples with bark and core samples only, 100% of the samples had higher tannins content in the bark than the core.

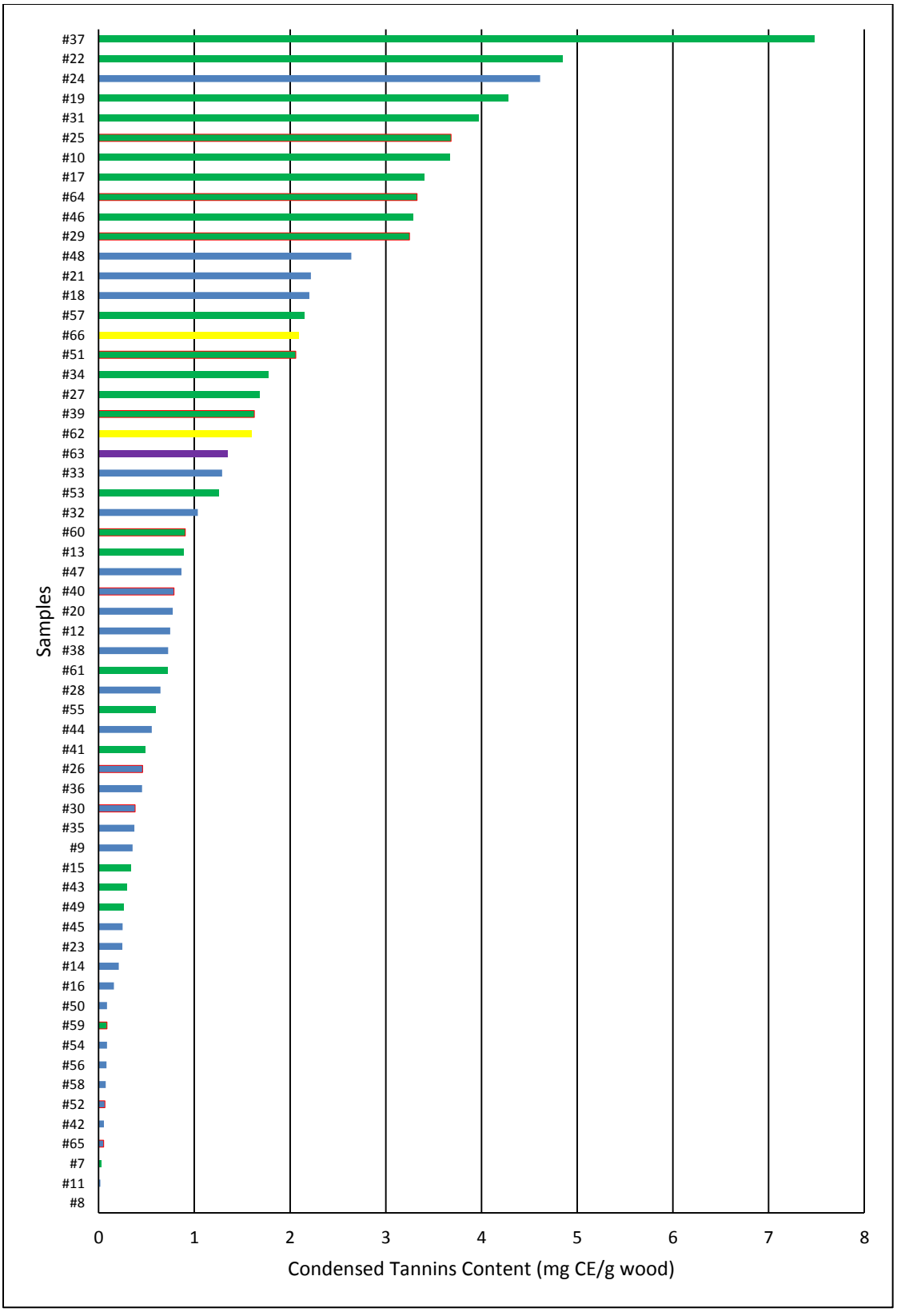

Figure 35: Overview of condensed tannins content from vanillin assay

Bark, Sapwood, and Heartwood

While bark is consistently higher than core samples regarding condensed tannins content, heartwood and sapwood also have their distinct trends. 87.5% of the time, heartwood was found to have higher condensed tannins content than sapwood.

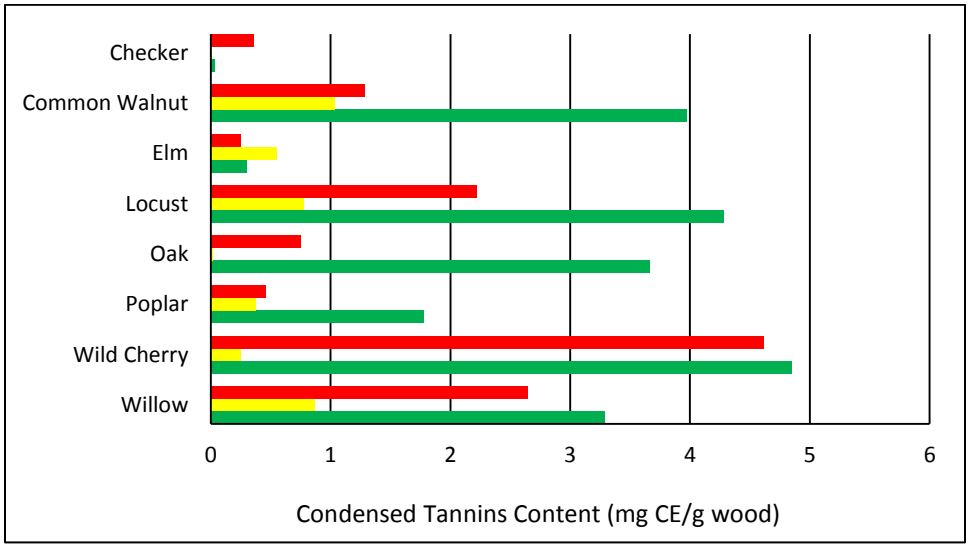

Figure 36: Vanillin assay condensed tannins content for multi-layer tree species

Hardwood vs Softwood

Condensed tannins content did not appear to differentiate between hardwood and softwood. While 20% of the total samples were softwood, their sum of mg CE/g wood were 18.6% of the total. This variation is small enough that it does not suggest a difference between softwood and hardwood tannins content.

Species

Table 11 is a comparison of the bark of each species listed from greatest to smallest condensed tannins content.

Table 11: Vanillin assay condensed tannins for exterior/bark samples

Species	mg CE/g wood
Ash	7.48
Wild Cherry	4.85
Locust	4.28
Common Walnut	3.97
Norway Spruce	3.68
Oak	3.67
Chestnut	3.41
Douglas Fir	3.33
Willow	3.29
European Larch	3.25
Birch	2.15
Boysenberry	2.09
Fir	2.06
Poplar	1.78
Service	1.69
Pine	1.63
Olive	1.60
Date Palm	1.35
Aspen	1.26
Aleppo Pine	0.907
Common Beech	0.892
Eucalyptus	0.721
Hornbeam	0.601
Lime	0.487
Maple	0.342
Elm	0.298
Alder	0.264
Maritime Pine	0.0881
Checker	0.0316

Discussion

The overarching message from the vanillin test was that bark contains a much higher condensed tannins content in comparison to core samples. As condensed tannins are a subset of

polyphenolic material, it would follow that these trends and implications would be the same. Condensed tannins are often cited as the main contributing factor to toxicity (Samis et al., 1999). They also have a very high oxygen demand, sometimes contributing to 50-60% of the COD of a leachate sample (Samis et al., 1999). Predicting condensed tannins content would be an important step at identifying industrial dangers to the natural ecosystem.

The analysis of condensed tannins content also begins to break down the makeup of the polyphenolic material. On average, the condensed tannins content makes up about 26.1% of polyphenolic material in the bark with 58.6% of the samples having less than 26.0% tannins ratio. Heartwood, which had a rather high polyphenolic material content, shows a different trend. On average, the condensed tannins content makes up about 23.3% of the polyphenolic material in the heartwood. However, 87.5% of the samples have less than 26.0% tannins ratio. Since the only tannins in heartwood are condensed tannins, this indicates another high concentration polyphenol that is currently unidentified in the heartwood (Samis et al., 1999).

Acidic Butanol Assay

Bark vs Core

As expected, there was a very strong trend between bark and core. 87.1% of the time, bark samples had higher condensed tannins content in comparison to the core or sapwood/heartwood samples. Although bark and exterior samples make up 48.3% of the total samples, they make up 71.8% of the sum of mg CyaE/g wood. Furthermore, for samples with bark and core samples only, 92.3% of the bark samples have a higher condensed tannins content.

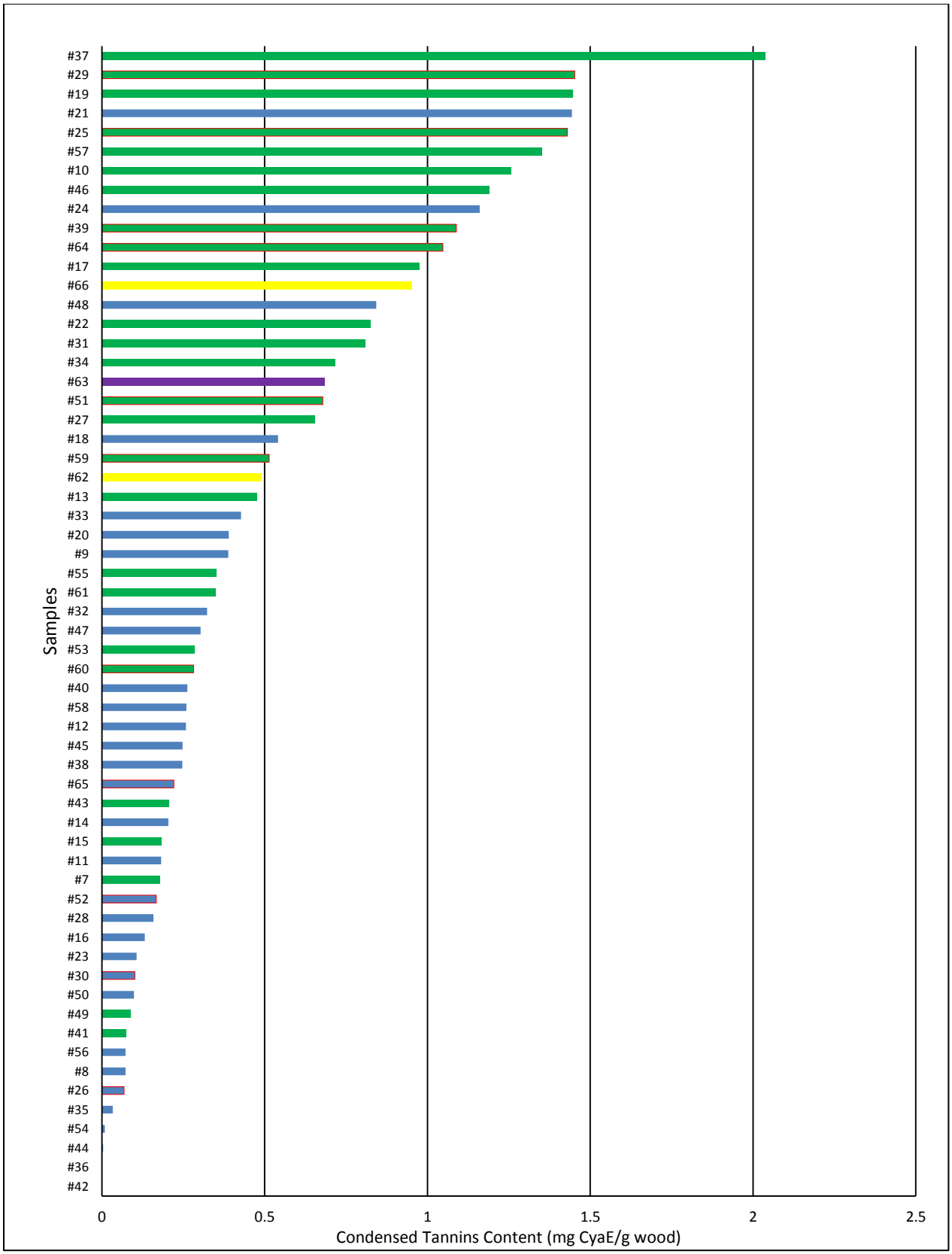

Figure 37: Overview of condensed tannins content from acidic butanol assay

Bark, Sapwood, and Heartwood

While bark is consistently higher than core samples regarding condensed tannins content, heartwood and sapwood also have their distinct trends. 87.5% of the time, heartwood was found to have higher condensed tannins content than sapwood.

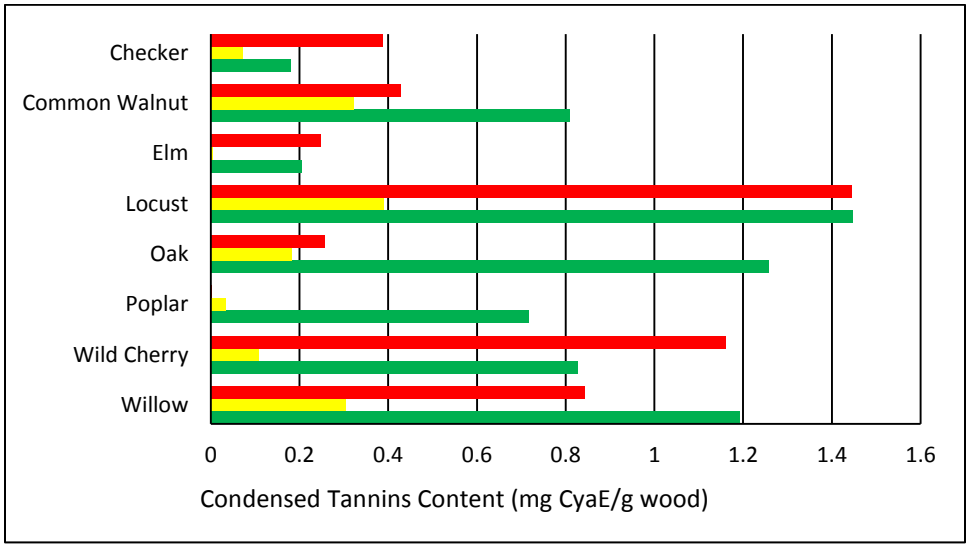

Figure 38: Acidic butanol assay condensed tannins content for multi-layer tree species

Hardwood vs Softwood

Condensed tannins content did not appear to differentiate between hardwood and softwood. While 20.0% of the total samples were softwood, their sum of mg CE/g wood were 23.8% of the total. This variation is small enough that it does not suggest a difference between softwood and hardwood tannins content.

Species

Table 12 is a comparison of the bark of each species listed from greatest to smallest condensed tannins content.

Table 12: Acidic butanol assay condensed tannins for exterior/bark samples

Species	mg CyaE/g wood
Ash	2.04
European Larch	1.45
Locust	1.45
Norway Spruce	1.43
Birch	1.35
Oak	1.26
Willow	1.19
Pine	1.09
Douglas Fir	1.05
Chestnut	0.976
Boysenberry	0.952
Wild Cherry	0.826
Common Walnut	0.809
Poplar	0.717
Date Palm	0.684
Fir	0.678
Service	0.656
Maritime Pine	0.514
Olive	0.491
Common Beech	0.478
Hornbeam	0.352
Eucalyptus	0.351
Aspen	0.285
Aleppo Pine	0.281
Elm	0.206
Maple	0.184
Checker	0.179
Alder	0.0878
Lime	0.0752

Discussion

In comparison to the vanillin assay test, the acidic butanol assay calculated a much lower concentration of condensed tannins. The concentrations were on average 2.7 times lower than those calculated in the vanillin assay test with some as much as 6.5 times lower. It is possible that the vanillin assay test created an overestimate due to its sensitivity to other monomeric flavonols (Schofield et al., 2001). On the other hand, some of the acidic butanol assay tests had negative or nearly zero values which could have meant that the test was not reacting sufficiently. Nevertheless, the trends between the samples are similar. Both tests found that bark had higher condensed tanning content than core wood and that heartwood had more condensed tannins than sapwood. Both tests also found that there were miniscule differences between softwood and hardwood.

When listing bark and exterior species from greatest to least, 17.2% of the samples were on the same place on both lists. 58.6% were at the same place or one spot above or below while 69% were at the same place of two spots above or below. This indicated that though the samples and tests varied, there was a general consistency in trends. Much more investigation would be needed to predict these trends accurately.

Error Analysis

Given that tree composition varies widely not only between trees of the same species, but within the same tree of a species, coefficients of variation of about 15-35% were expected in retesting these samples. The age and exact location of origin for each wood sample was unknown, providing two unknown variables in the tree samples. Additionally, in preparing the leachate samples, sources of potential error include cross-contamination from wood cutting tools and surfaces. In some species the boundary between the tree layers was irregular or undefined so there may have been some cross-contamination between consecutive tree layers. As the dry samples were prepared, an effort was made to test samples the same number of days after cutting up the wood pieces. However, some samples were prepared earlier than others, which may have affected moisture content in the wood pieces weighed. Small growths of mold were also observed on the last set of samples run. An effort was made to remove these growths before weighing samples, but some contamination may have persisted in samples.

When weighing out dry samples, the scale used was a potential source of error as any number in the hundredths place or smaller fluctuated greatly. The scale was most significant as an error source when measuring small sample sizes (less than one milligram) in analytical tests. All other machinery and lab equipment used appeared to be in good working condition so the standard amount of error from calibrations was assumed to be negligible.

To perform an error analysis on the various tests used in this research, six samples of Maritime Pine bark were prepared by the same procedure and incubated for just 24 hours before testing with all analytical tests, except for the chemical oxygen demand test. Error analysis was then performed on the results from this set of samples using standard deviation and the

coefficient of variation. The layers of four tree species were also retested for DOC and TDN. Sources of error specific to each test are discussed in the following sections.

Dissolved Organic Carbon and Total Dissolved Nitrogen

In order to determine the variability and error of these experiments, layers of four tree species (Ash, Checker, Locust, and Maple) were retested for DOC and TDN. Additionally, six samples of Maritime Pine bark were prepared and incubated for just 24 hours before testing in order to calculate variation over a larger number of samples. The standard deviation and coefficient of variation for each sample was calculated for each test performed. The results are given in Table 13.

Table 13: Error in DOC and TDN tests

Species	Layer	Standard Deviation of DOC (mg C/g sample)	Coefficient of Variation (%)	Standard Deviation of TDN (mg N/g sample)	Coefficient of Variation (%)
Ash	Bark	5.56	99.8	0.0062	90.2
Checker	Bark	3.11	73.9	0.0158	38.9
Checker	Sapwood	0.02	05.0	0.0005	04.3
Checker	Heartwood	0.44	35.1	0.0005	03.4
Locust	Bark	0.44	05.4	0.1136	28.1
Locust	Sapwood	0.17	04.7	0.0829	29.1
Locust	Heartwood	0.30	07.0	0.0055	21.5
Maple	Bark	0.50	17.9	0.0094	21.7
Maple	Core	0.02	03.7	0.0114	38.3
Maritime Pine	Bark	0.19	16.7	0.0035	15.1

All samples from the Locust tree tested within the acceptable range for the coefficient of variation for both DOC and TDN. Both the sapwood and heartwood of the Checker tree and the bark of the Maple tree also tested within the acceptable range of variation for DOC and TDN. The Maple tree core tested within the acceptable range of variation for DOC, but with a slightly higher variation in TDN. Based on the results from the DOC test and the fact that the Maple bark samples still produced leachate with a higher concentration of both DOC and TDN than that of

the core samples, the results are still considered acceptable since tree composition is expected to vary widely. The Checker tree bark sample tested with a high level of variation for DOC and a slightly higher than acceptable level of variation for TDN. This error may be attributed to the previously discussed sources of error.

The Ash tree bark sample displayed the greatest coefficient of variation in both DOC and TDN. This variation was expected because of the odd coloration of the leachate samples from the Ash tree in the first set of tests. The Ash tree bark sample did not produce a leachate darker than the Ash tree heartwood in the first set, but did in the second set. Therefore, it is supposed that there was a contaminant or mistake made with preparing the first Ash tree bark sample.

The six Maritime Pine samples experienced a coefficient of variation of about 15-17% for both the DOC and TDN tests. This suggests that over a large set of samples, both the DOC and TDN tests are reliable and reproducible with an assumed source of error based on the nature of trees.

Chemical Oxygen Demand

Due to time constraints, it was not possible to run additional tests for COD so a comprehensive error analysis is not feasible. However, based on the correlation of the COD data with DOC data, the error in COD data is assumed to be similar to the error in DOC data. Several possible sources of error in the COD test include machine error and error in preparing the acid solution and loading the vials. However, machine error is assumed to be negligible compared to the error that results simply from the variability of tree samples. When preparing the vials for the COD test, the acid solution was delivered to cleaned vials in two parts from a prescribed recipe. Typically, COD test vials are industrially prepared with the acid solution recipe loaded into a

fresh vial. Thus, the acid solution used was not up to industrial standards and may have presented a source of error.

UV-Visible Spectroscopy and Gauss Identification

SUVA and Gauss identification tests had many sources of error including multiple tests, fluctuating software, and creating subjective ranges of good fit. Overall, the coefficient of variation for the SUVA values was fairly low, ranging from 8% to 13%. The UV absorbance was determined by identifying peaks visually and with slope changes. The SUVA values took those absorbances and applied DOC, dilution factor, and sample weight. While the coefficient of variation is very good, it is also noted that in sample #1, there was a peak missing at 340nm. Since this sample had the highest DOC of the six, it is possible that important peaks might be hidden with samples of higher carbon content. On the other hand, the data values above 340nm were so small that changes and peaks were negligible. This test was limited to only looking at peaks below around 350nm.

Table 14: Error in UV-visible spectroscopy and Gauss identification tests

	UV Absorbance at ~280	UV Absorbance at ~340	SUVA280/g wood	SUVA340/g wood	B1 (conc./g wood)	B2 (conc./g wood)	B3 (conc./g wood)
#1	0.607		0.240		0.115	0.0184	0.00160
#2	0.580	0.250	0.320	0.138	0.219	0.0340	0.00775
#3	0.536	0.213	0.306	0.121	0.209	0.0327	0.00638
#4	0.660	0.273	0.322	0.134	0.207	0.0322	0.0186
#5	0.639	0.280	0.370	0.162	0.259	0.0214	0.0170
#6	0.520	0.231	0.329	0.146	0.191	0.00857	0.0102
STDEV	0.0508	0.0253	0.0387	0.0134	0.0434	0.00927	0.00593
Avg	0.590	0.250	0.314	0.140	0.200	0.0245	0.0102
CV	8.6%	10.1%	12.3%	9.6%	21.7%	37.8%	57.9%

For Gauss identification, equations are set to model the spectrum using software that aimed to minimize the distance between the data and the modelled spectrum. Three to five

fluorophores were identified at various wavelengths for each sample. With the synchronous fluorescence spectroscopy already set with a wavelength different of 50 nm, available literature limited the analysis to looking for specific identifying wavelengths. For each of those characteristic wavelengths from the literature, a range was needed to capture data from every sample. In order to capture B1, B2, and B3 values from 99.5% of the samples, ranges of 7 nm, 20 nm, and 30 nm were created. The larger coefficient of variation in B3 and B2 could be due to capturing different specific fluorophores. However, all fluorophores in those ranges should have similar characteristics in terms of water treatment or chemical reactivity.

UV Irradiation

When looking at the sample tests, the effect of UV irradiation appeared to be reproducible. The coefficient of variability depends on how the data is presented. If the values are presented as a decrease percentage, the values are smaller making the coefficient of variability larger. With the data from samples #1-6, both ways have coefficients of variability comfortably below 15%. UV irradiation was also run on ultra-pure water. This test was also consistent as the coefficients of variability are below the 35% max.

Table 15: Error in UV irradiation tests

	% Decrease	% of Original	Water % Decrease	Water % of Original
#1	0.282	0.718	0.127	0.873
#2	0.213	0.787	0.0716	0.928
#3	0.291	0.709	0.0905	0.909
#4	0.257	0.743	-	-
#5	0.279	0.721	-	-
#6	0.202	0.798	-	-
STDEV	0.0346	0.0346	0.0229	0.0229
Avg	0.254	0.746	0.0963	0.904
CV	13.6%	4.6%	23.8%	2.5%

Some of the possible sources of error include evaporation and time. As these tests lasted 24 hours, the volume in the cuvette dropped during the test. The evaporation could have changed the concentration of material in the water. Furthermore, some of the samples were tested immediately after the UV light was turned off while others sat for upwards of four hours. This could affect the comparability of tests from different sets and days.

Total Polyphenol Content and Condensed Tannins Content

The three analytical tests for polyphenols and condensed tannin content were fairly accurate. The first step in each of these tests was determining an absorbance. When the coefficient of variation (CV) was calculated for the absorbance at their respective wavelength, each of the values was below 25% as seen in the table below. The next step in each of these tests was to use an equation or calibration curve to create contextual values. For the Acidic Butanol Assay test, an equation was used and therefore the coefficient of variation had very little change. The coefficient of variation for the other two tests, using a gallic acid and catechin calibration, increased by a factor of 13 and 6 respectively. This demonstrates a larger source of error in the calibration than the UV absorbance.

Table 16: Error in analytical methods

	Folin-Ciocalteu (Absorbance at 760)	Total Polyphenol Content (mg GAE/g wood)	Vanillin Assay (Absorbance at 500)	Condensed Tannins (mg CE/g wood)	Acidic Butanol Assay (Absorbance at 530)	Condensed Tannins (mg CyaE/g wood)	Combined Tannins Tests
#1	0.0331	-0.426	0.126	0.0322	0.0392	0.357	
#2	0.0435	0.00828	0.114	0.0201	0.0293	0.265	
#3	0.0545	0.465	0.115	0.0209	0.0270	0.245	
#4	0.0541	0.449	0.111	0.0167	0.0201	0.183	
#5	0.0452	0.0792	0.108	0.0143	0.0370	0.337	
#6	0.0507	0.307	0.109	0.0151	0.0246	0.223	
STDEV	0.00740	0.308	0.00584	0.00602	0.00670	0.0613	0.132
Avg	0.0468	0.147	0.114	0.0199	0.0295	0.268	0.144
CV	15.8%	209.4%	5.1%	30.3%	22.7%	22.9%	91.3%

There were various sources of error when making the calibration curves. While the gallic acid used for the calibration was from the same initial sample, the samples were taken over a 10 day period. The color of the parent solution appeared to have changed by the time it was discarded meaning that the solutions used for each dilution could have been chemically different. The last samples to be run were the most diluted, presenting an unreliability for lower concentration samples. Samples #1-6 had lower concentrations than the majority of the samples due to their shorter incubation period. Therefore when the calibration is applied, it would be expected to calculate a large coefficient of variation.

When the catechin calibrations were run, the absorbance range was from .15 to .30. In sampled #1-6, the average absorbance was .11 while the overall average absorbance was 0.37. In order to maximize accuracy, the calibration was split into two linear equations with a coefficient of determination value of at least 0.80. As a result, this catechin equivalent values required extrapolation from the calibration data.

It should also be noted that both the catechin equivalents and cyanidine equivalents should be the same value. However, when the assumption that both tests are of the same sample, the coefficient of variation is 0.91. The tests use reactions with different reaction paths. The vanillin test is noted for being unreliable as it reacts with other monomeric flavonols. On the other hand, there is very little information about how other polyphenol groups react with the acidic butanol assay. Furthermore the acidic butanol test required a 95°C water bath. While the test was running, it was very hard to maintain the high temperature water bath. This could have reduced the reaction completion resulting in the lower condensed tannins content estimate.

Conclusions and Recommendations

Reliability of Tests

Overall, the tests performed in this study are considered to be reliable with the basis that trees are natural organisms and therefore highly variable. Based on time and resource constraints, this research was not able to run all tests in duplicate to ensure total accuracy. Additionally, there were sources of variation in the wood samples selected, such as age, location of origin, and moisture content, that were not able to be controlled during testing. It is recommended that these tests be repeated in duplicate with additional controls for these variables.

Toxicity of Trees by Layer

As confirmed by this research, tree bark produces leachate with higher concentrations of organic carbon, nitrogen, and polyphenols, particularly condensed tannins, than the inner layers of trees. Dissolved organic carbon testing showed that tree bark produced leachate containing up to 15 times more carbon than that of the leachate produced by its core. Similarly total dissolved nitrogen testing showed that tree bark from some species produced leachate containing up to 6 times more nitrogen than that of the leachate produced by its core. This indicates that bark has higher mass transfer of organic carbon, nitrogen, polyphenols, and condensed tannins into water than inner layers. One strong exception was the Chestnut tree core which produced leachate with the highest total polyphenolic content of all tree species tested. As neither the vanillin assay nor the acidic butanol assay indicated that the Chestnut core leached an extremely high content of condensed tannins, these polyphenols were most likely hydrolysable tannins or another source of polyphenolic material, of which there is little known.

For the constituents of tryptophan-like, humic-like, and fulvic-like materials, there was no strong trend in concentration when comparing bark and core leachate samples. Gauss identification analysis indicated that core samples may leach tryptophan-like and fulvic-like materials more readily than bark, but that there is essentially no difference in the concentration of humic-like materials when comparing bark and core leachate samples. UV-visible spectroscopy testing also confirmed the lack of significant difference in the concentration of humic-like materials between bark and core leachate samples.

When examining the inner layers of trees, heartwood generally leached more organic carbon, fulvic-like material, humic-like material, and polyphenols, especially condensed tannins, than sapwood. Both UV-visible spectroscopy and Gauss identification analysis supported the conclusion that heartwood leaches more humic-like material than sapwood. Gauss identification analysis showed no trend when comparing heartwood and sapwood for tryptophan-like material leaching. Thus, more testing by would be necessary to confirm a trend in tryptophan-like material leaching between heartwood and softwood. According to total dissolved nitrogen testing, heartwoods and sapwoods yielded leachate of similar nitrogen concentrations.

While heartwood generally leached the water toxins tested more readily than sapwood, the ability of bark to leach most materials more readily than either heartwood or sapwood is a greater concern, because of its exposure to the environment. In lumber yards or other forms of wood storage, tree samples are generally stored as whole logs with the bark intact. Thus, the bark would experience the most exposure to the environmental conditions and provide the most prevalent source of water toxins.

Toxicity of Trees by Hardwoods vs Softwoods

When comparing the toxin leaching ability of hardwoods vs softwoods, hardwood tree species appeared to transfer fulvic-like and humic-like material, as well as some polyphenols, to water more readily than softwood species. According to UV-visible spectroscopy, hardwoods leach more humic-like materials than softwoods. Analysis by Gauss identification showed that softwoods leached tryptophan-like material more readily than hardwoods, while confirming that hardwoods leach more humic-like materials as well as fulvic-like materials.

While hardwoods generally leached polyphenols more readily than softwoods, condensed tannin content testing by both the vanillin assay and acidic butanol assay methods revealed no trend between softwoods and hardwoods. This indicates that hardwoods probably leach hydrolysable tannins or other polyphenolic materials rather than condensed tannins. An analysis of the polyphenolic content in leachate samples would be necessary to confirm this.

Both hardwood and softwood tree species leached significant amounts of organic carbon and nitrogen though dissolved organic carbon and total dissolved nitrogen testing, respectively, showed no significant trend between hardwood and softwood species. Chemical oxygen demand testing revealed that softwoods may have a higher chemical oxygen demand than hardwoods, indicating a higher content of organic carbon in softwood leachate. Based on the limited number of samples which were analyzed by chemical oxygen demand testing, further research would be necessary to confirm or deny this trend.

Toxicity of Trees by Species

Several tree species appeared to be significantly more toxic than others based on their ability to leach several water toxins more readily than the other tree species tested. The Ash bark sample tested produced leachate with high concentrations of tryptophan-like material, humic-like

material, fulvic-like material, polyphenols, and condensed tannins. During chemical oxygen demand testing, the Ash bark leachate also had a COD value which surpassed the DR/2400 Portable Spectrophotometer's ability to measure the sample, indicating a high concentration of dissolved organic matter.

The Olive tree branch sample tested yielded leachate with the highest concentration of both organic carbon and nitrogen. Poplar bark leachate also contained a high organic carbon concentration in addition to a high concentration of tryptophan-like material, fulvic-like material, and polyphenols. Chestnut was another significant species in that its core sample produced the highest concentration of polyphenolic material in any leachate tested. Interestingly, the bark of the Chestnut tree did not yield significantly high concentrations of any other materials in its leachate. This means that the Chestnut tree's toxicity to waterways may be reduced simply by maintaining the bark on Chestnut lumber. This solution appears to be unique to Chestnut alone, as the bark for every other tree species generally produced more toxic leachate than that of its inner layers.

Environmental Conditions and Considerations

UV irradiation analysis provided a method to examine the potential effect of sunlight on leachate samples, specifically the concentration of tryptophan-like, humic-like, and fulvic-like materials. Generally, the UV irradiation performed had the strongest impact on humic and fulvic-like materials, but little affect to tryptophan-like materials. The UV irradiation analysis also indicated that sunlight would probably degrade these materials in bark leachate more easily than in core leachate. Sapwood leachate also experienced a strong effect from UV irradiation, but the heartwood experienced almost no effect. Once of the species which was most strongly impacted by UV irradiation was the Ash tree bark, which was also produced one of the most toxic

leachates. Thus, exposure to sunlight may be a good way to reduce the concentration of some materials, especially humic and fulvic-like materials, in leachate from Ash and other select trees.

The results of the UV irradiation test are limited in indicating the effect of sunlight as this test was analyzed at just one wavelength. Sunlight consists of varying wavelengths, which would have varying effects on leachate samples. Additionally, the UV irradiation test only accounts for sunlight exposure after leaching has occurred, rather than during the leaching process. It is recommended that more tests be run to examine the effect of a range of UV light as well as the effect of UV light before, after, and during the leaching process to provide a clearer vision of how sunlight impacts tree leachates.

Similarly, the leachate preparation process used in this study could be varied to examine a range of environmental conditions. Leachate samples could be prepared with varying ratios of wood and water to imitate different amounts of water exposure in the environment. The method of water exposure could be varied as water sprinkling or still water to imitate rain and water spraying or standing water in the environment. It is recommended that more tests be run, applying a variety of leachate production methods, to examine the impact of various environmental conditions on leachate toxicities.

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Appendix

Appendix I: Overview UV, DOC, TDN, COD

#	Weight (g)	Water (mL)	Dilution	UV Absorbance at ~280	UV Absorbance at ~340	DOC (mgC/L)	TDN (mgN/L)	COD (mg O2/g wood)
1	4.9902	150	1	0.6067	N/A	50.76	0.8077	N/A
2	5.0321	150	1	0.5794	0.2503	35.94	0.6575	N/A
3	5.0157	150	1	0.5359	0.2128	34.93	0.7476	N/A
4	5.006	150	1	0.6592	0.2733	40.86	0.9149	N/A
5	4.9968	150	1	0.6392	0.2803	34.65	0.8919	N/A
6	5.0166	150	1	0.5198	0.231	31.5	0.5997	N/A
7	4.9994	150	1	0.5393	0.145	36.55	0.8238	N/A
8	5.0363	150	1	0.156	0.0708	11.38	0.4096	N/A
9	4.98	150	10	0.0994	0.0456	56.38	0.4682	N/A
10	4.9567	150	20	0.4804	0.2049	313.5	1.547	25.37
11	5.0205	150	1	0.8163	0.3568	38.23	0.5948	N/A
12	5.0972	150	10	0.4223	0.1423	127.8	0.4867	N/A
13	5.0731	150	10	0.2015	0.0748	110.6	1.012	N/A
14	5.0968	150	1	0.3358	0.1283	21.81	0.4228	N/A
15	5.0234	150	1	0.9208	0.3897	76.17	1.132	N/A
16	4.9976	150	1	0.2474	0.1644	19.63	0.6128	N/A
17	5.0908	150	20	0.8252	0.3692	525.8	2.492	37.25
18	4.917	150	20	1.0024	0.1758	495.9	1.04	32.23
19	4.953	150	10	0.577	0.206	251.4	9.587	24.25
20	5.0459	150	10	0.2095	0.0915	125.2	6.784	N/A
21	4.9503	150	10	0.6503	0.2551	149.7	0.6628	11.91
22	5.0471	150	10	0.9405	0.4326	340.6	1.785	29.94
23	5.9698	150	1	0.7758	0.379	78.81	0.4607	N/A
24	4.9556	150	10	0.4604	0.1885	164.6	0.347	12.77
25	4.9636	150	10	0.5045	0.3312	339.4	1.015	30.07
26	5.0193	150	1	0.846	0.1546	56.46	0.3998	N/A
27	4.9525	150	10	0.257	0.128	306.5	0.614	24.49
28	5.0653	150	1	0.175	0.103	22.21	0.3113	N/A
29	4.9681	150	10	0.73	0.167	463.4	1.587	35.90
30	4.94	150	1	0.825	0.504	121.4	0.3499	N/A
31	4.911	150	10	1.4673	0.6302	285.6	0.8276	24.34
32	5.0439	150	10	0.2829	0.0994	105.7	2.119	N/A
33	5.035	150	10	0.7326	0.1472	138.1	0.4988	N/A
34	5.06	150	20	0.7317	0.361	802.3	1.345	41.95

35	4.9945	150	1	0.4693	0.2806	31.72	0.5935	N/A
36	5.0568	150	1	0.5208	0.3058	57.46	0.3807	N/A
37	5.0648	150	40	0.9021	0.8561	375.6	0.4406	181.36
38	5.0045	150	10	0.1264	0.127	94.36	0.3426	N/A
39	5.0895	150	10	0.1285	0.0693	347.8	2.665	28.30
40	5.0779	150	1	0.7748	0.296	189.3	0.6479	13.89
41	5.023	150	1	0.6994	0.3484	59.86	0.6209	N/A
42	5.0142	150	1	0.3813	0.3437	28.76	0.4047	N/A
43	5.8076	150	10	0.167	0.0787	83.18	0.8348	N/A
44	5.0777	150	1	0.4072	0.2472	27.35	0.2991	N/A
45	4.9872	150	10	0.1036	0.0761	56.92	0.4365	N/A
46	5.0321	150	10	0.612	0.224	213.2	1.192	16.39
47	4.9947	150	10	0.189	0.096	64.66	0.3577	N/A
48	4.9787	150	10	0.469	0.152	147.4	0.3876	N/A
49	4.9753	150	10	0.164	0.107	88.1	1.85	N/A
50	5.0762	150	1	0.503	0.13	29.51	0.2819	N/A
51	5.0568	150	10	0.361	0.125	180.5	0.8626	14.86
52	4.932	150	1	0.26	0.132	18.55	0.4471	N/A
53	5.032	150	10	0.415	0.206	309.9	1.808	27.28
54	5.04	150	1	0.567	0.305	33.21	0.47	N/A
55	4.9485	150	10	0.6001	0.3282	206.4	2.766	17.66
56	4.9408	150	1	0.7569	0.3203	27.53	1.91	N/A
57	5.014	150	10	0.3249	0.0883	228.4	0.572	17.40
58	4.937	150	1	0.3249	0.0883	29.46	0.2791	N/A
59	4.9475	150	1	0.6372	0.2322	46.58	0.3977	N/A
60	4.978	150	10	0.5576	0.234	196.4	11.96	19.87
61	4.995	150	10	0.4898	0.2475	154.5	2.155	12.90
62	5.0288	150	10	0.6572	0.2675	929.4	20.67	42.90
63	5.0566	150	10	0.57	0.2972	200.4	4.037	18.71
64	4.974	150	10	0.3007	0.1252	255.8	0.8202	N/A
65	4.9148	150	1	0.285	0.1521	14.88	0.4939	N/A
66	5.0412	150	20	0.543	0.283	272.4	1.54	N/A
67	5.9585	150	10	N/A	N/A	242.2	1.859	N/A
68	4.9833	150	1	N/A	N/A	10.18	0.3722	N/A
69	4.9268	150	1	N/A	N/A	26.78	0.4963	N/A
70	1.9792	60	20	N/A	N/A	279.9	17.07	N/A
71	2.1058	60	10	N/A	N/A	118.8	12.9	N/A
72	1.952	60	10	N/A	N/A	128.3	1.012	N/A
73	4.9781	150	10	N/A	N/A	108.5	1.745	N/A
74	4.9088	150	1	N/A	N/A	20.76	1.348	N/A
75	5.0716	150	20	N/A	N/A	0.4215	0.0228	N/A

Duplicate samples (#67 through 75) were only analyzed for a few tests to determine reproducibility.

Appendix II: Overview SUVA, Gauss, UV Irradiation

#	SUVA280/g wood	SUVA340/g wood	Gauss B1 (conc./g wood)	Gauss B2 (conc./g wood)	Gauss B3 (conc./g wood)	UV Irradiation (Global Decrease)
1	0.240	N/A	0.11483	0.01844	0.00160	0.718
2	0.320	0.138	0.21880	0.03398	0.00775	0.787
3	0.306	0.121	0.20894	0.03270	0.00638	0.709
4	0.322	0.134	0.20655	0.03216	0.01858	0.743
5	0.369	0.162	0.25917	0.02141	0.01701	0.721
6	0.329	0.146	0.19057	0.00857	0.01017	0.798
7	0.295	0.079	0.08681	0.01520	0.00780	0.809
8	0.272	0.124	0.08617	0.01509	0.00774	0.826
9	0.354	0.162	0.06084	0.00823	0.00602	0.740
10	0.618	0.264	0.01755	0.00161	0.00746	1.006
11	0.425	0.186	0.10935	0.04103	0.01374	0.700
12	0.648	0.218	0.03198	0.00334	0.00667	1.092
13	0.359	0.133	0.15296	0.04908	0.00611	0.821
14	0.302	0.115	0.50267	0.13204	0.01334	0.729
15	0.241	0.102	0.07823	0.03225	0.08918	0.422
16	0.252	0.168	0.15768	0.05443	0.17749	0.335
17	0.617	0.276	0.03359	0.01532	0.01198	0.831
18	0.822	0.144	0.05430	0.00793	0.00834	0.947
19	0.463	0.165	0.23420	0.01514	0.01656	0.609
20	0.332	0.145	0.14229	0.02358	0.00813	0.472
21	0.878	0.344	0.13535	0.00020	0.00525	1.609
22	0.547	0.252	0.18902	0.01248	0.16049	0.407
23	0.165	0.081	0.30035	0.02144	0.01541	1.004
24	0.564	0.231	1.22205	0.00020	0.00747	0.818
25	0.299	0.197	0.48916	0.01914	0.01874	1.015
26	0.299	0.055	2.05128	0.02729	0.00020	1.052
27	0.169	0.084	0.03069	0.00767	0.00687	0.950
28	0.156	0.092	0.07778	1.92447	0.00928	0.844
29	0.317	0.073	0.75260	0.03502	0.01167	0.981
30	0.138	0.084	0.94879	0.07955	0.00749	1.022
31	1.046	0.449	0.30503	0.01364	0.00692	0.991
32	0.531	0.186	0.17149	0.00059	0.00615	0.795
33	1.054	0.212	0.47428	0.00020	0.00735	1.082
34	0.360	0.178	1.38182	0.55435	0.08478	0.868
35	0.296	0.177	0.20182	0.04064	0.01782	0.921
36	0.179	0.105	0.17363	0.06783	0.01740	0.892

37	1.897	1.800	2.79182	0.27346	0.19152	0.295
38	0.268	0.269	0.32551	0.04516	0.02857	0.658
39	0.073	0.039	0.09156	0.00373	0.00766	1.088
40	0.081	0.031	0.38421	0.11816	0.01201	1.044
41	0.233	0.116	0.38383	0.01931	0.09656	0.575
42	0.264	0.238	0.21100	0.00279	0.07319	0.544
43	0.346	0.163	0.01240	0.00689	0.00740	0.459
44	0.293	0.178	0.53115	0.55675	0.03250	0.672
45	0.365	0.268	0.03950	0.02607	0.01043	0.599
46	0.570	0.209	0.10075	0.01729	0.00735	0.946
47	0.585	0.297	0.03063	0.00300	0.00801	0.801
48	0.639	0.207	0.36757	0.00221	0.00743	0.700
49	0.374	0.244	0.02633	0.00442	0.00904	0.708
50	0.336	0.087	0.46334	0.00098	0.01438	1.004
51	0.396	0.137	0.11114	0.00831	0.00653	1.042
52	0.284	0.144	0.11821	0.02048	0.01115	0.705
53	0.266	0.132	0.14348	0.04273	0.02742	0.897
54	0.339	0.182	0.21627	0.05377	0.02083	0.834
55	0.588	0.321	0.05577	0.00020	0.02708	0.280
56	0.556	0.235	0.21697	0.02712	0.01397	0.734
57	0.284	0.077	0.44715	0.00179	0.00698	1.059
58	0.223	0.061	0.24468	0.03018	0.02674	0.785
59	0.276	0.101	0.30015	0.01900	0.02304	0.719
60	0.570	0.239	0.07573	0.01286	0.03515	0.568
61	0.635	0.321	0.03383	0.01041	0.02422	0.486
62	0.141	0.057	1.20585	0.08849	0.03520	0.636
63	0.562	0.293	0.04509	0.02571	0.02828	0.530
64	0.236	0.098	0.17089	0.01448	0.00804	0.976
65	0.390	0.208	0.15911	0.02442	0.00834	0.900
66	0.791	0.412	0.03828	0.00079	0.01250	1.041
67	N/A	N/A	N/A	N/A	N/A	0.874
68	N/A	N/A	N/A	N/A	N/A	0.806
69	N/A	N/A	N/A	N/A	N/A	0.813
70	N/A	N/A	N/A	N/A	N/A	0.547
71	N/A	N/A	N/A	N/A	N/A	0.297
72	N/A	N/A	N/A	N/A	N/A	1.253
73	N/A	N/A	N/A	N/A	N/A	0.659
74	N/A	N/A	N/A	N/A	N/A	0.648
75	N/A	N/A	N/A	N/A	N/A	0.479

Appendix III: Overview Analytical Tests

#	Folin-Ciocalteu (Absorbance at 760)	Total Polyphenolic Content (mg GAE/g wood)	Vanillin Assay (Absorbance at 500)	Condensed Tannins (mg CE/g wood)	Acidic Butanol Assay (Absorbance at 530)	Condensed Tannins (mg CyaE/g wood)
1	0.033	-0.4258	0.126	0.0322	0.0392	0.357
2	0.043	0.0083	0.114	0.0201	0.0293	0.265
3	0.054	0.4652	0.115	0.0209	0.0270	0.245
4	0.054	0.4495	0.111	0.0167	0.0201	0.183
5	0.045	0.0792	0.108	0.0143	0.0370	0.337
6	0.051	0.3073	0.109	0.0151	0.0246	0.223
7	0.105	2.5836	0.125	0.0316	0.0197	0.179
8	0.070	1.1210	0.095	0.0002	0.0080	0.072
9	0.181	5.7438	0.218	0.3555	0.0425	0.388
10	0.311	11.2306	0.722	3.6660	0.1371	1.258
11	0.113	2.8716	0.111	0.0172	0.0201	0.182
12	0.585	22.1200	0.280	0.7485	0.0289	0.258
13	0.250	8.4761	0.302	0.8924	0.0533	0.478
14	0.076	1.3325	0.196	0.2102	0.0229	0.204
15	0.075	1.3064	0.216	0.3420	0.0203	0.184
16	0.063	0.8212	0.188	0.1597	0.0145	0.132
17	0.649	24.7792	0.696	3.4053	0.1092	0.976
18	1.624	66.9616	0.496	2.2027	0.0585	0.541
19	0.411	15.4704	0.815	4.2805	0.1575	1.447
20	0.124	3.3443	0.283	0.7735	0.0432	0.389
21	0.333	12.2004	0.501	2.2168	0.1571	1.444
22	0.297	10.4846	0.915	4.8454	0.0917	0.826
23	0.077	1.1691	0.208	0.2475	0.0140	0.107
24	0.516	19.8723	0.866	4.6135	0.1264	1.160
25	0.337	12.3398	0.725	3.6819	0.1560	1.430
26	0.167	5.1510	0.234	0.4583	0.0075	0.068
27	0.098	2.3136	0.420	1.6860	0.0714	0.656
28	0.051	0.2961	0.264	0.6473	0.0176	0.158
29	0.279	9.8671	0.659	3.2480	0.1586	1.452
30	0.084	1.6953	0.221	0.3827	0.0110	0.101
31	0.647	25.6228	0.763	3.9722	0.0873	0.809
32	0.311	11.0571	0.324	1.0361	0.0358	0.323
33	0.605	23.2498	0.363	1.2891	0.0473	0.427
34	0.728	28.1950	0.440	1.7768	0.0798	0.717
35	0.063	0.8092	0.221	0.3746	0.0037	0.034
36	0.054	0.4573	0.234	0.4549	0.0001	0.001

37	1.049	41.3845	1.328	7.4775	0.2270	2.039
38	0.116	3.0140	0.275	0.7272	0.0272	0.247
39	0.071	1.1175	0.418	1.6273	0.1218	1.089
40	0.117	3.0032	0.286	0.7884	0.0293	0.262
41	0.073	1.2360	0.238	0.4870	0.0083	0.075
42	0.106	2.6009	0.147	0.0543	-0.0051	-0.046
43	0.087	1.5712	0.216	0.2975	0.0263	0.206
44	0.100	2.3305	0.250	0.5541	0.0004	0.004
45	0.103	2.4897	0.201	0.2507	0.0272	0.248
46	0.304	10.7808	0.672	3.2874	0.1318	1.191
47	0.150	4.4380	0.296	0.8660	0.0333	0.303
48	0.351	12.8757	0.567	2.6410	0.0922	0.842
49	0.107	2.6757	0.203	0.2643	0.0096	0.088
50	0.104	2.4994	0.182	0.0884	0.0110	0.099
51	0.254	8.6764	0.484	2.0608	0.0754	0.678
52	0.076	1.3940	0.158	0.0661	0.0181	0.167
53	0.152	4.4921	0.358	1.2582	0.0315	0.285
54	0.071	1.1574	0.181	0.0880	0.0010	0.009
55	0.161	4.9384	0.255	0.6008	0.0383	0.352
56	0.111	2.8715	0.173	0.0813	0.0079	0.073
57	0.430	16.0509	0.495	2.1504	0.1490	1.352
58	0.112	2.8906	0.166	0.0743	0.0282	0.260
59	0.115	3.0360	0.179	0.0881	0.0559	0.514
60	0.229	7.7717	0.302	0.9068	0.0308	0.281
61	0.192	6.1812	0.274	0.7208	0.0385	0.351
62	0.488	18.4396	0.411	1.6016	0.0543	0.491
63	0.262	9.0229	0.373	1.3492	0.0760	0.684
64	0.237	8.1214	0.672	3.3271	0.1145	1.047
65	0.034	-0.3985	0.144	0.0520	0.0239	0.221
66	0.534	20.2622	0.487	2.0910	0.1055	0.952
67	0.115	2.4964	0.290	0.6899	N/A	N/A
68	0.035	-0.3261	0.150	0.0567	N/A	N/A
69	0.076	1.3912	0.171	0.0793	N/A	N/A
70	0.394	36.9152	0.836	11.0471	N/A	N/A
71	0.167	12.2479	0.308	2.2301	N/A	N/A
72	0.292	26.5433	0.368	3.4084	N/A	N/A
73	0.141	4.0887	0.224	0.3948	N/A	N/A
74	0.077	1.4387	0.151	0.0586	N/A	N/A
75	0.767	29.7120	1.285	7.1912	N/A	N/A

Appendix IV: COD Absorbance Tests

#	Absorbance 1	Absorbance 2	Absorbance 3	Average	COD (mgO2/L)
10	0.309	0.286	0.277	0.291	838.3
17	0.432	0.444	0.439	0.438	1264.2
18	0.349	0.369	0.381	0.366	1056.5
19	0.284	0.29	0.259	0.278	800.8
21	0.134	0.133	0.142	0.136	393.2
22	0.366	0.337	0.345	0.349	1007.5
24	0.151	0.147	0.141	0.146	422.0
25	0.339	0.339	0.357	0.345	995.0
27	0.276	0.281	0.284	0.280	808.5
29	0.418	0.412	0.407	0.412	1189.2
31	0.28	0.273	0.276	0.276	796.9
34	0.492	0.492	0.488	0.491	1415.1
37	2.144	2.106	2.12	2.123	6123.7
39	0.335	0.336	0.328	0.333	960.4
40	0.159	0.165	0.165	0.163	470.1
46	0.191	0.185	0.196	0.191	549.9
51	0.175	0.17	0.176	0.174	500.9
53	0.324	0.315	0.313	0.317	915.2
55	0.208	0.197	0.201	0.202	582.6
57	0.201	0.209	0.195	0.202	581.6
60	0.228	0.227	0.231	0.229	659.5
61	0.17	0.146	0.131	0.149	429.7
62	0.503	0.494	0.499	0.499	1438.2
63	0.211	0.226	0.219	0.219	630.6

Appendix V: Catechin and Gallic Acid Calibrations

Catechin (g CE/L)	Absorbance at 500nm
0.03	0.30090
0.015	0.23410
0.0075	0.19390
0.005	0.18860
0.00375	0.18650
0.003	0.17120
0.0015	0.15440
0.001	0.14840
0	0.08210

Gallic Acid (g GAE/L)	Absorbance at 760nm
0.84110	1.8777
0.42055	1.8299
0.21028	1.5824
0.10514	0.7934
0.08411	0.6483
0.07009	0.5364
0.05257	0.3990
0.04206	0.3202
0.02804	0.2799
0.02103	0.2421
0.01682	0.1593
0.01402	0.1380