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Investigating changes in the chemical composition of wood upon digestion by wood-boring Bivalves of the sub-family Xylophagaidae, and potential implications for marine biogeochemical cycles

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Abstract

The sub-family of molluscs Xylophagaidae are the primary degraders of wood in the oceans. They are part of an opportunistic ecosystem, which survives on temporary falls of large organic matter, such as carcasses and wood debris. After digesting the wood with the help of symbiotic bacteria, Xylophagaidae excrete a pulp like substance, which lines the walls of their burrows. The nutrients in the broken down wood becomes accessible to other forms of life, and growing populations create anoxic environments, which encourage chemoautotrophic activity. This research aims to identify the role that Xylophagaidae play in the biogeochemical cycles of certain elements, by investigating the changes in wood chemical composition after digestion by these wood-borers.

Introduction

The Chemistry of Wood

Wood is a unique, natural material with many uses to man and the natural world, so there has unsurprisingly been plentiful research on its structure. It is composed of cellulose, hemicellulose, lignin, and extractives (Ritter, 2008). Cellulose is one of the most abundant organic materials on earth, being present in every terrestrial and aquatic plant as well as in algae. It is a polymer made up of thousands of D-glucose monomer units (figure 1) which are mostly arranged into a crystalline structure; the degree of crystallinity of cellulose in cell walls has been linked to mechanical properties of wood like stiffness and strength (Fujimoto, et al., 2007) (Jiang, et al., 2007).

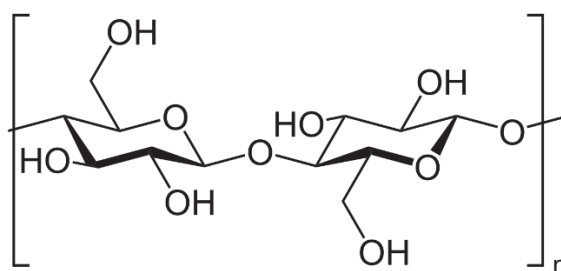


Figure 1: The structure of a cellulose chain, showing two D-glucose monomer units.

Hemicellulose is also composed of sugar monomer units, although these tend to be xylose molecules with side chains of other sugars like arabinose, galactose, mannose, and glucose, which forms cross links with pectin (Ritter, 2008). Within the spaces between these polysaccharide chains lies lignin, which is covalently bonded to hemicellulose, and made up of three monolignols: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Wang, et al., 2013). The chemical interactions of these three polymers create a complex matrix known as lignocellulose, which together contribute to woods mechanical properties, like viscosity, hardness, elasticity, and density, (Wang, et al., 2016).

Extractives refers to any other constituents within the wood, which are primarily lipophilic compounds such as fatty acids, resin acids, waxes, sterols, sterol esters and glycerides (Pandey, 1999) (Gutiérrez, et al., 2001); the functions of which range from biosynthesis, food reserves, plant protection, and cell membrane components (Holmbom, 1999). An early study also links certain extractives to the durability of wood (Hawley, et al., 1924).

Aside from these components, woody plants also contain trace elements. Like all living organisms, they need these for various biological functions. It is important to determine 'normal' element concentrations in environmental samples for comparison in cases of pollution or contamination, and for tracking environmental changes. The elemental composition of wood has been extensively explored, due to its uses in the engineering, paper and biofuel industries, and it is clear from the literature that the variation in trace element concentrations within wood is quite significant, even within each study (Esch, et al., 1996), (NYSERDA, 2013), (Oberberger & Thek, 2004),

(Nicewicz & Szczepkowski, 2008), (Baltreinaite, Booth, & Butkus, 2010). This is quite expected for environmental samples, and wood is indeed included in this as there are many factors that could affect its chemical makeup, such as geographic location, sample location within the tree, fluctuating pollution levels, and species variation (Simpson, 1998).

Analytical Techniques for Wood and their Importance

At the microscopic scale, light microscopy can provide an overall look at the anatomy of wood, being useful for studying anatomical changes, as well as for species identification – with uses from tracking illegal timber (WWF, 2014) to identifying antique wood carvings (Ruffinatto, et al., 2014). Beyond this, at the molecular and atomic scales, the vast literature on wood analysis has taken many forms, depending on the purpose of the analysis. Chemical wood pulping methods, such as Kraft pulping, yield almost pure cellulose fibers. However, analysis of the products from these processes has shown that they can be damaging to the environment; producing harmful airborne pollutants (Bordado & Gomes, 2002) (Hoffman, et al., 2017) and effluents which cause physiological disturbances in aquatic life (V, et al., 1992) (Sandström & Neuman, 2003) (Hewitt, et al., 2006).

As a safer, 'greener' alternative, there has been much research into attaining isolation of lignocellulosic components of wood using ionic liquids (Fort, et al., 2006); the dissolution properties of different species of wood has been inspected using various NMR techniques (Honglu & Tiejun, 2006) (Qu, et al., 2011) (Qu, et al., 2013). Beyond the isolation of these components, their structural properties have been quantitatively and qualitatively analysed using techniques such as X-ray diffraction (Thygesen, et al., 2005) (Howell, et al., 2009) (Ju, et al., 2015), FT-IR (Chen, et al., 2010) (Xu, et al., 2013), FT Raman spectroscopy (Agarwal, et al., 2011), and FT Raman microscopy (Edwards, et al., 1997).

In elemental analyses of wood, the most common analytical technique used seems to be ICP – likely due to the ease of obtaining data for many multiple elements at a time, and the large detectable concentration range between OES and MS instruments; general detection ranges for ICP-OES being between 0.5-100 $\mu\text{g L}^{-1}$, and ICP-MS between 0.0005-5 $\mu\text{g L}^{-1}$ (EAG Laboratories, 2014). Preparation of wood for ICP analysis requires acid digestion of the samples, which can be achieved through 'wet' acid digests of wood or wood ash (Queirolo, et al., 1990), and through microwave-assisted digestion (Obernberger & Thek, 2004) (Yang, et al., 2013) (Tafur-Marinos, et al., 2016), which is perhaps the simplest and definitely the quickest method. X-ray fluorescence spectroscopy has also been successfully used to determine concentrations of trace elements in wood (Block, et al., 2007) (Fellin, et al., 2014) (Morgan, et al., 2015), and has been useful when non-destructive analyses are needed, for example with research into the biodeterioration of wood (Illman & Bajt, 1997) (Kirker, et al., 2017).

A Novel Application of Wood Analysis

In terrestrial ecosystems, biological degradation of wood is carried out by wood-decay fungi, which have the ability to solubilize insoluble metal-containing minerals, as well as other compounds, through synthesising organic acids from the products of wood degradation (Liaud, et al., 2014). These organic acids act as ligands in complexes with metal cations, nutrients, and phosphates (Gadd, 1999), and there is

evidence of fungi exchanging some ions, such as Fe, K, Ca, Mn, and Zn, with the host wood (Jellison, et al., 1997) (Kirker, et al., 2017). Research has particularly focused on the application of this to environmental biotechnology, including bioremediation (Kaewdoun, et al., 2015), so it may not be far-fetched to consider these applications for mechanisms of wood degradation in marine organisms.

Marine fungi also have the ability to synthesise these organic ligands (Sunda & Gessner, 2015) (Pang, et al., 2011), but the contribution made by wood-boring bivalves of organic ligands within the ocean has not been investigated. This is significant, as woodborers are the primary degraders of wood in marine environments, and organic ligands can control the speciation, solubility, toxicity, and transport of free ions within the water and within organisms (Vraspir & Butler, 2009). The marine biogeochemical cycles of these elements have relations to global climate (Bargagli, 2000) and ocean circulation (Müller, 2012). Data is desperately needed, in order to understand: how the ocean works and responds to change; how to preserve and protect this vastly significant environment; and ultimately how the fate of the ocean affects human life. Our comprehension here is in some places limited, so further research is needed which focusses on sources and sinks, transport, transfer mechanisms, and chemical fates of trace elements (SCOR Working Group, 2007) (Achterberg, 2014).

The manner in which wood interacts within and affects the marine environment - a distinctive matrix of metal ions, loose sediment, particulate organic matter, and biological organisms, is largely uncharted. Knowledge of the distribution and geographic origin of natural wood falls could give insights into the types of wood that make their way to the deep-sea, and how different types of wood might cause species variation within the ecosystem of their degradation.

Xylophagaidae and their Role in Deep-Sea Wood Fall Communities

The sub-family Xylophagaidae defines deep-sea bivalves, which survive by boring into wood. It is composed of members within the genera *Xylophaga*, *Xylopholas*, and *Xyloredo*, of which a number of new species have recently been discovered (Voight, 2007) (Voight, 2009) (Romano, et al., 2014), almost two decades after the first classifications of *Xylophaga dorsalis* in the British Isles (Turton, 1822).

Xylophagaidae are highly opportunistic, surviving by exploiting marine wood falls in the deep-sea, which can originate from natural sources, such as mangrove habitats, or manmade sources, such as wrecked ships. The destruction of wooden structures in the early 1700s by members of the shallow-water sister family, Teredinidae, initiated research into the lives of these unique molluscs (Sellius, 1733). Since then, much has been discovered of the Teredinidae, also known as “shipworms”, such as knowledge of their growth, reproduction, and dispersal (Tyler, et al., 2007). Of particular note is the discovery of cellulolytic and nitrogen fixing endosymbiotic bacteria in their gills (Popham & Dickinson, 1973), which are presumed to aid in cellulose digestion and provide nitrogen to the shipworms (Distel, et al., 2002) (Luyten, et al., 2006), although the mechanism for the transfer of cellulases and nitrogen is not known.

In contrast, knowledge of Xylophagaidae is somewhat lacking, however there have been noteworthy advancements. In an interesting discovery, Haga and Kase (2013) suggest that *Xylophaga supplicata* go through a hermaphroditic stage - changing

from male to female, after which they begin to carry dwarf males, whose sole purpose is to provide spermatozoa to the female host. Other studies have recently begun to illustrate the role of Xylophagidae within deep-sea communities. Like Teredinidae, Xylophagidae have also been found to host endosymbiotic bacteria in their gills (Distel & Roberts, 1997), suggesting a similar cellulolytic and nitrogen fixing purpose to symbionts in Teredinidae. They also bore with their shell, and excrete digested material around them within their burrows (Fagervold, et al., 2014), rather than dispelling their fecal matter like the Teredinidae. This is vital for opportunistic ecosystems, as it has been found that this digested pulp provides sustenance for detritus feeders, which along with the woodborers themselves, become food for predators (Bienhold, et al., 2013). Diverse species start to inhabit the wood, including chemoautotrophs attracted to the sulphidic environment caused by increased respiration (Bienhold, et al., 2013). Furthermore, the discovery of closely related species at wood-fall communities, and cold-seep and hydrothermal vent communities- also known for their chemoautotrophic inhabitants- brought about a theory that wood falls may have acted as 'evolutionary stepping stones' for organisms from wood fall ecosystems to disperse to cold seeps and hydrothermal vents, or vice versa (Distel, et al., 2000).

The Role of Trace Elements in the Marine Environment

Phosphorus and sulphur are found in ocean sediments as part of their natural cycles. The concentration of dissolved phosphorus has been found a major predictor of oceanic primary productivity (Delaney, 1998) - being one of the most limiting nutrients for phytoplankton growth (Labry, et al., 2002), thus having the ability to affect entire marine ecosystems. Research on the role of sulphur in oligotrophic regions mainly focusses on ecosystems at hydrothermal vents, where microbial communities release energy through oxidation of geothermally sourced reduced sulphur compounds (Zierenberg, et al., 2000), (Sievert, et al., 2008). Away from hydrothermal vents, sulphur compounds are involved in redox reactions with other benthic sediment ions, mediating the speciation and bioavailability of some heavy metals (Jasinska, et al., 2012).

As well as phosphorus and Sulphur, bioactive trace metals are also of importance to primary production, according to their speciation and bioavailability (Bruland, et al., 1991). As previously stated (section 1.3), organic ligands are vital for ion transport and controlling things like the speciation and bioavailability of ions (Vraspir & Butler, 2009). Transition metals are particularly relevant in exploring this because of their ability to complex fairly easily with a range of organic ligands, due to their empty valence shells. The importance of them within all ecosystems also makes them an obvious choice for investigation. They are used in many biochemical processes, for example: iron within hemes, used for respiration, photosynthesis, and nitrate assimilation (Hogle, et al., 2014); copper, iron, vanadium, and zinc in metalloenzyme catalysts (Butler, 1998) (Kim, et al., 2008); and manganese in proteins relating to the oxidation of water during photosynthesis (Shutilova & Moiseev, 2010).

Aims

Preceding its main objective, this work investigates a few species of tree grown in the south west of the UK, through implementing and modifying existing methods of acid digestion to confirm their validity. It will compare elemental compositions in a few

select species of soft- and hard-woods, with the aim to support existing research showing variability within these.

Following on from this, this research seeks to support a collaborative project: “Biodiversity, connectivity and ecosystem function in organic-rich whalebone and wood-fall habitats in the deep sea”, led by Dr Craig Smith of the Benthic Ecology Lab at the University of Hawaii. This investigation will aim to provide a first look at the change in chemical composition of wood after digestion by the opportunistic *Xylophaga*. The analysis considers the impact of any changes of inorganic components on local marine biogeochemical processes, as well as potentially shedding some more light on the benefits of this process to the bivalves, and nutrient cycling within these delicate, yet resourceful ecosystems.

It may be acceptable on a first-look basis to assume that concentration changes in trace metals infers evidence on ligand availability in the organic portion of the wood - considering that ligand binding with free metal ions in the surrounding water will generally have occurred. The trace elements explored in this research include Vanadium (V), Cobalt (Co), Cadmium (Cd), Nickel (Ni), Phosphorus (P), Sulphur (S), Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), Magnesium (Mg), Potassium (K), and Sodium (Na).

Objectives

The specific objectives of this project are as follows:

- 1) To quantify and compare concentrations of elements in different species of hard and soft woods.
- 2) To compare ashed and dried methods for wood digestion, in terms of accuracy and precision of results and feasibility.
- 3) To quantify element concentrations in wood pulp excreted from wood boring bivalves of the genus *Xylophaga*, and compare this to element concentrations in surrounding undigested wood.
- 4) To experiment with milli-Q soaked samples to determine the portion of elements complexed with organic ligands within the wood.
- 5) To explore the potential effects and contributions of organic wood falls in marine biogeochemical cycles.

Experimental Method

Consumables, Equipment, and Instrumentation

Table 1 details specific information regarding the laboratory equipment and analytical instrumentation used, and table 2 details that regarding all chemicals used in the lab.

Table 1: Details of equipment and instrumentation used.

Type	Use	Manufacturer	Models and Identifiers
Analytical balance	Weighing of wood samples for CHN elemental analysis	Toledo	Mystic Mett AT201
Aluminium cups – 8 X 5 mm	Containing wood samples for CHN analysis	DEA Laboratories	C11470.250P
Elemental Analyser	CHN analysis	CE Instruments OEA Labs	EA 1110 CHNS
Analytical balance	Weighing of wood samples for ICP analysis	Oxford Analytical	XB220A
Drying Oven	Drying samples	Thermal Control	Status
Drying Oven	Drying PTFE vessels	Thermo Scientific	Heraeus
Filter paper	Filtering digest solutions	Whatman	Grade 40: 8 µm
Analytical balance	Calibration of pipettes	Oxford Analytical	A2204
Pipettes	Measuring stock solutions for ICP calibration standards	Thermo Scientific	MH36674
ICP-OES	Determination of trace elements in all sample digests	Thermo Scientific	iCAP
ICP-MS	Determination of trace elements in terrestrial sample digests	Thermo Scientific	X Series 2
ICP-MS	Determination of trace elements in marine-deployed sample digests	Thermo Scientific	iCAP RQ

Table 2: Details of chemical reagents and consumables used.

Reagent	Supplier and Grade
Cyclohexanone-2,4-dinitrophenylhydrazone	OEA Labs, Certified Organic Analytical Standard
L-cystine	OEA Labs, Certified Organic Analytical Standard
Nitric Acid 70%	Fisher Scientific - Analytical reagent grade
Hydrogen Peroxide >30% w/v	Fisher Scientific - Analytical reagent grade
Multi-element stock solution	LabKings
Individual Stock solutions of P and S	LabKings

ICP Calibration Standards

Calibration Series' were prepared for both ashed and dried wood digests. Before any standards were made, the analytical balance and pipettes were calibrated; the analytical balance was calibrated using weights with a known mass, and then pipettes were calibrated by weighing out measured samples of water using the calibrated balance. The concentrations of the calibration standards are shown in table 3. The standards made up for ICP-OES were diluted 100X for use with ICP-MS analysis. To the ICP-MS standards, 25 μL of 10000 mg L^{-1} Indium and Iridium were added for use as internal standards.

Table 3: Volumes and concentrations of elements in calibration standards for ICP-OES.

Element	Conc. of soln./ mg L^{-1}	Vol. Used / mL	Total Vol. / mL	Final Standard Conc. / mg L^{-1}
Fe, Mn, Mg, Zn, V, Cu, Co, Ni, Ca, Na, P, S	100	0.1; 0.25; 0.5; 1	25	0.4; 1; 2; 4
K	1000	0.1; 0.25; 0.5; 1		4, 10, 20, 40

Methods for Quality Control

For all analyses procedural blanks, solvent blanks, and three sample replicates were acquired. For each instrumental analysis, instruments were calibrated with suitable standards, to check that a suitable linear range was attained. For CHN elemental analysis, L-cystine was used as a certified reference material for comparison of results. For ICP-OES and -MS, standard checks were run between every 10 or so

samples to check that instrument performance was maintained at an acceptable level. All of the ICP instruments used automatically corrected measurements to account for the solvent blank. The procedural blank values for ashing and drying sample techniques were also taken from the relevant measured values to account for any procedural influences on element concentrations. As well as this, for ICP-MS analysis, Indium (In) and Iridium (Ir) were used as internal standards. The recovery factors in each sample were automatically calculated by the instrumentation and these were applied to concentration values to improve the accuracy of the results.

Cleaning of Equipment

All glassware was soaked over 12 hours in 10% HCl baths, before rinsing with deionised water, then Milli-Q water. After drying by air, glassware was stored in new plastic ziplock bags until use. To clean PTFE vessels, 3 mL 70% HNO₃ was added to each and microwaved for approximately 3 minutes. After leaving to cool for at least 15 minutes, vessels were thoroughly rinsed with Milli-Q water and dried at around 250 °C. The porcelain pestle and mortar used were cleaned by soaking in concentrated nitric acid – slightly diluted 70% HNO₃. After several hours of soaking, the pestle and mortar were thoroughly rinsed with Milli-Q water and left to dry in air overnight.

Analysis of Terrestrial Wood Samples

Dry wood samples from various species were used to practise, modify and optimise the methods of analysis found in the literature. The species analysed included: Sitka fir (SF), Hazel (HZ), Ash (AS), Douglas fir (DF), Willow (WL), Pine (PN) Oak (OA) and Moringa (MR). WL, DF, SF, AS, and HZ were sourced from trees at Nanswhyden Farm in Newquay. PN, OA, and MR were sourced from excess cuttings at a local timber yard. Pictures of some of these samples are shown in figures 2-5. The locations of the sample within each tree was not known, although it is clear from the figures that some included sapwood (closest to the bark), and some were most likely heartwood.



Figure 2: Sitka fir sample

Samples were prepared for acid digestion by sanding with a coarse grade, 40 grit, aluminium oxide sandpaper. For each species, three replicates of around 500 mg sawdust, and three replicates of around 1 g were collected on a clean sheet of paper. Pieces of sandpaper grit that had become free in the process picked out of the sample with clean, plastic tweezers, with care to make as little contact with the

sample as possible. Samples were then transferred to 50 mL centrifuge tubes and stored in a cool, dry place until use. Moisture content of the samples was measured by loss of mass after drying until constant mass. Ash content was measured by the loss of mass on ignition.



Figure 3: Willow sample



Figure 4: Hazel sample



Figure 5: Douglas Fir sample

CHN Elemental Analysis of Terrestrial Samples

Moisture content of the samples was measured by the loss of mass after drying until a constant mass was achieved, and the ash content was measured by the loss of mass on ignition.

Sawdust dried in an oven overnight at around 105 °C. Three replicates of around 2.50 mg of each sample, the standard CYC, and the CRM CYS, were weighed out into tin cups using a 5-figure balance. The balance was first tared with the tin cup, which was then removed to a clean glass plate for sample addition with a micro-spatula. The cups were carefully folded and crushed into balls using metal forceps and placed into a numbered sample tray. To inspect whether the crushed cups contained any holes, they were dropped onto the glass plate from a height of a few centimeters, and if any sawdust could be seen to drop onto the glass plate, a replacement was prepared.

A standard operating procedure was followed for the Elemental Analyser EA1110, based on the instruments operation and installation manual (EMASyst, 1996):

- 1) Compressed air and high purity oxygen supplies turned on.
- 2) Instrument taken out of stand-by mode by using the keypad on the front of the instrument to press: SPC FUN; (CNT.) ► (STBY); ▲ (YES) ▼ (NO); SPC FUN, then using the ◀► keys to check that helium flow rate is at 99 mL min⁻¹, and the column temperatures are correct – combustion tube at 1000 °C, reduction tube at 750 °C, and analytical column at 65 °C. It took some time (at least half an hour) for the instrument to reach these operating conditions, as flow rate and temperature are reduced in stand-by mode.
- 3) Boosted the DOS based operating system software “EMASYST”, by shutting down the computer but selecting “exit into DOS”. After a C prompt appeared, typed: CD\emasyst; ↵; emasyt .; ↵; (any key to continue).
- 4) Checked number of combustion and reduction cycles did not exceed 400 – in the event that they exceeded this value, a technician would need to be consulted to change combustion and reduction tubes.
- 5) The standards and samples were then loaded into the instruments auto-sampler tray in the order of: unweighed standard; blank; 3X weighed standard; 3X weighed CRM; 3X moringa samples; 3X pine samples; 3X oak samples; blank; 3X weighed standard.
- 6) Data saved and analysis terminated by first resetting the system –pressing F8 and changing “N” to “Y”. Then Emasyt program exited by pressing “SHIFT, F10” and again changing “N” to “Y”.
- 7) Compressed air and high purity oxygen supplies turned off and instrument put back into stand-by mode: SPC FUN; (CNT.) ► (STBY); ▲ (NO) ▲ (YES); SPC FUN.

ICP Analysis of Terrestrial Samples

For ashed samples, three replicates of ~1 g of dried sawdust ignited in a muffle furnace, for 16 hours at 450 °C. The ashed samples were microwave digested in PTFE vessels, with 4 mL Nitric Acid (70%). Microwave set to medium heat for 3 minutes, four vessels at a time. Vessels left to cool for 15 minutes before digests

quantitatively transferred to 25 ml volumetric flasks, and made up to the mark with milli-Q water. For dried samples, three replicates of ~0.3 g sawdust dried overnight at 105 °C. Samples transferred to PTFE vessels and left overnight in a fume cupboard in 4 mL Nitric Acid (70%) and 1 mL Hydrogen peroxide (30%). The same microwave programme and dilution steps were followed as for ashed samples. The operating conditions for each ICP instrument are detailed in table 4.

Table 4: Instrumental parameters for three ICP instruments used

Parameter	ICP-OES Thermo Scientific iCAP	ICP-MS Thermo Scientific X Series 2	ICP-MS Thermo Scientific iCAP RQ
RF power / W	1150	1400	1550
Coolant gas flow / L min⁻¹ argon	12	14	14
Auxiliary gas flow / L min⁻¹ argon	0.5	0.7	0.7
Nebuliser gas flow / L min⁻¹ argon	0.75	0.79	1.05
Exposure time / s	2	-	-
Dwell Time / ms	-	10	10
Sweeps	-	50	50
Viewing height	12 mm above load coil	-	-
Nebuliser	MiraMist (Burgener)	Concentric glass	Concentric glass
Spray chamber	Cyclone	Conical with impact bead	Cyclone (chilled to 4°C)
Collision Cell Gas	-	7% hydrogen in helium at a flow rate of 3.5 mL min ⁻¹	Helium at a flow rate of 5 mL min ⁻¹

The ICP-MS Thermo Scientific iCAP RQ instrument was used to determine elements in the marine deployed samples, and the ICP-MS Thermo Scientific X Series 2 was

used to determine elements in the terrestrial ashed and dried wood samples. The change in instrument was due to the X Series 2 being retired. Once the operating conditions were set, performance checks were run on each instrument (see appendices). The standards and sample names were then entered into the computer system and the program was started.

Analysis of Marine-deployed Samples

The Marine Study Site

The analysed wood blocks, of the species *Pseudotsuga menziesii* or ‘Douglas Fir’, were obtained as sub-samples from bone/wood lander deployments, or “BOWLS”, which were triangular frames with 3 open, 500 µm mesh bins on each side of the frame. Each bin and lid held a set of bone, wood and basalt control treatments. The positions of wood, bone and control within the bins and on the bin lids were random. The sub-samples analysed in this research originated from samples in BOWLS 2, 3, 5 and 6; there were three sub-samples per BOWL, taken from each side of the triangular frame. The samples were exposed to deep seas in the North Pacific Ocean for 15 months, in a range of locations off the coast of Seattle - the positions of which are detailed in table 5.

Table 5: Positions of BOWL deployments given in latitude and longitude values.

BOWL Number	Latitude (N)	Longitude (W)	Depth (m)
2	47°57.462	126°02.118	1596
3	47°16.201	127°35.573	2666
5	45°52.704	127°33.928	2917
6	43.54.522	125°10.238	1605

Table 6: The sub-samples from each BOWL used in the analysis.

	BOWL Number			
	2	3	5	6
Sample Identifier	W34	W28	W8	W19
	W5	W1	W21	W25
	W3	W26	W9	W6

Preparation of Marine-deployed Samples

The samples were stored in 500 mL centrifuge tubes at -20 °C. Samples were defrosted over two or three nights in a fridge before preparation. The components of interest were degraded pulp and non-degraded wood. Some of the sub-samples, such as those in figures 6-8, are almost completely degraded, whereas some, such as those in figures 9-11, were not well inhabited if at all.



Figure 6: Sub-sample W5 from BOWL 2



Figure 7: Sub-sample W3 from BOWL 2



Figure 8: Sub-sample W34 from BOWL 2

Where suitable sample sizes of pulp could not be obtained, sub-samples were left out of the analysis. The wood and pulp components were separated using clean plastic tweezers and transferred to separate plastic containers. Sample collection from outer faces of the wood blocks was avoided due to possible contamination from methods of sub-sample collection.

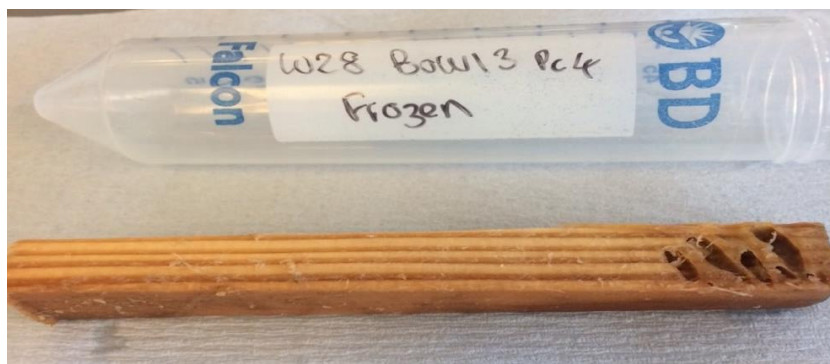


Figure 9: Sub-sample W28 from BOWL 3

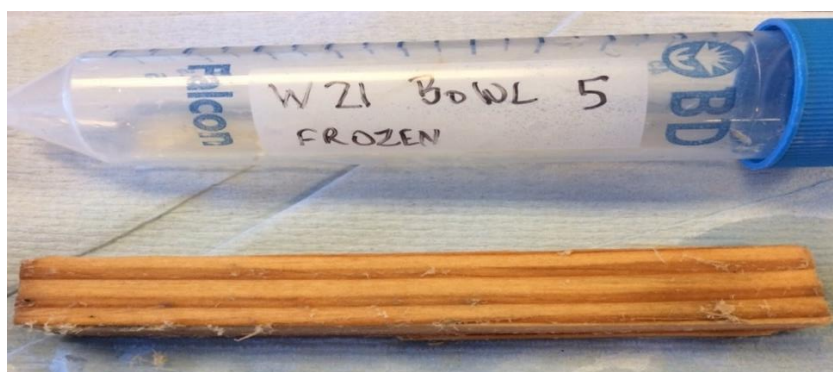


Figure 10: Sub-sample W21 from BOWL 5



Figure 11: Sub-sample W9 from BOWL 5

Collection of pieces of non-degraded wood suitable for acid digestion was achieved by either scraping off fibers in lightly degraded wood, or by easily breaking off small chunks in heavily degraded samples. To collect wood pulp, clean plastic tweezers were used for light scraping around the insides of the mollusc burrows. Care was taken to avoid collection of leftover shells of the molluscs (white, calcareous), and any sawdust – differentiated by its lighter colour and rougher texture. Once separated, samples re-frozen until final analysis.

ICP Analysis of Marine-deployed Samples

The analysis followed roughly the same procedures as the reference samples, including determination of moisture content, and straight acid digests of wood and pulp samples followed by ICP-OES and -MS determination of elements. The ash content and ashing digest methods were not carried out for these samples, due to a lack of suitable sample. Additionally, the deployed samples were analysed to determine the proportion of element concentrations present within ligands rather than components of the wood/pulp structure. For this, a comparative investigation between samples soaked in milli-Q water and un-soaked samples was carried out.

Results and Discussion

Quality Control

For CHN Elemental analysis, the instrument was calibrated with cyclohexanone-2,4-dinitrophenylhydrazone (CYC), and the technique yielded results within 1% of the theoretical result for carbon, hydrogen, and nitrogen (refer to appendices for all raw and additional data). The procedure was validated using the certified reference material L-cystine (CYS), for which expected values were calculated by dividing the molar mass of each element within the molecule by the molar mass of the molecule, as shown as an example in equation series (i). Using the expected values a percentage recovery factor for each element was calculated, summarized in table 7.

Table 7: Percentage error for carbon, nitrogen, and hydrogen in CRM L-cystine for CHN elemental analysis.

	Mean Value	Theoretical Value	Recovery Factor/ %
N	11.38	11.66	97.60
C	29.91	29.99	99.73
H	4.971	5.034	98.75

Theoretical % C in $C_6H_{12}N_2O_4S_2$:

$$= \frac{(6 \times 12.0107 \text{ u})}{240.292 \text{ u}} \times 100$$

$$= 29.99 \%$$

(i)

For ICP instruments performance reports were carried out, which showed that they were in suitable condition and were measuring accurate and precise values (see appendices). To measure the sensitivity of the ICP instrumentation, the limit of blank and the limit of detection were calculated (table 8) using equations (i) and (ii) according to Armbruster and Pry (2008). Concentration values below these limits were discredited for potential inaccuracy, and not used in any statistical analyses.

$$LoB = mean_{blank} + 1.645(SD_{blank}) \quad (ii)$$

$$LoD = LoB + 1.645(SD_{low\ concentration\ sample}) \quad (iii)$$

Table 8: Limit of blank and limit of detection of each element for ICP-OES and -MS

	LoB/ $\mu\text{g L}^{-1}$			LoD/ $\mu\text{g L}^{-1}$		
	ICP-OES Th. Sci. iCAP	ICP-MS Th. Sci. X Series 2	ICP-MS Th. Sci. iCAP RQ	ICP-OES Th. Sci. iCAP	ICP-MS Th. Sci. X Series 2	ICP-MS Th. Sci. iCAP RQ
P	85.12	-	-	85.15	-	
S	87.79	-	-	87.85	-	
Fe	4.093	-	-	4.104	-	
Mn	0.8235	-	-	0.8276	-	
Cu	1.9	0.02139	-	1.906	0.08226	
Zn	2.995	0.02632	-	3.003	0.4688	
Mg	0.5885	-	-	0.5933		
K	27	-	-	27.08		
Na	8.603	-	-	8.614		
V	6.598	0.01645	0.02401	6.605	0.1711	0.1576
Co	-	0.00658	0.002236	-	0.07074	0.09061
Cd	-	0.02139	0.001635	-	0.1201	0.07620
Ni	13.54	0.1908	0.099	13.55	0.3125	0.1869

Figures 12 and 13 show the calibration graphs for both ICP-OES and –MS instruments. The linearity of the results for the multi-element standards was proven to range from 0-4 mg L⁻¹ for ICP-OES and 0-40 µg L⁻¹ for elements except K.

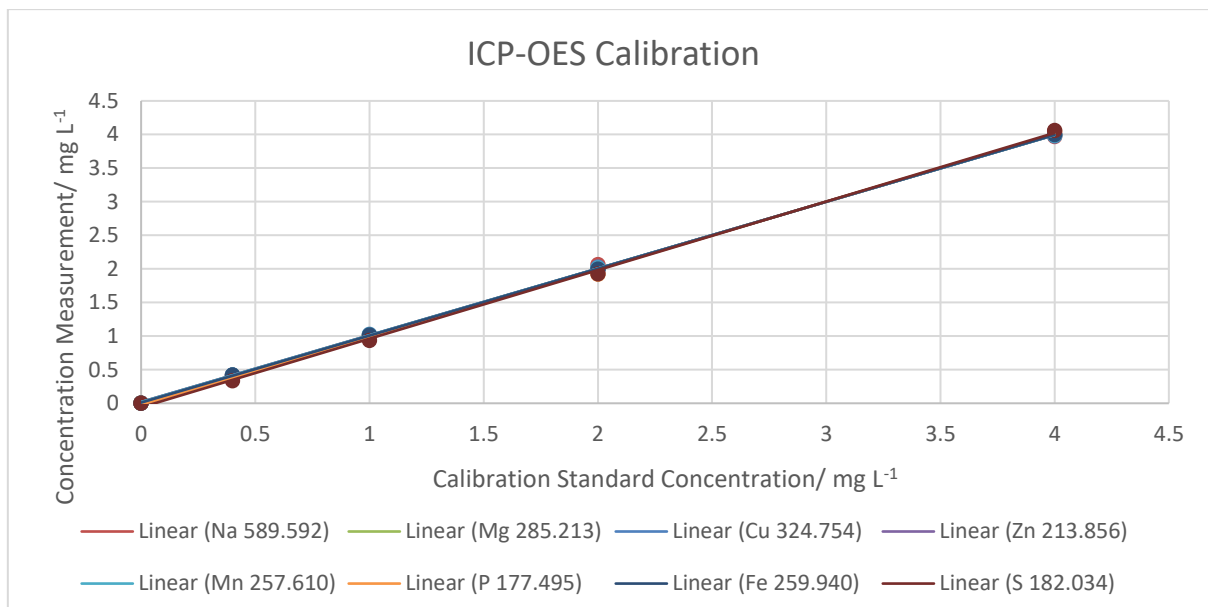


Figure 12: Calibration graph for ICP-OES showing the measured values of known concentration multi-element standards.

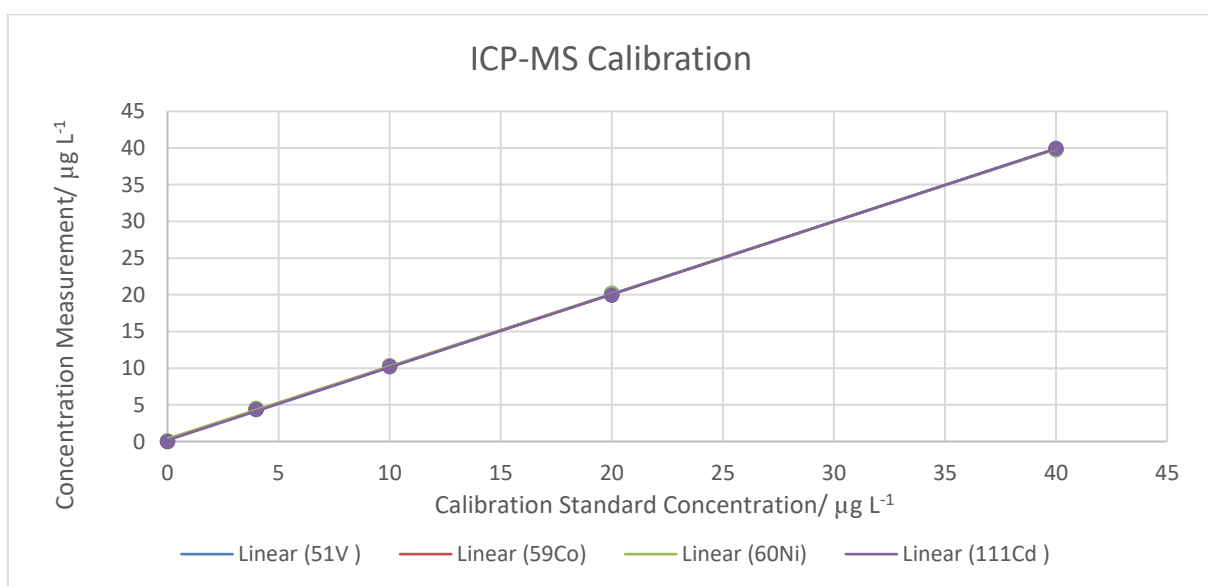


Figure 13: Calibration graph for ICP-MS showing the measured values of known concentration multi-element standards.

The linearity for K was proven from 0-40 mg L⁻¹ for ICP-OES. This is shown in table 9, which displays the Pearson's correlation coefficients (R²) for each element. The lowest value is 0.9992 proving strong linearity within these ranges. All statistical analyses were carried out using Microsoft Excel 2016.

Table 9: R² values obtained from the ICP-OES calibration standards.

Element	R2 Value
Na	0.9995
Mg	1
Cu	0.9999
Zn	1
Mn	0.9999
P	0.9993
Fe	1
S	0.9992
K	0.9995
V	0.9999
Co	0.9999
Ni	0.9999
Cd	0.9999

Terrestrial Sample Results

Moisture, Ash, and CHN Content of Various Wood Species

The moisture content was recorded for the wood of six species of tree: Sitka fir (SF), Hazel (HZ), Ash (AS), Douglas fir (DF), Willow (WL), and Pine (PN), shown in table 10. The low standard deviations (SD) and moderately low relative standard deviations (RSD) show that the data for each species did not vary much.

There does appear to be some variation between species, as displayed in figure 14, with a mean value of 7.26 % and a RSD of 24.6 %. The RSD is expected to be somewhat high for environmental samples, particularly here where wood structure is known to differ between species. A study from the US Department of Agriculture

(Simpson, 1998) calculated the equilibrium moisture content (EMC) of wood in over 300 locations worldwide, with the majority of results falling between 8 and 18 %.

Actual moisture content standards for fuel wood pellets have been recorded as being below 10% according to Austrian standard 'ÖNORM M 7135: compressed wood or compressed bark in natural state-pellets and briquettes' (Österreichisches Normungsinstitut, 2000), and Swedish standard 'SS 187120: biofuels and peat-fuel pellets-classification' (Swedish Standards Institution, 1998). Obernberger & Thek (2004) report an average moisture content of 7.7 % in 21 wood pellet samples and 8.0% in wood briquettes. A t-test for two samples with unequal variances showed no significant difference between moisture contents of hard- and softwoods, as the t_{stat} was lower than the t_{crit} value.

Table 10: Percentage moisture content in six species, including soft- and hardwoods.

Species	Wood Type	Moisture Content (%)	SD	RSD
Sitka Fir	Softwood	10.1	0.0805	0.801
Hazel	Hardwood	6.81	0.129	1.89
Ash	Hardwood	8.02	0.525	6.55
Douglas Fir	Softwood	8.16	0.542	6.65
Willow	Hardwood	6.23	0.338	5.42
Pine	Softwood	4.32	0.413	9.57

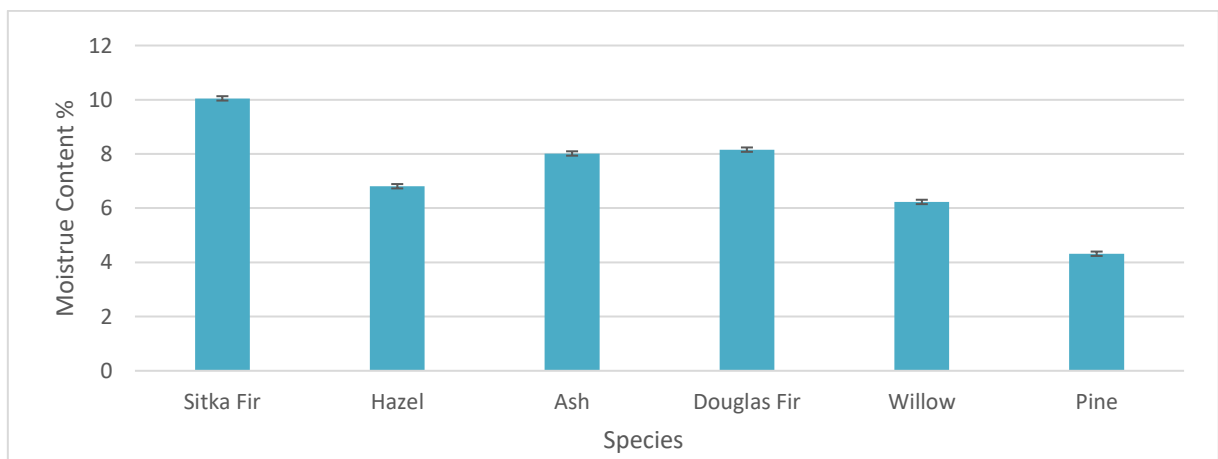


Figure 14: Moisture content of wood from various species.

The ash content for species SF, HZ, AS, and DF are shown in table 11 and figure 15. PN and WL samples were not ashed due to lack of sample at the time of ashing. The RSD values for each sample are slightly higher compared to those for the moisture content, showing variation within each species. Between species, a RSD of 50.4 % can mostly be attributed to species variation, as geographical factors such as soil mineral content should not have greatly affected these samples, as they all came from trees within the same forest. Using a t-test for two samples with unequal variances, there was determined to be a significant difference between hard- and softwoods, as the t_{stat} was greater than the t_{crit} value, which supports current knowledge that the ash content of hardwoods is higher than that of softwoods.

Table 11: Percentage ash content of two hardwood and two softwood species.

Species	Ash Content (%)	SD	RSD
Sitka Fir	0.772	0.208	27.0
Hazel	1.71	0.254	14.9
Ash	2.08	0.383	18.4
Douglas Fir	0.673	0.200	29.8

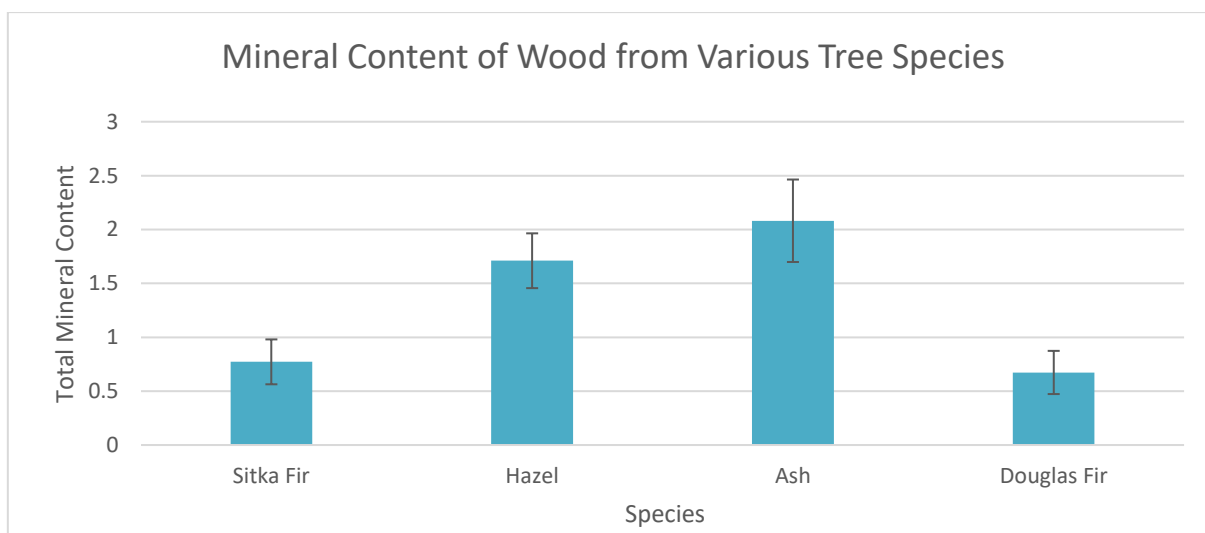


Figure 15: Mineral content of wood from various species.

The results from the CHN Elemental analysis of MR, PN, and OA are shown in table 12. Other species were not tested as samples had not yet been obtained, and the conformity of the data between these three species (two hard- and one softwood) and in the literature suggested there was not much need for further species. For example, between 41 species of tree analysed by Lamlom & Savidge (2003) C content ranged from 46.32 ± 0.17 to 49.97 ± 0.82 in hardwoods, and from 47.21 ± 0.35

to 54.66 ± 0.27 in softwoods. In the same study, N content ranged from 5.56 ± 2.10 to 8.74 ± 0.07 in both hard- and softwoods. The variation between species was also very low in these results, with RSD values for C and N being 1.89 and 1.59 respectively. As well as this, very low RSD values in table 13 show minimal variation within each species for both C and H. No N was detected in any samples – very low N content can be found in wood from some trees – as low as 0.03% in some species (Martius, 1992) – so detection may have been restricted by the detection limits of the instrument. The variation between species was also very low, with RSD values for C and N being 1.89 and 1.59 respectively.

Table 12: Percentage carbon, nitrogen, and hydrogen content in two hardwood species and one softwood species.

Species	C (%)	SD	RSD	H (%)	SD	RSD	N (%)	SD	RSD
Moringa	49.4	0.282	0.572	6.48	0.0705	1.09	0	0	0
Pine	47.3	0.406	0.860	6.43	0.0920	1.43	0	0	0
Oak	48.6	0.164	0.337	6.33	0.0812	1.28	0	0	0

Differences in Ashing and Drying Digestion Methods

The concentrations measured by the ICP instruments were converted from mass per volume concentration to mass per mass concentration as in equations (iii) and (v). An example calculation for P in an ashed SF sample is shown in calculation (vi).

$$\text{Mass of analyte (mg)} = \text{Conc. of analyte (mg L}^{-1}\text{)} \times \text{Vol. of Digest (L)} \quad \text{(iii)}$$

$$\text{Conc. of analyte in original sample (mg g}^{-1}\text{)} = \frac{\text{Mass of analyte in digest (mg)}}{\text{Mass of original sample (g)}} \quad \text{(v)}$$

$$2.70 \text{ mg L}^{-1}P \times 0.025 \text{ L} = 0.0675 \text{ mg}$$

$$\frac{0.0675 \text{ mg}}{0.649 \text{ g}} = 0.104 \text{ mg g}^{-1} P \quad \text{(vi)}$$

To test whether there was a significant difference in the results from the ashing and drying methods of digestion, a t-test was carried out for paired two sample means. The summary of these findings is shown in table 13. There was a significant

difference found for the majority of elements tested, with the exceptions being Nickel, Zinc, and Sulphur, which all had relatively low concentrations. Representations of the data (see appendices) are displayed in figures 16-19.

Table 13: Results of Paired T-Tests for elements in Ashed and Dried samples.

Metal	Tstat	Tcrit	Significant Difference?
V	3.27	2.23	YES
Co	5.15	2.20	YES
Ni	1.12	2.23	NO
Cd	5.85	2.31	YES
Cu	3.99	2.23	YES
Zn	1.85	2.20	NO
P	2.89	2.20	YES
S	1.40	2.20	NO
Fe	3.73	2.23	YES
Mn	3.58	2.20	YES
Mg	3.807	2.20	YES
K	4.092	2.20	YES
Na	4.185	2.20	YES

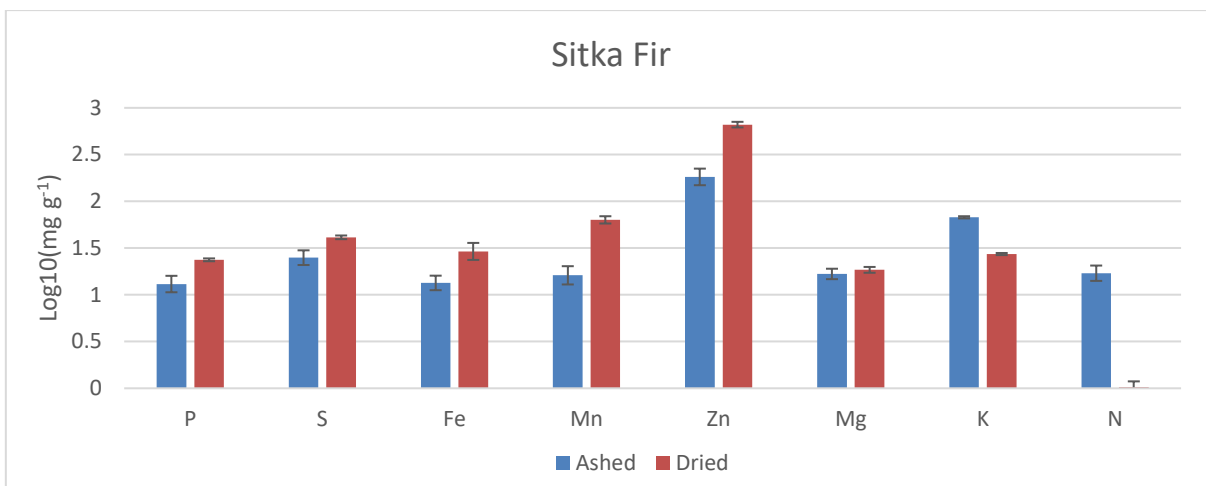


Figure 16: Comparison of log₁₀(element concentrations) from Ashing and Drying methods, for elements detected in SF samples by ICP-OES.

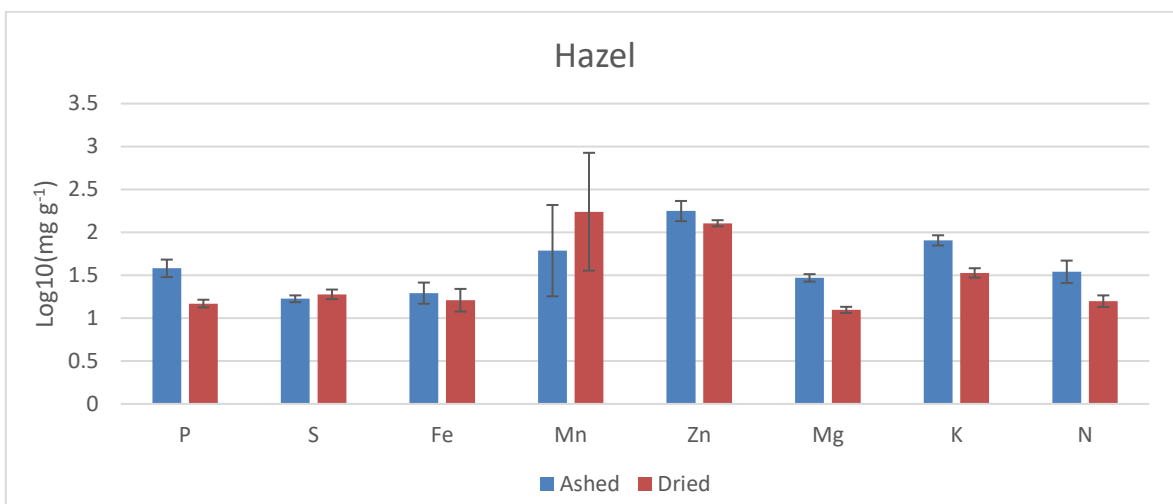


Figure 17: Comparison of log₁₀(element concentrations) from Ashing and Drying methods, for elements detected in HZ samples by ICP-OES.

In each species, the ashed and dried wood mostly showed similar concentrations of elements. In SF, DF, and AS, the dried samples generally yielded higher concentrations, which is contradicted in HZ where all elements, except for Mn, were of very similar or higher concentrations in ashed samples. One possible reason for this is that the digestion of ashed samples may have been more efficient in reducing the organic content of the samples, as this is completely burnt off in the ashing process as opposed to its destruction by hydrogen peroxide. This effect is expected to be minimal, though, as all terrestrial samples were left at least overnight in peroxide before further microwave digestion. Another possible cause of variation between the methods could be potential contamination of ashed samples from the muffle furnace, as the current model installed (5th floor laboratory in Davy Building at Plymouth University) has been in use for many years, and the vacuum caused when the furnace door opens can cause samples to 'jump' out of crucibles.

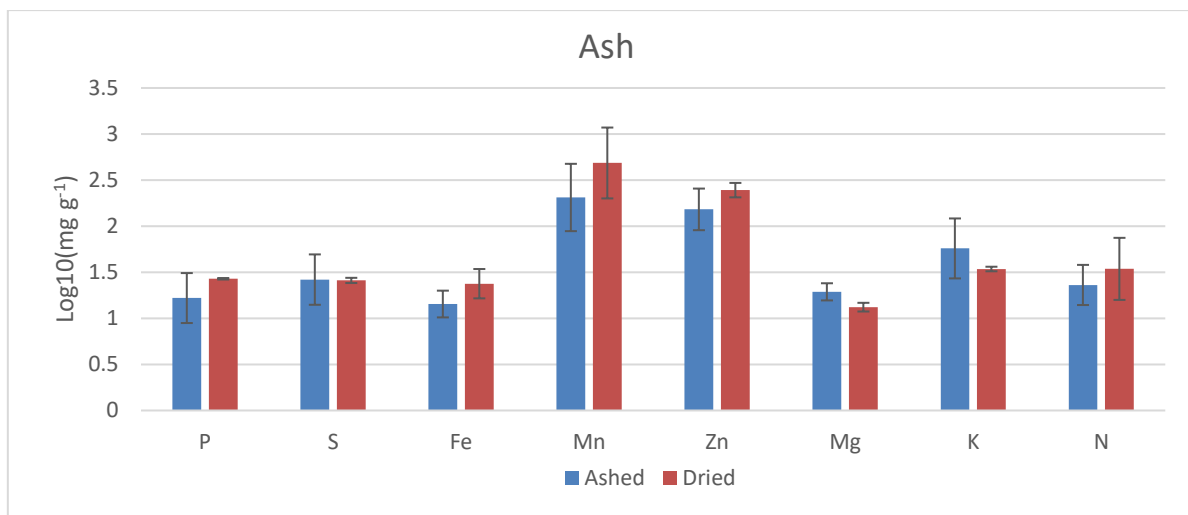


Figure 18: Comparison of log₁₀ (element concentrations) from Ashing and Drying methods, for elements detected in AS samples by ICP-OES.

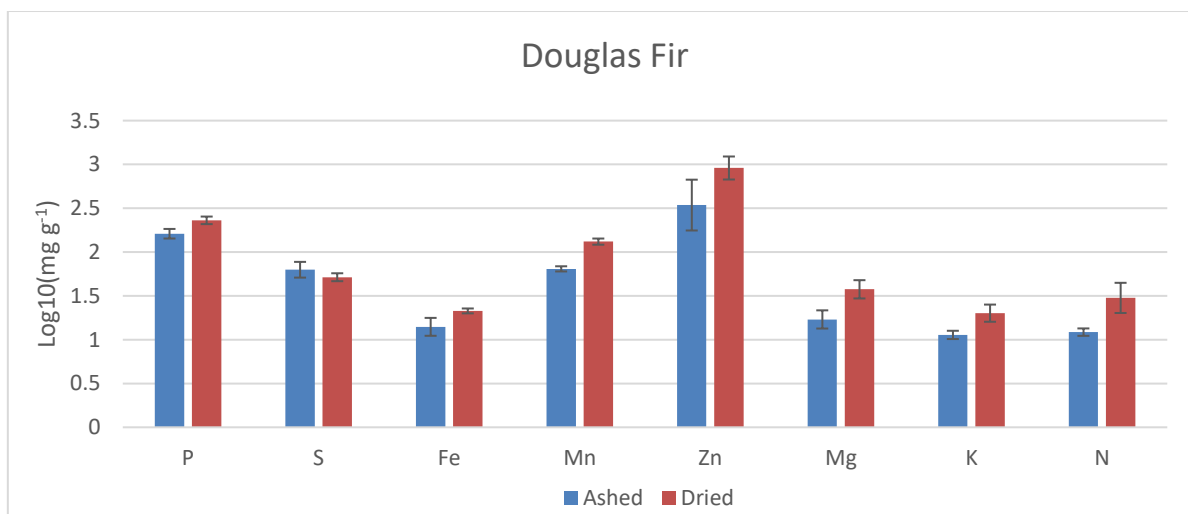


Figure 19: Comparison of log₁₀ (element concentrations) from Ashing and Drying methods, for elements detected in DF samples by ICP-OES.

This vacuum effect may also account for some minor sample losses, which could have contributed to the lower concentrations seen in SF, DF, and AS. On observing the ashed wood samples, there were noticeable differences between the densities of soft- and hard-wood sawdusts. The hardwoods AS and HZ were more compact in structure, and so created a very fine, lightweight sawdust. The softwoods on the other hand, DF and SF, formed larger particles. Both seemed to have some static attraction to crucibles once ashed or even dried, although this was more apparent in

the softwoods, in which particles could be seen to 'jump' around the crucibles, potentially making them more prone to sample loss.

While the differences between the methods were found to be significant, both showed good precision, with SD values all below 1 mg g⁻¹. Taking a closer look at the RSD values, the drying technique only had lower dispersion of values for Mn, and for other elements either similar, for example 22.0% for Mg in dried DF versus 24.7% in ashed DF, or much higher RSDs, for example 51.1% for K in dried AS versus 5.62% in ashed AS. This suggests that the ashing procedure may be a more precise technique; however, the drying procedure may have slightly greater accuracy.

As previously discussed, different structural characteristics of wood can affect mineral concentrations amongst other characteristics of wood, so small differences between species are completely expected. Looking at the data, variation between species is present in an expected range for environmental samples, but other than this appears insignificant.

Marine-deployed Sample Results

Visual Observations

Most samples were not well degraded- the only samples which held a significant amount of accessible pulp were those from BOWL 2; samples W3, W5, and W34. Pulp collected from other samples was not sufficient to gain accurate results.

Moisture Content of Deployed Wood Samples

Table 14 shows the average moisture content from samples W1, -3, -5, -25, -26, and -34. SD and RSD values for pulp from individual sample could not be calculated due to a lack of sample; however, it has been calculated from the average value of all samples. It is obvious that the pulp holds a much higher moisture content than the surrounding wood. The only exception to this seems to be W25, and it is suspected that this may be due to sawdust being falsely collected as pulp, as this was the first sample to be separated and was not that well degraded, so it is likely some mistakes were made. In the highly degraded samples, W3, -5, and -34, the moisture content of the pulp was slightly higher. Again, the sample collection process probably contributed to this slight variation, as it was easy to scrape relatively large clumps of pulp out of burrows in highly degraded wood, meaning the samples were less disturbed and so probably held slightly more moisture when in clumps. In comparison to terrestrial samples, the marine –deployed samples help greater moisture content in the wood as well as the pulp. This can definitely be expected, as wood is a porous material and so will have absorbed and held water whilst exposed to it.

Table 14: Average moisture content of wood and pulp samples from various BOWLS.

Sample Identity	Moisture Content (%)	SD	RSD	Sample	Moisture Content (%)	SD	RSD
Wood				Pulp			
W1	23.03	3.850	16.72	W1	75.51	-	-
W3	44.46	0.3828	0.8609	W3	78.25	-	-
W5	52.30	1.248	2.387	W5	77.66	-	-
W25	25.51	2.232	8.752	W25	30.20	-	-
W26	13.90	0.5218	3.753	W26	61.02	-	-
W34	50.91	2.956	5.807	W34	76.33	-	-
x	35.02	14.83	42.36	x	66.50	17.26	25.96

Differences in Elemental Composition between Wood and Pulp

The specific concentrations determined for each element can be found in the appendices. Paired t-tests were carried out to determine whether the difference in element concentration between wood and pulp samples was significant. Test statistics were calculated using excel and compared to critical values for two-tailed hypotheses. A summary of this is shown in table 15. Significant difference in concentrations was found in all elements except Ni and Zn – as in the terrestrial samples, these elements are found in very small concentrations in wood, and reaching near the limits of detection can affect the accuracy of readings. In terms of the direction of significance, the pulp samples showed higher element concentrations in all samples and for all elements. Figures 20-22 display comparisons between wood and pulp in different element concentrations.

Table 15: Results of paired T-Tests for elements in wood and pulp samples.

Metal	Tstat	Tcrit	Significant Difference?
V	3.84	2.57	YES
Co	4.57	2.78	YES
Ni	1.52	2.78	NO
Cd	6.39	2.78	YES
P	6.99	2.78	YES
S	15.6	2.57	YES
Fe	35.1	3.18	YES
Mn	3.44	2.57	YES
Zn	2.20	2.78	NO
Mg	15.9	2.57	YES
K	23.9	2.57	YES
Na	10.2	2.57	YES

Of the highest concentrations by far is sodium, which is known to be one of the most abundant trace elements in seawater, due to Na⁺ ions from dissolved NaCl. Other elements in relatively high concentrations include Mg, S, P, K and Fe, which are all classed as major ions in seawater, so this is quite an expected result. As previously discussed, these elements play major roles in biological systems, and there has been quite a lot of research focused on their roles within marine biogeochemical systems, which will be further explored in a later section.

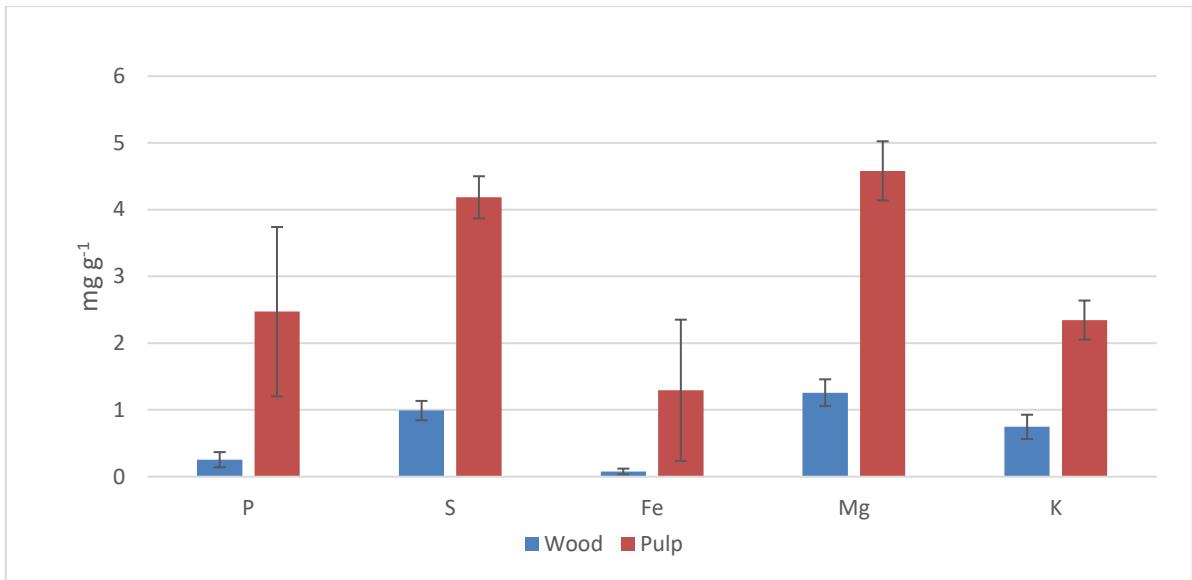


Figure 20: Comparison of P, S, Fe, Mg, and K concentrations in wood and pulp.

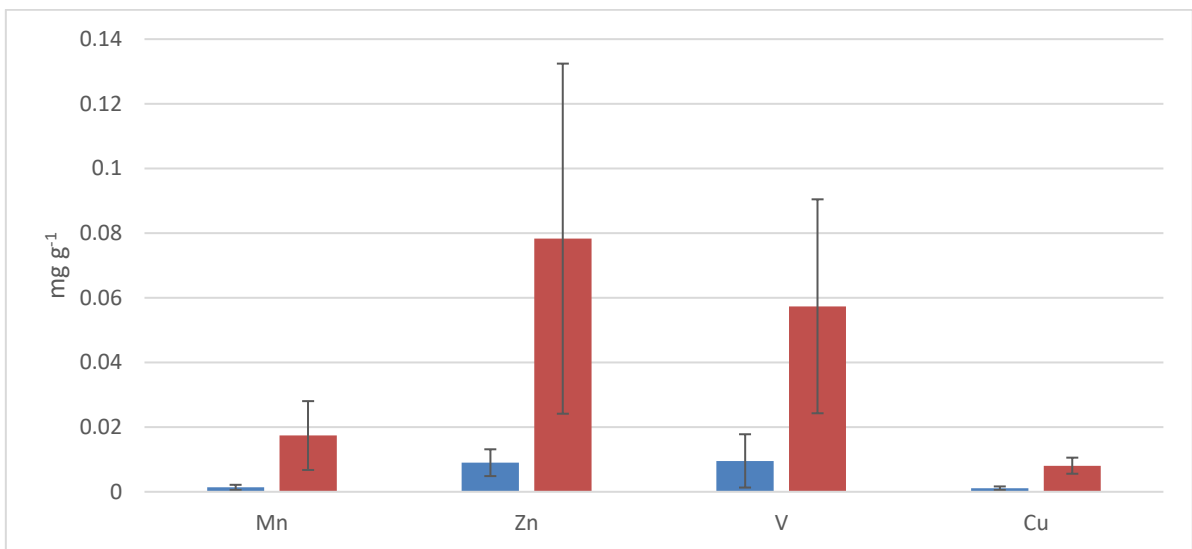


Figure 21: Comparison of Mn, Zn, V, and Cu concentrations in wood and pulp.

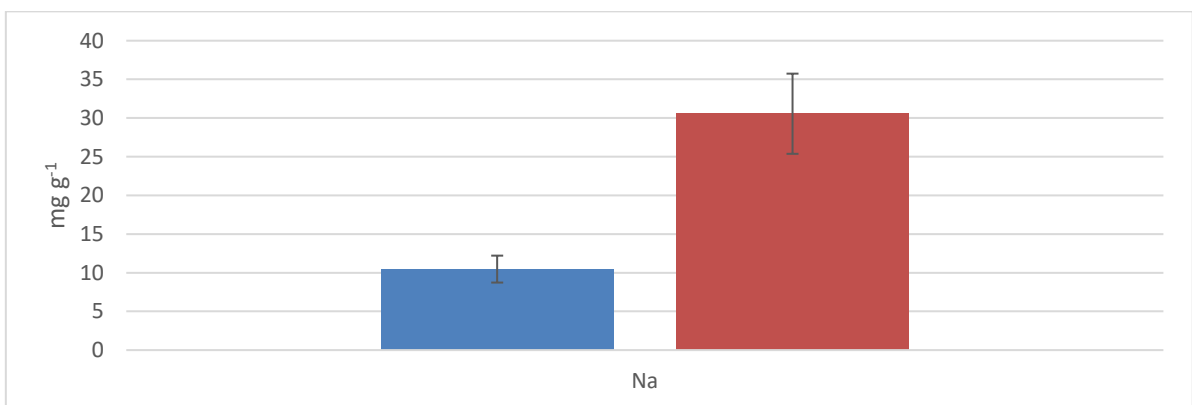


Figure 22: Comparison of Na concentrations in wood and pulp.

Changes in Elemental Composition of Wood and Pulp after Milli-Q Soaking

The effect of soaking wood samples overnight was tested for significance, again using t-tests with two-tailed hypotheses (table 16). No suitable results were obtained for Cobalt concentrations, but the results of the t-tests show that there was not a significant difference in any of the elements except for Mg, K, and Na, which all reduced in concentration after soaking.

Table 16: Results of paired T-Tests for elements in Soaked and Non-soaked wood samples.

Metal	Tstat	Tcrit	Significant Difference?
V	0.320	4.30	NO
Co	-	-	-
Ni	1.32	4.30	NO
Cd	0.376	4.30	NO
P	1.03	4.30	NO
S	3.30	4.30	NO
Fe	0.221	4.30	NO
Mn	1.58	4.30	NO
Zn	0.466	4.30	NO
Mg	5.38	4.30	YES
K	7.47	4.30	YES
Na	14.7	4.30	YES

Even for elements not showing significant concentration changes after soaking, concentrations still decreased in general, as can be seen in figures 23-25. This suggests that concentrations of elements in non-soaked samples - particularly in Mg, K, and N - are inaccurate, as soaking has proven to remove at least some free ions. This allows the determination of ions present within the actual wood. This in turn allows for a more accurate measurement of how Xylophagadidae might change the composition of the wood.

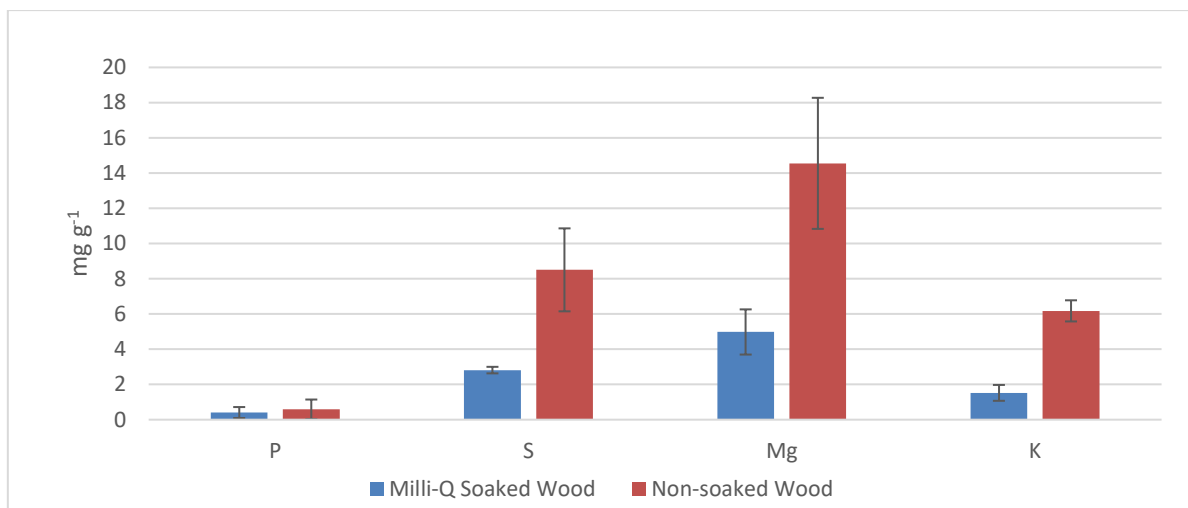


Figure 23: Comparison of in P, S, Mg, and K concentrations in Soaked and Non-soaked wood.

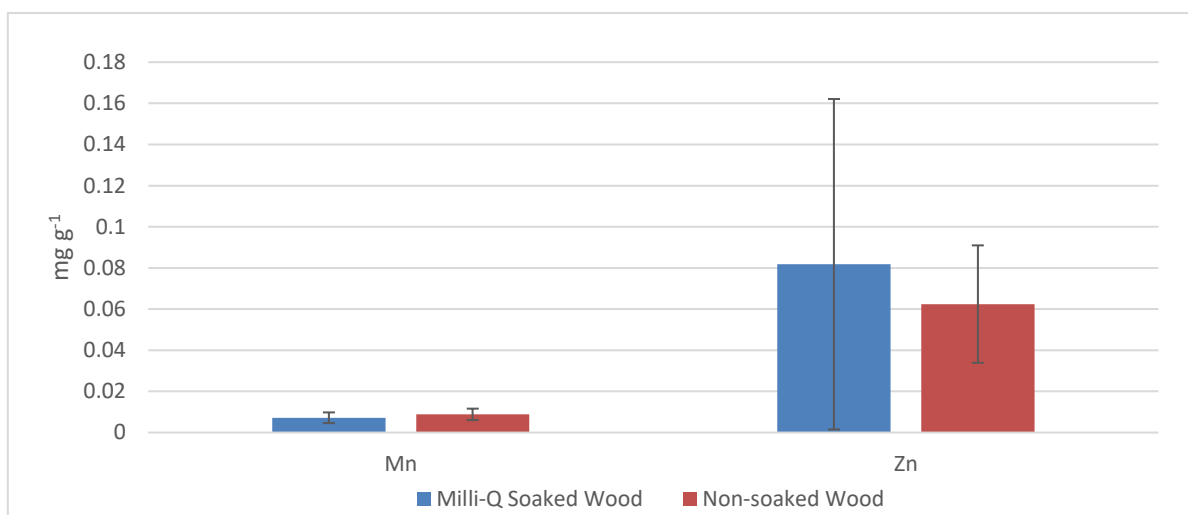


Figure 24: Comparison of Mn and Zn concentrations in Soaked and Non-soaked wood.

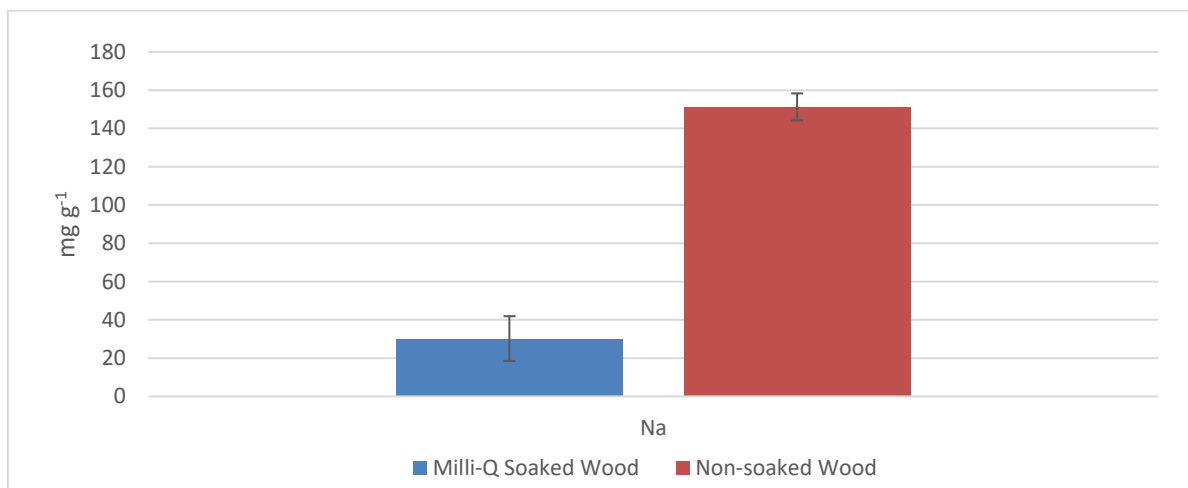


Figure 25: Comparison of Na concentrations in Soaked and Non-soaked wood.

As with the wood samples, the effect of Milli-Q soaking was also tested on pulp samples. A summary of the paired t-test results is shown in table 17. Again, Mg, K, and Na were amongst those significantly decreasing in concentration after soaking.

Table 17: Results of paired T-Tests for elements in Soaked and Non-soaked pulp samples.

Metal	Tstat	Tcrit	Significant Difference?
V	3.43	4.30	NO
Co	2.63	4.30	NO
Ni	1.05	4.30	NO
Cd	0.449	4.30	NO
P	5.24	4.30	YES
S	3.30	4.30	NO
Fe	1.75	4.30	NO
Mn	2.13	4.30	NO
Zn	2.91	4.30	NO
Mg	7.71	4.30	YES
K	47.4	4.30	YES
Na	14.7	4.30	YES

Additionally, in the soaked pulp samples, a significant decrease in P concentration occurred, as well as S decrease being close to significant.

This further supports the idea that concentrations of these elements in the marine-deployed wood can be partially attributed to the presence of free ions dissolved in water within the samples. The earlier presented data, showing a much greater moisture content in pulp samples compared to wood, likely contributes to the greater concentration change seen in soaked pulp samples- with a greater ability to hold water, free ion concentration should naturally be higher. Figures 26-28 shows the effect Milli-Q soaking pulp had on individual element concentrations.

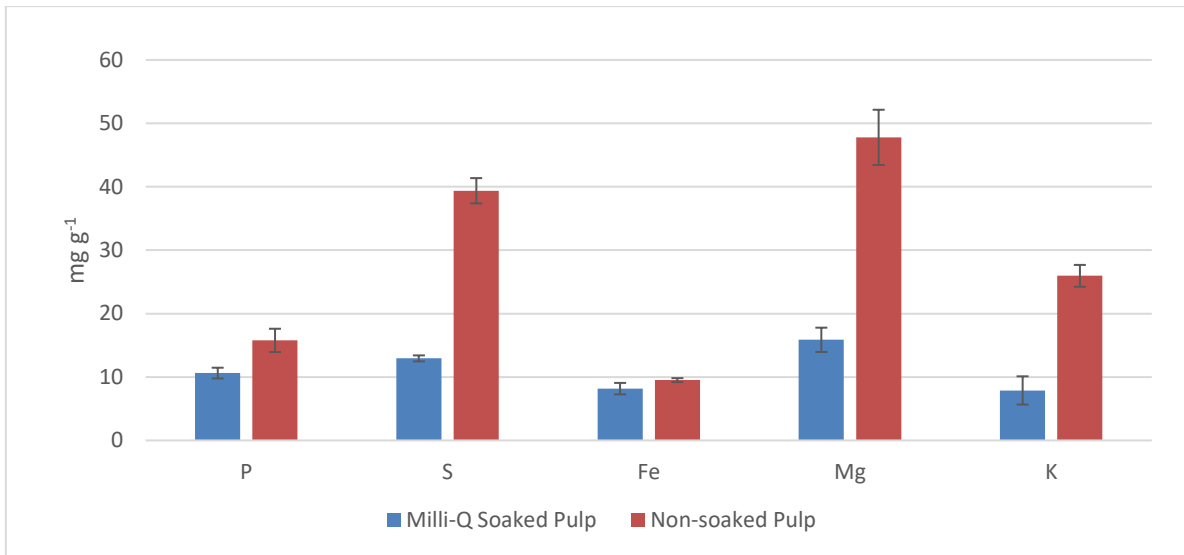


Figure 26: Comparison of P, S, Fe, Mg, and K concentrations in Soaked and Non-soaked pulp

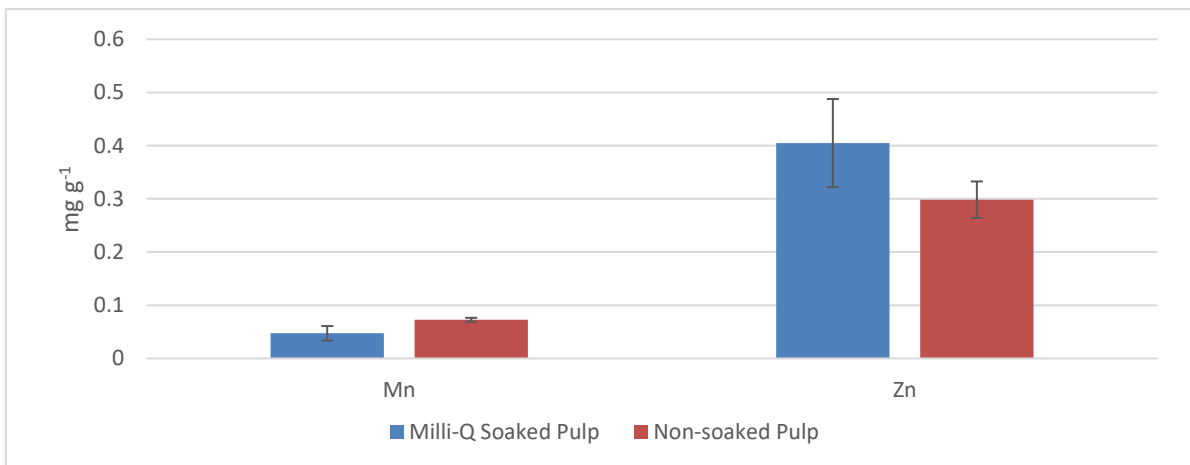


Figure 27: Comparison of Mn and Zn concentrations in Soaked and Non-soaked pulp.

It is clear that soaking the wood and pulp samples affected the results of some elements, particularly major seawater ions Na, Mg, and K. In order to determine the specific role of Xylophagaidae, comparisons were made between element concentrations determined in Milli-Q soaked wood and pulp samples. Table 18 details whether a significant difference for each element was found. The differences in both K and Na are no longer significant, showing that prior differences were likely caused only by the presence of free ions. The decrease in Mg also became much less significant. Referring to table 18, we can see that elemental changes in wood, upon digestion by Xylophagaidae, were significant for V, Cd, P, S, Fe, and Mg.

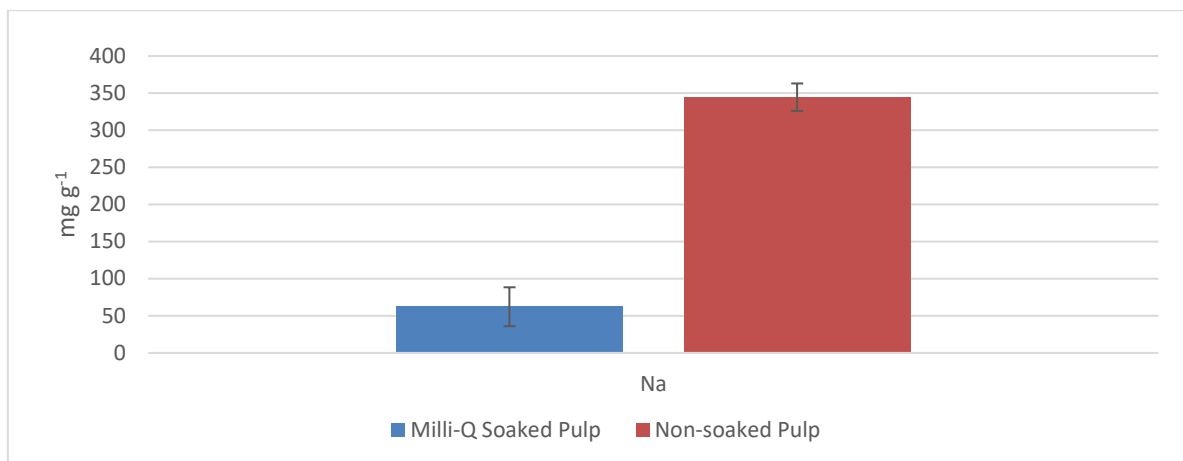


Figure 28: Comparison of Na concentrations in Soaked and Non-soaked pulp.

Table 18: Summary of t-test results displaying differences in Milli-Q soaked wood and pulp.

Metal	Tstat	Tcrit	Significant Difference?
V	12.8	4.30	YES
Co	N/A	N/A	N/A
Ni	1.70	4.30	NO
Cd	10.9	4.30	YES
P	17.1	4.30	YES
S	49.7	4.30	YES
Fe	12.6	4.30	YES
Mn	3.90	4.30	NO
Zn	3.59	4.30	NO
Mg	4.84	4.30	YES
K	3.93	4.30	NO
Na	1.93	4.30	NO

Conclusions

The research has compared two methods for elemental analysis of wood via ICP: microwave digestion of ash, and microwave digestion of dried wood. There was a statistically significant difference between the two methods in 10 out of 13 elements, presumably a result of differing digestion efficiency. Variation between species was minimal for moisture and CHN content, and variation in ash content fell within expected values from the literature.

For marine-deployed samples, wood was successfully separated from the pulp-like excretions of Xylophagidae. Initial ICP analysis showed significant differences for all elements except for Ni and Zn. Further investigation into the effects of soaking samples in Milli-Q water showed that initial determinations were inaccurate, particularly regarding Na, K, Mg, P, and S, as concentrations of these elements significantly reduced after soaking. This demonstrated the removal of free dissolved ions, in order to determine concentrations of elements within the actual wood structure. Thus, a clearer picture of the specific role of Xylophagidae biogeochemical cycles was generated. Elemental changes in the wood were significant for V, Cd, P, S, Fe, and Mg.

Further research should perhaps focus on the identification of organic ligands within this process, and their interactions with specific metals. On a simple scale, initial investigations could include CHN analysis of the Xylophagidae's pulp excretion, for quantification of inorganic carbon content, thus building more evidence for the presence of inorganic ligands in the pulp. This was not possible in this research due to a lack of pulp sample and an inability to analyse inorganic carbon content of wood in the undergraduate lab. More detailed investigating could be achieved with ligand exchange adsorptive cathodic stripping voltammetry (Gerringa, et al., 2016), or perhaps extracting and identifying organic acids from the samples via HPLC.

There should also perhaps be further investigations into patterns in species dispersion. The ability of species to survive is known to depend on the stability of factors like temperature and pH. The huge differences in habitation of wood blocks of the same species in this investigation suggest that there are variables affecting the ability of Xylophagidae to inhabit wood-falls and disperse to other ecosystems. It would also be useful to analyse habitation of different wood species, and to map the natural variation and influx of certain species.

Acknowledgements

Firstly, I would like to thank Dr Simon Ussher for being my project supervisor and beyond. You have been amazingly patient with me, and have shown me support through hard times. I am so grateful for your knowledge, kindness, and uplifting words.

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Finally, I thank my friends, Persi, and Lucinda, my housemates, Olivia, Tom and Ekene, and my sister, Emma. Thank you for keeping me sane (most of the time!), for making me laugh, and for your constant encouragement and love throughout the year.

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