Development of fluidic systems for water filtration and bio-separation

by

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B.Tech., Indian Institute of Technology Delhi (2013)
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Abstract

Biological contaminants and biomolecules play a major role in disease etiology and pathogenesis. In the context of disease prevention and management, removal of biological contaminants and biomolecules often relies on separations performed in fluidic systems. The design and operation of such systems relies fundamentally on understanding how fluidic transport phenomena are governed by material properties and effects such as sorption. This thesis focuses on understanding transport phenomena in two novel fluidic systems and leverages the insights to develop devices for water filtration and blood purification.

In the first section, we focus on the characterization and engineering of gymnosperm xylem for developing water filters. The xylem tissue, which transports water and nutrients in plants, has nanoscale pores that can remove contaminants from water. However, xylem's functional attributes as a water filter, such as flow rate, filtration capacity, rejection performance, susceptibility to foulants in water, etc., are not well-understood. Additionally, methods that can help tailor these attributes to suit practical needs have not been developed. We generate new insights into the mechanisms that govern the transport of water through xylem. These include the non-linear dependence of resistance to fluid flow on filter thickness explained using a percolation-based model, 'self-blocking' behavior governed by the dissolution and convective re-deposition of hemicellulose within the xylem conduits, and elevated propensity for fouling in the presence of large organic molecules and dust. We use these insights to develop methods for fabrication of practically useful xylem filters. We demonstrate that these filters have shelf-life >2 years and can provide >3 log removal of *E. coli*, MS-2 phage, and rotavirus from synthetic test waters and coliform bacteria from natural water sources. To show how xylem could be incorporated in filtration devices, we develop a gravity-operated functional device prototype for household drinking water treatment using user-centered design approaches. The findings related to the characterization, modeling, and engineering of xylem reported in the thesis fundamentally advance the state of knowledge about xylem tissue and lay the groundwork for the design and development of a wide variety of xylem-based devices in the future.

In the second section, we focus on modeling cytokine transport in an extracorporeal blood purification (EBP) device for managing hypercytokinemia. Traditional EBP methods, which focus on non-specific removal of broad-spectrum cytokines to regulate host immune response, have many disadvantages, such as potential immuno-suppression and elimination of desirable molecules. A cytokine-specific EBP method can overcome these drawbacks. We study the cytokine binding and transport characteristics in a device, where selective cytokine removal is achieved by pumping the blood through tubes coated with antibodies. Analogous to the Lévêque problem, we develop a mass transport model which can predict the rate of cytokine removal and volumetric clearance as a function of device geometry, operational conditions, and surface properties. These predictions matched *in vitro* experimental results. In the future, such devices could be used for creating flexible and highly selective blood-filtering platforms for elimination of individual, harmful cytokines as they are expressed, facilitating the development of personalized treatment strategies.

Thesis Supervisor: Rohit N. Karnik
Title: Professor of Mechanical Engineering
Associate Department Head for Education

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Chapter 1

Introduction

Biological contaminants and biomolecules play a major role in disease etiology, pathogenesis and management [4, 5]. Biological contaminants, which include microbes, allergens, and molds, can cause inflammations, infections, allergies etc. and have an adverse impact on human health [6]. Infectious diseases caused by pathogenic microbes were the third major cause of global death in 2016 [7]. Biomolecules comprise primarily of carbohydrates, proteins, nucleic acids and lipids [8]. While dysfunctional biomolecular interactions are known to be causative factors for several genetic diseases and disorders [9, 10], biomolecules are also an integral part of the immune system and significantly influence host response to a disease and its progression [11]. Further, biomolecules such as antibodies and peptides have also become increasingly popular as novel drug modalities for therapeutic applications.

In the context of disease prevention and management, the use of separation-based fluidic methods has been widely explored for the removal and isolation of biological contaminants and biomolecules [12, 13, 14]. Transport phenomena play a critical role in the design and development of such devices. Separation is typically achieved due to the variation in transport behavior between the target and bulk, which arises because of differences in physical, chemical, mechanical, and electrical properties. Understanding the nature of these variations is critical to guide the material selection, design, and operation of devices targeted towards separation-based applications. For example, deterministic lateral displacement, a technique where differences in hydrodynamic properties are exploited to modulate transport trajectory, has been used for cell sorting [15, 16]. Antibody-binding methods, which use differences in the chemical affinity of antigens expressed on cell surface for sorption-based separation, are also quite popular [17, 18]. Size-based separation is used in air filters to inhibit the transport of molds and air-borne pathogens [19]. The same principle is used in water filters for removal of microbial contaminants to make water safe for drinking [20] and in dialysis for removing excess solutes and toxins from blood to treat patients with dysfunctional kidneys [21].

Through the investigation of transport and sorption phenomena in two under-explored fluidic systems, this thesis focuses on the design and development of devices for water filtration and blood purification:

1. **Engineering and characterization of gymnosperm xylem for developing water filters:** Naturally-occurring membranes in gymnosperm xylem have nanoscale pores, which can be used for removing microbial contaminants from water. While the hydraulic properties of xylem have been well-characterized in the context of sap transport in plants [22, 23, 24], xylem's functional attributes as a water filter, such as flow rate, filtration capacity, and variation in flow rate over time, particularly with contaminated water as the fluid medium and in the absence of active transport mechanisms that regulate flow in plants, are currently not well-understood. The thesis aims to bridge this knowledge gap to determine if xylem is a suitable material for water filtration. Using these insights, the thesis also focuses on developing engineering methods for fabricating practically useful xylem filters and devices for household water treatment.

2. **Modeling transport behavior in extracorporeal blood purification (EBP) devices for managing hypercytokinemia:** Traditional EBP methods, which focus on non-specific removal of broad-spectrum cytokines to regulate host immune response, have many disadvantages, such as potential immuno-suppression and elimination of desirable molecules. A cytokine-specific EBP method can overcome these drawbacks. This thesis focuses on modeling the cytokine transport and binding behavior in a cytokine-specific EBP method, where selectivity is achieved by pumping blood through tubes coated with antibodies. The transport and binding characteristics are studied as a function of device geometry, operational conditions, and surface properties to develop a mass transport model which can predict the performance of the device and

guide device design.

A detailed description of the background and the work accomplished in the context of each of these aims has been provided in subsequent chapters. Part-I (Chapters 2-6) focus water filtration using xylem. Part-II (Chapter-7) focuses on removal of biomolecules using extracorporeal blood purification device. Additionally, the thesis also covers preliminary work on development of graphene-based device for analytical sample preparation in biotherapeutic applications, which has been described in Appendix D.

Part I

Water filtration using gymnosperm xylem

Chapter 2

Background to xylem filters

2.1 Motivation

Access to safe drinking water is a cause of global health concern. Diarrheal diseases caused by microbial contamination of water and poor sanitation are a global problem. In 2019, diarrheal diseases accounted for 1.5 million deaths per year, primarily in resource-limited settings amongst children under the age of five [7]. Majority of the deaths (57.8%) are caused by bacterial pathogens, while water-borne viruses and protozoa account for 33.8% and 8.3% of the fatalities respectively [25]. The number of deaths caused by different microorganisms and their size is depicted in Figure 2-1.

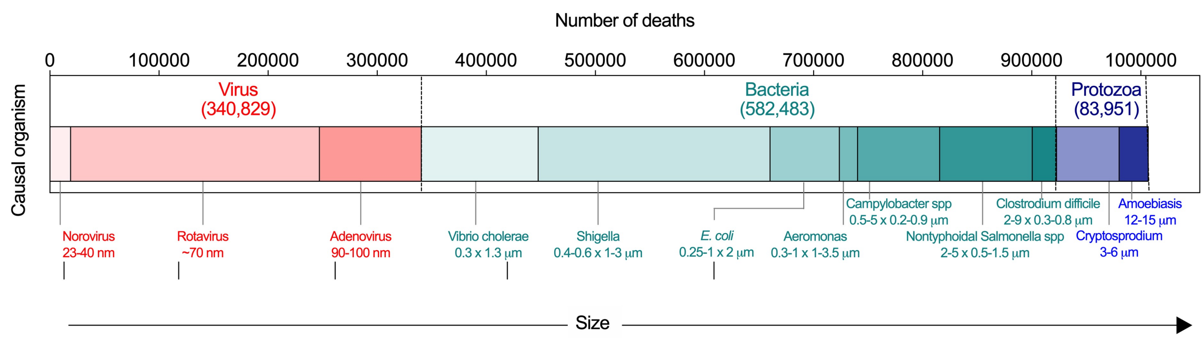

Figure 2-1: Diarrheal deaths caused by different microorganisms and their size

Household water treatment (HWT) methods like chlorination, solar disinfection, and filtration can significantly reduce the risk of diarrheal diseases [26, 27]. However, the adoption of these methods in resource-constrained settings is often hindered by their limited availability in remote locations, incompatibility with local socio-cultural practices, high cost, or lack of suitable financing schemes [26, 27, 28]. In addition, the common perception that water that appears clear is safe for drinking, and the difficulty in appreciating the link between diarrheal diseases and poor water quality, also impede uptake [26, 27, 28]. Novel water treatment technologies that are inexpensive, readily available, socially acceptable, and effective against water-borne pathogens have the potential to address these challenges and improve access to safe drinking water.

2.2 Background

Gymnosperm (non-flowering plants like conifers) wood, a common material that is widely available and traded across the globe [29], presents the intriguing possibility of creating inexpensive, sustainable, and socially-acceptable filters to address this challenge [30, 31, 32, 33]. The gymnosperm sapwood consists largely of xylem tissue that conducts sap, with longitudinally-oriented conduits called tracheids up to 10 mm long that are interconnected by 'pit membranes' with pore size ranging from 100–500 nm (Figure 2-2) [23].

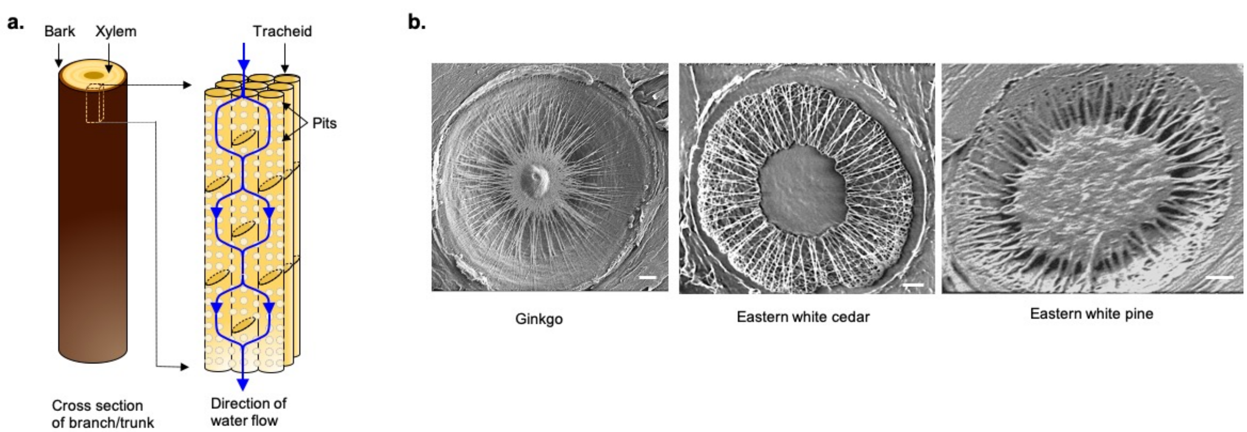

Figure 2-2: Structure of gymnosperm xylem **a.** Schematic illustration. **b.** SEM images of pit membranes in different gymnosperms [1]. Scale bar, 1 μm

Fluid flowing through a transverse section of a branch that is thicker than a single tracheid must therefore pass through the pit membranes, which can act as physical sieves that trap particulate contaminants present in water [30] (Figure 2-3). Compared to most angiosperms (flowering plants), the short length of tracheids and their high proportion in the cross-section makes gymnosperm sapwood better-suited to creating compact filters.

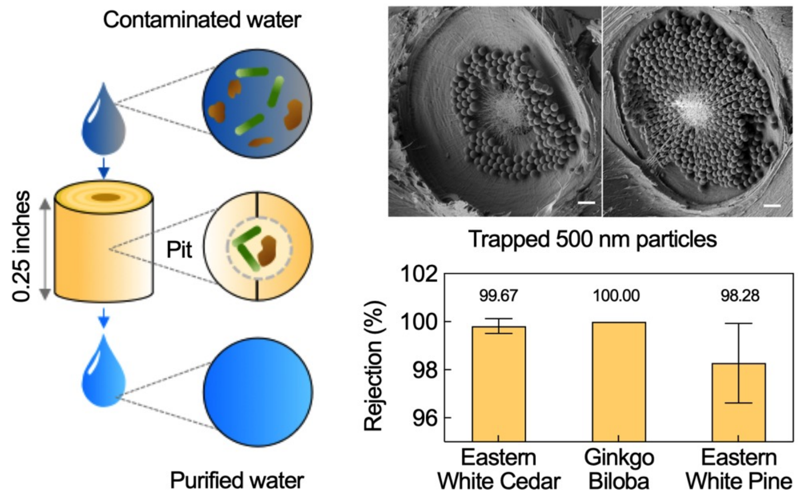

Figure 2-3: Schematic depiction and SEM images of filtration of 500 nm particles by xylem pit membranes in a section of a branch (ginkgo, 1 cm diameter, 0.25 inch thickness) [1]. Scale bar, 1 μm. The bar graph shows rejection of 500 nm particles (mean ± s.d., n=3) by fresh xylem filters made from different tree species (1 cm diameter, 0.25 inch thickness; see Method M4 in Appendix A for details).

The unique structure of gymnosperm xylem gives rise to two interesting questions: a) is the xylem a suitable material for water filtration, and b) if so, how can it be engineered to create practically useful water filters.

Previous studies have reported that pit membranes in pine xylem can filter bacteria from deionized water and incorporation of silver nanoparticles in xylem can enhance removal of bacteria [30, 33]. A known challenge with xylem filters is that their permeance (defined as flow rate per unit area per unit pressure difference) drops significantly upon drying, which limits their usability in dry state [30]. Wet filters have reasonable permeance, but have limited shelf-life due to their propensity for degradation and are heavy to transport. Thus, methods for preserving xylem in dry state are critical for their supply and distribution, particularly to remote, low-resource settings where they are most needed.

Previous studies have shown that the xylem filters can be preserved in a dry state by controlling their filter thickness [1]. The permeance in dried filters strongly depends on filter thickness. While the underlying reason for this behavior was not known, it was reported that permeance drops sharply upon increasing filter thickness beyond 0.25 inches. Since filters have to be thicker than the conduit length to ensure contaminant removal, the minimum filter thickness is bounded by the conduit length in gymnosperms (which is typically <0.22-inches long) [24]. To further improve rejection performance, multiple filters could be stacked in series (Figure 2-4). Two 0.25-inch thick water-dried filters in series were reported to have better rejection than a single 0.25-inch thick filter (1.70±0.24 log versus 0.95±0.16 log (p=0.015)), with the log rejection being additive, and higher rejection and permeance recovery than a 0.50-inch thick filter (1.70±0.24 log versus 1.26±0.06 log (p=0.04), and 48.8±4.9% versus 1.8±1.0% (p = 0.0013), respectively). Stacking could be also used in conjunction with solvent treatment to further improve the rejection performance of filters, which offers opportunities for tailoring the rejection capability of xylem to suit different applications.

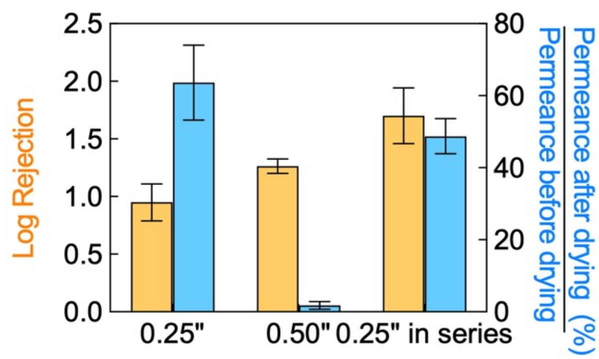

Figure 2-4: Two 0.25-inch water-dried filters stacked in series perform significantly better than 0.25-inch and 0.50-inch thick water-dried filters (n=3, mean±s.d). [1]

Preliminary evidence also suggests that treating xylem filters with alcohols like ethanol before drying could offer yet another method for preserving filters in dry format [1]. The blockage of xylem filters upon drying is related to the physiological function of pit membranes that have evolved to protect the plant against cavitation (i.e., nucleation of vapor bubbles) [23] that could severely disrupt sap flow. In gymnosperms, surface tension forces of a receding liquid meniscus (corresponding to an advancing vapor bubble) pull the pit membrane towards an aperture in the cell wall; water-mediated adhesive forces cause the pit membrane to seal against the cell wall, thereby isolating any cavitated conduits [34, 35]. While the exact mechanism underlying this phenomenon, referred to as 'pit aspiration', remains to be elucidated, it relies on the presence of water to mediate adhesion [35]. Similar to cavitation, drying induces the formation of liquid-vapor interfaces in the xylem, which triggers pit aspiration and reduces the permeance (with prior work reporting 100× drop in flow rate for 1-inch thick filters) [30]. Pit aspiration can be reduced by replacing the sap in the xylem with non-aqueous solvents, like alcohols, as it precludes water-mediated adhesion between the pit membrane and the cell wall during drying [34, 35, 36]. Leveraging these findings, a method for dry preservation of xylem filters involving treating them with ethanol before drying was developed [1]. Pit membranes in ethanol-dried filters were found to be unaspirated while those in water-dried filters were aspirated (Figure 2-5).

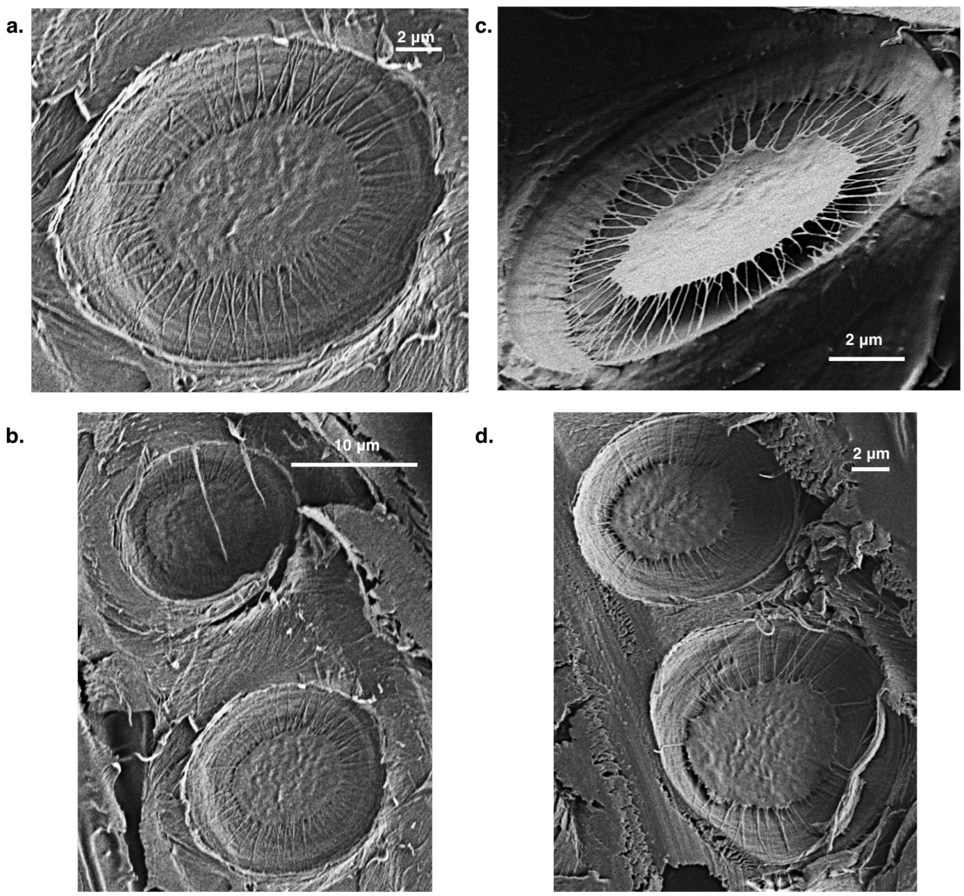

Figure 2-5: Effect of ethanol treatment on pit membranes. **a,b.** Aspirated pit membranes in water-dried filters. **c,d.** Unaspirated pit membranes in ethanol-dried filters. [1]

Preliminary experiments showed that ethanol treatment results in higher recovery (ratio of flow rate after and before drying) and the effect of ethanol treatment is more pronounced for thicker filters.

While significant progress has been made towards preserving xylem filters in dry state, the underlying reason for the strong non-linear dependence of permeance on length is not fully

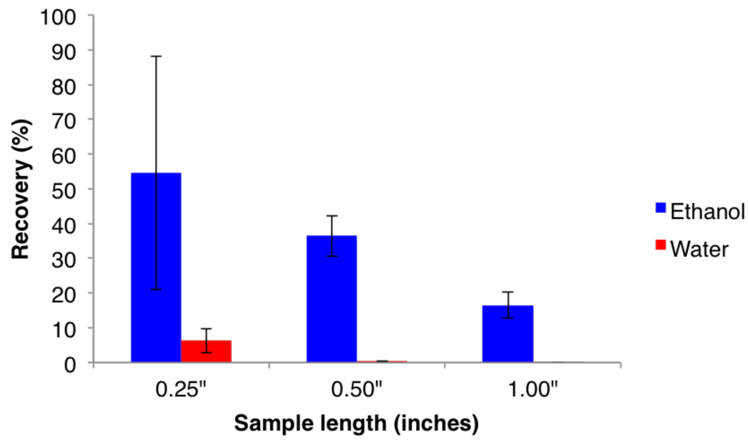

Figure 2-6: Effect of ethanol treatment on recovery for filters with different thickness [1].

understood. Experimental results from prior studies suggest that this dependence could potentially be explained using percolation theory, but further investigation is warranted to conclusively prove this hypothesis. Questions such as what is the shelf-life of ethanol-treated filters, how repeatable and reliable is ethanol treatment given that the structural characteristics of the xylem vary considerably within and across species, what is the effect of ethanol treatment on rejection performance of filters and whether other solvents can be used instead of ethanol remain to be answered. Further, several other material characteristics of xylem that are critical for practical water filtration applications, such as its structural stability over the course of its operational life, susceptibility to different foulants present in water, and mechanisms of fouling, etc. remain to be explored. While the hydraulic properties of xylem have been well-characterized in the context of sap transport in plants [22, 24, 37], xylem's functional attributes as a water filter, such as flow rate, filtration capacity, and variation in flow rate over time, etc., particularly with contaminated water as the fluid medium and in the absence of active transport mechanisms that regulate flow in plants, are currently not well-understood. Additionally, simple and inexpensive methods for filter design and manufacture that help tailor xylem's functional attributes to suit practical needs are required to facilitate technology translation.

2.3 Scope of thesis for Part-I

The thesis focuses on the following:

- Investigation of the reason underlying the strong non-linear dependence of permeance on length.
- Characterization of the effect of ethanol treatment on permeance, rejection performance, and shelf-life of xylem filters.
- Investigation of material and operational characteristics of xylem (structural stability of xylem over the course of its shelf- and operational-life, effect of contaminants on the permeance, lifetime and rejection performance, fouling behavior and mechanisms).
- Development of methods for designing and manufacturing practically useful xylem filters.
- Technology translation through field validation of filter performance, design and development of functional xylem filtration device prototype and identification of implementation pathways.

2.4 Outline for Part-I

- Chapter 3 will focus on elucidating the reason underlying the non-linear dependence of permeance on filter thickness.
- The stability of xylem filters over the course of their operational and shelf-life, fouling behavior of xylem filters in the presence of contaminants, and the the underlying fouling mechanisms are described in Chapter 4.
- Findings from field studies aimed at technology validation, understanding user preferences, and design and development of xylem filtration device prototypes have been presented in Chapter 5.
- Chapter 6 provides a summary and recommendations for future work with regards to xylem filter development.

Chapter 3

Understanding and characterizing dry preservation methods for xylem filters

This chapter focuses on understanding the underlying reason for the strong dependence of permeance on thickness in dried filters and characterizing the effect of ethanol treatment on the permeance, rejection performance, and shelf-life of xylem filters.

3.1 Non-linear dependence of permeance on thickness in dried filters

3.1.1 Background

Traditionally, Darcy's law, which is commonly applied to porous media and predicts a linearly inverse relation between thickness and permeance (i.e., permeability, defined as permeance normalized by thickness, is constant), has been used to model the permeance of xylem [38, 39]. While the Darcy's law was found to well followed in fresh xylem filters, the inverse dependence of permeance on thickness in dried filters was highly non-linear; permeability dropped abruptly on increasing filter thickness beyond 0.25 inches Figure 3-1 [1].

Percolation theory offers a potential explanation for such a behavior. Permeability of dried xylem filters is a function of not only the flow resistance of tracheids and pit membranes [22, 24] but also the tracheid interconnectivity [40]. The length scale over which tracheids

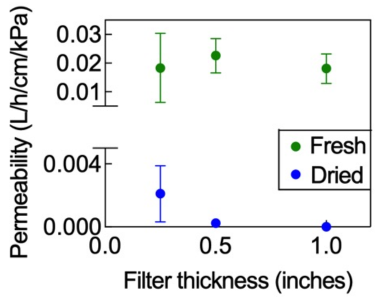

Figure 3-1: Permeability (permeance normalized by thickness) is constant with filter thickness in fresh filters, but drops abruptly with increasing thickness in the case of dried filters.). [1]

maintain connectivity depends on the degree to which the pit membranes get blocked during drying, and corresponds to cluster size in percolation theory [40, 41, 36]. Filters much thicker than this length scale of connectivity will be impermeable to flow, while those that are thinner, will have non-zero permeance (Figure 3-2). When filter thickness is comparable to this length scale, a highly non-linear dependence of permeance on thickness that deviates strongly from Darcy's law, is expected.

3.1.2 Simulation of percolation effects in xylem filters

To understand whether percolation theory can explain the strong non-linear dependence of permeance on filter thickness, a probabilistic model based on percolation theory [41] to capture the flow characteristics of a dried filter was developed.

Model details

The xylem was modeled as a 2-D node-edge network in Matlab, where the tracheids and pits correspond to the nodes and edges respectively (Figure 3-2). The model was designed to represent xylem filters made from Eastern white pine having 1 cm diameter and 1.5 inch thickness. Assuming that, on average, the tracheids are 4-mm long and 40 μm in diameter [42, 43] and that 80% of the filter cross section area is sapwood (typical for our filters), the model comprised a square lattice of 10×200 tracheids along the filter thickness and cross-section, respectively. For simplicity, it was assumed that each tracheid was connected to four other tracheids (Figure 3-2). The non-zero permeance of 0.25-inch thick sections in dried

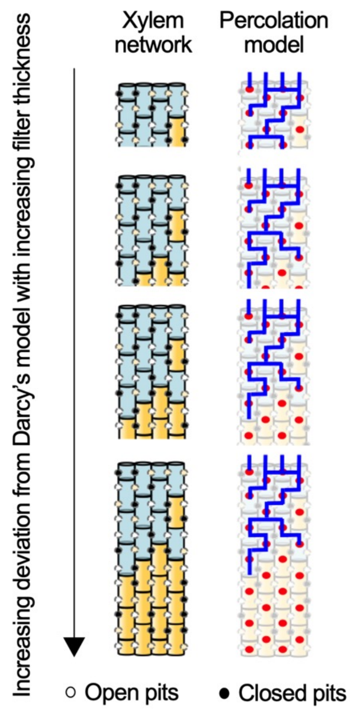

Figure 3-2: Schematic of the physical structure of xylem and the proposed percolation-based model (tracheids depicted as red dots) for filters of different thickness, illustrating percolation-governed length dependence. Black dots represent blocked tracheid connections (pit aspiration probability, p=0.35 for all filters). Blue shading and lines represent tracheids and flow pathways that are connected to the top surface; there is no flow pathway from the top to bottom surface for the two thickest filters.

filters indicate that some pit membranes remain open (unaspirated) even after drying, i.e., the probability of pit aspiration blocking off a tracheid-tracheid interconnection upon drying is <1.The model associated a variable probability p for an edge being broken [41], which in the case of a xylem filter represents the likelihood of connectivity between two tracheids being broken by pit aspiration. To simplify the problem, it was assumed that all tracheid-tracheid connections were either permeable (open) or aspirated (closed) with each tracheid-tracheid connection having an equal aspiration probability during drying. In general, the pit aspiration probability will depend on the condition of the neighboring tracheids and pit membranes, and may vary along the filter thickness depending on how the drying front propagates within the filter; such effects are not captured in the model.

This xylem structure was modeled using a 3-D matrix with the x and y indices representing the relative location of tracheids and the z indices representing the 'open' or 'closed' state (denoted by '1' and '0') of the four tracheid-tracheid connections after drying. A $10\times100\times4$

matrix representing alternate tracheid columns accounted for all the tracheid-tracheid connections. To assign open/closed status to the tracheid-tracheid connections, uniform random numbers in the range of 0 to 1 were generated for each connection, and connections with numbers higher than the aspiration probability were designated to be open while the rest were designated closed. Depending on which tracheid-tracheid connections were open, tracheids that were connected to at least one tracheid in the top row of the filter through one or more open pathways were identified. The relevant codes have been provided in Appendix A Method M18.

Results

Simulations of this percolation model in a simplified, 2-D xylem network using MATLAB corroborated experimental observations; for a given pit aspiration probability, the connectivity (and thus the permeance) dropped to zero beyond a critical filter thickness (Figure 3-3; to generate the figure, the fraction of tracheids in each row connected to the top row of the filter was computed and plotted against the row number on the x-axis after averaging over 100 simulations).

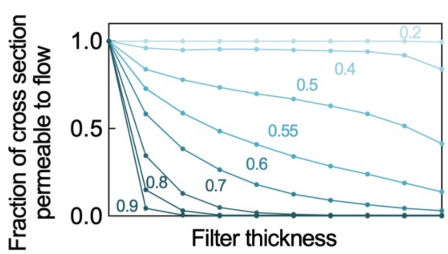

Figure 3-3: Variation of the filter cross-section that is permeable to flow (i.e., connected to the top face) along filter thickness for different pit aspiration probabilities (probability values are color-coded and specified next to the corresponding curves).

Further, the model suggested that the converse should also be true, i.e., for a given filter thickness, there exists a critical probability p=p_c, at which there is transition from zero to non-zero permeance (Figure 3-4; to generate this graph, the fraction of tracheids in the last row connected to at least one of the tracheids in the top row (row 10) was computed for different pit aspiration probabilities and averaged over 100 simulations. The insets in Figure 3-4 were generated for one simulation at a given pit aspiration probability using the ‘imagesc(xmap)’ function in MATLAB).

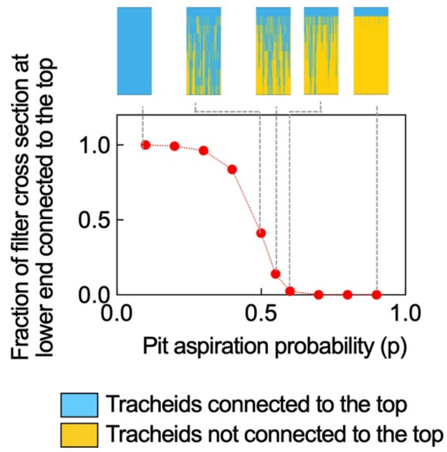

Figure 3-4: Variation in fraction of filter cross-section connected to the top with pit aspiration probability. Insets show interconnected tracheids in blue for different pit aspiration probabilities.

The results of the simulation explain the abrupt drop in filter permeance with increasing thickness and also why ethanol-dried filters have a higher permeance than water-dried filters. The length scale of connectivity in water-dried filters is ∼0.25 inches, which is why filters thicker than 0.25 inches have zero permeance (this finding corroborates previous experimental results where where 1.5-inch dried filters made from Eastern white pine were completely blocked, but 0.25-inch sections cut from the same blocked filters were permeable to flow [1]). Treatment with ethanol reduces the probability of pit aspiration and consequently, results in longer length scales for connectivity. This is evident from the non-zero permeance of 0.5- and 1-inch thick ethanol-dried filters.

3.2 Characterization of ethanol treatment for dry preservation of xylem filters

The effect of ethanol treatment on permeance, rejection, and shelf-life of dried filters was studied (see Methods M2 and M3). Consistent with previous findings [1], ethanol-dried filters exhibited higher permeance than their water-dried counterparts (Figure 3-5a); the effect of ethanol treatment was found to be more pronounced for thicker filters (0.5- and 1.0-inch thick) where water-dried filters were almost completely blocked whereas ethanol-dried filters retained permeance. The rejection performance of ethanol-dried filters with 1 μm microspheres was significantly better than water-dried filters (p <0.001 for 0.25-, 0.50-, and 1-inch thick filters respectively) and comparable to fresh filters (p = 0.02, 0.59, and 0.08 for 0.25-, 0.50-, and 1-inch thick filters respectively) (Figure 3-5b). The shelf-life of ethanol-preserved filters was at least 2 years (Figure 3-5c). Ginkgo filters (4 cm diameter, 0.375-inch thickness) stored for 2 years had a comparable permeance to those that were tested immediately post-drying with General Test Water (composition of General Test Water provided in Table 4.1). These filters were also able to achieve 3-log removal of *E. coli* (further details provided in Section 4.6).

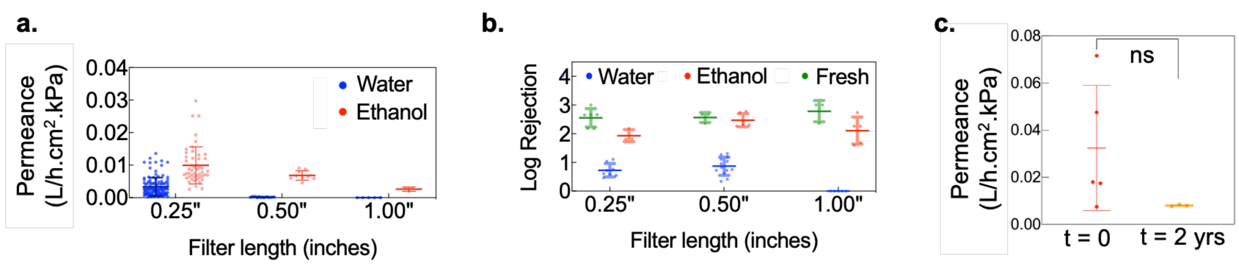

Figure 3-5: Effect of ethanol treatment on performance of dried filters. **a,b.** Ethanol-drying improves permeance and rejection over water-drying in a thickness-dependent manner. Individual data points and mean±s.d. are shown. 33 data points in **(a)** and 15 data points in **(b)** overlap with prior work [1]. **c.** Ethanol-dried filters have a shelf-life of at least 2 years. No significant different was observed between the permeance of ethanol-dried filters tested immediately after drying and those tested after 2 years.

3.3 Summary of findings

The strong dependence of permeance on filter thickness in dried filters was explained using the percolation-based model of xylem network. The length of scale of interconnectivity set by the pit aspiration probabilty established to a threshold filter thickness beyond which permeance sharply declined to zero. The effect of ethanol treatment on permeance, rejection, and shelf-life of dried filters was characterized. Filters treated with ethanol were found to have consistenly high permeance than water-dried filters and rejection performance comparable to fresh filters. The shelf-life of ethanol-preserved filters was found to be at least 2 years.

Chapter 4

Material and operational characteristics of xylem as a water filtration membrane

4.1 Introduction

The key performance characteristics important for a water filter include its flow rate or permeance (flow rate normalized by area), lifetime or volumetric capacity (the total amount of water that can be processed before the filter needs to be replaced), rejection of contaminants, and variation in permeance and rejection over the course of its operational life. This chapters explores the aforementioned characteristics of xylem filters and their suitability for practical use.

4.2 Criteria for practically useful water filters

Literature reports and our field trips to India (described in Chapter 5 in more detail) revealed that, to be useful in households in resource-limited settings, xylem filters should a) process at least 8 L of water to meet the daily drinking water requirement (see Section 5.2.1), b) have flow rates of at least 1 L/h, c) effectively remove contaminants [44], d) function reliably with contaminated water, e) operate under gravity with heads less than 1 m to minimize operation costs and space requirements, and f) be easy to access and use [44] (see Section 5.2.1).

4.3 Permeance of dry-preserved xylem filters

Dry preservation (using either solvent treatment or thickness control) is critical to preserve the permeance and rejection performance of dried xylem filters [1]. To evaluate whether dry preservation techniques can be used reliably to improve the shelf-life of xylem filters, the permeance of 47 filters made from different Eastern white pine trees in Cambridage, MA were tested over a two-year period. The permeance of these filters was comparable to commercial micro-filtration membranes with similar pore size; the permeance range for commercial membranes is 0.002–0.05 L/h.cm^2.kPa [45, 46, 47, 48, 49] while 95% of the ethanol-dried filters consistently had permeance greater than 0.005 L/h.cm^2.kPa (Figure 4-1).

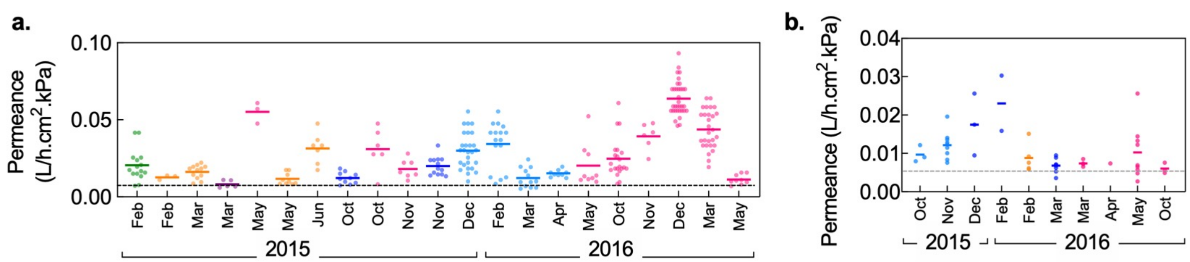

Figure 4-1: Permeance of (a) fresh filters (n = 260) and (b) ethanol-dried filters (n = 47) measured over a two-year period. Permeance of 95% of the filters exceeded 0.005 L/h.cm^2.kPa (shown by dotted line). Different colors denote different trees. Individual data points and their mean values are shown. Individual data points correspond to single measurements on different filters.

Further, ethanol-preserved filters had a shelf-life of at least one year. The permeance of filters stored for one year was 0.0074±0.0003 L/h.cm^2.kPa and the rejection of 1 μm microspheres was 99.92±0.05%). Ethanol-water mixtures with ethanol concentration $>$ 90% and other alcohols like isopropanol can also be used for dry preservation (Figure 4-2). The solvent used for dry-preservation must be certified as food-grade and the level of residual solvent in dried filters should be maintained within the permissible limits for human consumption as prescribed by food safety standards [50].

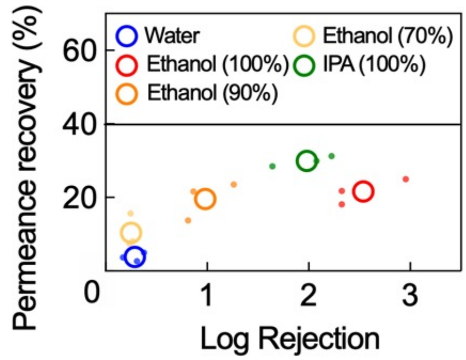

Figure 4-2: Effect of varying ethanol concentration and using isopropanol (IPA) on permeance recovery and rejection of xylem filters (filter thickness of 0.50 inches was used because the effect of alcohol treatment is more pronounced for thicker filters, facilitating comparison). Small, filled circles show individual data points while the large, open circles denote mean values. n = 3. Ethanol-water mixtures with concentration below 90% are ineffective in preserving permeance and rejection.

4.4 'Self-fouling' and its control

In membrane-based filters, fouling due to contaminants in the feed water determines the filter's volumetric capacity, i.e., the total amount of water that can be processed before the filter needs to be replaced [51]. Surprisingly, the flow rate of xylem filters was observed to decline, eventually resulting to blockage after a certain period of time even when filtering uncontaminated, deionized (DI) water (Figure 4-3).

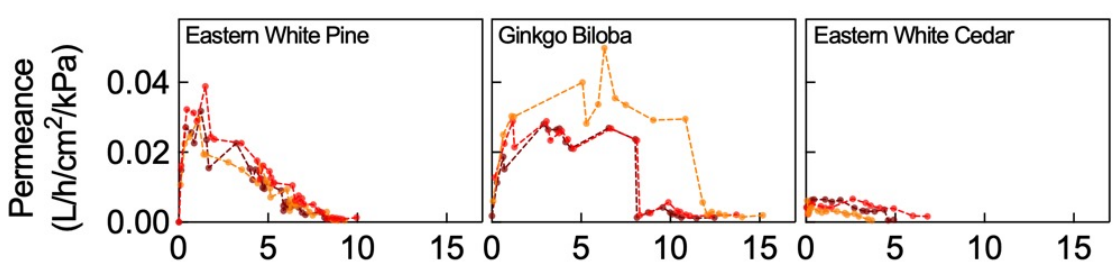

Figure 4-3: Permeance of 0.25-inch thick ethanol-dried filters made from different gymnosperm species decreases with permeate volume when filtering deionized water (n=3, denoted by different colors).

To explain the drop in permeance of xylem filters in the absence of external contaminants, the following hypotheses were explored:

1. **Pit aspiration:** Filters could get blocked due to pit aspiration induced by the

nucleation of gas bubbles (i.e., cavitation) at the low-pressure end of the filter, where gas solubility is lower than the high-pressure end. However, operating ethanol-dried Eastern white pine filters (1 cm diameter, 0.25 inch thickness) at lower pressures to reduce the variation in solubility across the filter thickness failed to preserve permeance (Figure 4-4a), suggesting that the underlying mechanism is not cavitation-driven.

2. Swelling of pit membrane fibrils: Previous studies have reported that the fibrils in the pit membranes swell to form a gel in the absence of Ca^{2+} and K^{+} ions normally present in sap [52, 53], which could result in a loss of permeable membrane area leading to a drop in permeance. However, addition of Ca^{2+} and K^{+} to DI water at physiological concentrations found in plants failed to improve the capacity of ethanol-dried Eastern white pine filters (1 cm diameter, 0.25 inch thickness), ruling out this hypothesis (Figure 4-4b)

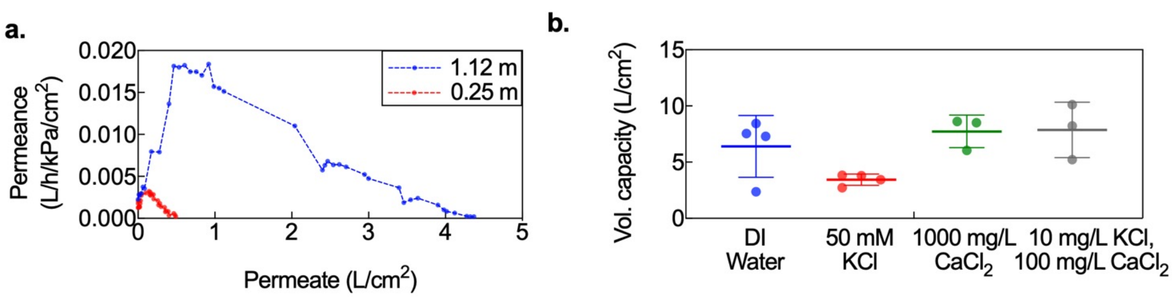

Figure 4-4: Effect of changing gravity head and adding ions on blocking of xylem filters (data reported with 1 cm diameter, 0.25-inch thick Eastern white pine filters). **a.** Use of smaller gravitational head to drive flow fails to prevent permeance from declining to zero, suggesting that cavitation does not play a major role in filter blockage. Each data set corresponds to measurements with a single filter. **b.** Effect of addition of Ca^{2+} and K^{+} ions in DI water on volumetric capacity. Mean±s.d., n = 3. Addition of ions to does not improve volumetric capacity.

3. Material deposition: After ruling out the aforementioned mechanisms, clogging of filters due to deposition of materials from within the filters was considered. In contrast to filters in operation, those soaked in DI water (without flow) over similar time durations were not blocked, indicating that fluid flow played an important role in the underlying mechanism leading to blockage (Figure 4-5a, b). SEM imaging revealed an apparent deposition of material on the pit membranes of the blocked filters (Figure 4-5c; compare this to pit membranes in unblocked ethanol-dried filters shown in Figure 3-5). Deposition of material even with DI water indicated that the material must originate from the filter itself. Xylem is composed of cellulose and hemicellulose fibers and hydrophobic lignin polymers, of which hemicellulose fibers are highly amorphous and relatively easily soluble in water [54]. It was therefore hypothesized that the dissolution of hemicellulose fibers in DI water and their convective re-deposition on the pit membranes gives rise to 'self-blocking' of xylem filters. Analysis of the water filtered through the xylem filters under atomic force microscopy revealed the presence of dissolved solids (Figure 4-5c) and further FTIR measurements confirmed the presence of hemicellulose, validating our hypothesis (Figure 4-5d) [55].

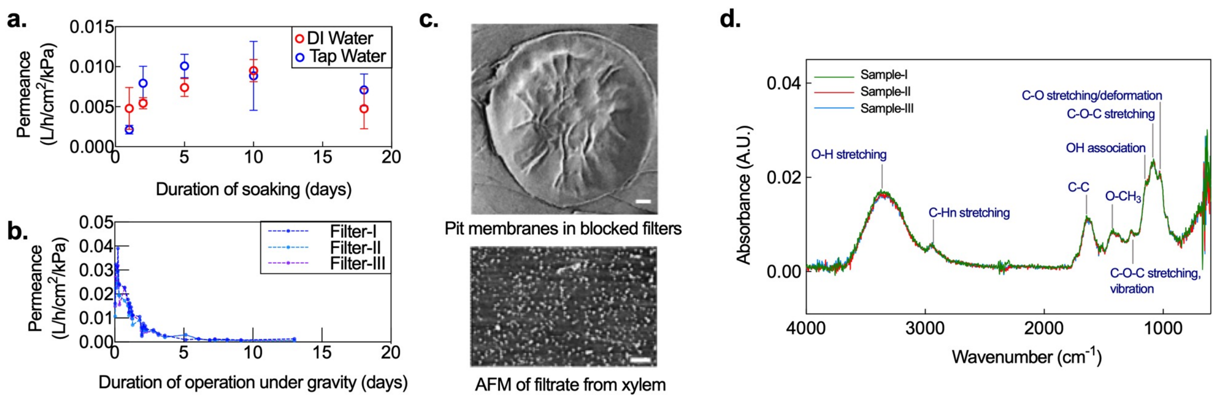

Figure 4-5: Self-blocking of xylem filters and its mitigation (data reported with 1 cm diameter, 0.25-inch thick Eastern white pine filters, unless otherwise specified). **a.** Use of smaller gravitational head to drive flow fails to prevent permeance from declining to zero, suggesting that cavitation does not play a major role in filter blockage. Each data set corresponds to measurements with a single filter. **b.** Effect of addition of Ca^{2+} and K^{+} ions in DI water on volumetric capacity. Mean±s.d., n = 3. Addition of ions to does not improve volumetric capacity. **c.** Microfibrils are covered by deposited material in pit membranes of blocked filters (SEM image, top) and filtrate dried on a surface contains particulates (AFM image, bottom), suggesting dissolution and deposition of organic material within the filter. Scale bars, 2 μm. **d.** FTIR spectra of different samples of filtered water indicate that hemicellulose leaches out of xylem filters. Modes corresponding to FTIR peaks are specified.

Self-blocking of xylem imposes an intrinsic limit on filter life and its volumetric capacity. However, it could also safeguard users against the risk of using a filter degraded by prolonged exposure to contaminated water or trapped microbes and signal the need for filter replacement. The ability to regulate self-blocking is therefore important, as it can help balance performance and safety. Broadly, self-blocking may be regulated by fixing the molecules within the xylem (which could also reduce degradation), or prior removal of the material responsible for the behavior. Effect on structural integrity of pit membrane (critical for rejection performance) and ease of implementation in low-resource settings are considerations that govern the choice of such methods.

The solubility of hemicellulose in water was leveraged to develop a simple process for mitigating self-blocking by soaking the filters in hot water to remove hemicellulose. The optimal temperature and duration of soaking was identified to improve volumetric capacity without compromising structural integrity of the pit membranes; soaking the filters in hot water at 60-65°C and atmospheric pressure for 1 h before ethanol-drying doubled the capacity while maintaining its ability for filtration (Figure 4-6a-c). In practice, the volumetric capacity of filters will also be limited by the fouling due to external water contaminants. Consequently, the necessity for measures to minimize self-fouling will be low if external contaminant load is high, and hot water soaking may not be needed. It is to be noted that this soaking process is different from industrial hydrolysis of hemicellulose that is typically performed at high temperature and pressure for extraction of chemical derivatives such as sugars [56].

Eastern white pine filters fabricated using hot water soaking and ethanol drying could maintain permeance >0.01 L/h.cm^2.kPa while filtering at least 11 L/cm^2 of DI water (Figure 4-6d). Thus, in the absence of fouling due to constituents in the feed water, filters with 10 cm^2 area (3.6 cm diameter) would achieve flow rates >1 L/h and volumetric capacity of ∼100 L under gravity-driven operation with 1 m head (see Figure 4-6e for variation in permeance with permeate filtered for intermittent and continuous operation and Figure 4-6f for scaling of flow rate with filter area).

However, the rejection performance of 0.25-inch thick filters was sensitive to the variability in filter thickness, which is expected if the filter thickness approaches the length of the xylem conduits (tracheids) in Eastern white pine [43]. To circumvent this issue, the filter thickness was increased to 0.375 inches for all filters in subsequent studies.

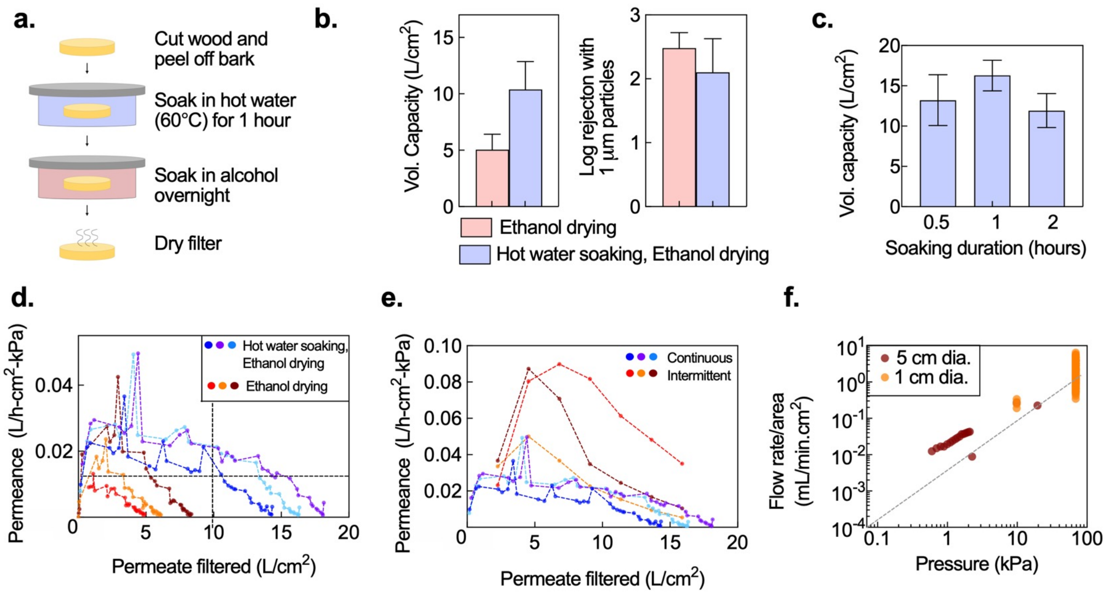

Figure 4-6: Mitigation of self-blocking **a,b.** Hot water soaking improves volumetric capacity and retains rejection (0.375-inch thick filters; n=3, mean±s.d. are indicated in (b). **c.** Variation in volumetric capacity with duration of soaking in hot water at 60-65°C. Mean±s.d., n = 3. **d.** Effect of hot water treatment on permeance and volumteric capacity. Different colors denote different filters. Data were obtained with 1 cm diameter Eastern white pine filters operated under 1 m gravity head. The horizontal dashed line denotes the permeance (0.01 L/h/cm^2/kPa) corresponding to the target flow rate of 1 L/h with a 10 cm^2 filter area and 1 m gravity head, whereas the vertical dashed line corresponds to a volumetric capacity of 100 L, which is achieved by the hot water soaked and ethanol-dried filters while maintaining the target permeance. **e.** Filters operated intermittently show a qualitatively similar trend as those operated continuously with values in the same range for both permeance and permeate filtered. Data for continuous operation were taken as is from (e.) for filters treated with hot water soaking and ethanol drying. For intermittent operation, ginkgo filters (4 cm diameter, 0.375 inch thickness) were used (details in Methods section M2 in Appendix A). Different colors denote different filters. **f.** Flow rate normalized by area varies linearly with pressure for ethanol-dried filters with diameters 1 cm (n = 295) and 5 cm (n = 20). Dotted line shows a linear fit.

4.5 Effect of contaminants on xylem filter performance

4.5.1 Xylem filter performance with synthetic test waters

Constituents in water such as humic acids or colloids typically cause fouling of membrane filters, reducing the flow rate with time. Understanding how such constituents affect the flow rate and volumetric filtration capacity of xylem filters is therefore essential to better inform how xylem filters would perform in practical settings. The World Health Organization (WHO) prescribes two kinds of synthetic test waters to evaluate the performance of household water treatment technologies [57]: a general test water (GTW) representing

Table 4.1: Composition of General Test Water (GTW) and Challenge Test Water (CTW).

	General Test Water	**Challenge Test Water**
Sea salts	275±225 mg/L	1500±150 mg/L
Sodium bicarbonate	80±120 mg/L	100±20 mg/L
Turbidity	<1 NTU	40±10 NTU
Organics	Tannic acid (1.05±0.95 mg/L)	Humic acid (15±5 mg/L)

high-quality groundwater or rainwater, and a challenge test water (CTW) with aggressive water specifications to represent turbid surface water. The composition of GTW and CTW has been specified in Table 4.1.

With GTW, the volumetric capacity and peak permeance (highest permeance over the course of operation) of xylem filters (fabricated by hot-water soaking and ethanol-drying) were sufficient to meet the target metrics (flow rate >1 L/h and volumetric capacity >8 L). However, filter performance varied significantly with water quality; both peak permeance and capacity with CTW (0.022±0.020 L/h.cm^2.kPa and 6.07±4.40 L/cm^2 respectively) were an order of magnitude lower than those with GTW (0.002±0.001 L/h.cm^2.kPa and 0.58±0.47 L/cm^2 respectively; Figure 4-7).

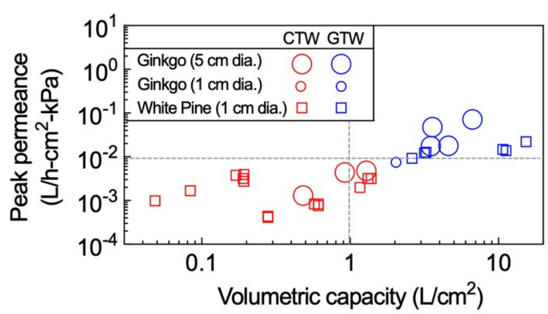

Figure 4-7: Peak permeance and volumetric capacity normalized by area for GTW and CTW. Dotted lines show the minimum peak permeance and capacity required to yield flow rate >1 L/h and capacity >10 L for a filter area of 10 cm^2 and thickness of 0.375 inches operated under 1 m gravity head. Each data point represents a single measurement.

4.5.2 Fouling mechanisms

The deterioration in performance with CTW could be attributed to one or many of the water quality parameters that differ between CTW and GTW, which are a) higher turbidity, b) higher concentration of organics, and c) the larger size of organic contaminant in CTW. To identify the key foulants that cause deterioration in performance, we measured filtration capacity of xylem while selectively adding different constituents at varying concentrations. Xylem filters were most susceptible to fouling by humic acids (present in decomposed organic matter) followed by particulates (dust) (Figure 4-8). By contrast, tannic acid did not impact filter capacity significantly, demonstrating that the filters have a low susceptibility for fouling with small, homogeneous organic molecules.

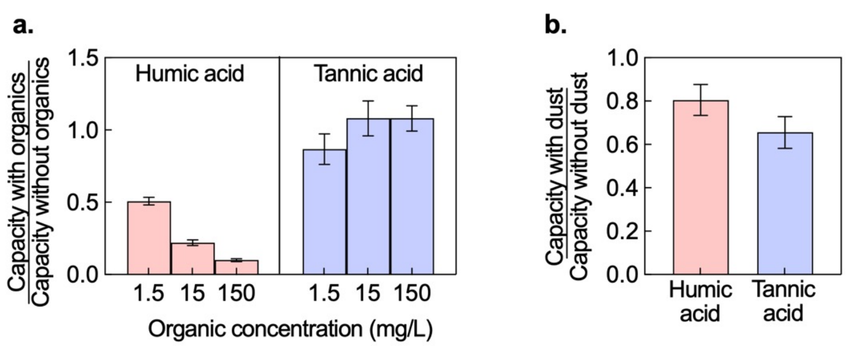

Figure 4-8: Filter capacity is most susceptible to humic acid, followed by dust and tannic acid (1 cm diameter, 0.375-inch thick Eastern white pine filters operated under 1 m gravity head; n=3, mean±s.d.) (see Method M12 in Appendix A for experiment details). In (a), either humic acid or tannic acid is added to water. In (b), water contains 70 mg/L dust or no dust in either 15 mg/L humic acid or tannic acid.

Fouling is a well-researched topic in membrane filtration and several fouling models have been developed to understand the nature of interaction between the foulants and membrane surface and aid membrane design, operation, and fouling control [58]. To model the fouling mechanisms in xylem filters, three different mechanisms that are conventionally used to model fouling in micro- and ultra-filtration membranes were considered [58]:

1. Complete blocking model: In the complete blocking model, each foulant particle blocks a pore completely without depositing over previously deposited particles. The number of open pores, p, therefore decreases linearly with the filtrate volume, V, as follows [58]:

$$p = p_0 - \rho_n V$$

where, p_0 is the number of initially open pores in the filter, and ρ_n is the number density of foulant particles per unit volume of the feed. As the flux through the filters is directly proportional to the number of open pores, the expression above can be written as follows:

$$J = J_0(1 - K_{cb}V)$$

where, J and J_0 represent the flux through filter in the fouled and initial state, respectively, and K_{cb} is called the blocking constant for the complete blocking model.

2. Intermediate blocking model: Here, the foulant particles deposit directly on the pores blocking them completely, or land on previously deposited foulant particles [58]. The number of open pores therefore decays exponentially with the filtered volume [59]:

$$p = p_0 e^{(-K_{ib}V)}$$

where, K_{ib} is the intermediate blocking constant. The flux can be written as follows:

$$J = J_0 e^{(-K_{ib}V)}$$

3. Cake filtration model: In cake filtration, the foulant particles deposit on the pores forming a permeable cake. The hydraulic resistance of this cake layer is in series with the resistance of the pores and increases linearly with the amount of foulant deposited (and hence with the filtered volume). The total resistance of each pore, R is then represented by the following equation:

$$R = R_{pore} + R_{cake} = R_{pore} + r\frac{V}{p_0} = R_{pore}(1 + \frac{r}{R_{pore}}\frac{V}{p_0}) = R_{pore}(1 + K_{cf}V)$$

where, R_{pore} is the resistance of the pores in the absence of fouling, R_{cake} is the resistance of the cake layer, and r is the resistance added by foulant deposition on the pore per unit volume of fluid filtered. The flux, J, can then be written as follows:

$$J = \frac{\Delta P}{\Delta R} = \frac{\Delta P}{R_{pore}(1 + \frac{r}{R_{pore}} \frac{V}{p_0})} = \frac{J_0}{1 + K_{cf} V}$$

where, ΔP is the pressure difference applied across the filter.

Of the three models, the intermediate fouling model provided the best fit to experimental data for Challenge Test Water and General Test Water (Figure 4-9a-c). The models differed primarily near the filters' end-of-life, where the permeance gradually tailed-off in alignment with the intermediate blocking model whereas the complete blocking model predicted a much sharper, linear decline in permeance to zero, and the predictions of cake filtration model overshot experimentally observed values. The intermediate fouling model has commonly been used to represent the fouling of polymeric micro/ultrafiltration membranes by biological and organic contaminants [60, 61, 62, 63]. In this model, foulant particles deposit randomly on the pit membranes and result in exponential decrease in permeance. SEM images of partially fouled filters were in agreement with this fouling mechanism (Figure 4-9d). The fouling model helps predict the change in filter permeance with time for a given contaminant load; consequently, it can be used for estimating volumetric capacity, filter lifetime, and replacement frequency for different water qualities.

4.5.3 Pre-filtration methods for mitigating fouling

Knowledge that humic acid and dust particles adversely impact filter performance offers the possibility of mitigating their impact through approaches ranging from pre-treatment of water to chemical modification of xylem. To keep filter manufacturing simple and inexpensive, and accommodate variations in contaminant type and load, pre-treatment methods that can be easily integrated in-line with xylem filters when the water quality is poor were explored. Specifically, cloth pre-filtration and granular activated carbon (GAC) adsorption were invetsigated to reduce the load of dust and humic acid respectively [64]. Both these methods have been commonly used for household water treatment, but have limited efficacy in removing bacterial or viral pathogens from water [65, 66].

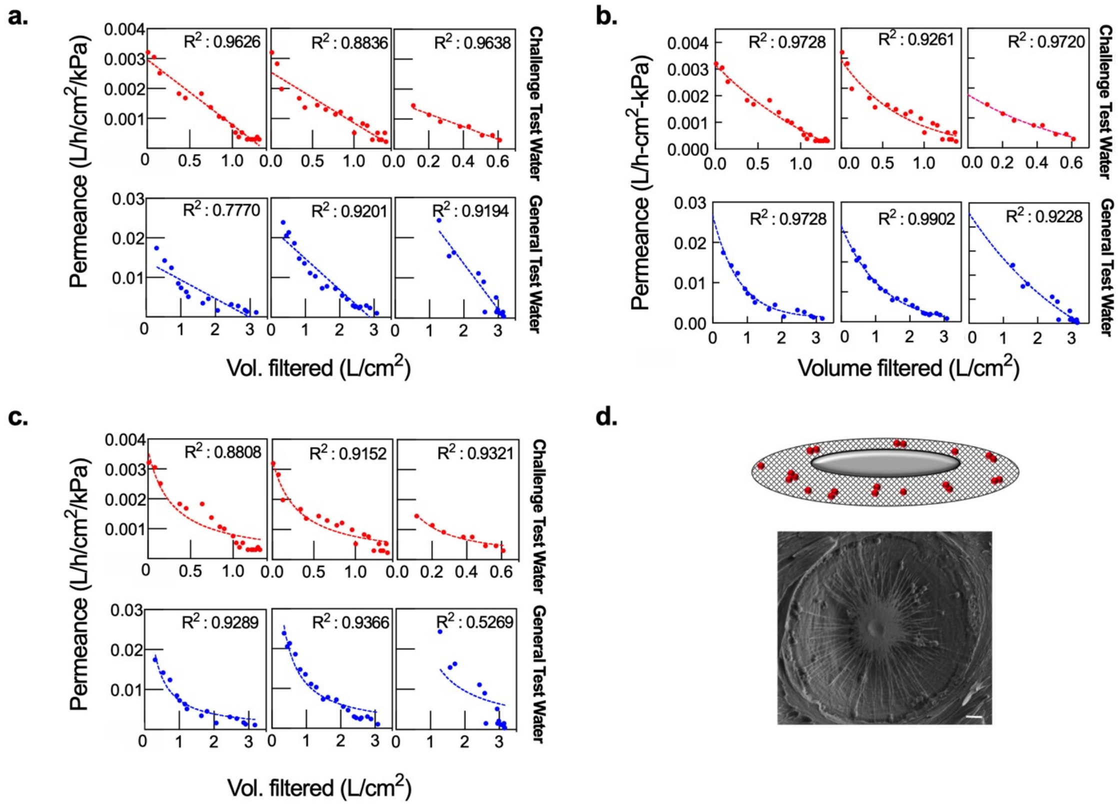

Figure 4-9: Fouling mechanisms in xylem filters **a-c.**Complete blocking model (a), intermediate fouling model (b) and cake filtration model (c) fits (dashed lines) to experimentally observed variation of permeance with volume filtered (symbols) for CTW (red) and GTW (blue). Each graph corresponds to measurements with a single filter. All measurement were performed under 1 m gravitational head with eastern white pine filters (1 cm diameter, 0.375 inch thickness) except for plot (vi) where a ginkgo filter (4 cm diameter, 0.375 inch thickness) was used.**d.**Schematic illustrating the deposition of foulant particles (red) on the pit membrane in the intermediate fouling model, which is consistent with foulant deposition observed by SEM in a partially-fouled ginkgo filter. Scale bar, 1 μm.

Design of GAC column

GAC is a porous carbon-based material that removes contaminants (organic, as well as some inorganic compounds) by adsorption [67, 64]. GAC is commonly used in municipal wastewater treatment plants as well as in household drinking water treatment [67, 64]. The efficacy of contaminant removal depends on a) the carbon source, which determines the pore size and specific area and thus, the types of contaminant that can be removed, b) the size of GAC granules, which governs adsorption kinetics, and c) the contact time of feed water with GAC, which determines the time available for adsorption to take place [64].

To design an effective GAC pre-filtration column, the adsorption characteristics of different commercially available GACs were investigated using humic acid as a model organic contaminant. Coal-based and coconut shell-based GACs with grain sizes of 0.6–2.4 mm, 0.4–1.7 mm, and 0.4–0.6 mm (characterized by sieving meshes of size 8×30, 12×40, and 30×40 respectively; 8×30 denotes granule sizes that pass through a mesh of size 8 but not through a mesh of size 30) were studied. GAC granules were packed in a 5 cm diameter, 15 cm long cylindrical pre-filtration column and humic acid removal from DI water at the same alkalinity and salinity as CTW was measured for different empty bed contact times (time for which the water is nominally in contact with the GAC; calculated by dividing the volume of the column by flow rate). Coal-based GAC was able to adsorb more humic acid for the same contact time than coconut shell-based GAC, and grains with a mesh size of 30×40 showed 20× faster adsorption than those with a mesh size of 12×40 (Figure 4-10). Based on these results, coal-based GAC column (5 cm diameter, 15 cm length) consisting of 30×40 granules that could be operated at flow rates of ∼2 L/h (flow rates controlled by a valve) was designed to reduce humic acid concentration in CTW by 95% (corresponds to concentration <1 mg/L) (details on cost and replacement frequency of GAC are provided in Section 5.4.

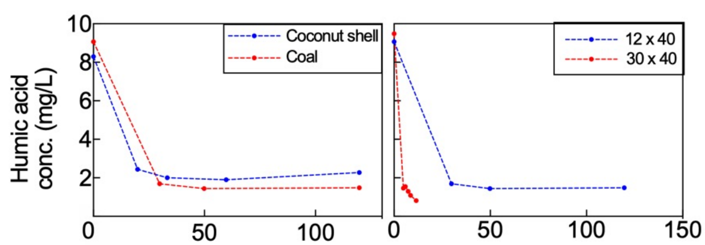

Figure 4-10: Humic acid removal by coconut shell-based and coal-based GAC.

Effect of pre-filtration on filter performance

When used in conjunction with cloth pre-filtration, the GAC column improved the performance of xylem filter with CTW significantly (Figure 4-11a); on average, capacity and flow rates increased by a factor of ∼3× and 5× respectively.

In practice, pre-filtration is not essential for operation of the filter; it is an option which, in conjunction with water quality, determines the flow characteristics. The decision whether

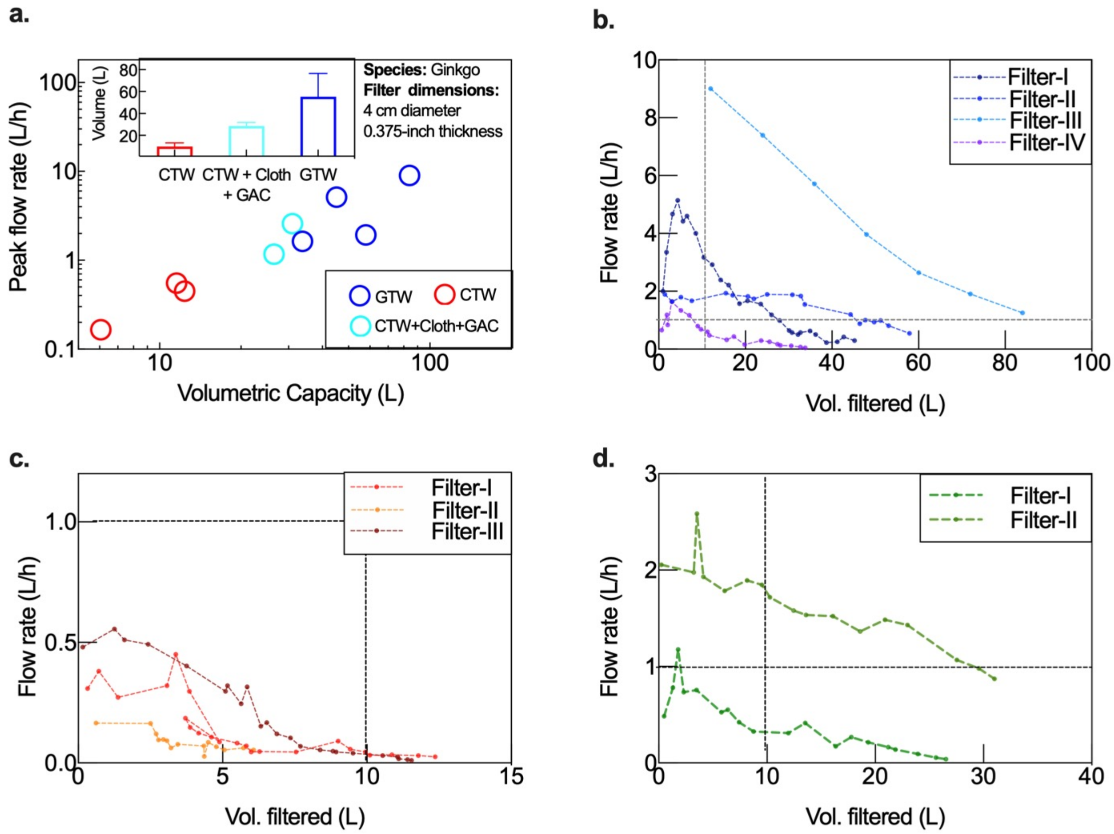

Figure 4-11: Effect of pre-filtration on filter performance. **a.** Pre-treatment with cloth and GAC improves the peak flow rates and volumetric capacity of ginkgo filters with GTW and CTW at 1 m gravity head. Each data point represents a single measurement. Inset shows mean±s.d. of the volumetric capacity. **b-d.** Variation of flow rate with volume filtered for 4 cm diameter, 0.375-inch thick ginkgo filters for GTW (b), CTW (c) and CTW with cloth and GAC pre-filtration (d). Different colors denote different filters.

to incorporate pre-treatment and the choice of pre-treatment would be governed by the tradeoff between the added convenience of longer filter lifetime or lower filter replacement frequency, cost, and the complication of an added replaceable component, plus the need to remove any chemical contaminants that may be present in the water. The replacement frequency of the cloth or the GAC module would vary depending on the type of cloth/GAC used, configuration of GAC module, and water quality. While the cloth pre-filter could be washed or replaced once it is dirty, the GAC might need replacement once every few months (1.5-6 months; see for estimates on GAC replacement frequency). The reduced lifetime or slower flow rates even with newly-replaced xylem filters could be used as an indicator for pre-filtration module replacement.

In summary, these studies demonstrate that xylem filters offer promise for practical translation. Filters made from Ginkgo biloba (ginkgo) with an area of 13 cm^2 (4 cm diameter) using the fabrication protocol shown in Figure 4-6a, operated under a 1 m gravity head could a) process $\sim$55±21 L of GTW without pre-filtration and 28±3 L of CTW with GAC and cloth pre-filtration, which is more than sufficient to meet the daily drinking water requirement of a household, b) yield peak flow rates of 1.5–9 L/h depending on water quality (see Figure 4-11b-c for variation of flow rates over filter lifetime), c) reject 99.76±0.25% of 1 μm particles.

4.5.4 Effect of fouling on rejection performance of filters

Deposition of foulants on the pit membranes over the course of filter operation is likely to further improve rejection. Ginkgo biloba filters rejected 94.01±3.31% of 100 nm particles, and the deposition of merely 0.13 mg of foulant (humic acid) per cubic centimeter of the filter volume improved the rejection to 98.73±±0.41%.

4.6 Microbiological performance of xylem filters

To assess the potential health impact of xylem filters and their effectiveness in reducing the risk of diarrheal diseases, the filters' ability to remove *E. coli*, MS-2 phage, and rotavirus (the single largest causal organism of diarrhea [25]) from water was tested. Xylem filters (4 cm diameter, 0.375 inch thickness, stored for 2 years, no pre-filtration) made from ginkgo were operated under a 1.2 m gravity head with General Test Water containing WHO-prescribed concentrations of *E. coli* ($\sim 10^6$ CFU/mL) and MS-2 phage ($\sim 10^5$ CFU/mL) [57] and NSF-prescribed concentrations of rotavirus ($\sim 10^4$ PFU/mL) [68]. *E. coli* and MS-2 phage were dosed simultaneously in the same test solution while rotavirus removal was tested separately. The bacterial and virus removal was tested at the start of filter operation and when permeance declined to 75%, 50%, and 25% of the initial value. After the first sampling point at the start of filter operation, dust was added to the test solution to accelerate clogging [69, 57] (refer to Methods section M20 in Appendix A for further details on test procedure). The filters showed >4-log removal of rotavirus and >3-log removal of *E. coli* and MS-2 phage (Figure 4-12, data provided in Table 4.2 and 4.3).

With such rejection performance, xylem filters would fall under the ‘comprehensive protection (high pathogen removal)’ category (⋆⋆) as per the WHO scheme for classifying water treatment technologies (Table 4.4) [57]. Since the virus particles are smaller than the expected pore size of the filters (MS-2 phage and rotavirus are 24 nm [57] and 70 nm [70] in diameter respectively, while the pore size is 100-500 nm [23]), the results suggest that the mechanism of virus removal is likely to be adsorption-driven. Virions can adsorb on cellulose-based materials [71], with cellulose nitrate reported to remove virions that are much smaller than the filter pore size [72]. It is possible that the relatively slow flow rate and the large thickness of xylem filters facilitates adsorption and removal of viruses.

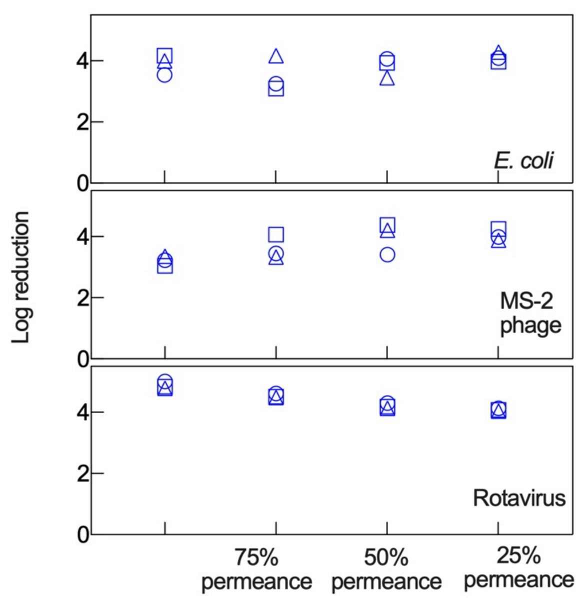

Figure 4-12: Microbial removal performance of xylem filters (ginkgo, 4 cm diameter, 0.375 inch thickness, no pre-filtration) when operated under 1.2 m gravity head with General Test Water containing *E. coli* ($\sim 10^6$ CFU/mL) and MS-2 phage ($\sim 10^5$ PFU/mL) dosed simultaneously, or rotavirus ($\sim 10^5$ PFU/mL). Rejection was measured at the start of filter operation and when permeance dropped to 75%, 50%, and 25% of initial permeance (see Method M20 for further details). Different symbols indicate different filters.

Table 4.2: Rejection performance with microbial contaminants

E.coli rejection							
Sample point	Influent conc. (CFU/L)	Effluent conc. (CFU/L)			Log removal		
		I	II	III	I	II	III
Start	3.60×10^9	1.02×10^6	2.40×10^5	3.65×10^5	3.548	4.177	3.995
75% permeance	3.85×10^9	2.14×10^6	3.10×10^5	2.60×10^5	3.256	3.095	4.171
50% permeance	4.10×10^9	3.50×10^5	4.80×10^5	1.45×10^6	4.069	3.932	3.452
25% permeance	5.20×10^9	4.20×10^5	5.60×10^5	2.70×10^5	4.093	3.968	4.285
MS-2 phage rejection							
Sample point	Influent conc. (CFU/L)	Effluent conc. (CFU/L)			Log removal		
		I	II	III	I	II	III
Start	4.25×10^8	2.52×10^5	3.78×10^5	1.87×10^5	3.227	3.051	3.357
75% permeance	5.30×10^8	1.88×10^5	4.56×10^4	2.45×10^5	3.451	4.066	3.336
50% permeance	6.22×10^8	2.40×10^5	2.60×10^4	3.86×10^4	3.414	4.379	4.208
25% permeance	3.52×10^8	3.65×10^4	1.96×10^4	4.56×10^4	3.985	4.255	3.888
Rotavirus rejection							
Sample point	Influent conc. (CFU/L)	Effluent conc. (CFU/L)			Log removal		
		I	II	III	I	II	III
Start	1.03×10^7	1.00×10^2	1.50×10^2	1.70×10^2	5.011	4.835	4.781
75% permeance	1.03×10^7	2.50×10^2	3.20×10^2	3.40×10^2	4.613	4.506	4.48
50% permeance	1.02×10^7	5.00×10^2	6.70×10^2	7.50×10^2	4.308	4.181	4.132
25% permeance	1.02×10^7	7.50×10^2	8.50×10^2	9.20×10^2	4.132	4.078	4.044

Table 4.3: Flow rates corresponding to different sampling points for rejection experiments.

Sample point	Flow rates (mL/min)					
	E. coli and MS-2			Rotavirus		
	I	II	III	I	II	III
Start	24.5	25.0	26.0	25.0	26.0	26.0
75% permeance	18.4	18.8	19.5	18.8	19.5	19.5
50% permeance	12.3	12.5	13.0	12.5	13.0	13
25% permeance	6.1	6.3	6.5	6.3	6.5	6.5

Table 4.4: WHO scheme for classification of household water treatment technologies

Performance Classification	Log_{10} reduction required			Interpretation (with correct, consistent use)
	Bacteria	Virus	Protozoa	
⋆ ⋆ ⋆	≥4	≥5	≥4	Comprehensive protection
⋆ ⋆	≥2	≥3	≥2	
⋆	Meets at least 2-star criteria for two classes of pathogens			Targeted protection
-	Fails to meet WHO performance criteria			Little or no protection

4.7 Summary of findings

The material and operational characteristics of xylem as a water filtration membrane were studied and methods to engineer xylem filters' functional attributes for practical applications were developed. Xylem filters (fresh as well as ethanol-dried) were found to have comparable permeance to commercial membranes with equivalent pore size. These filters had a unique tendency of self-blocking, which arose due to the dissolution of loose hemicellulose fibers and their convective re-deposition on the pit membranes. Soaking filters in hot water for an hour was found to be effective in mitigating self-blocking. With regards to external contaminants, xylem filters were found to be most susceptible to fouling by humic acid and dust. Pre-filters made of granular activated carbon and dust helped improve the performance of xylem filters in the presence of these foulants. Xylem filters, treated by hot water soaking and ethanol-drying, were able to achieve >3-log rejection of *E. coli* and MS-2 phage and >4-log removal of rotavirus. With this rejection performance, xylem filters would fall under the 2-star category of WHO's scheme of household water treatment technologies.

Chapter 5

Technology translation

We assessed filter performance in the field, developed a functional prototype device through user-centric design, and examined aspects of social acceptance and user preferences to gauge the potential of xylem filters to lower existing barriers for HWT adoption.

5.1 Validation of filter performance with natural water sources

To assess the ability of xylem filters to function with natural water and facilitate access to safe drinking water in resource-constrained settings, field studies were conducted in India. India has the highest water-borne illness mortality rate in the world with more than 160 million people lacking access to safe and reliable water [25, 73]. In particular, low-income communities in urban slums (Delhi and Bangalore) and rural villages (Uttarakhand) were targeted. Xylem filters made from gingko trees in US and those manufactured in India with indigenous *Pinus roxburghii* (chir pine) using local resources for all fabrication steps such as cutting, hot water soaking, and dry preservation, were tested with water from natural springs, municipal taps, and tubewells (groundwater) (which were the primary sources of drinking water in Uttarakhand, Delhi and Bangalore respectively; see Table 5.1 for water quality information).

Xylem filters with 4 cm diameter mounted by simply clamping the xylem filters in a

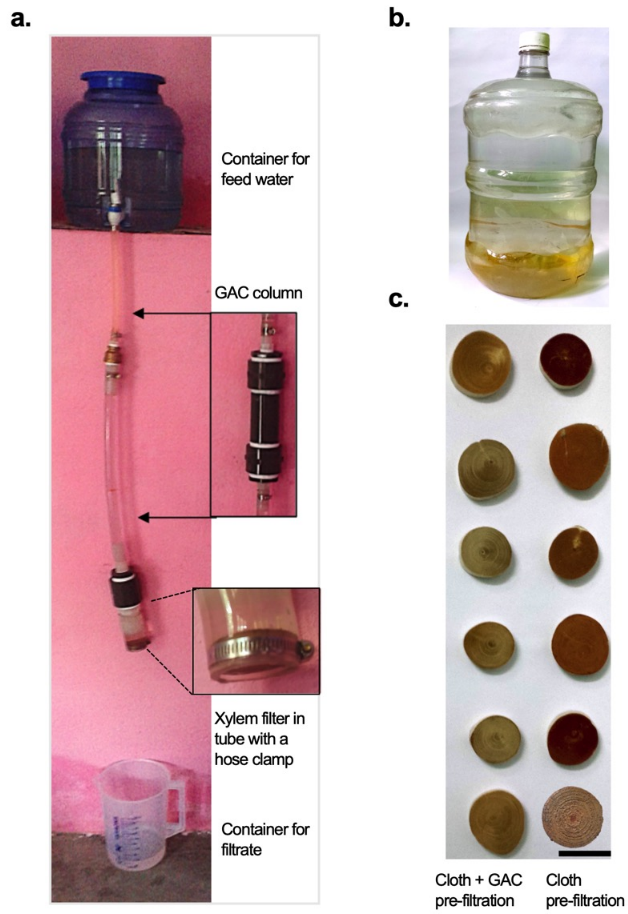

Figure 5-1: Field tests in India. **a.** Field set-up for testing filter performance. **b.** Tap water sample (New Delhi, India) used for testing. **c.** Xylem filters used with GAC show reduced deposits after filtration with tap water. Scale bar, 4 cm.

tube (Figure 5-1) and operated under 1 m gravity head yielded peak flow rates exceeding 1 L/h and filtration capacities exceeding 10 L in most cases, with either cloth pre-filtration or cloth and GAC pre-filtration (Figure 5-2 a,b,d-g). With cloth pre-filtration, xylem filter capacity ranged from ~40 L with groundwater to 12-30 L with turbid tap water. The benefits of adding a GAC pre-filtration module varied with water quality; GAC improved filter capacity from 38 L to 102 L with groundwater and yielded a capacity of ~30 L with spring water, but did not improve xylem filter performance with tap water. No total or fecal coliform bacteria were detected in the filtrate for 5 out of the 6 xylem filters tested (3 filters operated with GAC and 3 filters operated without GAC) (Figure 5-2c, 5-3). These results confirmed that xylem filters could remove coliform bacteria and function in realistic settings with replacement on a daily to weekly basis depending on the operating conditions.

Table 5.1: Water quality parameters for field tests

Parameter	Water Source			Desirable Test Limit (BIS)	Permissible limit (BIS)	Standard test method
	Spring A (Uttarakhand)	Groundwater (Delhi)	Municipal tap water (Delhi)			
pH	8.03			6.5-8.5	No relaxation	IS 3025 (Part 11, 2002)
Total Dissolved Solids (TDS), mg/L	49.1			500	2000	IS 3025 (Part 16, 2006)
Turbidity (NTU)	5.02	<2		1	5	IS 3025 (Part 10, 2006)
Total alkalinity (as $CaCO_3$), mg/L	50			200	600	IS 3025 (Part 23, 2003)
Total hardness (as $CaCO_3$), mg/L	40			200	600	IS 3025 (Part 21, 2002)
Fluoride, mg/L	0.9			1	1.5	APHA 22nd Ed.-4500-F-D
Nitrate, mg/L	0.5			45	No relaxation	APHA 22nd Ed.-4500-NO3-B
Sulfate, mg/L	Not detected			200	400	APHA 22nd Ed.-4500-SO42-E
Residual free chlorine, mg/L	Not detected			0.2	1	APHA 22nd Ed.-4500-CI-G
Taste	Agreeable			Agreeable	Agreeable	APHA 22nd Ed.-2160-C
Color	Not detected			1	5	APHA 22nd Ed.-2120-C
Conductivity, uS/cm	81.3			-	-	APHA 22nd Ed.-2510-B
Chloride, mg/L	8.3			250	1000	APHA 22nd Ed.-4500-Cl-B
Iron (as Fe), mg/L	0.5			0.3	No relaxation	APHA 22nd Ed.-3500-Fe-B
COD		246.01	7.71			IS 3025 (Part 58)
Associated figures	Figure 5-2a,d	Figure 5-2a,e	Figure 5-2c,f,g			
Spring A is depicted by a red circle in Figure 5-2a,d. Water quality data for Spring B (Bhainswari village, Uttarakhand) denoted by the orange circle in Fig. 5f is unavailable. Data for Figure 5-3 was obtained using the water from Spring A (Kith, Uttarakhand) but water quality parameters other than fecal and using the water from Spring A (total coliform were not tested when the experiments were conducted.						

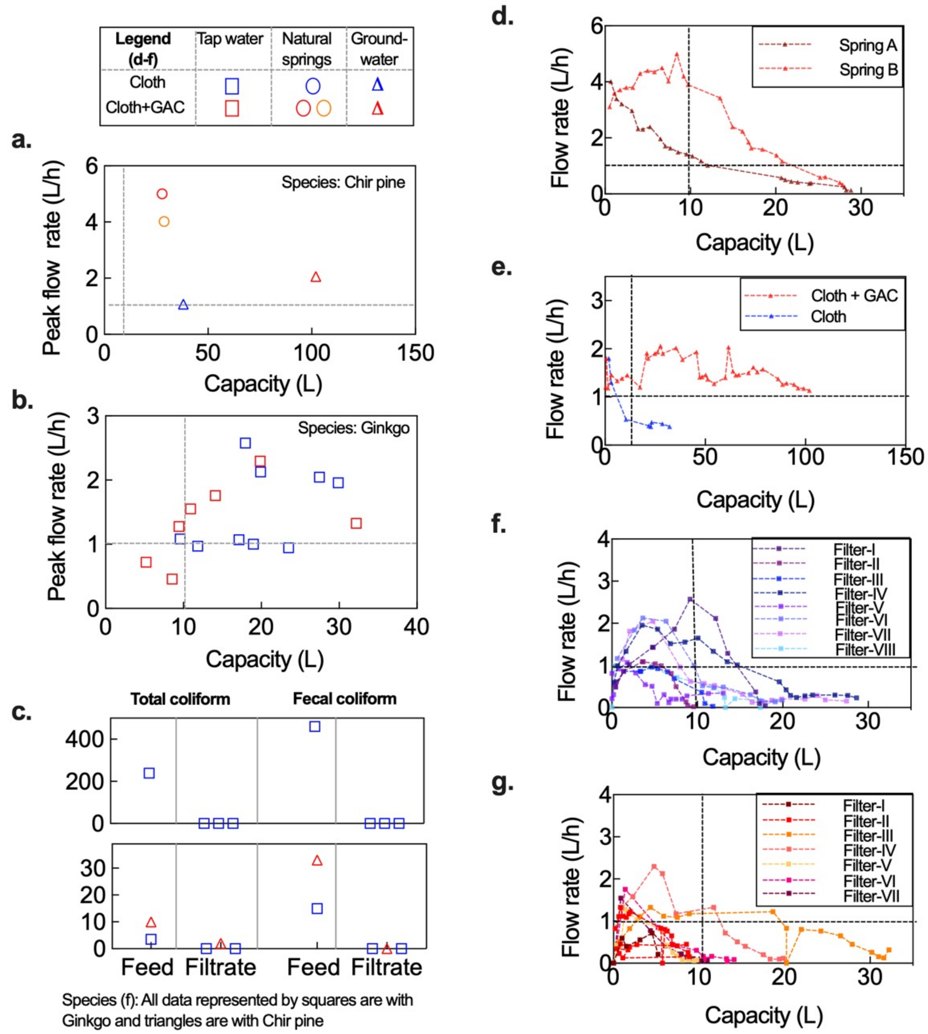

Figure 5-2: Xylem filter performance with natural water sources. **a-c.** Chir pine and ginkgo filters show peak flow rates and capacity exceeding 1 L/h and 10 L, respectively, indicated by dashed lines in a and b, and absence of coliform in the filtered water. Legend is shown at the top. In c, chir pine and ginkgo filters were used for tap water and groundwater studies, respectively. **d-g.** Variation of flow rate with capacity for (d) chir pine filters with water from two natural springs in Uttarakhand, with cloth + GAC pre-filtration (e) chir pine filters with groundwater (obtained from tubewells) in Delhi with cloth and cloth + GAC pre-filtration, (f) ginkgo filters with municipal tap water in urban slums in Delhi with cloth pre-filtration, and (g) ginkgo filters with municipal tap water in urban slums in Delhi with cloth + GAC pre-filtration. Data is reported with 4 cm diameter, 0.375-inch thick filters processed using hot water soaking and ethanol treatment and operated under 1 m gravity head (see Method M15 in Appendix A for details on field testing of filters).

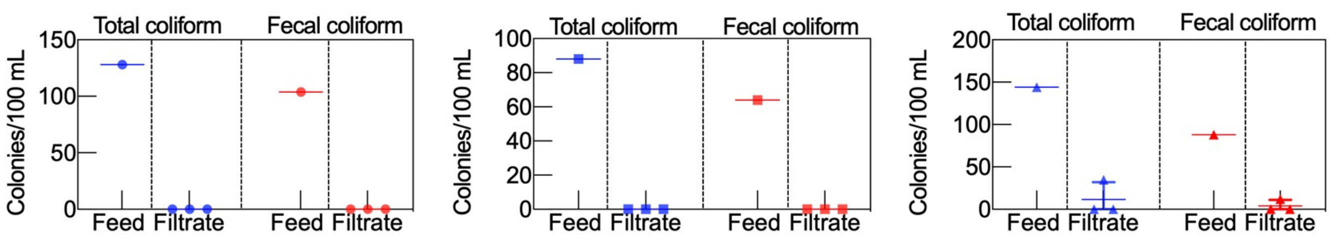

Figure 5-3: Microbiological performance of ethanol-preserved xylem filters **a-c.** Removal of coliform bacteria by ethanol-preserved xylem filters (1 cm diameter, 0.25 inch thickness) operated under 1 m gravity head with water from a natural spring in Kith village, Uttarakhand. Filters were made from a) Eastern white pine, b) ginkgo and c) chir pine.

5.2 Design of xylem filtration devices

Analogous to other membrane filters, xylem filters have to be housed in a device for HWT. A wide range of device configurations could be designed to suit different use cases, water quality, resources available, and user preferences. As an illustrative example, a functional, first-generation device prototype was built based on the feedback gathered from potential users on preferences for household water treatment and response to xylem filters (user studies were conducted in collaboration with MIT D-Lab).

5.2.1 Findings from user studies and literature on design of point-of-use devices

The criteria for practically useful water filters were formulated based on existing literature on design of point-of-use water treatment devices [44] and user research studies in the rural villages of Uttarakhand state and urban slums of New Delhi and Bengaluru in India conducted over a period of two years in collaboration with D-Lab. These studies involved interactions with over 1000 potential users, water filter manufacturers, and NGOs (Himmotthan, Essmart, Pan-Himalayan Grassroots, Shramyog, Peoples Science Institute) that distribute water filters through semi-structured/key informant interviews, focus group discussions, and design workshops. The key findings from literature and user studies have been summarized below:

1. Although targets for microbiological performance of water filters and minimum flow rates for practical use of water filters have been well-documented in literature [74, 75], filter lifetimes are relatively non-standardized and vary from 3-6 months for membrane-based filter cartridges to 1-2 years for ceramic filters [76, 77, 78, 79, 80, 81, 82, 83, 84]. Since xylem filters (in their present form) have a finite lifetime and require daily to weekly replacement that is very different from existing water treatment products, literature reports were not helpful in assessing user reception or setting filter lifetime targets for xylem filters. Consequently, we relied on data gathered from user studies (focus group discussions and individual household interviews) in India for developing

a target filter lifetime. The preferred filter replacement frequency was found to be closely tied to other product attributes such as cost, ease of filter replacement, ease of availability of replacements, etc., but a minimum lifetime of one day was found to be critical for uptake. The filter capacity was thus determined based on a daily filter replacement frequency and estimates on the daily drinking water need for an average household. An average household in low-income countries of Asia and Africa comprises 4–5 members [85]. Given a recommended daily water intake for an individual of 2–3.5 L [86], the drinking water requirement for such households is 8-17 L per day. Xylem filters operated overnight (8–10 h) could meet this requirement if they have flow rates of at least 1–2 L/h; this flow rate is also the minimum useful flow rate reported in literature [75].

2. Household water treatment systems in resource-constrained areas should preferably be independent of electricity or tap pressure [44], which makes gravity-driven devices well-suited for such settings. The water head in these devices should be sufficient to achieve the desired flow rate, but not so high that it is inconvenient for users to fill the device with untreated water. 79 of the 100 participants in the user interviews reported that they would be comfortable with a total device height of 0.9–1 m.

3. Potential users in villages as well as slums mentioned that the natural appeal and the simplicity of xylem filters distinguished them from other products in the market. 40% of the 300 respondents cited these as the primary attributes they liked about xylem filter devices.

4. Consistent with literature [87, 88], preliminary interactions with low-income households suggested that they prefer cheaper, frequent replacements to more expensive, long-term replacements. Of the 120 respondents in the urban slums of New Delhi, 50–60% reported an average household spending of $3 (INR 200) per week for purchasing reverse osmosis water cans and strongly indicated a willingness to switch to cheaper devices if they are available.

5. Users cited aesthetic appeal, ease of usage, and availability of devices at local shops as

key factors for adoption of water filters.

6. Co-design workshops and interviews were conducted to identify holder designs that enable easy replacement of filter cartridges and are simple to use. Four different holder designs were tested (prototypes developed in collaboration with MIT D-Lab): a) a holder for rectangular filters with an O-ring-based face-seal mechanism and side latch for clamping the filter in place (Figure 5-4a), b) a holder for circular filters with a union design comprising of three parts: a lower part for filter insertion, an upper part that connects to tubing and water reservoir from one end and face-seals against the filter using an O-ring insert on the other, and a sleeve that couples the upper and lower parts; this design allows for rotation of the lower part for inserting/removing the filter cartridge while maintaining the upper part and the associated tubing in a stationary position (Figure 5-4b) and prevents tube twisting, c) a holder for circular filters comprising of a plastic tubing for filter insertion and a hose clamp that seals against the sides of the filter; this mechanism enables utilization of the entire filter surface area (Figure 5-4c), d) a two-part screw-on holder for circular filters where the filter is inserted into the lower part, and both parts containing O-rings inserts thread onto one another to face seal against the filter (Figure 5-4d). Of the different holder mechanisms tested with 175 potential users, 64% of the respondents preferred the screw-on mechanism to other designs due to its relative ease of usage.

5.2.2 Design of xylem-filtration of device prototype for household water treatment

Product attributes desired by users revealed through these field studies included: a) ease of operation (filling and extracting water from the device, replacement of filter cartridge, etc.), b) low cost, and c) aesthetic appeal. Combining these inputs with existing guidelines for fabricating household water filters [44], a device consisting of the following components was developed:

- Top container for feed water
- Screw-on holder that incorporates xylem filter and allows for easy replacement

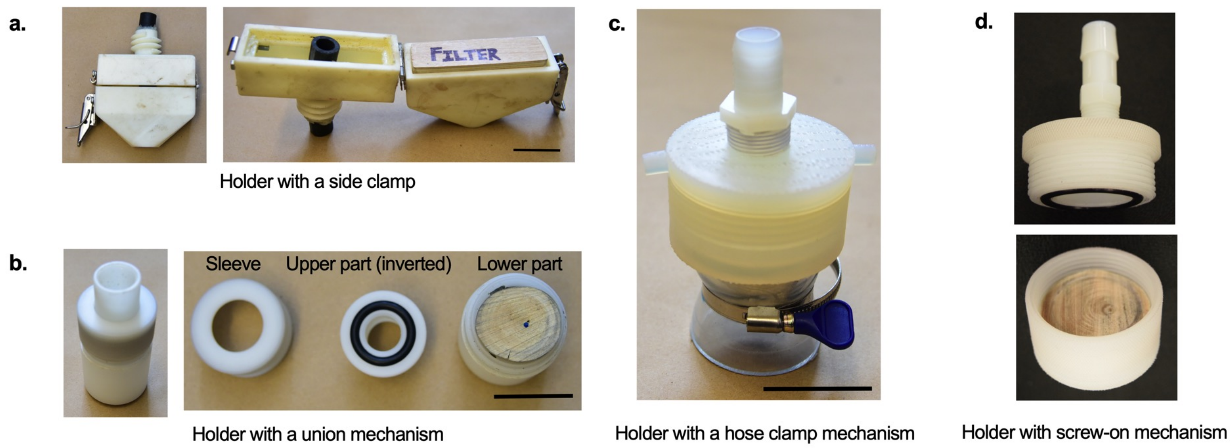

Figure 5-4: Holder configurations. **a-d.** Different filter holder mechanisms tested with users. These include a holder with a latch on the side, a holder with a three-part union mechanism, a holder with a hose clamp for sealing the filter from the side and holder with a screw-on mechanism where the upper and lower parts thread together. Scale bar, 4 cm

- A bottom receptacle with a dispenser to extract filtered water and a cover to minimize the risk of re-contamination

The device height was optimized such that users could fill the water in the top receptacle conveniently and the water head was sufficient to yield adequate flow rates (Figure 5-5a-c). Some challenges encountered during device design included obtaining a leak-proof seal between the holder and xylem filter due to irregularities on the wood surface, and preventing air entrapment in the tubing and filter holder that could obstruct flow. These challenges were overcome by cutting the wood at high speeds using a cold saw to obtain a smooth surface finish, using O-rings with appropriate compliance to conform to the wood surface, proper sizing of tubing and connectors to avoid bubble entrapment, and providing a vent in the holder for releasing any trapped air. This device showed 99.76±0.41% rejection of 1 μm particles in lab studies and filtered 10 L of tap water in Delhi at flow rates >1 L/h (Figure 5-5d). Flow rates can be improved further by clamping the filter on the side instead of the face, which prevents complete utilization of the peripheral filter cross-sectional area containing the xylem (the effective filtration area in the device was 7 cm^2.

A list of resources required for filter fabrication in low-resource settings is provided in Table 5.2.

Table 5.2: Resource requirement for fabricating xylem filters and filtration devices

Task	**Resources needed**
	Xylem filter cartridge
Extraction of branches	A pruner or sickle could be used for cutting branches. A chain saw might be used for cutting trunks.
Peeling of bark	The bark in the branches can be usually peeled by hand. Gloves should be used to avoid contact with resin. Band saws/hand saws might be needed for removing the bark from trunks.
Cutting the wood to desired sizes	Wood can be cut using a band saw, cold saw or hand saw. The surface roughness of the filter will vary with the equipment used. Smooth surfaces are required to prevent leaks while using filters with a face seal holder (as shown in Figure 5-4d), but surface roughness might be a cause of lesser concern when a side sealing mechanism is used (5-1a). To obtain a smooth surface, filters should be cut with a cold saw.
Hot water treatment	Clean (tap) water, vessels for soaking the filters in water, stove (gas/electric/induction) or just fuel wood for heating the water to 60°C, and a thermometer for monitoring temperature.
Alcohol treatment	Certified, food-grade alcohol (methanol, ethanol, etc.) with >99% purity and alcohol-compatible vessels for soaking the filters. The level of residual alcohol in dried filters should be maintained within the permissible limits for human consumption as prescribed by food safety standards [50].
Drying	Filters could be dried at room/ambient temperatures of 25–40 °C or using an oven.
Filtration device (The table below enlists the resource requirement for the filter designs depicted in Figure 5-5a-c. Although these device were fabricated in the US, they are amenable to local manufacture in India. The containers, O-rings, valves, tubing, dispenser and metal rods used in the device are commonly available items and can be sourced locally. The processes necessary for device fabrication (cutting, drilling, injection molding, etc.) are also well-established in the manufacturing industry. The device design could also be tuned as per the local availability of resources.	
Storing unfiltered water	Food-grade container with appropriate capacity should be used. In Figure 5-5a,b, the container capacity is 5 L.
Providing gravitational head	The container with the unfiltered water has to be placed at a suitable height to provide the gravitational head to drive the water through the filter (lower head may be compensated for by larger filter area). While this could be achieved in different ways, a mechanisms have been illustrated in Figure 5-5a,b, where the container is placed on a sturdy stand. Food-grade tubing is needed to connect the container at the top to the filter holder.
Holding the filter in the device	Potential users in India highly preferred a holder with a screw-on mechanism (shown in Figure 5-5a-c). The particular holder, designed for 5 cm diameter, 0.375-inch thick filters, was machined from High Density Polypropylene, but the design is amenable to mass manufacture using injection molding. The key criteria that determine the successful functioning of the holder include: a) An O-ring of appropriate hardness, such that it conforms to the wood surface to seal it effectively. For the holder in Figure5-5a,b, silicone O-rings with a shore hardness of 70 (procured from The Hope Group in Massachusetts, USA) were used. b) Appropriate depth and width of the O-ring grooves is critical. Excessively deep grooves compromise the ability of the O-ring to conform to the wood surface, while shallow grooves may cause the O-ring to fall out, making user handling difficult. Further, O-rings that are smaller than the holder diameter reduce the effective cross-section area available for filtration (while accommodating a larger size range of filters), but larger O-rings increase the chance of leakage (when variations in filter shape cause the O-ring to sit on or beyond the filter edge). For the holder in Figure 5-5a,b, the depth and width of the O-rings was 76% and 124% of their thickness respectively (conventional design values are 80% and 120%) and the O-ring diameter was 25% smaller than that of the nominal diameter of the filter (as shown in the inset in Figure 5-5d). The inner diameter of the holder was 4.3 cm and the O-ring was designed to seal a filter with a diameter 4 cm or more. c) Preventing entrapment of air bubbles. Air trapped in the tubes and connectors can disrupt water flow. Using tubes and connectors with sufficiently large diameters and avoiding narrow constrictions in the flow pathway can avoid trapping of air. In Figure 5-5a,b, 0.25 inch diameter tubes and the connectors were used, and a vent consisting of a small hole plugged with an insert made of styrene butadiene rubber (SBR) procured from McMaster Carr (part number 9545K38) was also provided to release any air trapped within the holder.
Controlling water flow from the top container	A valve may be used between the top container and the filter, such that users can turn off the water supply when the device is not in use or while replacing the xylem filter cartridge.
Storing the filtered water	Food-grade container with appropriate capacity and a lid to prevent re-contamination should be used. The container should also have a dispenser to access the filtered water. This container should be placed at a height of at least 10-15 cm above the floor such that glasses, bottles, or other utensils can be placed below the dispenser.
Filter stand	The filter stand in Figure 5-5a was fabricated using 0.25-inch thick aluminum angle rods (90°). However, stands could be fabricated in several other ways.

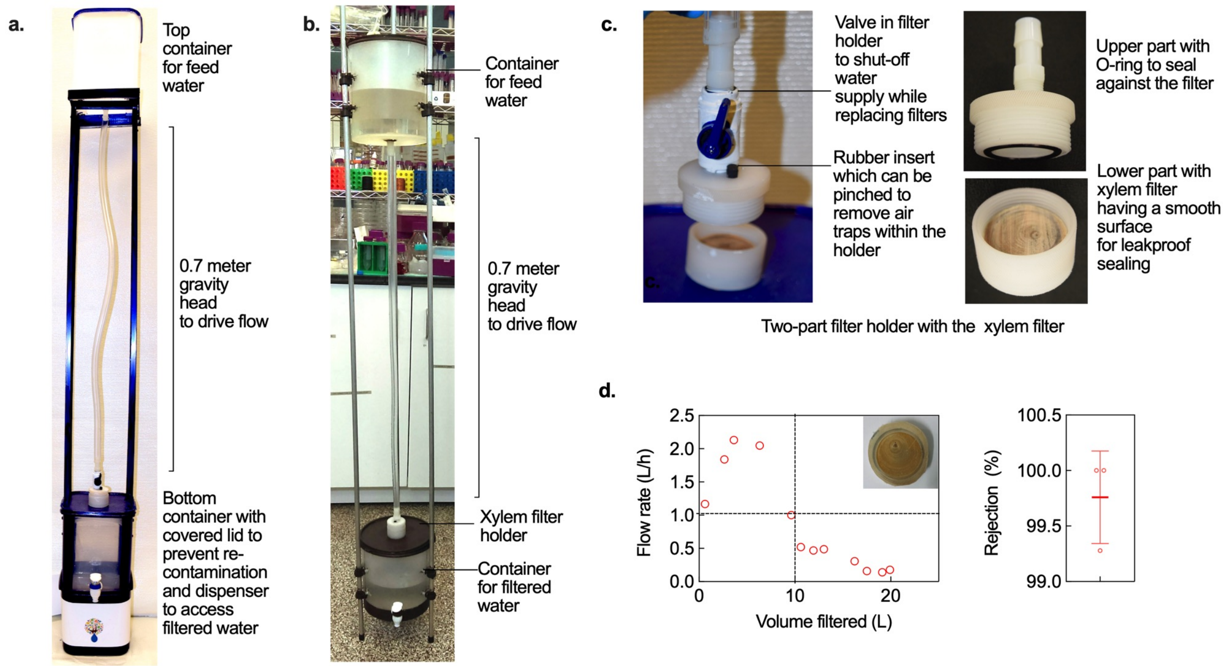

Figure 5-5: Xylem-based filtration device prototype. **a-c.** Device prototype and its components. **b.** Device prototype with which lab-based rejection tests and field-based capacity and flow rate tests were conducted. **d.** Device flow rate with tap water in New Delhi and rejection of 1 μm particles measured in the laboratory are consistent with prior characterization of xylem filters. Filters were made from ginkgo of 4 cm diameter and 0.375 inch thickness.

5.2.3 Xylem filters for emergency use

During disasters and emergency situations such as floods, disease outbreaks, contamination of public water supply network, etc., access to safe drinking water is a major cause of concern. Some common methods employed to provide safe drinking water to the affected population under such situations include distribution of bottled/packaged water procured from government agencies or commercial vendors, delivering water through water tankers, using neighboring water systems and using water treatment systems at point-of-entry or point-of-use [89]. Point-of-use, household water treatment (HWT) methods can be useful during the acute phase of an emergency when responders cannot reach the affected population and in the recovery phase when longer term solutions are still under development [90]. Effective use of HWT treatment methods, including water filtration, has been shown to effectively reduce the incidence of water-borne diseases during emergencies [90]. Examples of studies where filtration methods were evaluated during emergencies include the following:

use of ceramic filters after the 2004 tsunami in Sri Lanka [91] and 2003 floods in Dominican Republic [92], distribution of ceramic/biosand after the 2010 earthquake in Haiti [93, 94], and use of membrane/ceramic filters during an emergency in Pakistan in 2007 [95]. The key learnings from these studies, in conjunction with guidelines enlisted in the Sphere handbook (the primary reference tool for NGOs, UN agencies, and governments to respond to emergencies and disasters) that can help guide filter design and its implementation for emergency use include the following: a) the device should be able to meet minimum drinking water requirement for survival, which is 2.5-3 liters per person per day [96] and meet the minimum drinking water quality requirements during emergencies ($<$ 10 CFU/100 mL, turbidity $<$ 5 NTU) [96], b) the target price for emergency filters can be benchmarked against reported costs of filtration devices distributed during emergencies; during the 2003 floods in Dominican Republic, ceramic filters had an upfront cost of $15 (though they were distributed free of charge) with $4.50 recurring cost every 6 months for candle replacement [92], c) major factors affecting HWT usage rates amongst the affected population included quality of source water, prior experience with using HWT methods, need for training associated with device use, availability of replacement parts, level of programmatic support, ease of portability of device, and the living environment (usage rates varied between people living in permanent shelters and those moving between temporary shelters)[91, 92, 93, 95, 97, 95]. Based on findings presented in the manuscript, xylem filters could be designed to meet the performance and cost targets specified above and due to their light weight, could be easy to transport and distribute. Other factors which could affect product adoption such as user training, filter lifetime, supply and distribution strategy, and user-perceived need for product would need to be further investigated.

Figure 5-6 depicts a potential design for a xylem-based emergency used filter. It comprises of two plastic bags heat sealed around the xylem filter; the plastic bags act as receptacles for the feed and filtrate water respectively. Plastic containers have often been distributed for safe water storage and bucket chlorination during disasters and emergencies [98, 99, 100, 101]. Several membrane filters designed for emergency use are available commercially [95, 102, 103], and some of these are membranes are housed in a plastic bag/container [95, 103]. The robustness and longevity of the plastic bags would depend on the use case; filters could be designed to last for a single use or for a few days, weeks or months with provisions for cartridge replacement.

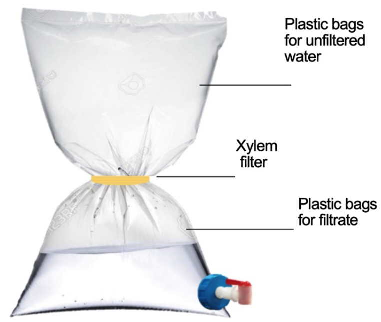

Figure 5-6: Illustration of possible configuration of xylem-based emergency use filter

5.3 Factors affecting xylem filter adoption

Although performance is important to the success of any technology, its adoption by the target population is critical to achieve impact. In addition to lack of awareness, the adoption of water treatment technologies in resource-constrained settings faces three main barriers: access, affordability, and acceptance. Xylem filters could lower these barriers in the following ways. First, the abundant availability of gymnosperms, local access to other raw materials required for filter fabrication (primarily alcohol for dry preservation), simplicity of the manufacturing process, and open access to this technology (filter fabrication has not been patented to facilitate adoption) could provide an opportunity for local manufacture of these filters, making them more accessible to local communities (see Table 5.2 for a list of equipment that can be used in low-resource settings). Due to their low weight (<10 g) and volume (∼13 cm^3), xylem filters could be shipped easily (even by post to remote locations) and stocked in local shops to facilitate access. Second, preliminary cost estimation studies suggest that, in comparison to conventional filter cartridges that cost USD 5-10 and require replacement every 3-6 months, xylem filters could cost USD 0.06–0.10 and require replacement every 1–5 days with a comparable cost per liter water filtered (see Section 5.4 for cost estimation details and Figure 5-7 for cost comparison with currently available commercial filters in India). Such amortization of filter replacement costs could significantly lower the barrier to affordability for low-income households, where ‘pay-as-you-go’ or ‘buying less, but more often’ model is preferred over longer-term filter replacements [88]. In Indian urban slums where Reverse Osmosis (RO) filtered water cans costing USD 0.28-0.56 per 20 L or government-operated water booths which provide RO water at USD 0.06-0.10 per 20 L are the only options, xylem filters could provide a viable HWT alternative for those who cannot afford or access these options easily. They could also facilitate sustained usage of HWT amongst those who are unable to purchase cans or fetch water from the booths on a regular basis to realize effective health outcomes. Furthermore, it could offer the opportunity to involve local suppliers, e.g., in small stores in the daily distribution of filters. Finally, the traditional comfort associated with using wood for fuels and utensils could facilitate the social acceptance of xylem filters. During field studies, 40% of the 300 survey respondents cited natural appeal and simplicity as the primary attributes they like about xylem filters, suggesting positive prospects for user reception.

The need for frequent filter replacement creates the risk of lack of user compliance and disruption of sustained usage, which could be exacerbated if pre-filtration units that require replacement at a different frequency than the xylem filters are used. Technological and product design improvements (such as engineering filters with longer lifetime, designing holders for easy filter replacement) or supply-related solutions (such as filters being available at stores that provide groceries or other regularly-purchased items) could help mitigate the risk of disruption of sustained usage, whereas the decrease in flow rate of filters with time mitigates the risk of lack of user compliance in replacing the filters. These efforts would nevertheless have to be complemented with behavior change interventions. The motivators for behavior change and thus, the nature of these interventions, would depend on the local social, cultural and environmental characteristics, such as water quality (whether water has visible coloration, odor or poor taste), perceived health risk associated with water quality, education level of the population, etc. [104, 105, 106, 107, 108, 109, 28]. The influence of these characteristics on HWT adoption has been extensively studied in literature and this knowledge, in addition to field studies with user groups, could be leveraged to facilitate sustained adoption of xylem filters. [104, 105, 106, 107, 108, 109, 28].

5.4 Estimation of xylem filter cost

Affordability is one of the major barriers impeding the major factor affecting the adoption of HWT technologies amongst low-income communities. To understand whether xylem filters could help lower this behavior, the cost of xylem filters was estimated.

The manufacturing costs of xylem filters (5 cm diameter and 0.375-inch thickness) are divided into four categories: raw material, wood processing, packaging, and transportation.

1. **Raw material:** Globally, the typical price of commercial softwood ranges from USD 40-140 per cubic meter [110]. Assuming that a filter uses ~20 cm^3 of wood, the cost of wood required for fabricating a filter is 0.08-0.30 ¢. It is to be noted that the sapwood used for making the filters is a waste by-product of the timber industry due to its low mechanical strength and durability, and may be available at lower cost.

2. **Processing:** The processing of xylem filters involves cutting the wood into desired sizes, soaking the filter in hot water, soaking the filters in alcohol and drying the filters. Assuming that filters are fabricated in batches of 500, the cost estimates for each of these steps has been provided below:

   (a) *Wood cutting cost:* This comprises of three components:

   - Equipment cost for cutting each filter calculated at a band saw cost of USD 900 [111, 112], equipment lifetime of 5 years, duration of operation of 8 hours/day for 5 days a week, and a processing time of 10 s per filter: 0.03 ¢
   - Utilities cost calculated for a band saw with a power rating of 1 kW [112] at an electricity tariff of 10 ¢per kWh [113]: 0.03 ¢
   - Labor cost calculated at USD 5 per day (cost based on field visits in India; could vary with geography) and a processing time of 5 s per filter: 0.09 ¢
   - Total wood cutting cost per filter: 0.15 ¢

   (b) *Hot water soaking:*

   - Cost of water calculated based on a water requirement of 0.5 L for every filter (can be further optimized) and an average water tariff of USD 1.98 per cubic meter [114]: 0.1 ¢

- Cost of utilities for hot water treatment assuming a heat consumption of 100 kJ, gas stove energy efficiency of 10%, and charges of 8 ¢/h for a gas stove with a standard capacity of 10,000 kJ/h: 0.8 ¢
- Labor cost assuming a labor charge of USD 5 per day and time requirement of 3 s for handling: 0.05 ¢
- Total cost of hot water treatment: 0.95 ¢

(c) **Alcohol treatment:** While filters were manufactured primarily using ethanol in this study, other alcohols like methanol and isopropanol may also be used. Methanol is less expensive than ethanol. However, since methanol is toxic, appropriate food safety standards should be consulted to determine the grade of methanol that can be used, and the level of residual methanol in the dried filters should be maintained within the permissible limits for human consumption as prescribed by safety standards [50].

- Cost of methanol treatment calculated at as requirement of 0.1 L per filter at a price of USD 0.6-1/kg : 5-8 ¢
- Labor charge for alcohol treatment estimated based on a labor charge of USD 5 per day and processing time of 3 s per filter: 0.05 ¢
- Cost of alcohol treatment for 1 filter: 5.05-8.05 ¢
- The cost for alcohol treatment can be substantially reduced if the alcohol is reused/recycled. Compared to immersion in a single alcohol bath, the alcohol consumption may be reduced by immersing the filters successively in multiple alcohol baths of smaller volumes.
- Total processing cost: ~6-9 ¢

3. **Packaging:** Cost of packaging filters individually in heat-sealed HDPE (high density polythene) pouches are estimated as follows:

- Cost of plastic required for packaging calculated using HDPE price of USD 2-5/kg [115], HDPE density of 930-970 kg/m^3 [116], plastic requirement of 1 cm^3 per filter (200 cm^2 area, 50 μm thickness): 0.19-0.5¢
- Cost of heat-sealing the plastic around the filter calculated based on a cost esti-

mate of USD 40 for the heat sealer [117], equipment lifetime of 1 year, operation duration of 8 h/day for 5 days a week and packaging time of 5 s per filter: 0.008 ¢

  - Labor cost for filter packaging estimated based on a labor wage of USD 5 per day and processing time of 5 s per filter: 0.09 ¢
  - Total packaging cost: 0.3-0.6 ¢

4. **Transportation:** Assuming no infrastructure for transportation, the filters can be sent by post from the manufacturing location to the customer base. The postal charges for sending filters as a package of 200 is 0.03–0.07 ¢(based on field visits in India, will vary with geography).

The total estimated cost of the xylem filters is therefore 6.5-10 ¢(INR 4.5–7). As evidenced by the performance studies on the field, the capacity of each filter could range anywhere between 10–100 L, depending on the water quality and nature of pre-filtration.

If used, a cloth filter would add $1 to the cost (taken from report of surveys conducted in India [66]) and could be washed or replaced once it is dirty.

The GAC needs replacement after its adsorption capacity is expended, the frequency of which depends on the amount of GAC used, water quality, and adsorption capacity of the GAC. A GAC column for pre-filtration is expected to cost USD 0.67–5 per 1000 L of water filtered and require a replacement every 1.5-6 months based on the following estimates:

- Cost of coal-based GAC in a column measuring 5 cm in diameter, 15 cm length (as described in Section 4.5.3) calculated using the following estimates:
  - (In the US) Price and density of USD 7-10.5 per kg and 0.5 g/cm^3 respectively for coal-based GAC [118]: USD 2-3
  - (In India) Price and density of INR 100 per kg and 0.5 g/cm^3 respectively for coal-based GAC [119]: INR 15
- Adsorption capacity of GAC for organic matter (estimated based on equilibrium adsorption data for dissolved organic matter (DOM) and humic acids on coal-based GAC (granule size 0.3-0.4 mm) reported in literature; input concentrations of DOM/humic

acid were in the range of 5.5-12 mg/L; adsorption capacity of GAC was determined based on the amount of humic acid adsorbed per unit mass of GAC at saturation, i.e., when the residual concentration in the treated solution equaled initial concentration [120]): 30-100 mg (DOM or humic acid)/ g GAC

- Total amount of organic matter that can be adsorbed by a coal-based GAC column with aforementioned dimensions: 4.5-12 g for DOM/humic acid
- Volumetric capacity of GAC column (total volume of water the GAC column can process while effectively removing organic contaminants) estimated based on adsorption capacity at different DOM/humic acid concentrations in water ranging from 5.5 mg/L to 12 mg/L [120]: $\sim$500 – 2000 L
- Cost of GAC column per 1000 liters of water: USD 0.5-3 or INR 0.008-0.03
- Replacement frequency of GAC columns assuming each household comprises of 4 members, each consuming 3 L of drinking water per day [85, 86]: 1.5-6 months

The recurring (cartridge) cost of a xylem filter is compared with different commercial filters in the Indian market in Figure 5-7. The rated capacity and the costs of filtration units and replacement cartridges was obtained from product websites [77, 76, 78, 79, 80, 81, 83, 82]. The cartridge cost was normalized by the rated capacity to obtain the cost per liter of filtered water. The recurring cost for xylem filters was estimated using the above xylem filter cost (INR 4.5–7). Cost of cartridge per liter water filtered was obtained by dividing the recurring cost with the filter capacity for different water qualities. Capacity was estimated by using the data presented for ginkgo filters in Figure 4-7, 4-11 to obtain the permeate filtered per unit area for different water qualities and pre-filtration methods and then scaling the permeate per unit area for filters with 5 cm diameter; capacity ranges and average values for different scenarios have been specified in the legend. The average cartridge cost per liter water filtered for each scenario was estimated by dividing the average filter price (INR 5.5) with the corresponding average volumetric capacity. The cost of the GAC column was evenly amortized across the cartridge replacement costs as follows:

- Assuming that the daily drinking water requirement for an average household is 8-17 L per day (as estimated in Section 5.2.1), the replacement frequency of the xylem filter cartridge was estimated for different water qualities based on the corresponding filter capacity (as specified in the legend for Figure 5-7).

- The total number of xylem filter cartridge replacements required over the lifetime of the GAC column (1.5-6 months) was estimated.
- The cost of the GAC column was evenly amortized across the total number of replacements and added to the filter cartridge cost.

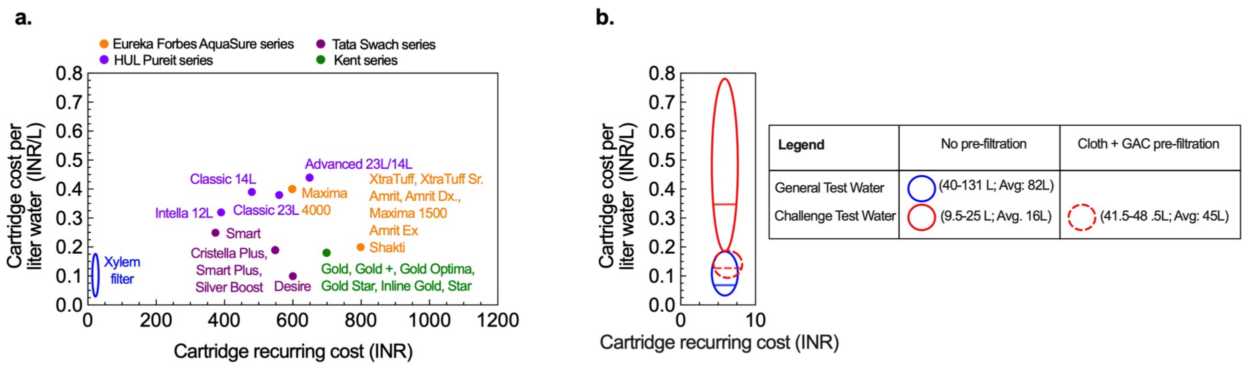

Figure 5-7: Cost comparison of xylem filters with other commercial filters. **a.** Comparison with commercial filters belonging to four major brands (denoted by different colors) in India shows the potential to offer unprecedentedly low cartridge replacement cost for a comparable or lower cost of cartridge per liter water filtered. Product names are included. **b.** Xylem filter cartridge cost per liter water filtered for CTW is greater than that for GTW; use of cloth and Granular Activated Carbon (GAC) pre-filtration can reduce this cost substantially for a marginal increase in recurring cost. Capacity ranges and average values corresponding to different water qualities and pre-filtration methods have been specified in the legend and are based on volumetric capacity measured for ginkgo filters in Figure 4-7 4-11 . The horizontal lines indicate average estimated cartridge cost per liter (calculated as ratio of average filter price (INR 5.5) and average volumetric capacity specified in the legend).

5.5 Environmental impact of creating xylem filters

Experiments conducted in the lab and the field suggest that xylem filter measuring 4 cm in diameter and 0.375-inches in thickness can process >10 L of water when operated under a 1 meter gravity head and can cater to the minimum drinking water requirement of an average household. Thus, the amount of wood needed to create a filter that can be used on a daily basis is $\sim$ 12 cm^3. If 1 billion people use the filters on a daily basis, then the amount of wood required to create 300 billion filters is $\sim 3.6 \times 10^6$ m^3. The volume of softwood traded on an annual basis is $\sim 100 \times 10^6$ m^3 [110]. So the amount of wood needed for 1 billion people is 3.6%, which is a small fraction of the global softwood trade. It is to be noted that xylem filters can be made from only the sapwood present in commercially traded timber and not the heartwood. In addition to wood from trunks which is traded commercially, xylem filters can also be made from tree branches that are discarded during the wood cutting process. Further, cultivating trees specifically for the purpose of creating xylem filters might be yet another option to sustainably produce them while minimizing environmental costs [121, 122, 123].

5.6 Design guide for selecting tree species for making xylem filters

To aid technology translation and the manufacture of xylem filters, a design guide for selecting tree species for making xylem filters has been provided below.

Gymnosperm xylem exhibits great morphological variability across and within species, and even within the same tree. A description of how the filter performance (i.e., permeance and rejection) may vary with the structural characteristics of the xylem is provided below. Appendix B and C list these characteristics in detail [42, 43, 124, 125, 126, 127, 128, 22, 129, 130, 131, 132, 133, 134] and also provides information on geographic availability, pricing, and decay resistance for a wide range of gymnosperms.

1. **Flow rate:** In general, for a given xylem structure without significant pit aspiration, the flow rate is expected to be proportional to sapwood area and driving pressure, and inversely proportional to filter thickness. In the absence of fouling, the flow rate through a xylem filter depends on:

    ○ *Fraction of xylem-containing sapwood present in the filter cross-section:* Flow rate increases with sapwood area. Inclusion of impermeable heartwood in filters (e.g., when a filter is made from a branch cross-section) can reduce the sapwood area available for filtration. Sections of branches used for filtration tend to have less effective area for filtration due to the impermeable heartwood present in the center. Filters made exclusively from the sapwood in trunks have their entire area available for filtration but are limited in size by the width of the sapwood in the trunk. Some tree species, like ponderosa pine (Pinus ponderosa), ocote pine (Pinus oocarpa), Douglas fir (Pseudotsuga menziesii), pond pine (Pinus serotina), red pine (Pinus resinosa), spruce pine (Pinus glabra), Virginia pine (Pinus virginiana), and sitka/yellow/western/silver spruce (Picea sitchensis) have a wider sapwood and are well-suited for creating large area filters [135].

- *Tracheid and pit membrane properties:* Wider and longer tracheids can yield higher flow rates [22]. A high fraction of the tracheid wall area covered by pit membranes and higher pit membrane porosity increase fluidic conductivity. The tracheid conductivity for some tree species is provided in Appendix B. The tracheid length plays a key role in determining filter thickness. Filters should ideally comprise not more than 2-3 tracheids along their thickness to minimize flow resistance without compromising rejection ability. Tracheid lengths could vary significantly within a tree. For example, tracheids in trunks are typically longer than those in stems, and tracheid length also decreases with tree height. However, tracheid lengths are typically less than 5.6 mm [24].

2. The rejection of the pit membranes depends on the following parameters:

   - *Pore size of pit membranes:* Smaller pores have better rejection performance. The pit membrane pore size varies considerably across and within a plant. The pores in latewood (summerwood) are typically smaller than those in earlywood (spring wood) [136]. Intra-species variability is most prevalent amongst pines [136], where reported pore size measurements vary from 200-400 nm [42, 43, 136]. The pit membranes in ancient gymnosperms (belonging to genus Cycas and Welwitschia), Ginkgo, and cedars (which belong to genus Thuja in the cypress family), are very dense and are expected to be capable of excellent rejection [136].
   - *Resin canals/ducts:* Resin canals/ducts are cylindrical intercellular spaces in the xylem oriented in the axial (longitudinal) direction [137]. Their inner surface is lined by epithelial cells, which secrete resins for defense against pests and pathogens [138]. If not filled with resin, these canals could act as leakage path-

ways in xylem filters and hence, attention should be paid to their presence. They could be several centimeters in length and are typically longer in the trunks than branches [139, 140]. Resin canals are generally present in *Picea* (spruce), *Larix* (larch), *Pseudotsuga* (Douglas fir), and *Pinus* (pine); those in pines being numerous, large and evenly spaced while the ones in spruces, larches, and firs are few, small, and evenly spaced [141, 142]. These canals are generally absent in *Abies* (fir), *Tsuga* (hemlock), *Pseudolarix* (golden larch), Cedrus (cedar), *Taxus* (yew, caution: yews are toxic), *Juniperus* (juniper), *Cupressus* (cypress) and *Ginkgo* (ginkgo) unless formed in response to external stimuli or stress [142, 143, 144].

3. **Preservation of xylem filters:** Filters made from tree species whose pit membranes are more resistant to cavitation may be less susceptible to drying-induced loss of performance during operation. Prior studies suggest that the resistance to cavitation depends on tracheid properties, the thickness or the rigidity of the torus, and ratio of the torus diameter to that of the cell wall aperture [128]. Junipers and cypress-pines tend to have high resistance to cavitation [128].

In addition to the aforementioned characteristics, the following notes could be useful for filter manufacture across species:

1. *Selection of branches:* Branches that do not have leaves undergo inactivation of the xylem through a process called compartmentalization [145] and have lower permeance. Junctions (where branches connect to one another) have a bent xylem vasculature and a lower hydraulic conductivity [146]. The wood from such branches and junctions should preferably not be used for manufacturing filters. Further, branches or trunks that show signs of decay (black marks, spots, etc.) should also be avoided.

2. *Standardization of filter size:* Xylem filters made from branches suffer from variability in shape and size, presenting challenges for designing compatible filter holders that can house these filters and enable their practical use. Coring out fixed-diameter circular discs from branches could enable standardization; but such a process can result in wastage of the xylem-containing sapwood present in the periphery while preserving the

impermeable heartwood in the center, thereby reducing the effective area available for filtration. Standardized filters with a high fraction of sapwood area can be manufactured by cutting rectangular or circular sections from the peripheral sapwood in tree trunks. Certain species like Ponderosa pine, which have wider sapwood in the trunk cross-section, are more suited for the creation of such filters [135]. It is to be noted that the length of xylem conduits could vary across trunks and branches; as a result, the filter thickness may need to be adjusted to maintain rejection performance [147]. 0.375-inch thick filters made from branches of Eastern white pine showed 98.8±1.2% rejection of 1 μm microspheres. However, 0.5-inch thick filters made from partly-dried trunks (procured after two weeks of felling) also showed comparable rejection (98.57±0.86%), whereas 0.75-inch thick filters showed a rejection of 99.21±0.73%.

3. Safety: Selection of tree species for filter fabrication should be preceded by a thorough investigation of the potential toxic effects of its sap and methods to eliminate them (if any). Sap of some trees is consumed by humans [148, 149], and sapwood occurs naturally in surface waters. Plants belonging to genus *Pinus* (pine), *Tsuga* (hemlock), and *Picea* (spruce) have been characterized as non-toxic [150, 151], though the needles of *Pinus ponderosa* (ponderosa pine) and *Pinus contorta* (lodgepole pine) and can be toxic to cattle during gestation [152, 150]. The oil extract of genus *Juniperus* (juniper) are commonly used in cosmetics [151]. Plants that are known to be non-toxic include *Norfolk pine* (Australia hemlocks). All parts of plants belonging to genus *Taxus* (yews) and *Cycas* (cycads) are known to be poisonous [153]. *Abies balsamia* (balsam fir) is known to be a skin irritant, and the seeds of *Ginkgo biloba* (ginkgo) are known to be poisonous [153]. Confirming that the sapwood is nontoxic is essential for use of the filters for filtering drinking water for human consumption. In addition, appropriate safety certifications and approvals may be procured before distributing the filters for human use. International NSF/ANSI standards are commonly employed to certify point-of-use drinking water filters. The following certifications could be useful before marketing the product:

   - *NSF/ANSI 53:* This standard is used to certify health-related microbiological contaminant reduction claims.

- *NSF/ANSI 42:* This standard is used to certify the chemical and material safety of filters. We note that drinking water standards typically do not have health-related constraints on natural organic matter in water (although there are constraints related to aesthetics, such as color).
- *NSF/ANSI 61:* This standard is intended to cover materials or products that come into contact with drinking water, drinking water treatment chemicals or both.

In the future, it may be useful to develop certifications specifically for xylem filters.

5.7 Quality control of xylem filters

Due to the variability in xylem structure across species, trees within the same species, and even across the same tree, reliable quality control methods are essential to ensure that the manufactured filters can achieve the desired performance. Test solutions that are made using commonly available, inexpensive materials and produce a visual output can be very useful for quality control. Examples of such test solutions include aqueous solution of turmeric and diluted yogurt (Figure 5-9, work performed in collaboration with Bettina Arkhurst [2]).

While such tests could be simple to perform, they involve wetting the filters with water, which renders the filters unusable. Such methods are therefore more suited for batch-based quality control protocols.

Other non-destructive methods, which do not involve wetting the filter, could also be developed for quality control of each manufactured filter. An example is the use of air seeded with dust or pollens particles with a known size distribution as a fluid medium for testing (Figure 5-9). The particle count in the feed and filtrate could be measured with the help of an air quality sensor to test the rejection performance of the filters.

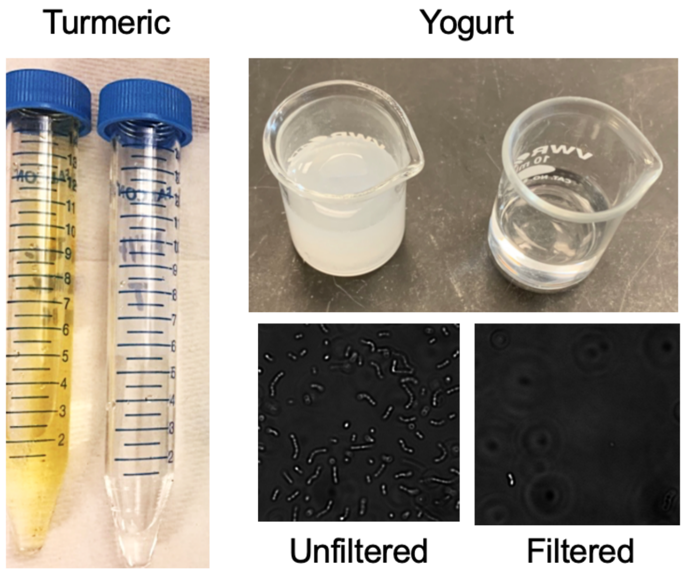

Figure 5-8: Test solutions for quality control of manufactured xylem filters. The images on the left and right show the use of turmeric solution and diluted yogurt as test solutions respectively and present a comparison between the feed and filtrate. The images on bottom right show images of a yogurt solution before and after filtration when examined under an optical microscope.

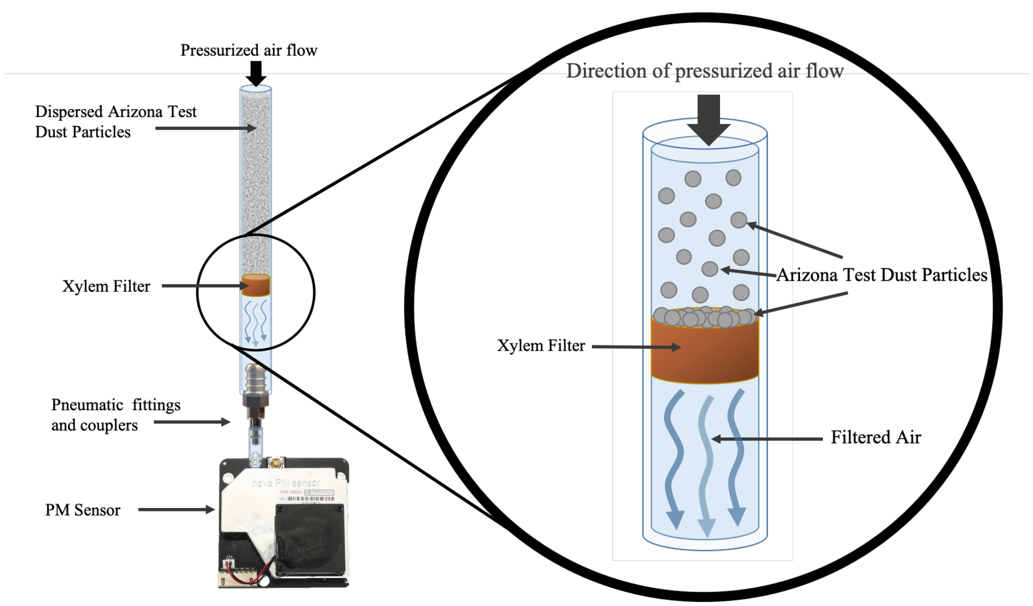

Figure 5-9: Schematic of set-up for quality control of xylem filters where air seeded with dust particles is used as fluid medium for testing [2].

5.8 Summary of findings

Tests conducted with natural water sources revealed that xylem filters were able to cater to the minimum drinking water need of an average household (8 L). 5 out of the 6 filters tested were also able to remove coliforms completely. A xylem filtration device prototype was developed using a user-centric design approach. Preliminary findings from market research and user studies in India suggest that the organic nature and simplicity of xylem filters are attributes that appeal to users and could promote user acceptance. Further, preliminary cost estimation studies suggest that xylem filters can amortize the cost for water treatment, which could lower the affordability barrier for low-income households, where high-frequency, inexpensive replacements are preferred over low-frequency, high cost replacements. A design guide for selecting tree species for fabrication of xylem filters was developed to aid entrepreneurs and NGOs in taking the technology to the users.

Chapter 6

Conclusion

This work provides new insights into gymnosperm xylem from the perspective of its use as a material for water filtration – namely, the interplay between permeance and rejection governed by percolation, the intriguing 'self-blocking' behavior arising from dissolution and re-deposition of hemicellulose, and elevated propensity for fouling in the presence of large organic molecules and dust. These insights were leveraged to develop engineering methods for preserving xylem filters in dry state, mitigating self-blocking, and obtaining practically useful performance with different water qualities (see Table 6.1 for a summary of the effect of the key design, manufacturing, and operating parameters on filter performance). To demonstrate potential for practical utility and translation, filters were fabricated using locally available gymnosperms in India and filter performance was validated with natural water sources used for drinking. As an example for how xylem could be incorporated in filtration devices, a gravity-operated, functional device prototype for household drinking water treatment was developed using user-centered design approaches. Finally, evidence gathered from user research and preliminary cost estimation studies was used to show how xylem filters could potentially reduce the barriers of access, affordability, and social acceptance to serve as an attractive HWT option for low-income communities that are at the highest risk of water-borne diseases.

Table 6.1: Effect of filter geometry, fabrication process, and operating conditions/parameters on xylem filter performance (flow rate, volumetric capacity (or filter lifetime), and rejection)

Performance metrics	**Xylem filter geometry**		**Fabrication methods**			**Operating conditions/parameters**	
	Sapwood area (A)	Thickness (t)	Dry preservation		Hot water treatment⋆ ⋆	With cloth or GAC pre-filtration	Pressure used for driving flow (P)
			Alcohol treatment⋆	Stacking multiple filters in series⋆			
Flow rate (Q)	$Q \propto A$	$Q \propto \sqrt{t}$, if tracheids are well-connected, else decreases more rapidly with thickness.	Increases	$Q \propto \frac{1}{n}$, where n is the number of filters	No significant effect.	Generally increases; depends on water quality and pre-filtration process.	$Q \propto P$
Volumetric capacity (V)	$V \propto A$ is expected for the same driving pressures	Unknown	Increases	Unknown; may be comparable to a single filter.	Increases if self-blocking is more dominant than fouling due to contaminants in water, else significant effect is not expected.	Generally increases; depends on water quality and pre-filtration process.	Unknown; depends on how pressure affects fouling.
Rejection							
Filtration-based	No effect	Depends on particle size. Typically increases rapidly till thickness exceeds tracheid length; may not increase further if sapwood has resin canals, else theoretically expected to increase with length.	Increases	Increases; theoretically log-additive, if rejection of each filter is identical.	Depends on temperature and duration of treatment; may decrease rejection. Soaking at high temperatures or for long durations compromises rejection performance.	Depends on pre-filtration method used; GAC can augment performance by removal of chemical contaminants.	No significant effect expected.
Adsorption-based (viruses)	No significant effect expected if permeate flux is constant	Expected to increase for the same driving pressure. $\tau \propto \frac{1}{t^2}$, where τ is contact time.	Unknown	Expected to increase; theoretically log-additive, if rejection of each filter is identical.	Unknown		Expected to decrease. $\tau \propto \frac{1}{P}$, where τ is contact time.

Benchmarked against water-dried filters⋆. Hot water treated, alcohol-dried filters compared to filters that are just alcohol-dried⋆⋆.[1]

With >3-log removal for bacteria and virus (>4-log removal for rotavirus), xylem filters can provide 'comprehensive protection' against water borne pathogens as per WHO's performance criteria for household water treatment technologies [57] and have potential for reducing the health burden of water-borne diseases (Figure 6-1). The contaminant removal ability of xylem filters could be improved further due to fouling, by stacking filters or using other approaches. Use of silver nanoparticles in xylem to enhance the removal of bacteria and methylene blue (a commonly used industrial dye that causes water pollution) using xylem [33] and chemical modification of xylem surface for copper adsorption has been reported [32]. Incorporating suitable sorbents such as zeolites or ferrous oxide into pre-filtration modules could enhance removal of viruses, arsenic, or other pollutants [154, 155]. More generally, xylem filters could be used synergistically with other water treatment methods, e.g., in conjunction with chlorine to remove protozoan cysts that are relatively chlorine-resistant. Beyond gymnosperms, plants like primitive angiosperms that have short xylem conduits and nanoscale pit membrane pores and are not prone to aspiration [156], or ubiquitous bamboo nodes64, could be explored for filtration applications.

Some limitations of xylem filters for household water treatment currently are: a) the higher replacement frequency of xylem in comparison to current filtration technologies could deter sustained usage, b) heterogeneity in xylem structure within and across gymnosperm species could create variability in filter performance, and c) a possibility that degradation of filters under certain conditions could cause their rejection performance to deteriorate and prevent them from getting blocked posing a health risk to users (timescales for degradation would depend on the feed water quality, type of wood used for manufacturing the filter, and local environmental factors and could be of the order of 1-2 weeks [157, 158] or less since bacteria are directly seeded on pit membranes during filtration). Mitigation of these risks will require: a) pilot studies of filter performance under different operational conditions (and measures such as increasing filter thickness or providing guidance as to when xylem filters are suitable for use if filter degradation is found to be an issue), b) standardization of sources of wood and development of methods to test and assure quality control [2], c) safety assessment of xylem filters for human use (e.g., assessing the filtrate for presence of organic compounds leached from xylem and evaluating their safety for human consumption,

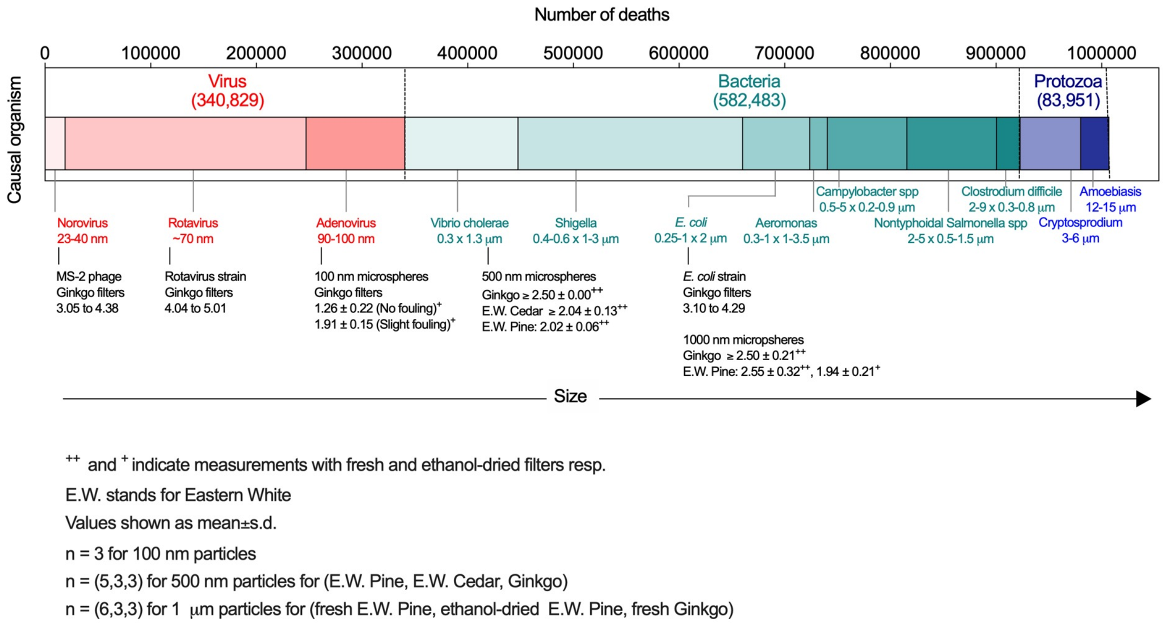

Figure 6-1: Comparison of diarrheal mortality burden due to different waterborne pathogens [25] arranged by size to the measured rejection of particles of different size by single 0.375-inch thick xylem filters (except for rejection experiments with 500 nm microspheres, where filter thickness was 0.25 inches) made from different tree species.

noting that cellulose, hemicellulose, lignin, and pectin occur naturally in many foods, and that plant material contributes to organic content in drinking water), d) designing filters holders for maximal ease of replacement along with ready local availability of filters, and e) behavior change interventions to facilitate sustained usage. To facilitate uptake and further advances in xylem filter technology, this technology has not been patented and a design guide for fabricating xylem filters and compendium enlisting the geographic availability, structural characteristics, and degradation properties of different gymnosperms have been created (Appendix B and C). It is important to use non-toxic sapwood for making xylem filters.

Xylem filters present a HWT solution with unique characteristics (see Table 6.2 for comparison with other low-cost water treatment technologies).

The availability of wood is not a bottleneck for scaling and large-scale dissemination – the sapwood used for making these filters is a low-grade by-product of the timber industry; we estimate that 0.01 % of the timber used in softwood global trade could be used to

Table 6.2: Comparison of xylem filters with other low-cost water treatment technologies

Technology	**Level of protection**	**Advantages**	**Limitations**
Chlorination [159]	Targeted (bacteria and viruses only)	• Portable, light-weight • Simple to use, no maintenance	• Less effective in inorganic-rich/turbid water • Unpleasant taste and odor • Dosage depends on water quality • Harmful organic by-products
Solar disinfection [159]	Targeted to comprehensive (bacteria, virus, protozoa)	• Little/no maintenance • Minimal chance of re-contamination • Simple to use	• Poor efficacy with turbid water • Dependent on weather • Volume to treat depends on availability of intact container • Long treatment time
Ceramic filters [160]	Targeted (bacteria and protozoa only)	• Local production • Minimal chance of re-contamination	• Performance heterogeneity due to manufacturing variability and susceptibility to cracks • Low flow rate: 1-3 L/h • Difficult to transport
Flocculation-disinfection [160, 159]	Comprehensive (bacteria, virus and protozoa)	• Residual protection against re-contamination • Easy to transport (typically available as sachets) • Reduction of some heavy metals and particle-associated pesticides	• Need for multiple steps and additional user support • Can have a negative effect on odor and taste • Requires reliable supply chain
Xylem filters	Comprehensive (>3-log removal of bacteria and virus)	• Biodegradable • Light weight • Minimal chance of re-contamination • Local production	• Frequency of replacement is relatively high (maintenance concerns) • Needs pre-filtration or large filter sizes with water having high turbidity or organic content • Low flow rate: 1-3 L/h

make a billion filters annually [161]. The compactness, light weight, and long shelf life of xylem filters could enable easy shipping to remote locations and bulk storage for prolonged use. Due to the worldwide availability of gymnosperms and simplicity of the filter fabrication process, xylem filters offer potential for local manufacture of a wide variety of water treatment and other filtration products, ranging from compact filter pouches for use in emergencies to household filtration devices. This opens up the possibility for involvement of micro-entrepreneurs at a global scale [162], implementation of innovative business models, and engagement of local communities in filter distribution [163, 164] and manufacture to raise awareness about importance of safe drinking water and encourage adoption. In addition, the characterization, modeling, and engineering of xylem filters is not limited to household water filters and has the potential to be applied to different filtration needs, such as for disaster and emergency use, microfiltration for assessment of water quality, and as an alternative to synthetic microfiltration membranes for some applications.

Part II

Removal of biomolecules using extracorporeal blood purification device

Chapter 7

Development of predictive mass transport model for an extracorporeal blood purification system to regulate Systemic Inflammatory Response Syndrome (SIRS)

This chapter is based on a paper by McAlvin *et al* [165].

7.1 Introduction

Systemic inflammatory response syndrome (SIRS) is a physiologic response of the body to a variety of insults [166] and involves complex interactions between platelets, leukocytes, coagulation system and multiple pro- and anti-inflammatory mediators (such as cytokines, histamines etc.) [167]. While SIRS is a result of innate immunity, it can have deleterious effects when it becomes excessive or uncontrolled and can result in multiple organ failure [167]. Sepsis, which is the systemic inflammatory response to a confirmed infection, is the primary cause of death in intensive care units [168]. The mortality associated with

SIRS can be attributed to at least two main causes - severely impaired immunity due to prolonged release of inflammatory mediators and the potential cytotoxic effects of cytokines [169, 170]. Prior studies have reported that septic patients tend to have high concentrations of circulating inflammatory cytokines (a condition referred to as hypercytokinemia), and mortality is highest when both, pro- and anti-inflammatory, cytokine levels are high [169, 171, 172].

In order to improve outcomes and survival rates for septic patients, extracorporeal blood purification (EBP) therapies where inflammatory mediators are removed to regulate host immune response have been proposed [169]. Examples of such techniques include high volume hemofiltration, high adsorption hemofiltration, high cut-off membrane filtration, plasma exchange, and hybrid systems like coupled plasma filtration adsorption [169, 173]. A vast majority of these therapies have focused on the non-specific removal of a broad-spectrum of inflammatory mediators to restore immune homeostasis [169, 173]. However, some of the drawbacks of these methods are that non-specific removal eliminates harmful as well as potentially beneficial cytokines [174] without accounting for the fact that specific cytokines can be deleterious at one stage but beneficial at others [175]. Non-specific cytokine removal during the pro-inflammatory phase could result in immunosuppression later [169]. Further, such processes also result in removal of desirable molecules, like therapeutic antibiotics [176] and clotting factors [177], which are required for patients with hypercytokinemia. A cytokine-specific, temporally controlled method for cytokine removal could be potentially beneficial in treating sepsis and a wide range of other conditions associated with SIRS [178, 179].

7.2 Background

EBP devices that selectively remove cytokines by passing the blood through tubes coated with antibodies against target cytokines (called Antibody-Modified Conduits or AMCs) have been developed (Figure 7-1) [180]. If installed in parallel and for patient-specific durations, AMCs could allow temporally controlled manipulation of specific cytokines during the inflammatory cascade in SIRS. From an implementation standpoint, the efficacy of such a device in arresting SIRS progression would depend on the identification of the right cytokine(s), and the extent, specificity, and rate of cytokine removal. The specificity will be largely governed by the choice of a suitable antibody and the extent and rate of removal will be determined by the transport properties of the AMC, which will in turn depend on antibody concentration, tube geometry, flow rate and other such parameters.

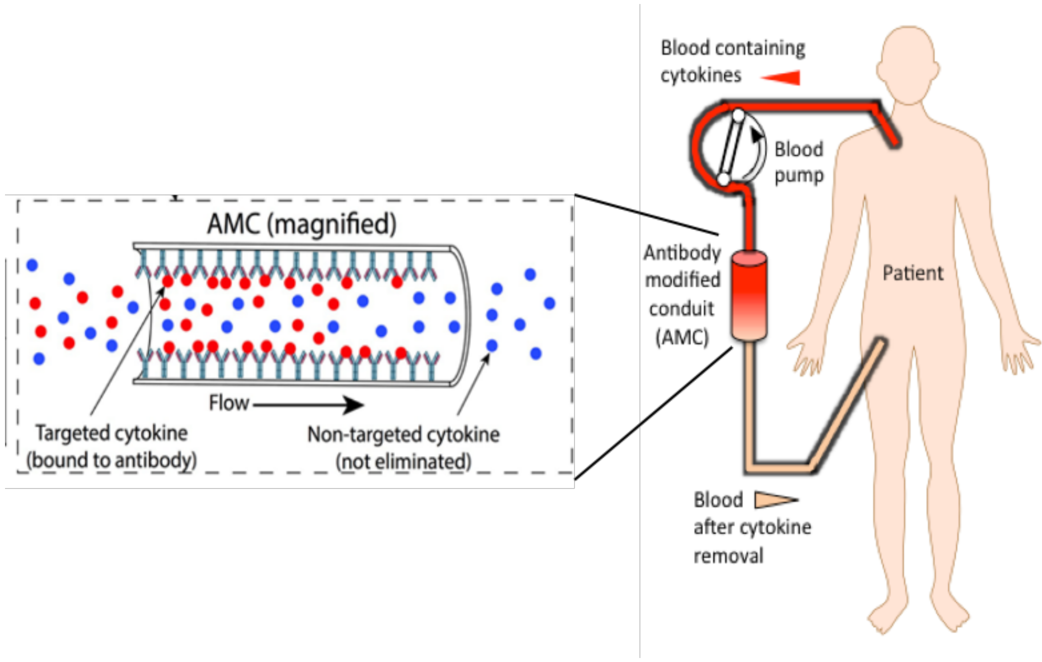

Figure 7-1: Schematic of extracorporeal blood purification with AMCs

7.3 Scope of thesis work for Part-II

Developing an understanding of the transport processes that govern the rate and effectiveness of cytokine clearance in AMCs is critical for designing effective devices for SIRS treatment. As a second goal, this thesis attempts to study the binding and transport kinetics of cytokine clearance from blood as a function of device geometry, operational conditions and surface properties to develop a predictive mass transport model that captures these interactions. This predictive model could then be used for building devices capable of achieving the desired performance. This research was conducted in collaboration with Boston Children's Hospital, where all experiments were performed. The thesis focuses on only the modeling part.

7.4 Modeling transport kinetics in AMCs

To identify the critical parameters affecting transport in AMCs, experimental data was gathered for different AMC geometries and operating conditions (experiments conducted by researchers in Boston Children's Hospital). AMCs were functionalized with antibodies against two model cytokines, human vascular endothelial growth factor A (VEGF-A) [181], which known to be an important determinant of sepsis morbidity and mortality and tumor necrosis factor α (TNF-α) that has been difficult to eliminate via non-selective EBP methods [182, 183, 184]. The efficacy of cytokine clearance was studied for 5 different variables: flow rate through AMCs, antibody concentration used during conjugation, AMC geometry (aspect ratio; length/inner diameter), circulating pH of fluid, molecular weight of PEG spacer molecules, and AMC surface area. These experiments were conducted using an *in vitro* apparatus where solutions of cytokines in PBS were circulated through the AMC and their concentration in the reservoir was measured over time (Figure 7-2).

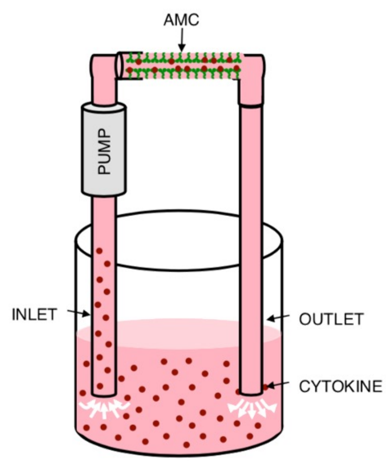

Figure 7-2: Schematic of the experimental setup for selective elimination of cytokines by AMCs. Cytokine-containing solutions were circulated through AMCs by a peristaltic pump and cytokine concentrations in the fluid were measured over time. Arrows represent direction of flow.

The different conditions under which the AMC performance was evaluated have been described in Table 7.1. The PEG spacer length and reservoir volume were held constant at 10 kDa and 20 mL in all cases.

Cytokine elimination from circulating fluid occurs when cytokines in the fluid bind to surface antibodies and is described by the Langmuir adsorption isotherm. The fraction of

Table 7.1: Experimental conditions for studying AMC performance *in vitro*

Parameter varied	**Inner diameter (mm)**	**Length (mm)**	**Surface area (mm2)**	**Aspect ratio (length\diameter)**	**Flow Rate (ml/min)**	**Antibody concentration (mg/ml)**
Conduit Geometry	1.6	886.0	4451.3	553.8	20.0	1
	1.6	444.5	2233.2	277.8	20.0	1
	3.2	222.3	2233.7	69.5	20.0	1
	4.8	148.1	2232.2	30.9	20.0	1
	4.8	98.6	1486.1	20.5	20.0	1
	6.4	111.3	2236.7	17.4	20.0	1
	6.4	55.4	1113.3	8.7	20.0	1
	9.5	74.2	2213.4	7.8	20.0	1
	9.5	24.6	733.8	2.6	20.0	1
	0.8	150.0	376.8	187.5	20.0	0.5
	0.8	400.0	1004.8	500.0	20.0	0.5
	0.8	400.0	1004.8	500.0	20.0	0.5
	0.8	400.0	1004.8	500.0	20.0	0.5
	0.8	800.0	2009.6	1000.0	20.0	0.5
	0.8	800.0	2009.6	1000.0	20.0	0.5
	1.6	150.0	753.6	93.8	20.0	0.5
	1.6	400.0	2009.6	250.0	20.0	0.5
	1.6	400.0	2009.6	250.0	20.0	0.5
	3.2	150.0	1507.2	46.9	20.0	0.5
	3.2	200.0	2009.6	62.5	20.0	0.5
	3.2	200.0	2009.6	62.5	20.0	0.5
	3.2	200.0	2009.6	62.5	20.0	0.5
	3.2	400.0	4019.2	125.0	20.0	0.5
Antibody Packing Density	3.2	222.3	2233.7	69.5	20.0	2
	3.2	222.3	2233.7	69.5	20.0	1.5
	3.2	222.3	2233.7	69.5	20.0	1
	3.2	222.3	2233.7	69.5	20.0	0.5
	3.2	222.3	2233.7	69.5	20.0	0.1
	3.2	222.3	2233.7	69.5	20.0	0.05
Flow Rate	3.2	222.3	2233.7	69.5	10.0	1
	3.2	222.3	2233.7	69.5	20.0	1
	3.2	222.3	2233.7	69.5	30.0	1
	3.2	222.3	2233.7	69.5	40.0	1

antibodies with bound cytokines (θ) depends on the concentration of cytokines at the conduit surface (c_s) and the affinity of surface antibodies for those cytokines (dissociation constant, K_d) according to the following relation [185]:

$$\theta = \frac{1}{1 + K_d/c_s}$$

Effective cytokine elimination (large θ) occurs when the antibody affinity for a given cytokine is high (small K_d) and the initial concentration of that cytokine (c_0) significantly exceeds K_d. Otherwise most of the antibodies will remain un-bound (small θ). A second necessary condition for large θ is that the quantity of surface antibodies must be greater than or equal to the quantity of cytokine molecules in the solution. Under these conditions, nearly complete clearance of the cytokine is expected (Figure 7-3, Case A). If the affinity is high, but the number of antibodies is less than the number of cytokine molecules (either from low surface antibody packing density or insufficient AMC surface area with optimal antibody packing density), then the cytokine concentration will decay until AMC capacity is saturated (Figure 7-3, case B).

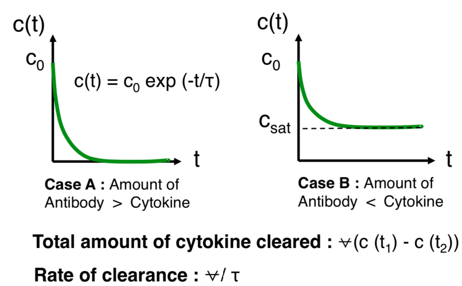

Figure 7-3: Two theoretical cytokine elimination curves representing either complete (Case A) or incomplete cytokine elimination (Case B); the latter represents AMC saturation with cytokine (C_{sat}).

Experimental data showed both patterns, where the cytokine concentration either decreased to zero or a steady value [165]. In the latter case, the amount of unbound cytokine remaining in circulation is given by $M = M_0 - C$ where M_0 is the initial amount of cytokine in the reservoir and C is AMC capacity. Provided $c_0 >> K_d$, then the number of antibodies available for binding is a good approximation of capacity.

Cytokine uptake by antibodies involves two main steps: transport of cytokine to surface antibodies and subsequent binding. The flow of cytokine containing fluid through the AMC is accompanied by the formation of boundary layers, which are regions close to the wall where, there exists a concentration and velocity gradient (Figure 7-4).

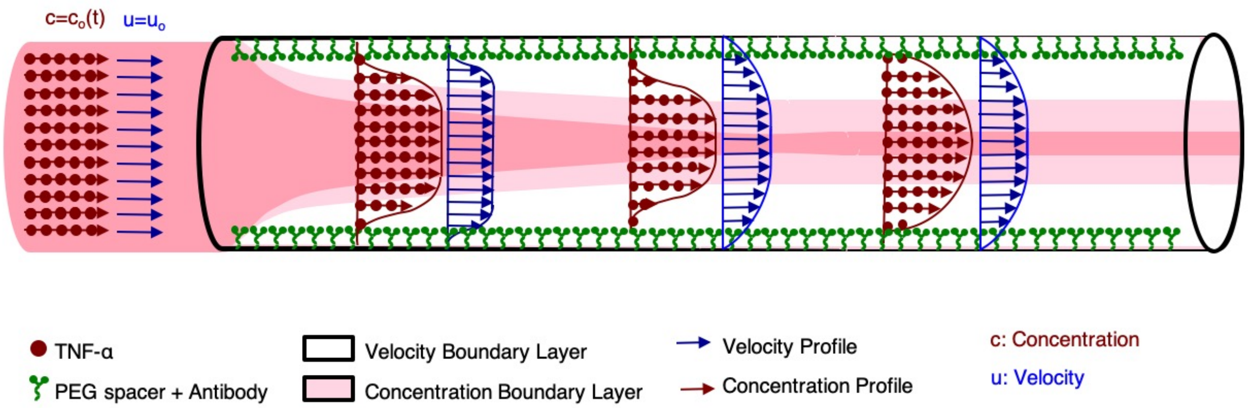

Figure 7-4: Schematic depicting transport in AMC. Thickness of concentration and velocity boundary layers (δC and δV respectively) vary along the length of an AMC until flow is fully developed, referred to as concentration (L_C) and hydrodynamic (L_H) entrance lengths respectively.

The concentration boundary layer is formed because of the presence of antibodies, which reduce the cytokine concentration near the surface to zero establishing a concentration gradient across the cross section of the AMC. The thickness of this boundary layer (δ_c) depends on diffusivity (D), or the ability of the cytokine to diffuse from the bulk solution to the wall where it can interact with the antibodies. The velocity boundary layer arises because the fluid in contact with the wall has zero velocity in comparison to the bulk fluid, which is being circulated at a certain flow rate. The velocity gradient arises because of viscosity, or relative motion between fluid molecules and gives rise to shear in the fluid. The thickness of this boundary (δ_v) layer depends on fluid viscosity. Both, the velocity and concentration boundary layers vary in their thickness up to a certain length, referred to as the hydrodynamic entrance length (L_H) and concentration entrance length (L_C) respectively, beyond which their thickness is constant and the velocity/concentration profile across the length remains unchanged (Figure 7-4). If the length of the AMC is much more than the entrance length, the flow is referred to as developed. Depending upon the flow parameters, tube geometry and diffusivity of the species involved in mass transport, the flow may or may not be developed velocity-wise, concentration-wise or both.

If the antibody affinity is high and antibodies are available for binding, uptake of cytokine by the antibody depletes cytokine within the concentration boundary layer near the wall, the thickness of which is governed by the combination of laminar flow in the axial direction and cytokine diffusion in the radial direction. The time scale for diffusion across the concentration boundary layer to the AMC wall where binding takes place can be estimated to be δ^2/D based on Fick's law of diffusion. For binding to occur between the antibody and cytokine, the diffusion time scale must be at least equal to (if not shorter than) the time scale for fluid flow from one end of the AMC to the other, as otherwise, the cytokine will cross the AMC without interacting with the antibody-coated surface. The time scale for fluid flow can be estimated to be L/V, where V is the fluid velocity, and L is the AMC length. Equating the two time scales gives Equation 1.

Equation 1:

$$\delta^2 \approx \frac{DL}{V}$$

Further, in fully-developed pressure driven laminar flows, it can be assumed that the velocity within the boundary layer scales linearly with the distance from the wall (Equation 2). For the conditions under which the experiments were conducted, the velocity profile was predicted to be fully developed, except in a very few cases and the flow was found to be laminar (based on Reynolds Number calculations). This approximation is valid as long as the ratio of the concentration boundary layer thickness to the AMC diameter is much smaller than 1, which for all experimental conditions tested was of the order of 10^{-2}.

Equation 2:

$$V \sim \gamma\delta$$

Here, V is the fluid velocity within the boundary layer and γ is the fluid shear at the wall. Equation 1 and 2 were then combined to give an expression for the concentration boundary layer thickness (Equation 3).

Equation 3:

$$\delta \sim \sqrt[3]{\frac{DL}{\gamma}}$$

As the fluid velocity varies roughly between zero to the average flow velocity, U_0(which can be expressed in terms of the volume flow rate $\dot{V}$ and AMC diameter d), an order of magnitude of estimate for γ can be obtained using Equation 4.

Equation 4:

$$\gamma \sim \frac{U_0}{d} \sim \frac{\dot{V}}{\pi d^3}$$

Combining Equation 3 and 4, an expression for δ can be obtained in terms of all known parameters (Equation 5).

Equation 5:

$$\delta \sim \sqrt[3]{\frac{\pi d^3 DL}{\dot{V}}}$$

As described in in the main text, the elimination of cytokines will be limited to an annular region near the AMC surface having a thickness of δ. The mass clearance rate of cytokines $\dot{m}$ can therefore be expressed as shown in Equation 6, where A is the area of the annulus, C is the cytokine concentration and V is the velocity.

Equation 6:

$$\dot{m} = ACV = (\pi d\delta)CV \sim (\pi d\delta)C\frac{U_0}{D}\delta$$

Substitution of Equation 5 in 6 gives Equation 7.

Equation 7:

$$\dot{m} \sim \pi(\frac{\pi d^3 DL}{4\dot{V}})^{2/3}U_0 \sim C\sqrt[3]{4\pi^2 D^2 L^2 \dot{V}}$$

The rate of change in cytokine concentration, $\dot{C}$ depends on the mass clearance rate $\dot{m}$ and reservoir volume V_r, as shown in Equation 8, which in combination with Equation 7 gives Equation 9.

Equation 8:

$$\dot{C} = \frac{dC}{dt} = -\frac{\dot{m}}{V_r}$$

Equation 9:

$$\frac{dC}{dt} \sim -\frac{C}{V_r}\sqrt[3]{4\pi^2 D^2 L^2 \dot{V}}$$

Equation 9 can be integrated to predict the variation in cytokine concentration in the reservoir over time (Equation 10 and 11), which can be then used to estimate the rate of cytokine removal by the AMC or the cytokine half-life (Equation 12).

Equation 10:

$$\frac{dC}{C} \sim -\frac{\sqrt[3]{4\pi^2 D^2 L^2 \dot{V}}}{V_r}dt$$

Equation 11:

$$C = C_0 e^{-\frac{t}{\tau}}$$

Equation 12:

$$\tau = \frac{V_r}{\sqrt[3]{4\pi^2 D^2 L^2 \dot{V}}}, T_{\frac{1}{2}} = 0.693\tau = 0.693\frac{V_r}{\sqrt[3]{4\pi^2 D^2 L^2 \dot{V}}}$$

The rate at which the cytokine concentration is reduced in the reservoir, can be estimated with Equation 13.

Equation 13:

$$\dot{V}_r = \frac{V_r}{\tau} = \sqrt[3]{4\pi^2 D^2 L^2 \dot{V}}$$

The expression used above can be validated with the solution to a very commonly studied flow and transport problem for laminar fluid flow between two surfaces separated by a constant thickness, referred to as the Lévêque problem [186]. The solution applies to a mass transfer problem, where the solute in the incoming fluid reacts with the surface to reach a constant concentration, different from the inflow. For our purpose, the cytokine concentration at the surface could be zero or non-zero depending on whether the antibodies are saturated or not. The Sherwood number (Sh), which is indicative of the volumetric clearance rate, is given by Equation 14. Here, Re denotes the Reynolds number and Pr denotes the Prandtl number. Expressions for Re and Pr have been given in Equation 15, where ρ denotes fluid density and μ denotes fluid viscosity.

Equation 14:

$$Sh = 1.615\sqrt[3]{RePr\frac{d}{L}}$$

Equation 15:

$$Re = \frac{\rho U_0 d}{\mu}, Pr = \frac{\mu}{\rho D}$$

Substitution of Equation 15 in 14 gives the following expression for Sh (Equation 16).

Equation 16:

$$Sh = 1.615\sqrt[3]{\frac{d^2 U_0}{DL}} = 1.615\sqrt[3]{\frac{4\dot{V}}{\pi DL}}$$

The total volumetric clearance rate across the entire AMC can then be expressed as shown in Equation 17.

Equation 17:

$$\dot{V}_r = \pi D L S h = \sqrt[3]{4\pi^2 D^2 L^2 \dot{V}}$$

It can be seen that Equation 12 and 17 are similar in terms of their dependence on the parameters involved. We note that Equations 12 and 17 ($T_{\frac{1}{2}} = \frac{V_r}{\sqrt[3]{4\pi^2 D^2 L^2 \dot{V}}}$ and $\dot{V}_r = \sqrt[3]{4\pi^2 D^2 L^2 \dot{V}}$) hold only in the case where the AMC capacity is much greater than the cytokine amount (i.e. $M_0 < C$). If not, the upstream end of the conduit will be saturated with cytokines and shorten the effective length L of the conduit, slowing cytokine clearance).

The rate of change in cytokine concentration predicted by the model (Equation 13) correlated linearly (R^2 value = 0.8) to that obtained by fitting exponential curves to the data (Figure 7-5A). The model also predicted volumetric clearance (i.e. the volume of solution from which the target cytokine is completely eliminated per unit time; m^3/s). Predicted volumetric clearance (Equation 17) correlated linearly with measured values (R^2 value = 0.9; Figure 7-5B). The measured volumetric clearance ($\dot{V}_r$) was obtained by dividing the fluid volume in the reservoir (V) by the time constant τ. Thus, the theoretical model accurately describes (and quantifies) AMC function, and can be used to predict, cytokine half-life and the volume of blood a given AMC will clear based on input parameters for that AMC.

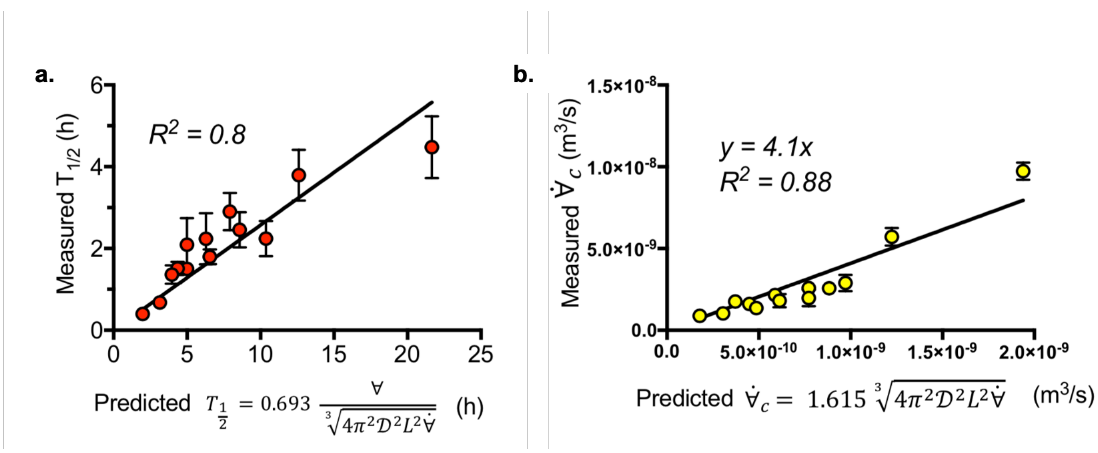

Figure 7-5: Comparison of theoretical cytokine elimination ($T_{\frac{1}{2}}$) and volumetric clearance from a predictive model (x-axis) to measured cytokine elimination rate and rate of volumetric clearance (m^3/s) (y-axis).

Further, in addition to the rate of cytokine clearance, the total amount of cytokines that can be removed is an important performance parameter of practical relevance. This would depend on the number of binding sites on the AMC surface that are accessible to cytokines, which, in turn, would vary with the antibody concentration, AMC surface area and the method used for coating antibodies on the AMC surface. If the number of accessible binding sites exceeds the number of cytokines, the cytokine concentration in the reservoir will eventually reduce to zero or else, the cytokine concentration in the reservoir will saturate at a non-zero value. AMCs have to be designed such that the cytokine concentration in the reservoir is reduced to the desired level without over-saturating the AMC surface with antibodies, as it would drive up device costs significantly. In order to understand the dependence of total cytokine clearance on antibody concentration and surface area,the variation in total cytokine removal for different tube geometries (or surface areas) coated with a certain antibody concentration was studied. The total cytokine clearance varied linearly with the surface area (Figure 7-6). This allowed calibration of the total cytokine clearance against a specified AMC geometry and antibody concentration. In conjunction with the mass transport model, this knowledge can help predict the rate of clearance for different operating conditions.

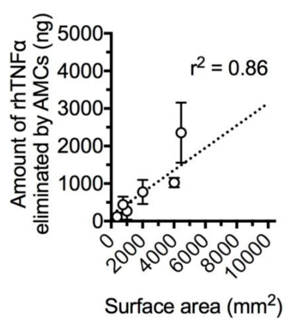

Figure 7-6: Variation of total cytokine clearance with AMC surface area

7.5 Conclusion

A predictive mass transport model was developed to guide the design of AMCs, that selectively remove cytokines from the bloodstream. The model captures the dependence of rate of cytokine clearance on device geometry and operating conditions. The predictions of the model correlated to experimental data with 80% accuracy. In the future, AMCs could be used for creating flexible and highly selective blood-filtering platforms for elimination of individual, harmful cytokines as they are expressed, without eliminating other cytokines best left in the circulation. This could greatly facilitate the development of personalized treatment strategies.

Appendix A

Methods for design and development of xylem filters

M1. Cutting of xylem filters from branches: Straight sections of branches of appropriate diameters were excised from trees (Eastern white pine, Eastern white cedar, ginkgo or chir pine) using a hand pruner and immediately immersed in water to prevent drying (in winter, foliate branches were excised and stored indoors at room temperature for 2-3 days with one end immersed in water to restore xylem activity and improve permeance). Branches without leaves or sections near branch attachments were avoided due to their low hydraulic conductivity [146, 145]. The natural direction of water flow in the branch was marked with a sharpie and the same flow direction was maintained in experiments. The branches were subsequently cut into smaller sections with the desired filter thickness using a band saw. The outer bark and the cambium were peeled off by hand to obtain a fresh xylem filter. The filters were stored in deionized (DI) water for up to 8 h before experiments or subsequent processing steps.

M2. Water flow rate and volumetric capacity measurements: Xylem filters were mounted in PVC (polyvinyl chloride) tubes with 0.375, 1.5 and 1.875 inch inner diameters (McMaster-Carr, part numbers 5233K63, 5233K77, and 53945K22) for 1, 4, and 5 cm diameter filters respectively. The tubing was connected to a nitrogen tank and regulator set at 10 psi (69 kPa) pressure, or to a water-filled tank providing a one-meter head (specified in figure captions). For nitrogen gas-driven measurement of permeance of 1 cm diameter filters, the tube was filled with 5 mL of DI water and the time required by the filter to process the water was used to obtain the flow rate. In gravity-driven experiments to measure permeance and capacity of 4-5 cm diameter filters, the volume of fluid processed by the filters was monitored with time by collecting it in a cylinder with 20 mL graduations to obtain flow rates. For experiments with DI water containing Ca^{2+} and K^{+} ions, calcium chloride dehydrate (ACS reagent grade, CAS number 10035-04-8, Sigma Aldrich) and potassium chloride (ACS reagent grade, CAS number 7447-40-71, Sigma Aldrich) were dissolved in DI water at the desired concentrations. For experiments involving intermittent filter operation, filters made from ginkgo (4 cm diameter, 0.375 inch thickness) were operated for a duration of 7 days while processing 5 L of DI water per day. The filters were mounted in the device and operated under a gravitational pressure head of 1 m with 5 L of DI water added into the device every day. The filters stopped producing water after the 5 L water was filtered, till 5 L of water was again added on the following day. The time required to filter the 5 L of water was measured and used to calculate the average permeance to filter 5 L of water on a given day. Permeance was calculated by normalizing the flow rate by the filter cross-section area and the driving pressure. The permeate processed by the filter per unit area was calculated by normalizing the volume of the fluid processed by the filter by the filter cross-section area.

M3. SEM Imaging: Xylem structure was visualized using scanning electron microscopy (SEM, Zeiss Merlin High Resolution). Samples were prepared by cutting thin slices of dried filters in the direction of the flow (longitudinally) using a razor blade. No coating was applied on the samples. The slices were imaged under an extra high tension (EHT) voltage of 0.7-1.2 kV and a probe current of 75-90 pA. The depth-of-resolution mode was used for acquiring images. The images in 2-3 were obtained from ethanol-dried xylem filters made from ginkgo (1 cm diameter and 0.25 inch thickness) after filtration of DI water spiked with 500 nm carboxylate-modified latex microspheres with yellow-green fluorescence (Thermo Fischer Scientific).

M4. Rejection of fluorescent microspheres: Carboxylate-modified latex microspheres with yellow-green fluorescence (Thermo Fischer Scientific) with diameters of 100 nm, 500 nm and 1 μm were used. The microspheres were sonicated for 1 min using a VWR Ultrasonic Mixer at room temperature (25°C), suspended in DI water at 10^6 mL^{-1} concentration, and vortexed for 1–2 min to obtain a homogenous feed solution. The feed solution was filtered through xylem filters mounted in PVC tubing at 10 psi pressure as described above. The filtrate was collected in clean glass vials; a feed volume 3–4× of the filter volume was used. 10 μL of the feed or filtrate solutions was introduced into a hemacytometer (Neubauer-modified C-Chip, InCyto) and imaged with a Nikon TE2000U epifluorescence microscope using a 20× objective for the 1 μm microspheres or 40× objective for the 500 and 100 nm microspheres. Due to the limited depth of focus of the 40× objective, images were acquired (by Andor iXon camera) with the focus at the mid-plane of the C-chip, and the particle count in this plane was used to estimate the concentration of the 100 nm microspheres. Particle counts over three draws of the feed/filtrate solution were averaged to calculate rejection as follows, where C_{feed} and $C_{filtrate}$ are microsphere counts in the feed and filtrate solutions, respectively.

$$Rejection = \frac{(C_{feed} - C_{filtrate})}{C_{feed}}$$

$$Log - rejection = -log_{10}(1 - Rejection)$$

Rejection tests with the xylem filter device were conducted by mounting the xylem filter in holder Figure 5-5c,d, connecting the holder to PVC tubing, filling the tubing and the holder with a fluospheres solution (concentration of 10^6 mL−1) of volume 3-4× that of the filter and pressurizing the fluid through the holder at 10 psi gas pressure. The filtrate was collected in a glass beaker. The particle count in the feed and the filtrate were evaluated as above. As the mechanism of bacterial removal in xylem filters is physical sieving [30], we expected the contaminant size to be the key determinant of the filtration characteristics and used 1 μm latex microspheres as surrogates for *E. coli*. Latex microspheres have been used as proxies to study the transport of *E. coli* in porous media due to the similarity in size and zeta potential [187, 188].

M5. Treatment of xylem filters: For hot-water treatment, filters were soaked in covered glass containers filled with deionized water maintained at 60 °C using a temperature-controlled water bath for 1 h (tap water and a simple stove was used for filters fabricated in India). A wood to water volume ratio of 1:20 was used. Subsequently, filters were immersed in room-temperature DI water 4-5 min before subsequent processing. 200-proof ethanol (CAS number 64-17-5, Koptec) or isopropyl alcohol (ACS reagent grade, CAS number 67-63-0, Macron Chemicals) were used for alcohol treatment; ethanol with 99.9% purity (CAS number 64-17-5, Merck) was used for field studies in India. Ethanol-water mixtures of different concentrations were prepared by adding DI water to 200-proof ethanol (CAS number 64-17-5, Koptec). Xylem filters (freshly cut or hot-water soaked) of 1 cm and 4-5 cm diameter were treated by flushing and soaking, respectively (filter to alcohol volume ratio of 1:6–8). For flushing, 10 psi gas pressure was used to drive the desired volume of alcohol loaded in plastic tubing in which xylem filters were mounted, similar to permeance measurements described above. For soaking, filters were placed edge-on in appropriately sized glass vessels and completely immersed in ethanol for 24–48 h. Most of the filters (90%) were soaked for more than 24 hours, in which case the alcohol in the vessel was typically replaced with fresh alcohol after 24 h. Filters manufactured in the field were treated with alcohol only by soaking.

M6.Drying of xylem filters: In laboratory experiments, all filters (soaked in water or in alcohol) were dried by placing edge-on on aluminum foil in an oven (VWR 1410 vacuum oven) at atmospheric pressure and 45 °C. To ensure complete drying, the filter weights were monitored (using Mettler Toledo AL104 scale) till they stabilized. Water-dried filters with 1 cm diameter and 0.25, 0.5 and 1.5 inch thickness required around 10, 25, and 50 h for complete drying. Ethanol treatment resulted in faster drying (6-8 h for 1 cm diameter, 0.25-inch thick filters). In field studies, filters were dried at 45 °C and atmospheric pressure for equivalent durations (at least 48 h) using an oven (Bajaj Majesty 1603 TSS Oven Toaster Grill) but weights were not monitored due to lack of access to appropriate instruments.

M7. Atomic Force Microscopy (AFM): DI water was filtered through Eastern white pine filter (1 cm diameter, 0.375 inch thickness) at a gas pressure of 10 psi using the method described above. The filtrate was collected in a clean glass vial, dispersed and dried on a substrate and analyzed under an Asylum-2 MFP-3D Coax AFM.

M8. FTIR Eastern white pine xylem filter (1 cm in diameter, 0.375 inch thickness) was placed in a glass vial filled with 20 mL deionized water maintained at 60 °C for 1 h. The filter was removed and the residual water was evaporated until the volume reduced to 5 mL to concentrate any extracts from the filter. The concentrate was analyzed using a Perkin Elmer FT-IR spectrometer.

M9. Preparation of synthetic test waters General Test Water (GTW) and Challenge Test Water (CTW) were prepared by adding the WHO-prescribed dosage of sea salts (Sigma Aldrich, product number S9883), sodium bicarbonate (BioXtra, 99.5-100.5%, CAS number 144-55-8 procured from Sigma Aldrich), and tannic acid (ACS reagent grade, CAS number 1401-55-4 obtained from Sigma Aldrich,), or humic acid (50-60%, CAS number 68131-04-4 procured from Alfa Aesar) in DI water Table 4.1 [57]. To achieve a turbidity of 40 NTU in CTW, Arizona Test Dust (ISO 12103-1, A2 fine test dust obtained from Powder Technologies Inc.) was added to DI water at a concentration of 70 mg/L.

M10. Susceptibility of xylem filter to different contaminants: To determine the susceptibility of volumetric capacity to different organics, DI water having same alkalinity, salinity, and turbidity as CTW (1.5 g/L sea salts, 100-120 mg/L sodium bicarbonate, 70 mg/L Arizona test dust) and varying dosages (0 mg/L, 1.5 mg/L, 15 mg/L or 150 mg/L) of tannic or humic acid was used. To evaluate the effect of dust on capacity, DI water with the same alkalinity and salinity as CTW (1.5 g/L sea salts and 100–120 mg/L sodium bicarbonate), 15 mg/L of humic or tannic acid, and varying dosages of dust was used (0 mg/L and 70 mg/L). All measurements were performed with Eastern white pine filters (1 cm diameter and 0.375 inch thickness) operated under a 1 m gravity head. Volumetric capacity was measured as per the protocol specified above.

M11. Cloth pre-filtration A sediment pre-filter manufactured by Hindustan Unilever Limited (PureIt microfiber mesh) was used for removing visible dust particles. Feed water was first passed through the pre-filter, collected in a separated beaker and subsequently poured into the container connected to the xylem filter for further filtration.

M12. Activated carbon pre-filtration Granular Activated Carbon (GAC, Activated Carbon Corporation) of 12×40 grain was sieved in a fume hood to obtain 30×40 grain size. Prior to usage, the GAC was soaked in tap water overnight and rinsed thoroughly under running tap water to remove dust that otherwise clogged the filters. The GAC column was fabricated by assembling off-the shelf components. The GAC was packed in a 5 cm diameter, 15 cm long pipe threaded at both ends (MPT (male pipe thread) × MPT, product number

4677T45, procured from McMaster Carr). A circular mesh with 5 cm diameter was cut from the PureIt microfiber mesh manufactured by Hindustan Unilever Limited and fixed to the lower end of the pipe using epoxy (3M Scotch-Weld Epoxy Adhesive DP100 Plus) to keep the GAC granules in place. The top end of the pipe was attached to 0.375 inch diameter PVC tubing (McMaster-Carr, part numbers 5233K63) that was connected to the feed water container through a set of connectors, which consisted of a 2-inch coupling (FPT (female pipe thread) × FPT, part number 9499, Metropolitan Pipe and Supply), a 2-inch × 0.75-inch reducer (MPT × FPT, part number 9530, Metropolitan Pipe and Supply), and a 0.75-inch × 0.375-inch reducer (McMaster-Carr, product number 5372K154). The lower end of the pipe was threaded on to a cap (McMaster-Carr, part number 4880K807) and connected to a standard port valve (McMaster-Carr, part number 45975K32) to regulate the flow rate (and thus the GAC contact time). The valve was connected to the xylem filter. During experiments, care was taken to avoid formation of air bubbles. If formed, air bubbles were removed by squeezing the tubing. The humic acid concentration in the feed and the filtrate was measured by UV-Vis spectrometry (Agilent Cary 60). The absorbance was averaged over 300-500 nm wavelengths and absorbance at 700 nm was subtracted. Calibration curves were generated using different concentrations of humic acid in DI water (curves were linear). Dust was not used in experiments involving measurement of humic acid concentration due to interference with the UV-vis measurement. DI water with 1.5 g/L of sea salts, 120 mg/L of sodium bicarbonate, and ∼10 mg/L of humic acid was used as the test solution for comparing the performance of different GACs (Figure 4-10).

M13. Testing xylem filters in field studies Experiments in India were conducted with locally fabricated filters made from chir pine (Pinus roxburghii), as well as ginkgo (Ginkgo biloba) and Eastern white pine (Pinus strobus) filters fabricated in Massachusetts. Filters made in Massachusetts were transported to India by air in zip-locked pouches containing desiccant (Silica gel manufactured by Dry Packs, serial number 1203-61) to prevent condensation of moisture and blocking. Water was collected from different water sources in clean 20 L plastic cans. Before starting the experiment, the tube, holder, and device container surfaces were wiped with cotton soaked in isopropyl alcohol to prevent contamination of the feed water. The feed was introduced after 5–10 min to allow sufficient time for alcohol evaporation. For microbiological testing, the feed and filtrate were collected in glass or plastic bottles that were previously disinfected in boiling hot water for 15–20 min. The filtrate for microbiological testing was collected during the filtration process as follows. Prior to collecting the filtrate for microbiological analysis, the bottom surface of the xylem filter, the hose clamp, and lower end of the tubing were wiped with cotton soaked in isopropyl alcohol (while filtration continued). Collection of the filtered sample was started after 10 min to allow sufficient time for any residual alcohol to be flushed or evaporated. The microbiological tests were conducted by certified third-party labs (Uttarakhand Jal Sansthan for all the tests conducted in Uttarakhand Figure 5-3 and Delhi Analytical Research Laboratory for tests conducted in Delhi Figure 5-2c. The methods used for sampling and microbiological examination conformed to Indian Standards, IS:1622 (1981) for data in Figure 5-2c and American Public Health Association (APHA) 22nd edition for data in Figure 5-3.

M14. Construction of xylem filters from trunks: Eastern white pine (Pinus strobus) trunks for constructing xylem filters were procured from a local sawmill in Essex, MA. Due to logistical issues, logs were used 2 weeks after the tree was felled (partial drying of the wood within this duration could have compromised rejection). The bark was removed using a band saw. 10×4 cm2 sections were cut from the sapwood (lighter in color than the heartwood) to obtain xylem filters. Hot water soaking and ethanol treatment were performed as described above. Rejection performance of these filters was tested as described above on cylindrical 1-cm diameter filters cored out from the dried filters using a hammer-driven small hole punch with 0.375 inch hole diameter (McMaster-Carr, part number 3424A25). To avoid leaks, the xylem filter were sealed in the tubing using epoxy.

M15. Statistical information All graphs were plotted using GraphPad Prism v8. The error bars in all figures represent positive and negative standard deviations from the mean and calculated using in-built functions in GraphPad Prism v8. The n values correspond to measurements taken with different filters. The n values for the data have been included in the figure captions for several plots.

M16. Field Studies Information on user needs and preferences for water filtration devices and feedback on xylem filtration device prototypes was gathered through 600 individual semi-structured interviews, 53 focus group discussions, and 2 hands-on co-design workshops with over 1000 potential users. The research sample for the field studies included low income rural households in the mountainous state of Uttarakhand, India and the urban slum households from Bangalore and Delhi and other stakeholders in the water filter supply chain, including, filter vendors, manufacturers, NGO staff, and local health officials. The 'National Ethical Guidelines for Biomedical and Health Research Involving Human Participants' guidelines published by the Indian Council of Medical Research (ICMR) were followed while conducting user research in India. These guidelines suggest that social and behavioral research for health applications should be approved by the ethics committee for the researchers' institution, which in our case would be the Massachusetts Institute of Technology Committee on the Use of Human Subjects (MIT COUHES). All human-subjects research procedures were approved by MIT COUHES under protocol 1612798762.

Different sampling strategies were followed for different data collection methods. Potential segments with potentially different needs were stratified based on a variety of factors including the geography, types of water sources, proximity to town, and heterogeneity of the population in terms of water related practices. Villages were selected based on these criteria with the assistance of local partner NGOs. For semi-structured interviews, simple random sampling was used within the selected villages. In general, 20-30 subjects were interviewed for each segment [189]. For focus group discussions and design workshops, a combination of non-probability sampling techniques such as convenience sampling and snowball sampling techniques were used. For key informant interviews, a purposeful sampling strategy was used to select participants. Data saturation was also considered in the studies. The homogeneity of the population with regards to water usage practices was assessed with the help of local experts and data was considered saturated when no new information or themes were observed. Pen and paper were used to record the data. Photos were taken and voice recorders were also used with participant consent. A translator was present during data collection. Researchers were not blind to experimental condition and study hypothesis. No data points were omitted while performing data analysis.

M17. Microbiological performance as per WHO protocol The microbiological performance of xylem filters was tested by a third party laboratory (Quality Filter Testing (QFT) Laboratory LLC, 1041 Glassboro Road Suite E-4, Williamstown, New Jersey

08094, USA; ISO 17025 laboratory certified by International Association of Plumbing and Mechanical Officials (IAPMO) to test water filters as per National Sanitation Foundation (NSF)/American National Standards Institute (ANSI) standards). Xylem filters (4 cm diameter, 0.375 inch thickness) made from ginkgo (Ginkgo biloba) wood using hot water treatment and ethanol-based dry preservation at Massachusetts Institute of Technology (MIT), USA and stored for 2 years were mounted in 1.5 inch diameter PVC (polyvinyl chloride) tubes (McMaster-Carr, part number 5233K77) and secured in place using hose clamps, and were shipped to the third-party laboratory. In the third-party laboratory, the tubing was connected to a water-filled tank providing a 1.2 m head. *E. coli* (ATCC 11229) and MS-2 phage (ATCC-15597-B1, with host organism *E. coli* ATCC-15597) were dosed in General Test Water at concentrations $\sim 10^6$ CFU/mL and $\sim 10^5$ PFU/mL respectively as per the 'WHO International Scheme to Evaluate Household Water Treatment Technologies' [57]. For rotavirus removal, rotavirus strain SA-11 (ATCC-VR-899) was spiked in General Test Water at concentration $\sim 10^4$ PFU/mL (as specified by NSF Protocol P231 for Microbiological Water Purifiers) [68]. The removal of bacteria and virus were tested at the start of filter operation and when permeance declined to 75%, 50%, and 25% of the initial value. Flow rates were measured by monitoring the volume of fluid processed by the filters over time. After the first sample at the start of filter operation, dust was added to achieve accelerated clogging of the filter (dust concentration raised turbidity to 120 NTU and 130 NTU for *E. coli*/MS-2 phage and rotavirus respectively, which is above what is required for Challenge Test Water). Prior to collecting the filtrate for microbiological analysis, the bottom surface of the xylem filter, the hose clamp, and lower end of the tubing were wiped with cotton soaked in ethanol (while filtration continued). Collection of the filtered sample was started after 10 min to allow sufficient time for any residual alcohol to be flushed or evaporated. *E. coli* and MS-2 phage were assayed using Standard Method 9222 and 9224 published by American Public Health Association (APHA) for the Examination of Water and Wastewater. The log reduction values were determined by measuring the bacteria/virus count in the feed solution (C_{feed}), and filtrate ($C_{filtrate}$) using the following equation:

$$log - removal = -log_{10} \frac{C_{filtrate}}{C_{feed}}$$

M18. Code for percolation model

```
clc;                                         % Number of tracheid rows
length=10;
n_col=200;                                          % Every second column of tracheids is↙
counted in the node network, number of tracheids = n_col+2
n_connect=4;                                        % Number of tracheids each tracheid can↙
connect to
xylem_ref=zeros(length,n_col);                      % Reference matrix for node positions
p_open=0.5;                                        % Probability that a xylem pit will remain↙
open (solvent-dependent)
n_trials=100;
cluster_size=zeros(n_trials,1);
row_max=zeros(n_trials,n_col);
fraction=zeros(n_trials,n_col);

%******~~~~~~~~~Initializing pit status randomly post-drying~~~~~~~~~~~~*****%
%***The algorithm currently assumes that each pit is associated
%with******
%***a random probability of a pit remaining open. In actual practice******%
%*this will be a function of the pressure changes with drying progression*%
%**************The distribution of random numbers is normal***************%

n_row=length;
row_hist=zeros(n_row,n_trials);
 for t=1:n_trials

    xylem=rand(n_row,n_col,n_connect);
    xylem=(xylem <= p_open);

    %Checking connection between 21/34
    xylem_dry=zeros(n_row,n_col,n_connect*2);
    xylem_1=xylem(:,:,1);
    xylem_1(:,1)=[];
    xylem_3=xylem(:,:,3);
    xylem_3(:,1)=[];

    connect_21=or(([xylem_1,zeros(n_row,1)].*xylem(:,:,2)),([xylem_3,zeros(n_row,1)].↙
*xylem(:,:,4)));
    array=n_row+1:n_row*n_col;
    ref_2_1=reshape(array,[n_row,n_col-1]);
    ref_2_1=[ref_2_1,zeros(n_row,1)];
    ref_21=ref_2_1.*connect_21;
    xylem_dry(:,:,1)=ref_21;

    connect_12=connect_21;
    connect_12(:,n_col)=[];
    ref_1_2=reshape(1:n_row*(n_col-1),[n_row,n_col-1]);
    ref_12=[zeros(n_row,1),connect_12.*ref_1_2];
    xylem_dry(:,:,2)=ref_12;

    %Checking connection between 13/24

    xylem_1=xylem(:,:,1);
    xylem_1(1,:)=[];
    xylem_2=xylem(:,:,2);
    xylem_2(1,:)=[];

    connect_31=or((xylem(:,:,3).*[xylem_1;zeros(1,n_col)]),(xylem(:,:,4).*[xylem_2;zeros↙
(1,n_col)]));
```

```
ref_3_1=reshape(1:n_row*n_col,[n_row,n_col]);
ref_3_1(1,:)=[];
ref_3_1=[ref_3_1;zeros(1,n_col)];
ref_31=ref_3_1.*connect_31;
xylem_dry(:,:,3)=ref_31;

ref_1_3=reshape(1:n_row*n_col,[n_row,n_col]);
ref_13=ref_1_3.*connect_31;
ref_13(n_row,:)=[];
ref_13=[zeros(1,n_col);ref_13];
xylem_dry(:,:,4)=ref_13;

%Checking connection along diagonal-32 connections

xylem_3=xylem(:,:,3);
xylem_3(:,1)=[];
xylem_3=[xylem_3,zeros(n_row,1)];

xylem_2=xylem(:,:,2);
xylem_2(1,:)=[];
xylem_2=[xylem_2;zeros(1,n_col)];

connect_32=xylem_3.*xylem_2;

%Upper connection
array=n_row:n_row*(1+n_col)-1;
ref_upper=reshape(array,[n_row,n_col]);
ref_upper(1,:)=[];
ref_upper=[ref_upper;zeros(1,n_col)];
ref_upper_ind=ref_upper.*connect_32;
ref_upper_ind(n_row,:)=[];
ref_upper_ind=[zeros(1,n_col);ref_upper_ind];
xylem_dry(:,:,5)=ref_upper_ind;

%Lower connection
ref_lower=reshape(2:n_row*n_col+1,[n_row, n_col]);
ref_lower(n_row,:)=zeros;
ref_lower(:,n_col)=zeros;
ref_lower_ind=connect_32.*ref_lower;
ref_lower_ind(:,n_col)=[];
ref_lower_ind=[zeros(n_row,1),ref_lower_ind];
xylem_dry(:,:,6)=ref_lower_ind;

%Checking along diagonal - 14 connections
xylem_1=xylem(:,:,1);
xylem_1(1,:)=[];
xylem_1=[xylem_1;zeros(1,n_col)];
xylem_1(:,1)=[];
xylem_1=[xylem_1,zeros(n_row,1)];

xylem_4=xylem(:,:,4);

connect_14=xylem_1.*xylem_4;

array=n_row+2:n_row*(1+n_col)+1;
ref_upper=reshape(array,[n_row,n_col]);
ref_upper_ind=ref_upper.*connect_14;
xylem_dry(:,:,7)=ref_upper_ind;
```

```
ref_lower=reshape(1:n_row*n_col,[n_row,n_col]);
ref_lower(:,n_col)=[];
ref_lower=[zeros(n_row,1),ref_lower];
ref_lower(n_row,:)=[];
ref_lower=[zeros(1,n_col);ref_lower];

connect_14_m=connect_14;
connect_14_m(n_row,:)=[];
connect_14_m=[zeros(1,n_col);connect_14_m];
connect_14_m(:,n_col)=[];
connect_14_m=[zeros(n_row,1),connect_14_m];

ref_lower_ind=ref_lower.*connect_14_m;
xylem_dry(:,:,8)=ref_lower_ind;

% Finding node connections-Method I
xylem_op=nonzeros(unique(xylem_dry));
xylem_open=reshape(1:n_row*n_col,[n_row,n_col]);
xylem_open(intersect(xylem_op,xylem_open))=-1;
xylem_open(xylem_open~=(-1))=0;
xylem_open=abs(xylem_open);
open_row=sum(xylem_open~=0,2);

%Finding node connections- Method II
x=cat(3,reshape(1:n_row*n_col,[n_row,n_col]),xylem_dry);
xo=zeros(n_row*n_col,n_col);
k=zeros(1,n_col);

for i=1:n_col
    temp=nonzeros(xylem_dry(1,i,:));
    s=size(temp,1);
    if (ismember((i-1)*n_row+1,xo))||(size(temp,2)==0)
        continue
    end
    loc=1:s;
    xo(loc,i)=temp;
    k(i)=s;
    j=1;
    while (j<=k(i))
        row=mod(xo(j,i),n_row)+n_row*(mod(xo(j,i),n_row)==0);
        column=ceil(xo(j,i)/n_row);
        temp1=nonzeros(xylem_dry(row,column,:));
        temp2=setdiff(temp1,xo(:,i));
        s=size(temp2,1);
        if (isempty(temp2))
            j=j+1;
            continue
        end
        loc=(k(i)+1):(k(i)+s);
        xo(loc,i)=temp2;
        k(i)=k(i)+s;
        j=j+1;
    end
end

%Mapping connected nodes
xmap_ref=nonzeros(unique(xo));
```

```
    xmap=reshape(1:n_row*n_col,[n_row,n_col]);
    xmap(intersect(xmap_ref,xmap))=-1;
    xmap(xmap~=(-1))=0;
    xmap=abs(xmap);
    xmap(1,:)=1;
    row_hist(:,t)=sum(xmap,2);
 end

row_hist_avg=mean(row_hist,2);
bar(1:n_row,row_hist_avg)
imagesc(xmap)
set(gcf, 'PaperUnits', 'inches');
set(gcf, 'PaperSize', [4 2]);
```

Appendix B

Geographic availability and pricing of gymnosperms

References

- Information on family, species names, common name, continent of availability and specific geographic availability has been obtained from https://www.wikipedia.org/ and https://www.conifers.org/.

- References for in the information in other columns have been provided in the last column. These include the following papers:

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Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Araucariaceae	Agathis atropurpurea	Black kauri or blue kauri	Australia	Queensland				
Araucariaceae	Agathis australis	Kauri	Australia	New Zealand				
Araucariaceae	Agathis australis	Kauri	Australia	New Zealand				
Araucariaceae	Agathis microstachya	Bull kauri	Australia	Queensland				
Araucariaceae	Agathis ovata	Mountain kauri	Australia	New Caledonia				
Araucariaceae	Agathis ovata	Mountain Kauri	Australia	New Caledonia				https://www.wood-database.com/wood-finder/
Araucariaceae	Agathis robusta	Queensland kauri pine or smooth-barked kauri	Australia	Queensland				
Araucariaceae	Agathis spp. (A. australis, A. alba, and A. vitiensis)	Kauri, Ancient Kauri	Australia	Primarily New Zealand, Australia, and Oceania	Sapwood typically same color as heartwood.	Pale yellowish white to golden brown heartwood.		https://www.wood-database.com/wood-finder/
Araucariaceae	Araucaria angustifolia	Parana Pine	South America	Southern Brazil, parts of Argentina	Sapwood is yellow.	Heartwood is light to medium brown, commonly with red streaks.	Extensively logged in the past, Parana Pine is very seldom available. Expect prices to be much higher than comparable domestic softwoods.	https://www.wood-database.com/wood-finder/
Araucariaceae	Araucaria araucana	Monkey Puzzle, Chilean Pine	South America, Australia	Chile, Argentina, southern Brazil, New Caledonia, Norfolk Island, Australia, and New Guinea	Paler sapwood isn't clearly defined. Sometimes afflicted with blue/gray fungal staining, particularly if not dried properly.	Heartwood is light brown, sometimes with a yellow or red hue.	International trade in Monkey Puzzle is highly restricted, with no trees being harvested from its natural range. Occasional blanks are available from downed ornamental trees planted outside its natural range. Expect prices to be high for a domestic softwood.	https://www.wood-database.com/wood-finder/
Araucariaceae	Araucaria araucana	Monkey puzzle tree or Chilian pine	South America, Australia	Chile, Argentina, southern Brazil, New Caledonia, Norfolk Island, Australia, and New Guinea				
Araucariaceae	Araucaria bidwillii	Bunya pine	Australia	Queensland				
Araucariaceae	Araucaria cunninghamii	Hoop pine, Colonial pine, Queensland pine, Dorrigo pine, Moreton Bay pine and Richmond River pine	Australia	Coastal tropical and subtropical rainforests from northern Queensland to Coffs Harbour in New South Wales, New Guinea	Heartwood is light brown, sometimes with a yellow or red hue.	Paler sapwood isn't clearly defined. Sometimes afflicted with blue/gray fungal staining, particularly if not dried properly.	Plantation grown trees are regularly harvested for lumber, though the wood is seldom imported to North America. Expect prices to be higher than comparable domestic softwoods.	https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Araucariaceae	Araucaria cunninghamii	Hoop pine, Colonial pine, Queensland pine, Dorrigo pine, Moreton Bay pine and Richmond River pine	Australia	Coastal tropical and subtropical rainforests from northern Queensland to Coffs Harbour in New South Wales, New Guinea				
Araucariaceae	Araucaria heterophylla	Norfolk Island Pine	Australia	Norfolk Island, a popular ornamental plant in Australia, New Zealand, Hawaii, California, and other places	Paler sapwood isn't clearly defined. Sometimes afflicted with blue/gray fungal staining, particularly if not dried properly.	Heartwood is light brown, sometimes with a yellow or red hue.	Not generally harvested within its native range, occasional turning blanks and short craft lumber is available in areas where the tree has been planted as an ornamental. Expect prices to be in medium to high for an imported softwood.	https://www.wood-database.com/wood-finder/
Araucariaceae	Araucaria heterophylla	Norfolk Island pine	Australia	Norfolk Island, a popular ornamental plant in Australia, New Zealand, Hawaii, California, and other places				
Araucariaceae	Araucaria hunsteinii	Klinki or Klinkii	Australia	New Guinea				
Araucariaceae	Araucaria laubenfelsii	De Laubenfels' araucaria	Australia	New Caledonia				
Araucariaceae	Wollemia nobilis	Wollemi pine	Australia	New South Wales				
Callistophytaceae	Callistophyton poroxyloides	Family of seed ferns, which formed an important component of Late Pennsylvanian vegetation						
Cephalotaxaceae	Cephalotaxus fortunei	Chinese plum yew	Asia	North Myanmar and China				
Cephalotaxaceae	Cephalotaxus harringtonia	Korean plum yew, Japanese plum-yew, Harrington's cephalotaxus, or Cowtail pine	Asia	Japan				
Cephalotaxaceae	Cephalotaxus wilsoniana	Taiwan plum yew, Taiwan cow's-tail pine, and Wilson plum yew	Asia	Taiwan				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Cordaitaceae	Cordaites sp.	No common names, Genus of extinct gymnosperms						
Cordaitaceae	Cordaites sp.	No common names, Genus of extinct gymnosperms						
Cordaitaceae	Cordaites sp.	No common names, Genus of extinct gymnosperms						
Cordaitaceae	Cordaites sp.	No common names, Genus of extinct gymnosperms						
Cordaitaceae	Cordaites sp.	No common names, Genus of extinct gymnosperms						
Cupressaceae	Actinostrobus pyramidalis	Swamp cypress, Swan River cypress and King George's cypress pine	Australia	Western Australia				
Cupressaceae	Athrotaxis cupressoides	Pencil pine	Australia	W. Tasmania				
Cupressaceae	Athrotaxis laxifolia	Tasmanian Pencil Pine, Summit Cedar (reference: https://landscapeplants.oregonstate.edu/plants/athrotaxis-laxifolia)	Australia	Tasmania				
Cupressaceae	Austrocedrus chilensis	Chilean cedar (reference: https://www.iucnredlist.org/species/31359/2805519)	South America	Chile and Argnetina				
Cupressaceae	Callitris columellaris	Coast cypress pine, (reference: https://www.conifers.org/cu/Callitris_columellaris.php)	Australia	All states except Tasmania				
Cupressaceae	Callitris columellaris (= C. glaucophylla)	Australian Cypress, White Cypress Pine	Australia	All states except Tasmania	Pale yellow or pinkish sapwood.	Heartwood color can vary between boards, ranging from light tan to darker brown, commonly with darker reddish brown streaks.	Most commonly offered as flooring, lumber prices should be moderate for an imported species.	https://www.wood-database.com/wood-finder/
Cupressaceae	Callitris endlicheri	Black cypress pine	Australia	ueensland, New South Wales, and Victoria				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Cupressaceae	Callitris glaucophylla	Australia's 'White Cypress Pine'	Australia	All states except Tasmania				
Cupressaceae	Callitris gracilis	Southern cypress pine (reference: https://www.stateflora.sa.gov.au/about-us/latest-articles/cypress-pine)	Australia					
Cupressaceae	Callitris intratropica	Blue cypress	Australia					
Cupressaceae	Callitris oblonga	South Esk pine, pigmy cypress pine, river pine, or Tasmanian cypress pine	Australia	New South Wales				
Cupressaceae	Callitris preissii	Rottnest Island pine, Murray pine, maroong, slender cypress pine	Australia	New South Wales, Victoria, South Australia, and Western Australia				
Cupressaceae	Callitris rhomboidea	Oyster Bay pine	Australia	Queensland, New South Wales, Victoria, Tasmania, & South Australia				
Cupressaceae	Calocedrus decurrens (syn. Libocedrus decurrens)	Incense Cedar, California White Cedar	North America	Western North America (primarily California)	Sapwood is differentiated from heartwood and is light tan to off-white	Heartwood is light to medium reddish brown.	Prices likely to be moderate for a domestic species.	https://www.wood-database.com/wood-finder/
Cupressaceae	Calocedrus formosana	Taiwanese incense cedar (reference: https://conifersociety.org/conifers/calocedrus-formosana)	Asia	Taiwan				
Cupressaceae	Chamaecyparis lawsoniana	Port Orford Cedar, Lawson's Cypress	North America	From Coos Bay in SW Oregon to the Klamath River in NW California near the coast and locally to 1700 m in the Siskiyou Mountains and Mt. Shasta area	Sapwood is pale yellow-brown to almost white and isn't clearly distinguished from the heartwood.	Heartwood is a light yellowish brown.	Due to the limited growing range, Port Orford Cedar's demand usually exceeds its supply. Expect availability to be limited, and prices to very high for a domestic softwood wood species.	https://www.wood-database.com/wood-finder/
Cupressaceae	Chamaecyparis obtusa	Japanese cypress, hinoki cypress or hinoki	Asia	Southern Japan, Taiwan				
Cupressaceae	Chamaecyparis pisifera	Sawara cypress or Sawara	Asia	Japan				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Cupressaceae	Chamaecyparis thyoides	Atlantic White Cedar, Southern White Cedar	North America	Maine, New Hampshire, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Delaware, Pennsylvania, Maryland, Virginia, North Carolina, South Carolina, Georgia. Some subspecies also occur in Florida, Alabama and Mississippi	Narrow sapwood is pale yellow-brown to almost white and is clearly demarcated from the heartwood.	Heartwood is a light reddish brown.	Due to the limited growing range and relatively small tree size, Atlantic White Cedar is more expensive than most other conifers in the eastern United States. Expect prices to be in the medium to high range for a domestic softwood.	https://www.wood-database.com/wood-finder/
Cupressaceae	Cryptomeria japonica	Sugi, Japanese Cedar	Asia	China, Japan	Sapwood is straw colored and clearly demarcated from the heartwood.	Heartwood is typically reddish brown.	Not widely imported, Sugi is generally available in Asia as siding or other light construction purposes, as well as veneer. Smaller craft lumber is sometimes imported and made available. Prices are in the mid range for an imported softwood.	https://www.wood-database.com/wood-finder/
Cupressaceae	Cryptomeria japonica	Sugi, Japanese Cedar	Asia	China, Japan	Sapwood is straw colored and clearly demarcated from the heartwood.	Heartwood is typically reddish brown.	Not widely imported, Sugi is generally available in Asia as siding or other light construction purposes, as well as veneer. Smaller craft lumber is sometimes imported and made available. Prices are in the mid range for an imported softwood.	https://www.wood-database.com/wood-finder/
Cupressaceae	Cryptomeria japonica	Japanese cedar or sugi	Asia	China, Japan				
Cupressaceae	Cunninghamia lanceolata	China fir (`https://www.conifers.org/cu/Cunninghamia.php`)	Asia	China, Vietnam, Laos, perhaps Cambodia; widely introduced in Japan, and widely planted throughout China				
Cupressaceae	Cupressus dupreziana	Saharan cypress, Moroccan cypress, or tarout	Africa	Algeria				
Cupressaceae	Cupressus funebris	Chinese weeping cypress	Asia	Vietnam and China				
Cupressaceae	Cupressus glabra	Arizona smooth bark cypress or smooth Arizona cypress	North America	USA (Arizona)				
Cupressaceae	Cupressus goveniana	Gowen Cypress	North America	USA (California)	Narrow sapwood is paler and usually clearly demarcated from the heartwood	Heartwood is a pale yellowish or reddish brown	Because of its small size and very limited distribution, Gowen Cypress isn't used for lumber commercially.	https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Cupressaceae	Cupressus lusitanica	Mexican Cypress, Cedar of Goa	Central America	Belize, Guatemala, Honduras, El Salvador and Mexico	Narrow sapwood is paler and usually clearly demarcated from the heartwood.	Heartwood is a pale yellowish or reddish brown.	Not commonly exported for sale, Mexican Cypress is oftentimes used locally for utility purposes. Prices are likely to be moderate for an imported softwood.	https://www.wood-database.com/wood-finder/
Cupressaceae	Cupressus macrocarpa	Monterey Cypress	North America	USA (California)	Narrow sapwood is paler and usually clearly demarcated from the heartwood.	Heartwood is a pale yellowish or reddish brown.	Not commonly seen for sale, Monterey Cypress is occasionally harvested/available locally for utility purposes. Prices are likely to be high for a domestic softwood.	https://www.wood-database.com/wood-finder/
Cupressaceae	Cupressus macrocarpa	Monterey cypress	North America	USA (California)				
Cupressaceae	Cupressus nootkatensis	Alaskan Yellow Cedar, Nootka Cypress	North America	USA, Canada (northwest coast)	Sapwood is a similar whitish/pale yellow and isn't distinct from the heartwood. Color tends to darken with age upon exposure to light, (though when left exposed outdoors it weathers to a uniform gray)	Heartwood is a light yellow	Supply of this wood is limited. Expect prices to be high for a domestic species, particularly for clear pieces free of knots.	https://www.wood-database.com/wood-finder/
Cupressaceae	Cupressus sargentii	Sargent's cypress	North America	USA (California)				
Cupressaceae	Cupressus sargentii	Sargent's cypress	North America	USA (California)				
Cupressaceae	Cupressus sempervirens	Mediterranean Cypress, Italian Cypress	Europe	Eastern Mediterranean region	Narrow sapwood is paler and usually clearly demarcated from the heartwood.	Heartwood is a pale yellowish or reddish brown.	Not commonly exported for sale, Mediterranean Cypress is oftentimes used locally for utility purposes. Prices are likely to be moderate for an imported softwood.	https://www.wood-database.com/wood-finder/
Cupressaceae	Cupressus sempervirens	Mediterranean Cypress, Italian Cypress	Europe	Eastern Mediterranean region				
Cupressaceae	Cupressus torulosa	Himalayan cypress or Bhutan cypress	Asia	Western Himalayas, China and Vietnam				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Cupressaceae	Cupressus x leylandii	Leyland Cypress	Europe	Originated as a hybrid in Wales, UK; no natural range	Narrow sapwood is paler and usually clearly demarcated from the heartwood.	Heartwood is a pale yellowish or reddish brown.	Not commonly seen for sale in lumber form, most cultivated trees are used for nursery stock.	https://www.wood-database.com/wood-finder/
Cupressaceae	Diselma archeri	Chestnut pine	Australia	Tasmania				
Cupressaceae	Fitzroya cupressoides	Alerce, Lahuan, Patangonian cypress	South America	Chile and Argnetina				
Cupressaceae	Juniperus chinensis	Chinese cypress	Asia	China				
Cupressaceae	Juniperus communis	Common juniper	Asia, Europe, North America					
Cupressaceae	Juniperus deppeana	Alligator Juniper	North America, Central America	USA (Arizona, New Mexico, Texas) and Mexico (Aguascalientes, Chiapas, Chihuahua, Coahuila, Durango, Hidalgo, Jalisco, México, Michoacán, Nuevo León, Puebla, San Luis Potosí, Sonora, Tamaulipas, Tlaxcala, Veracruz, and Zacatecas) and Guatemala				https://www.wood-database.com/wood-finder/
Cupressaceae	Juniperus monosperma	One-seed juniper	North America	USA (Arizona, Colorado, New Mexico, Oklahoma and Texas)				
Cupressaceae	Juniperus osteosperma	Utah juniper	North America	USA (Arizona, California, Colorado, Idaho, Montana, New Mexico, Nevada, Utah and Wyoming)				
Cupressaceae	Juniperus osteosperma	Utah juniper	North America	USA (Arizona, California, Colorado, Idaho, Montana, New Mexico, Nevada, Utah and Wyoming)				
Cupressaceae	Juniperus osteosperma	Utah juniper	North America	USA (Arizona, California, Colorado, Idaho, Montana, New Mexico, Nevada, Utah and Wyoming)				
Cupressaceae	Juniperus scopulorum	Rocky Mountain juniper	North America	Canada, USA and Mexico				
Cupressaceae	Juniperus scopulorum	Rocky Mountain juniper	North America	Canada, USA and Mexico				
Cupressaceae	Juniperus scopulorum	Rocky Mountain juniper	North America	Canada, USA and Mexico				
Cupressaceae	Juniperus scopulorum Sarg.	Rocky Mountain juniper	North America	Canada, USA and Mexico				
Cupressaceae	Juniperus silicicola (Juniperus virginiana var. silicicola)	Southern Redcedar	North America	USA (Florida, Georgia, South Carolina and North Carolina)				https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Cupressaceae	Juniperus virginiana	Aromatic Red Cedar, Eastern Redcedar	North America	Canada (Ontario, Québec), USA(Alabama, Arkansas, Connecticut, Delaware, District of Columbia, Florida, Georgia, Iowa, Illinois, Indiana, Kansas, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Vermont, Virginia, West Virginia, and Wisconsin)	Sapwood is a pale yellow color, and can appear throughout the heartwood as streaks and stripes.	Heartwood tends to be a reddish or violet-brown.	Large and/or clear sections of Aromatic Red Cedar are much less common, but smaller, narrower boards with knots present are readily available at a modest price.	https://www.wood-database.com/wood-finder/
Cupressaceae	Juniperus virginiana	Red cedar, Eastern red cedar, Virginian juniper, Eastern juniper, Red juniper, Pencil cedar, and Aromatic cedar	North America	Canada (Ontario, Québec), USA(Alabama, Arkansas, Connecticut, Delaware, District of Columbia, Florida, Georgia, Iowa, Illinois, Indiana, Kansas, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Vermont, Virginia, West Virginia, and Wisconsin)				
Cupressaceae	Metasequoia glyptostroboides	Dawn redwood	Asia	China				
Cupressaceae	Metasequoia glyptostroboides	Dawn redwood	Asia	China				
Cupressaceae	Metasequoia glyptostroboides	Dawn redwood	Asia	China				
Cupressaceae	Papuacedrus papuana	-						
Cupressaceae	Platycladus orientalis	Oriental thuja or Oriental arborvitae	Asia	Korea, E. Russia, and China				
Cupressaceae	Sequoia sempervirens	Coast redwood, coastal redwood and California redwood	North America	USA (SW Oregon and NW California)				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Cupressaceae	Sequoia sempervirens	Redwood, Sequoia, Coast Redwood, California Redwood, Vavona (burl)	North America	USA (SW Oregon and NW California)	Sapwood is a pale white/yellow.	Heartwood color can range from a light pinkish brown to a deep reddish brown.	Should be in the mid to upper price range as a construction lumber, though clear and/or figured woodworking lumber is likely to be much more expensive.	https://www.wood-database.com/wood-finder/
Cupressaceae	Sequoia sempervirens	Coast redwood, coastal redwood and California redwood	North America	USA (SW Oregon and NW California)				
Cupressaceae	Sequoia sempervirens	Coast redwood, coastal redwood and California redwood	North America	USA (SW Oregon and NW California)				
Cupressaceae	Sequoia sempervirens (D. Don) Endl./	Coast redwood, coastal redwood and California redwood	North America	USA (SW Oregon and NW California)				
Cupressaceae	Sequoia sempervirens (D. Don) Endl./ Cupressaceae	Coast redwood, coastal redwood and California redwood	North America	USA (SW Oregon and NW California)				
Cupressaceae	Sequoiadendron giganteum	Giant sequoia; also known as giant redwood, Sierra redwood, Sierran redwood, Wellingtonia or simply big tree	North America	USA (California)				
Cupressaceae	Taiwania cryptomerioides	Taiwania	Asia	Myanmar, Vietnam, and Taiwan				
Cupressaceae	Taxodium distichum	Bald cypress	North America	USA(Alabama, Arkansas, Delaware, Florida, Georgia, Illinois, Indiana, Louisiana, Mississippi, Missouri, Kentucky, Maryland, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia),Mexico,Guatemala				
Cupressaceae	Taxodium distichum	Cypress, Bald-cypress	North America	USA(Alabama, Arkansas, Delaware, Florida, Georgia, Illinois, Indiana, Louisiana, Mississippi, Missouri, Kentucky, Maryland, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia),Mexico,Guatemala	Sapwood is nearly white	light, yellowish brown.	Prices ought to be in the mid-range for domestic woods, with clear, knot-free boards for woodworking applications costing more than construction-grade lumber.	https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Cupressaceae	Taxodium distichum	Bald cypress	North America	USA(Alabama, Arkansas, Delaware, Florida, Georgia, Illinois, Indiana, Louisiana, Mississippi, Missouri, Kentucky, Maryland, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia),Mexico,Guatemala				
Cupressaceae	Taxodium distichum (L.)	Bald cypress	North America	USA(Alabama, Arkansas, Delaware, Florida, Georgia, Illinois, Indiana, Louisiana, Mississippi, Missouri, Kentucky, Maryland, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia),Mexico,Guatemala				
Cupressaceae	Taxodium mucronatum	Montezuma bald cypress, Montezuma cypress, sabino, or ahuehuete	North America, Central America	Guatemala, Mexico (Chiapas, Coahuila, Distrito Federal, Durango, Guanajuato, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Nayarit, Nuevo León, Oaxaca, Puebla, Querétaro, San Luis Potosí, Sinaloa, Sonora, Tabasco, Tamaulipas, Tlaxcala, Veracruz, Zacatecas), USA (S Texas, along the Rio Grande valley)				
Cupressaceae	Tetraclinis articulata	Arartree, alerce, sandarac gum tree, gharghar [Maltese], thuya d'Algérie, thuya de Barbarie, bois de titre, Barbary arborvitae, alerce; Mediterranean alerce; citronwood tree; and African juniper	Europe, Africa	S Spain, Morocco, N Algeria, N Tunisia, Malta, perhaps Libya	Color is generally an orangish or reddish brown. Color tends to darken with age to a medium to dark reddish brown.		Most commonly sold as root burls. Expect prices to be very high, particularly on pieces with premium figuring exhibiting numerous tightly-packed burl eyes.	https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Cupressaceae	Tetraclinis articulata	Arartree, alerce, sandarac gum tree, gharghar [Maltese], thuya d'Algérie, thuya de Barbarie, bois de titre, Barbary arborvitae, alerce; Mediterranean alerce; citronwood tree; and African juniper	Europe, Africa	S Spain, Morocco, N Algeria, N Tunisia, Malta, perhaps Libya				
Cupressaceae	Thuja occidentalis	Northern White Cedar, Eastern Arborvitae	North America	Canada (Manitoba, Ontario, Québec; Prince Edward Island, New Brunswick, Nova Scotia), USA (Minnesota, Michigan, Wisconsin, Illinois, Indiana, Ohio, Kentucky, Tennessee, North Carolina, Virginia, West Virginia, Maryland, Pennsylvania, New York, Connecticut, Massachusetts, Vermont, New Hampshire, Maine)	narrow sapwood is nearly white	Heartwood is pale brown or tan	Generally available in smaller sizes of lumber. Prices should be in the mid range for a domestic softwood.	https://www.wood-database.com/wood-finder/
Cupressaceae	Thuja occidentalis	Northern white-cedar or Eastern arborvitae	North America	Canada (Manitoba, Ontario, Québec; Prince Edward Island, New Brunswick, Nova Scotia), USA (Minnesota, Michigan, Wisconsin, Illinois, Indiana, Ohio, Kentucky, Tennessee, North Carolina, Virginia, West Virginia, Maryland, Pennsylvania, New York, Connecticut, Massachusetts, Vermont, New Hampshire, Maine)				
Cupressaceae	Thuja plicata	Western Redcedar, Western Red Cedar	North America	USA (Alaska, Montana, Idaho, Washington, Oregon and California), Canada (British Columbia, Alberta)	Narrow sapwood is pale yellowish white, and isn't always sharply demarcated from the heartwood.	Heartwood reddish to pinkish brown, often with random streaks and bands of darker red/brown areas.	Should be moderately inexpensive for construction-grade lumber, though higher grades of clear, straight-grained, quartersawn lumber can be more expensive.	https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Cupressaceae	Thuja plicata	Western redcedar or Pacific redcedar, Giant arborvitae or Western arborvitae, giant cedar, or shinglewood	North America	USA (Alaska, Montana, Idaho, Washington, Oregon and California), Canada (British Columbia, Alberta)				
Cupressaceae	Thujopsis dolabrata	Hiba arborvitae	Asia	Japan				
Cupressaceae	Widdringtonia nodiflora	Mountain cypress	Africa	South Africa, Mozambique, and Zimbabwe				
Cupressaceae	x Cupressocyparis leylandii	Leyland cypress	North America	USA (California)				
Cupressaceae	Xanthocyparis nootkatensis	Nootka cypress, Alaska-cedar or yellow cypress	North America	USA, Canada (Pacific Coast area)				
Cycadaceae	Cycas thuarsii	Madagascar cycad						
Cycadeoidaceae	Cycadeoidea spp.	Extinct species						
Ginkgoaceae	Ginkgo beckii	-						
Ginkgoaceae	Gingko biloba L	Ginkgo, Maidenhair tree	Asia, Europe, North America	Native to China, cultivated in other continents also				
Ginkgoaceae	Ginkgo biloba	Ginkgo, Maidenhair tree	Asia, Europe, North America	Native to China, cultivated in other continents also				
Ginkgoaceae	Ginkgo biloba	Ginkgo, Maidenhair tree	Asia, Europe, North America	Native to China, cultivated in other continents also				
Ginkgoaceae	Ginkgo biloba	Ginkgo, Maidenhair tree	Asia, Europe, North America	Native to China, cultivated in other continents also				
Pinaceae	Abies alba	European silver fir or silver fir	Europe	France, Italy, Switzerland, Germany, Austria, Bulgaria, Ukraine				
Pinaceae	Abies alba	European silver fir	Europe	France, Italy, Switzerland, Germany, Austria, Bulgaria, Ukraine	pale sapwood that isn't clearly distinguished from the heartwood.	Heartwood is usually white to reddish brown	Prices should be moderate throughout its natural range in Europe when harvested for construction lumber. Though clear, quartersawn, or other such specialty cuts of fir lumber are likely to be more expensive.	https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Abies amabilis	Pacific silver fir	North America	US (SE Alaska, W Washington and Oregon, NW California), Canada (W British Columbia)	pale sapwood that isn't clearly distinguished from the heartwood.	Heartwood is usually white to reddish brown	Pacific silver fir is used as construction lumber and is commonly grouped together with other species of fir and hemlock and sold under the more generic label "HEM-FIR." Prices should be moderate for such utility lumber, though clear, quartersawn, or other such specialty cuts of fir lumber are likely to be more expensive.	https://www.wood-database.com/wood-finder/
Pinaceae	Abies balsamea	Balsam fir	Europe, North America	Canada (Alberta, Saskatchewan; Manitoba, Ontario, Québec, Prince Edward Island, New Brunswick, Nova Scotia, Newfoundland), France (St. Pierre and Miquelon), USA (Minnesota, Michigan, Wisconsin, Iowa, West Virginia, Virginia, Pennsylvania, New York, Connecticut, Massachusetts, Vermont, New Hampshire, and Maine)		Heartwood is usually white to reddish brown	Balsam fir is used as construction lumber and is commonly grouped together with other species of spruce and pine and sold under the more generic label spruce-pine-fir, or simply SPF. Prices should be moderate for such utility lumber, though clear, quartersawn, or other such specialty cuts of fir lumber are likely to be more expensive.	https://www.wood-database.com/wood-finder/
Pinaceae	Abies balsamea	Balsam fir	Europe, North America	Canada (Alberta, Saskatchewan; Manitoba, Ontario, Québec, Prince Edward Island, New Brunswick, Nova Scotia, Newfoundland), France (St. Pierre and Miquelon), USA (Minnesota, Michigan, Wisconsin, Iowa, West Virginia, Virginia, Pennsylvania, New York, Connecticut, Massachusetts, Vermont, New Hampshire, and Maine)				
Pinaceae	Abies bracteata	Bristleccone fir, Santa Lucia fir	North America	USA (California)				https://www.wood-database.com/wood-finder/
Pinaceae	Abies cephalonica	Greek fir	Europe	Mountainous regions of Greece				https://www.wood-database.com/wood-finder/
Pinaceae	Abies concolor	White fir, Concolor fir	North , Central America	USA (Idaho, Oregon, California, Nevada, Utah, Colorado, New Mexico, Arizona), Mexico (Baja California Norte, Sonora)	pale sapwood that isn't clearly distinguished from the heartwood.	Heartwood is usually white to reddish brown	White fir is used as construction lumber and is commonly grouped together with other species of fir and hemlock and sold under the more generic label "HEM-FIR." Prices should be moderate for such utility lumber, though clear, quartersawn, or other such specialty cuts of fir lumber are likely to be more expensive.	https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Abies concolor	White fir, Concolor fir	North , Central America	USA (Idaho, Oregon, California, Nevada, Utah, Colorado, New Mexico, Arizona), Mexico (Baja California Norte, Sonora)				
Pinaceae	Abies forrestii	Forrest fir	Asia	China and Tibet				
Pinaceae	Abies fraseri	Fraser fir	North America	USA (Virginia, Tennessee, North Carolina and Georgia)				https://www.wood-database.com/wood-finder/
Pinaceae	Abies grandis	Grand fir, giant fir, lowland white fir, great silver fir, western white fir, Vancouver fir, or Oregon fir	North America	Canada (British Columbia), USA (Montana, Idaho, Washington, Oregon and California)	pale sapwood that isn't clearly distinguished from the heartwood.	Heartwood is usually white to reddish brown,	Grand fir is used as construction lumber and is commonly grouped together with other species of fir and hemlock and sold under the more generic label "HEM-FIR." Prices should be moderate for such utility lumber, though clear, quartersawn, or other such specialty cuts of fir lumber are likely to be more expensive.	https://www.wood-database.com/wood-finder/
Pinaceae	Abies grandis	Grand fir, giant fir, lowland white fir, great silver fir, western white fir, Vancouver fir, or Oregon fir	North America	Canada (British Columbia), USA (Montana, Idaho, Washington, Oregon and California)				
Pinaceae	Abies lasiocarpa	Subalpine fir, alpine fir, balsam fir, white fir, mountain balsam fir, white balsam, western balsam fir, Rocky Mountain fir	North America	Canada (Yukon, Northwest Territories, British Columbia, Alberta), United States (Washington, Oregon, California, Idaho, Montana, Wyoming, Colorado, New Mexico, Arizona, Utah and Nevada)				
Pinaceae	Abies lasiocarpa	Subalpine fir, alpine fir, balsam fir, white fir, mountain balsam fir, white balsam, western balsam fir, Rocky Mountain fir	North America	Canada (Yukon, Northwest Territories, British Columbia, Alberta), United States (Washington, Oregon, California, Idaho, Montana, Wyoming, Colorado, New Mexico, Arizona, Utah and Nevada)	pale sapwood that isn't clearly distinguished from the heartwood.	Heartwood is usually white to reddish brown	Subalpine fir is used as construction lumber and is commonly grouped together with other species of spruce and pine and sold under the more generic label spruce-pine-fir, or simply SPF. Prices should be moderate for such utility lumber, though clear, quartersawn, or other such specialty cuts of fir lumber are likely to be more expensive.	https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Abies lasiocarpa	Subalpine fir, alpine fir, balsam fir, white fir, mountain balsam fir, white balsam, western balsam fir, Rocky Mountain fir	North America	Canada (Yukon, Northwest Territories, British Columbia, Alberta), United States (Washington, Oregon, California, Idaho, Montana, Wyoming, Colorado, New Mexico, Arizona, Utah and Nevada)				
Pinaceae	Abies magnifica	California red fir, silvertip fir, red fir	North America	USA (Oregon, California and Nevada)	pale sapwood that isn't clearly distinguished from the heartwood.	Heartwood is usually white to reddish brown	California red fir is used as construction lumber and is commonly grouped together with other species of fir and hemlock and sold under the more generic label "HEM-FIR." Prices should be moderate for such utility lumber, though clear, quartersawn, or other such specialty cuts of fir lumber are likely to be more expensive.	https://www.wood-database.com/wood-finder/
Pinaceae	Abies pinsapo	Spanish fir	Europe	Spain				
Pinaceae	Abies procera	Noble fir	North America	USA (Washington, Oregon and California)	pale sapwood that isn't clearly distinguished from the heartwood.	Heartwood is usually white to reddish brown	Noble fir is used as construction lumber and is commonly grouped together with other species of fir and hemlock and sold under the more generic label "HEM-FIR." Prices should be moderate for such utility lumber, though clear, quartersawn, or other such specialty cuts of fir lumber are likely to be more expensive.	https://www.wood-database.com/wood-finder/
Pinaceae	Abies religiosa	Sacred fir, oyamel	Central America	Mexico (S Chihuahua, Colima, Guerrero, Jalisco, C Michoacán, Mé, S Nuevo León, Oaxaca, Puebla, San Luis Potosí, Sinaloa, W Tamaulipas, Veracruz), Guatemala				https://www.wood-database.com/wood-finder/
Pinaceae	Abies sachalinensis	Sakhalin fir	Asia	Russia (Sakhalin, the southern Kuril Islands, and one small stand on Kamchatka), Japan (Hokkaido)				
Pinaceae	Cedrus atlantica	Atlas cedar	Africa	Morocco, Algeria				
Pinaceae	Cedrus deodara	Deodar cedar, Himalayan cedar, or deodar/devdar/devadar/devadaru	Asia	India, Pakistan				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Cedrus libani	Cedar of Lebanon	Europe	Turkey, Syria and Lebanon	Narrow sapwood is a pale yellowish white.	Heartwood is a cream to light reddish brown color.	Not commonly seen for sale, Cedar of Lebanon is generally only available in smaller blocks and turning blanks, and occasionally as veneer. Prices are moderate for an imported lumber.	https://www.wood-database.com/wood-finder/
Pinaceae	Larix decidua	European Larch, Common Larch	Europe	SE France, Switzerland, N Italy, S Germany, Austria, Czech Republic, Slovakia, Poland, Ukraine, and Romania	Narrow sapwood is nearly white and is clearly demarcated from the heartwood.	Heartwood ranges from yellow to a medium reddish brown.	European Larch is harvested for construction lumber; prices should be moderate within its local range.	https://www.wood-database.com/wood-finder/
Pinaceae	Larix decidua	European Larch, Common Larch	Europe	SE France, Switzerland, N Italy, S Germany, Austria, Czech Republic, Slovakia, Poland, Ukraine, and Romania				
Pinaceae	Larix gmelinii	Dahurian larch	Asia	Russia (the typical variety occurs in E Siberia, Sakhalin, the inner valley of Kamchatka), NE Mongolia, NE China (Heilongjiang, Jilin, Nei Mongol), and Korea				
Pinaceae	Larix kaempferi (syn. L. leptolepis)	Japanese Larch	Asia	Japan, also grown on plantations in Japan and Europe	Narrow sapwood is nearly white and is clearly demarcated from the heartwood.	Heartwood ranges from yellow to a reddish brown.	Japanese Larch is grown on plantations and is harvested in both Japan and Europe for construction lumber. Prices should be moderate within its local range(s).	https://www.wood-database.com/wood-finder/
Pinaceae	Larix larcina	Tamarack, American Larch, Eastern Larch	Europe, North America	Canada (Yukon, Northwest Territories, British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Québec, Prince Edward Island, New Brunswick, Nova Scotia, Newfoundland), France (St. Pierre and Miquelon), USA(Alaska, Minnesotsa, Michigan, Wisconsin, Illinois, Indiana, Ohio, Pennsylvania, West Virginia, Maryland, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire and Maine)	Narrow sapwood is nearly white and is clearly demarcated from the heartwood.	Heartwood ranges from yellow to a medium orangish brown.	Lumber production of Tamarack is very small, and wood is very seldom available commercially. Expect prices to be moderate.	https://www.wood-database.com/wood-finder/
Pinaceae	Larix occidentalis	Western Larch	North America	Canada (British Columbia), USA (Montana, Idaho, Washington and Oregon)	Narrow sapwood is yellowish white and is clearly demarcated from the heartwood.	Heartwood ranges from yellow to a reddish brown.	Western Larch trees grow much larger than the closely related Tamarack, and the species is much more commercially important as well. Western Larch is frequently mixed with Douglas Fir and sold as construction lumber and stamped with the initials "DF-L." Prices should be moderate.	https://www.wood-database.com/wood-finder/
Pinaceae	Larix occidentalis	Western larch	North America	Canada (British Columbia), USA (Montana, Idaho, Washington and Oregon)				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Picea abies	Norway spruce or European spruce	Europe	Albania, Austria, Belarus, Bosnia & Herzegovina, Bulgaria, Croatia, Czech Republic, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Macedonia, Montenegro, Norway, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Sweden, Switzerland, and Ukraine				
Pinaceae	Picea abies	Norway Spruce, European Spruce, German Spruce	Europe	Albania, Austria, Belarus, Bosnia & Herzegovina, Bulgaria, Croatia, Czech Republic, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Macedonia, Montenegro, Norway, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Sweden, Switzerland, and Ukraine	Norway Spruce is typically a creamy white, with a hint of yellow and/or red.		Construction grade spruce is cheap and easy to find. Although Norway Spruce is native to Europe, it has also been planted in the northeast, Rocky Mountains, and Pacific coast areas of the United States. Species of construction-grade spruce will vary by locale. However, Quartersawn billets of instrument-grade Norway Spruce, (frequently sold under more "sophisticated" names such as German Spruce, Yugoslavian Spruce, etc.) can easily exceed the cost of most all domestic hardwoods in terms of per board-foot cost.	https://www.wood-database.com/wood-finder/
Pinaceae	Picea engelmanni	Engelmann spruce,white spruce,mountain spruce,or silver spruce	North America	Canada (Alberta, British Columbia), USA (Washington, Idaho, Montana, Wyoming, Colorado, Oregon, California, Nevada, Utah, Arizona, New Mexico)				
Pinaceae	Picea engelmannii	Engelmann spruce, white spruce, mountain spruce, or silver spruce	North America	Canada (Alberta, British Columbia), USA (Washington, Idaho, Montana, Wyoming, Colorado, Oregon, California, Nevada, Utah, Arizona, New Mexico)				
Pinaceae	Picea engelmannii	Engelmann spruce, white spruce, mountain spruce, or silver spruce	North America	Canada (Alberta, British Columbia), USA (Washington, Idaho, Montana, Wyoming, Colorado, Oregon, California, Nevada, Utah, Arizona, New Mexico)				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Picea engelmannii	Engelmann Spruce	North America	Canada (Alberta, British Columbia), USA (Washington, Idaho, Montana, Wyoming, Colorado, Oregon, California, Nevada, Utah, Arizona, New Mexico)	Engelmann Spruce is usually a cream to almost white color, with an occasional hint of red		Construction grade spruce is cheap and easy to find. However, old growth and/or quartersawn clear pieces—free from knots—can be more expensive. Quartersawn billets of instrument-grade Engelmann Spruce can easily exceed the cost of most all domestic hardwoods in terms of per board-foot cost.	https://www.wood-database.com/wood-finder/
Pinaceae	Picea engelmannii	Engelmann spruce, white spruce, mountain spruce, or silver spruce	North America	Canada (Alberta, British Columbia), USA (Washington, Idaho, Montana, Wyoming, Colorado, Oregon, California, Nevada, Utah, Arizona, New Mexico)				
Pinaceae	Picea engelmannii	Engelmann spruce, white spruce, mountain spruce, or silver spruce	North America	Canada (Alberta, British Columbia), USA (Washington, Idaho, Montana, Wyoming, Colorado, Oregon, California, Nevada, Utah, Arizona, New Mexico)				
Pinaceae	Picea glauca	White spruce	Europe, North America	Canada (Yukon, North West Territories, British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Québec, Prince Edward Island, New Brunswick, Nova Scotia, Newfoundland), France (St. Pierre and Miquelon), USA (Alaska, Montana, Wyoming, South Dakota, Minnesota, Wisconsin, Michigan, New York, Vermont, New Hampshire, Maine)				
Pinaceae	Picea glauca	White spruce	Europe, North America	Canada (Yukon, North West Territories, British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Québec, Prince Edward Island, New Brunswick, Nova Scotia, Newfoundland), France (St. Pierre and Miquelon), USA (Alaska, Montana, Wyoming, South Dakota, Minnesota, Wisconsin, Michigan, New York, Vermont, New Hampshire, Maine)				
Pinaceae	Picea glauca	White Spruce	Europe, North America	Canada (Yukon, North West Territories, British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Québec, Prince Edward Island, New Brunswick, Nova Scotia, Newfoundland), France (St. Pierre and Miquelon), USA (Alaska, Montana, Wyoming, South Dakota, Minnesota, Wisconsin, Michigan, New York, Vermont, New Hampshire, Maine)	White Spruce is typically a creamy white, with a hint of yellow.		Construction grade spruce is cheap and easy to find. However, quartersawn clear pieces—free from knots—can be more expensive. White Spruce is occasionally used for piano soundboards, requiring clear quartersawn pieces.	https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Picea glauca	White spruce	Europe, North America	Canada (Yukon, North West Territories, British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Québec, Prince Edward Island, New Brunswick, Nova Scotia, Newfoundland), France (St. Pierre and Miquelon), USA (Alaska, Montana, Wyoming, South Dakota, Minnesota, Wisconsin, Michigan, New York, Vermont, New Hampshire, Maine)				
Pinaceae	Picea likiangensis	Luiang spruce	Asia	Bhutan, China				https://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?taxonid=291750&isprofile=0&
Pinaceae	Picea mariana	Black spruce	Europe, North America	Canada (all provinces), France (St. Pierre and Miquelon), USA (Alaska, Minnesota, Wisconsin, Michigan, Pennsylvania, New York, New Jersey, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire and Maine)				
Pinaceae	Picea mariana	Black Spruce	Europe, North America	Canada (all provinces), France (St. Pierre and Miquelon), USA (Alaska, Minnesota, Wisconsin, Michigan, Pennsylvania, New York, New Jersey, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire and Maine)	Black Spruce is typically a creamy white, with a hint of yellow.		Construction grade spruce is cheap and easy to find. However, quartersawn clear pieces—free from knots—can be more expensive. Quartersawn billets of instrument-grade Black Spruce can easily exceed the cost of most all domestic hardwoods in terms of per board-foot cost.	https://www.wood-database.com/wood-finder/
Pinaceae	Picea mariana	Black spruce	Europe, North America	Canada (all provinces), France (St. Pierre and Miquelon), USA (Alaska, Minnesota, Wisconsin, Michigan, Pennsylvania, New York, New Jersey, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire and Maine)				
Pinaceae	Picea mariana	Black spruce	Europe, North America	Canada (all provinces), France (St. Pierre and Miquelon), USA (Alaska, Minnesota, Wisconsin, Michigan, Pennsylvania, New York, New Jersey, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire and Maine)				
Pinaceae	Picea mariana	Black spruce	Europe, North America	Canada (all provinces), France (St. Pierre and Miquelon), USA (Alaska, Minnesota, Wisconsin, Michigan, Pennsylvania, New York, New Jersey, Connecticut, Rhode Island, Massachusetts, Vermont, New Hampshire and Maine)				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Picea rubens	Red Spruce, Adirondack Spruce	Europe, North America	Canada (Ontario, Québec, Prince Edward Island, New Brunswick, Nova Scotia), France (St. Pierre and Miquelon), USA (Maine, New Hampshire, Vermont, Massachusetts, Connecticut, New York, Pennsylvania, New Jersey, Maryland, Virginia, West Virginia, North Carolina and Tennessee)	Red Spruce is typically a creamy white, with a hint of yellow and/or red.		Construction grade spruce is cheap and easy to find. However, quartersawn clear pieces—free from knots—are much more expensive. Quartersawn billets of instrument-grade Red (Adirondack) Spruce can easily exceed the cost of most all domestic hardwoods in terms of per board-foot cost.	https://www.wood-database.com/wood-finder/
Pinaceae	Picea sitchensis	Sitka Spruce	North America	Northwestern coast	Sapwood not clearly demarcated from heartwood.	Ranges from cream/white to yellow; heartwood can also exhibit a subtle pinkish red hue in some instances.	Construction grade spruce is cheap and easy to find. However, old growth and/or quartersawn clear pieces—free from knots—can be more expensive. Quartersawn billets of instrument-grade Sitka Spruce can easily exceed the cost of most all domestic hardwoods in terms of per board-foot cost.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus albicaulis	Whitebark pine, White pine, Pitch pine, Scrub pine, and Creeping pine	North America	US and Canada				
Pinaceae	Pinus banksiana	Jack Pine	North America	Canada (North West Territories, British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Québec, Prince Edward Island, New Brunswick and Nova Scotia), USA (Minnesota, Wisconsin, Michigan, Illinois, Indiana, Pennsylvania, New York, Vermont, New Hampshire, and Maine)		Heartwood is orangish brown	When sold as lumber, Jack Pine is mixed with other softwood species under various lumber grade groups; one common grouping consists of various species of spruce, pine, and fir and is stamped with the abbreviation "SPF." In this form, Jack Pine should be widely available as construction lumber for a modest price.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus banksiana	Jack pine	North America	Canada (North West Territories, British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Québec, Prince Edward Island, New Brunswick and Nova Scotia), USA (Minnesota, Wisconsin, Michigan, Illinois, Indiana, Pennsylvania, New York, Vermont, New Hampshire, and Maine)				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Pinus banksiana	Jack pine	North America	Canada (North West Territories, British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Québec, Prince Edward Island, New Brunswick and Nova Scotia), USA (Minnesota, Wisconsin, Michigan, Illinois, Indiana, Pennsylvania, New York, Vermont, New Hampshire, and Maine)				
Pinaceae	Pinus caribaea	Caribbean pine	Central America	Caribbean (Bahamas and Turks-Caicos Islands), W Cuba (Pinar del Rio and Isla de la Juventud), Mexico (S Quintana Roo), N Guatemala, Belize, Honduras incl. Islas de la Bahía; El Salvador; Nicaragua				
Pinaceae	Pinus caribaea	Caribbean Pine	Central America	Caribbean (Bahamas and Turks-Caicos Islands), W Cuba (Pinar del Rio and Isla de la Juventud), Mexico (S Quintana Roo), N Guatemala, Belize, Honduras incl. Islas de la Bahía; El Salvador; Nicaragua	sapwood is yellowish white and is distinct from the heartwood.	Heartwood is reddish brown	Caribbean Pine is an important commercial species which is widely grown on plantations worldwide. Caribbean Pine should be available throughout its native range for a modest price.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus cembra	Swiss stone pine or arolla pine	Europe	Austria, Czech Republic, France, Germany, Italy, Poland, Romania, Switzerland, Ukraine				
Pinaceae	Pinus clausa	Sand Pine	North America	USA (Alabama and Florida)	wide sapwood is yellowish white.	Heartwood is reddish brown	Generally only larger trees are harvested for lumber, with most specimens being smaller shrubs. It is sold and mixed interchangeably with other species as Southern Yellow Pine, which is widely available as a construction lumber for a modest price.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus contorta	Lodgepole pine and Shore pine	North, Central America	W USA, W Canada, Mexico				
Pinaceae	Pinus contorta	Lodgepole pine, Shore pine, Twisted pine, Contorta pine	North, Central America	W USA, W Canada, Mexico				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Pinus contorta	Lodgepole Pine, Shore Pine	North, Central America	W USA, W Canada, Mexico	sapwood is yellowish white	Heartwood is light reddish/yellowish brown	Lodgepole Pine should be widely available as construction lumber for a modest price. Some Lodgepole Pine is mixed with Ponderosa Pine and sold together as construction lumber under the stamp "PP/LP". It is also mixed with various species of spruce, pine, and fir and sold under the group abbreviation "SPF."	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus contorta	Lodgepole pine, Shore pine, Twisted pine, Contorta pine	North, Central America	W USA, W Canada, Mexico				
Pinaceae	Pinus contorta	Lodgepole pine, Shore pine, Twisted pine, Contorta pine	North, Central America	W USA, W Canada, Mexico				
Pinaceae	Pinus contorta	Lodgepole pine, Shore pine, Twisted pine, Contorta pine	North, Central America	W USA, W Canada, Mexico				
Pinaceae	Pinus contorta	Lodgepole pine, Shore pine, Twisted pine, Contorta pine	North, Central America	W USA, W Canada, Mexico				
Pinaceae	Pinus echinata	Shortleaf Pine	North America	USA (New York, Pennsylvania, Ohio, Illinois, Arkansas, Missouri, Kentucky, Tennessee, West Virginia, Maryland, Delaware, Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, Oklahoma and Texas)	sapwood is yellowish white.	Heartwood is reddish brown	Should be widely available as construction lumber for a modest price.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus edulis	Pinyon Pine, Two-needle Pinyon, Colorado Pinyon	North, Central America	USA (widespread in Arizona, Utah, Colorado, and New Mexico, with small outlier populations in extreme eastern Nevada, southern Wyoming, extreme western Oklahoma, trans-Pecos Texas), Mexico (Chihuahua)		Heartwood is a yellowish brown	Because of their small size and short trunks, Pinyon Pines aren't harvested for lumber commercially. Availability is likely to be limited to specialty/hobbyist projects within the tree's natural range.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus edulis	Colorado pinyon, two-needle piñon, pinyon pine, or simply piñon	North, Central America	USA (widespread in Arizona, Utah, Colorado, and New Mexico, with small outlier populations in extreme eastern Nevada, southern Wyoming, extreme western Oklahoma, trans-Pecos Texas), Mexico (Chihuahua)				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Pinus elliottii	Slash Pine	North America, Africa	Eastern US, South Africa, Zimbabwe	sapwood is yellowish white.	Heartwood is reddish brown	Should be widely available as construction lumber for a modest price.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus flexilis	Limber Pine, Rocky Mountain White Pine	North America	Canada (SE British Columbia and SW Alberta), USA (Oregon, Idaho, Montana, North Dakota, South Dakota, Nebraska, Wyoming, Colorado, Utah and Nevada to N New Mexico,N Arizona, S California)	narrow sapwood is a pale yellow to nearly white. Color tends to darken with age	Heartwood is a light brown, sometimes with a slightly reddish hue	Because the trees are small, slow-growing, and with an irregular growth form, there is virtually no commercial value for Limber Pine lumber. The tree is sometimes harvested incidentally along with other, more commercially valuable species.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus flexilis	Limber Pine, Rocky Mountain White Pine	North America	Canada (SE British Columbia and SW Alberta), USA (Oregon, Idaho, Montana, North Dakota, South Dakota, Nebraska, Wyoming, Colorado, Utah and Nevada to N New Mexico,N Arizona, S California)				
Pinaceae	Pinus glabra	Spruce Pine	North America	USA (South Carolina, Georgia, Florida, Alabama, Mississippi & Louisiana)	sapwood is yellowish white.	Heartwood is reddish brown	Spruce Pine has a lower density than most other species in the Southern Yellow Pine grouping, hence its distribution is more generally restricted to local lumber needs and plywood production. The wood is sometimes sold as Southern Yellow Pine for a moderate price.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus halepensis	Aleppo pine, Jerusalem's oren	Africa, Asia, Europe, North America	Mediterreanean, W Asia (Morocco, Algeria, Tunisia, Libya, Israel, Jordan, Syria, Lebanon, Turkey, Greece, Albania, Montenegro, Bosnia & Herzegovina, Croatia, Italy, Malta, France, and Spain), USA (California), South Africa				
Pinaceae	Pinus hartwegii	Hartweg's pine	Central America	Guatemala (departments of Chimaltenango, El Quiché, Guatemala, Huehuetenano, Quezaltenango, Sacatepequez, San Marcos, Sololá, and Totonicapán), Honduras (on Cerro Santa Bárbara), Mexico (states of Chiapas, Chihuahua, Coahuila, Colima, Distrito Federal, Durango, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Nuevo León, Oaxaca, Puebla, Tamaulipas, Tlaxcala, and Veracruz)				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Pinus jeffreyi	Jeffrey Pine	North, Central America	USA (SW Oregon, California Nevada), Mexico (N Baja California Norte)	sapwood is yellowish white.	Heartwood is reddish brown	The wood of Jeffrey Pine is anatomically indistinguishable from that of Ponderosa Pine, and no commercial distinction is made between the lumber of the two species (both are simply sold as Ponderosa Pine). It should be widely available as construction lumber for a modest price. Some Ponderosa Pine is mixed with Lodgepole Pine and sold together as construction lumber under the stamp "PP/LP".	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus kesiya (syn. P. insularis)	Khasi Pine, Benguet Pine	Asia	India, Thailand, Myanmar, Laos, China (Xizang, Yunnan), Vietnam (Lai Chau, Lang Son, Cao Bang, and Quang Ninh), Philippines	sapwood is pale yellow and isn't clearly demarcated from the heartwood.	Heartwood is light reddish brown	Frequently mixed with Sumatran Pine and sold as construction lumber.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus lambertiana	Sugar Pine	North, Central America	USA (Oregon, California), Mexico (Baja California Norte, Sierra San Pedro Mártir)	Sapwood is a pale yellow to nearly white.	Heartwood is a light brown, sometimes with a slightly reddish hue	Sugar Pine is widely harvested for construction lumber (particularly in California). It's a member of the White Pine group, and is sold with other species interchangeably. Prices should be moderate to high for a domestic softwood.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus merkusii	Sumatran Pine, Merkus Pine	Asia	Vietnam, Laos, Cambodia, Philippines, Malaysia and Indonesia	demarcated sapwood is pale yellow to nearly white. Color tends to darken with age.	Heartwood is light reddish brown	Frequently mixed with Khasi Pineand sold as construction lumber.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus monophylla	Single-leaf pinyon	North, Central America	USA (Idaho, Utah, Nevada, Arizona, California); Mexico (Baja California Norte)				
Pinaceae	Pinus monticola	Western White Pine, Idaho White Pine	North America	USA (Washington, Montana, Idaho, Nevada, Oregon, California), Canada (Alberta, British Columbia)	sapwood is a pale yellow to nearly white. Color tends to darken with age.	Heartwood is a light brown, sometimes with a slightly reddish hue	Western White Pine is widely harvested for construction lumber and is sometimes sold interchangeably with Sugar Pine. Prices should be moderate to high for a domestic softwood.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus mugo	Mugo pine or Swiss mountain pine	Europe, North America	Central and Southeastern Europe, Canada (Alberta, British Columbia, Ontario, and Québec), USA (Massachusetts, Michigan, Minnesota, New Hampshire, and Wisconsin)				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Pinus nigra	Austrian pine or black pine	Australia, Europe, North America	Albania, Algeria, Andorra, Austria, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, France, Greece, Italy, Macedonia, Montenegro, Morocco, Romania, Russia, Slovenia, Spain, Turkey, Ukraine, Australia, Canada (Alberta, British Columbia, Ontario, and Québec), Great Britain, New Zealand, Portugal, USA (Illinois, Maine, Massachusetts, Michigan, Missouri, New Jersey, New York, Ohio, Pennsylvania, and probably Washington)	wide sapwood is pale yellow to nearly white	Heartwood is light reddish brown	Austrian Pine is commonly harvested for construction lumber and pulpwood. Expect prices to be moderate within its natural growing range.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus nigra	Austrian pine or black pine	Australia, Europe, North America	Albania, Algeria, Andorra, Austria, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, France, Greece, Italy, Macedonia, Montenegro, Morocco, Romania, Russia, Slovenia, Spain, Turkey, Ukraine, Australia, Canada (Alberta, British Columbia, Ontario, and Québec), Great Britain, New Zealand, Portugal, USA (Illinois, Maine, Massachusetts, Michigan, Missouri, New Jersey, New York, Ohio, Pennsylvania, and probably Washington)				
Pinaceae	Pinus oocarpa	Ocote Pine, Mexican Yellow Pine	Central America	Mexico, Guatemala, Honduras, El Salvador and NW Nicaragua	sapwood is a paler yellowish white.	Heartwood is light brown	Ocote Pine is widely grown on plantations, though it is certainly not as widely available as Radiata Pine. Lumber usage is usually for local needs or as wood pulp for papermaking. If available as dimensioned wood, prices are likely to be moderate for an imported timber.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus palustris	Longleaf Pine	North America	USA (Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana and Texas)	sapwood is yellowish white.	Heartwood is reddish brown	Should be widely available as construction lumber for a modest price.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus patula	Patula Pine	Central America, Africa	Mexico (Tamaulipas, Querétaro, Hidalgo, México, Distrito Federal, Morelos, Tlaxcala, Puebla, Veracruz, Oaxaca and Chiapas), grown in plantations in tropical areas like South Africa	sapwood is a paler yellowish white	Heartwood is light pinkish brown	Much like Radiata Pine, Patula Pine has a somewhat narrow natural distribution which is greatly expanded through plantation trees. Patula Pine is grown for basic construction purposes and should be available in tropical and sub-tropical regions for a modest price.	https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Pinus pinaster	Maritime pine or Cluster pine	Europe	Algeria, France (S of the Gironde and along the French Mediterranean coast; Corsica), Gibraltar, Italy (Sardinia; Sicily; the W coast), Malta, Monaco, Morocco, Portugal, Spain (Alicante. Balearic Islands. Barcelona. Castellón. Gerona. Lérida. Tarragona. Valencia), and Tunisia	demarcated sapwood is pale yellow to nearly white.	Heartwood is light reddish brown	Maritime Pine is extensively grown on plantations in France, Spain, and Portugal for use as construction lumber. Prices within its natural range should be moderate.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus pinaster	Maritime pine or Cluster pine	Europe	Algeria, France (S of the Gironde and along the French Mediterranean coast; Corsica), Gibraltar, Italy (Sardinia; Sicily; the W coast), Malta, Monaco, Morocco, Portugal, Spain (Alicante. Balearic Islands. Barcelona. Castellón. Gerona. Lérida. Tarragona. Valencia), and Tunisia				Bouche et al., (2014), Jansen et al. (2012), Delzon et al. (2010)
Pinaceae	Pinus pinea	Italian stone pine, umbrella pine and parasol pine	Europe, North America, Africa	Mediterranean region, USA (California), South Africa				Bouche et al. (2014), Delzon et al. (2010)
Pinaceae	Pinus ponderosa	Ponderosa pine, Bull pine, Blackjack pine, or Western yellow-pine	North, Central America	Canada (British Columbia), US (North Dakota, trans-Pecos Texas, California); Mexico (Baja California and Sonora)	sapwood is yellowish white.	Heartwood is reddish brown	Ponderosa Pine has a very wide distribution throughout western North America, and is one of the most important lumber species in the western United States. It should be widely available as construction lumber for a modest price. Some Ponderosa Pine is mixed with Lodgepole Pine and sold together as construction lumber under the stamp "PP/LP".	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus ponderosa	Ponderosa pine, Bull pine, Blackjack pine, or Western yellow-pine	North, Central America	Canada (British Columbia), US (North Dakota, trans-Pecos Texas, California); Mexico (Baja California and Sonora)				
Pinaceae	Pinus ponderosa	Ponderosa pine, Bull pine, Blackjack pine, or Western yellow-pine	North, Central America	Canada (British Columbia), US (North Dakota, trans-Pecos Texas, California); Mexico (Baja California and Sonora)				
Pinaceae	Pinus pungens	Table Mountain Pine	North America	USA (Pennsylvania, New Jersey, Delaware, Maryland, Virginia, West Virginia, North Carolina, Tennessee, South Carolina, and Georgia in the Appalachian Mountains and associated Piedmont)	sapwood is yellowish white.	Heartwood is reddish brown	Table Mountain Pine is sold and mixed interchangeably with other species as Southern Yellow Pine, which is widely available as a construction lumber for a modest price.	https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Pinus radiata	Radiata Pine, Monterey Pine, Insignis Pine	North, Central America	USA (coast of central California) Mexico, Islas Guadalupe, Cedros, e, Mexico (at 600-1200 m elevation); widely planted throughout the southern hemisphere	wide sapwood is a paler yellowish white, and is distinct from the heartwood.	Heartwood is light brown	Radiata Pine is grown almost exclusively on plantations—most notably in Chile, Australia, and New Zealand. Prices should be moderate for an imported lumber, though most likely more expensive than domestic pines/softwoods.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus radiata	Radiata Pine, Monterey Pine, Insignis Pine	North, Central America	USA (coast of central California) Mexico, Islas Guadalupe, Cedros, e, Mexico (at 600-1200 m elevation); widely planted throughout the southern hemisphere				
Pinaceae	Pinus resinosa	Red Pine, Norway Pine	North America	Canada (Manitoba, Ontario, Québec, Prince Edward Island, New Brunswick, Nova Scotia and Newfoundland), USA (Minnesota, Wisconsin, Illinois, Michigan, West Virginia, Pennsylvania, New Jersey, New York, Connecticut, Massachusetts, Vermont, New Hampshire, and Maine)	sapwood is pale yellow to nearly white	Heartwood is light reddish brown	Red Pine is sometimes mixed with various species of spruce, pine, and fir and is stamped with the lumber abbreviation "SPF." In this form, Red Pine should be widely available as construction lumber for a modest price.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus resinosa	Red Pine, Norway Pine	North America	Canada (Manitoba, Ontario, Québec, Prince Edward Island, New Brunswick, Nova Scotia and Newfoundland), USA (Minnesota, Wisconsin, Illinois, Michigan, West Virginia, Pennsylvania, New Jersey, New York, Connecticut, Massachusetts, Vermont, New Hampshire, and Maine)				
Pinaceae	Pinus rigida	Pitch Pine	North America	Canada (Ontario and Québec), USA (Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Delaware, Pennsylvania, Maryland, Ohio, Virginia, West Virginia, Kentucky, Tennessee, North Carolina, South Carolina and Georgia)	sapwood is yellowish white.	Heartwood is reddish brown	Pitch Pine is sold and mixed interchangeably with other species as Southern Yellow Pine, which is widely available as a construction lumber for a modest price.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus serotina	Pond Pine, Marsh Pine	North America	USA (New Jersey, Delaware, Maryland, Virginia, North Carolina, South Carolina, Georgia, Florida & Alabama)	wide sapwood is yellowish white.	Heartwood is reddish brown	Pond Pine is sold and mixed interchangeably with other species as Southern Yellow Pine, which is widely available as a construction lumber for a modest price.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus strobus	Eastern white pine, Northern white pine, White pine, Weymouth pine, and Soft pine	North, Central America	Canada (Newfoundland, Nova Scotia, New Brunswick, Prince Edward Island, Québec, Ontario, and Manitoba), France (St. Pierre and Miquelon),USA (All states E from Minnesota, Iowa, Illinois, Kentucky, Tennessee and Georgia to the Atlantic Ocean (excepting Florida)), Mexico, Guatemala				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Pinus strobus	Eastern white pine, Northern white pine, White pine, Weymouth pine, and Soft pine	North, Central America	Canada (Newfoundland, Nova Scotia, New Brunswick, Prince Edward Island, Québec, Ontario, and Manitoba), France (St. Pierre and Miquelon),USA (All states E from Minnesota, Iowa, Illinois, Kentucky, Tennessee and Georgia to the Atlantic Ocean (excepting Florida)), Mexico, Guatemala	sapwood is a pale yellow to nearly white	Heartwood is a light brown, sometimes with a slightly reddish hue	Eastern White Pine is widely harvested for construction lumber. Prices should be moderate for a domestic softwood.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus strobus	Eastern white pine, Northern white pine, White pine, Weymouth pine, and Soft pine	North, Central America	Canada (Newfoundland, Nova Scotia, New Brunswick, Prince Edward Island, Québec, Ontario, and Manitoba), France (St. Pierre and Miquelon),USA (All states E from Minnesota, Iowa, Illinois, Kentucky, Tennessee and Georgia to the Atlantic Ocean (excepting Florida)), Mexico, Guatemala				
Pinaceae	Pinus sylvestris	Scots pine	Asia, Australia, Europe, North America	Albania, Andorra, Armenia, Austria, Azerbaijan, Belarus, Bosnia & Herzegovina, Bulgaria, China, Croatia, Czech Republic, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Italy, Kazakhstan, Latvia, Lithuania, Macedonia, Mongolia, Montenegro, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, and the United Kingdom, also planted in New Zealand and Northeastern and Midwestern United States.				
Pinaceae	Pinus sylvestris	Scots Pine	Asia, Australia, Europe, North America	Albania, Andorra, Armenia, Austria, Azerbaijan, Belarus, Bosnia & Herzegovina, Bulgaria, China, Croatia, Czech Republic, Estonia, Finland, France, Georgia, Germany, Greece, Hungary, Italy, Kazakhstan, Latvia, Lithuania, Macedonia, Mongolia, Montenegro, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, Ukraine, and the United Kingdom, also planted in New Zealand and Northeastern and Midwestern United States.	demarcated sapwood is pale yellow to nearly white	Heartwood is light reddish brown,	Scots Pine is commonly harvested for construction lumber and pulpwood. Expect prices to be moderate within its natural growing range.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus taeda	Loblolly Pine	North America	USA (New Jersey, Delaware, Maryland, Virginia, North Carolina, Tennessee, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, Arkansas, Oklahoma, and Texas)	sapwood is yellowish white.	Heartwood is reddish brown	Should be widely available as construction lumber for a modest price.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus uncinata	Mountain pine	Europe	Sierra de Gúdar, Sierra Cebollera, Pyrenees, Massif Central, and the western Alps, Switzerland, Austria, east Germany				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Pinus virginiana	Virginia Pine, Scrub Pine	North America	USA (New York, New Jersey, Pennsylvania, Ohio, Indiana, Kentucky, Tennessee, Mississippi, Alabama, Georgia, South Carolina, North Carolina, West Virgina, Virginia, Maryland and Delaware), Canada (Alberta, British Columbia, and Ontario)	wide sapwood is yellowish white.	Heartwood is reddish brown	Virginia Pine is sold and mixed interchangeably with other species as Southern Yellow Pine, which is widely available as a construction lumber for a modest price.	https://www.wood-database.com/wood-finder/
Pinaceae	Pinus wallichiana	Himalayan pine or Himalayan white pine	Asia	Afghanistan, Pakistan, India, Tibet (China: Xizang), Nepal, Bhutan, Myanmar				
Pinaceae	Pseudolarix amabilis	Golden larch	Asia	China				
Pinaceae	Pseudotsuga menziesii	Douglas fir, Douglas-fir, Oregon pine, and Columbian pine	North America	Canada (British Columbia and Alberta), United States (Washington, Oregon, California, Idaho, Montana, Wyoming, Nevada, Utah, Colorado, Arizona, New Mexico, and Texas), Mexico (Chihuahua, Coahuila, Durango, Hidalgo, Nuevo León, Oaxaca, Sonora, and Zacatecas)	Can vary in color based upon age and location of tree. Usually a light brown color with a hint of red and/or yellow, with darker growth rings.		Should be widely available as construction lumber for a modest price. Old growth or reclaimed boards can be much more expensive.	https://www.wood-database.com/wood-finder/
Pinaceae	Pseudotsuga menziesii	Douglas fir, Douglas-fir, Oregon pine, and Columbian pine	North America	Canada (British Columbia and Alberta), United States (Washington, Oregon, California, Idaho, Montana, Wyoming, Nevada, Utah, Colorado, Arizona, New Mexico, and Texas), Mexico (Chihuahua, Coahuila, Durango, Hidalgo, Nuevo León, Oaxaca, Sonora, and Zacatecas)				
Pinaceae	Pseudotsuga menziesii	Douglas fir, Douglas-fir, Oregon pine, and Columbian pine	North America	Canada (British Columbia and Alberta), United States (Washington, Oregon, California, Idaho, Montana, Wyoming, Nevada, Utah, Colorado, Arizona, New Mexico, and Texas), Mexico (Chihuahua, Coahuila, Durango, Hidalgo, Nuevo León, Oaxaca, Sonora, and Zacatecas)				
Pinaceae	Tsuga canadensis	Eastern Hemlock, Canadian Hemlock	North America	Canada (All provinces east from Ontario, except Newfoundland) USA (All states E from Minnesota, Wisconsin, Indiana, Kentucky, Tennessee and Alabama except Florida)	Sapwood may be slightly lighter in color but usually isn't distinguished from the heartwood.	Heartwood is light reddish brown.	Eastern Hemlock is one of the two primary commercial species of hemlock harvested in North America—with the other being Western Hemlock (Tsuga heterophylla). Hemlock is used primarily as a construction timber, and is in good supply. Expect prices to be moderate for a domestic softwood.	https://www.wood-database.com/wood-finder/

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Pinaceae	Tsuga heterophylla	Western Hemlock	North America	Canada (Alberta and British Columbia), USA (Alaska, Montana, Idaho, Washington, Oregon and California)	Sapwood may be slightly lighter in color but usually isn't distinguished from the heartwood. Occasionally contains dark streaks caused by bark maggots.	Heartwood is light reddish brown.	Western Hemlock is one of the two primary commercial species of hemlock harvested in North America—with the other being Eastern Hemlock (Tsuga canadensis). Western Hemlock is used as construction lumber and is commonly grouped together with other species of fir and hemlock and sold under the more generic label "HEM-FIR." Expect prices to be moderate for a domestic softwood.	https://www.wood-database.com/wood-finder/
Pinaceae	Tsuga mertensiana	Mountain Hemlock	North America	Canada (British Columbia), USA (Alaska, Washington, Idaho, Montana, Oregon, California and Nevada)	Sapwood may be slightly lighter in color but usually isn't distinguished from the heartwood.	Heartwood is light reddish brown.	Because many trees are difficult to access, Mountain Hemlock isn't harvested nearly as extensively as its close relative, Western Hemlock, which is another hemlock species that is found in nearly the same geographic distribution. Mountain Hemlock is used for the same construction purposes as Western Hemlock; expect prices to be moderate for a domestic softwood.	https://www.wood-database.com/wood-finder/
Podocarpaceae	Acmopyle pancheri	-	Australia	New Caledonia				
Podocarpaceae	Afrocarpus gracilior	East African yellow wood	Africa	Ethiopia, Kenya, Tanzania, Uganda				
Podocarpaceae	Dacrycarpus dacrydioides	kahikatea	Australia	New Zealand, Stewart Islands				
Podocarpaceae	Dacrydium araucarioides	-	Australia	New Caledonia				
Podocarpaceae	Dacrydium cupressinum	Rimu	Australia	New Zealand, Stewart Islands				
Podocarpaceae	Falcatifolium taxoides	New Caledonian sickle pine	Australia	New Caledonia				
Podocarpaceae	Halocarpus bidwillii	Bog pine or Mountain pine	Australia	New Zealand, Stewart Islands				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Podocarpaceae	Lagarostrobos franklinii	Huon pine or Macquarie pine	Australia	Tasmania	Varies from light yellow to golden or reddish brown. Darker reddish brown streaks are common in Dacrydium species.		Being that Huon Pine is slow growing, with the trees not reproducing until they are several hundred years old, supplies are limited and expected to only decrease in the future. The wood is occasionally exported; expect prices to be medium to high for an imported softwood.	https://www.wood-database.com/wood-finder/
Podocarpaceae	Lagarostrobos franklinii	Huon pine or Macquarie pine	Australia	Tasmania				
Podocarpaceae	Manoao colensoi	Manoao, Silver pine, Westland pine, or White silver pine	Australia	New Zealand				
Podocarpaceae	Phyllocladus trichomanoides	Tanekaha	Australia	New Zealand				
Podocarpaceae	Phyllocladus trichomanoides	Tanekaha	Australia	New Zealand				
Podocarpaceae	Podocarpus cunninghamii	Hall's tōtara, Mountain tōtara or Thin-barked tōtara	Australia	New Zealand, Stewart Islands				
Podocarpaceae	Podocarpus elatus	Plum pine, Brown pine or the Illawarra plum	Australia	New South Wales and Queensland				
Podocarpaceae	Podocarpus lawrencei	Alpine plum pine, mountain plum pine (`https://www.conifers.org/po/Podocarpus_lawrencei.php`)	Australia	Tasmania, Victoria, and New South Wales				
Podocarpaceae	Podocarpus rubens	-	Asia, Australia	Central to South Sumatra, Celebes, Lesser Sunda Islands (Timor: G. Mutis), and New Guinea (incl. Normanby & New Britain)				
Podocarpaceae	Podocarpus salignus	Willow-leaf podocarp	South America	Chile				
Podocarpaceae	Podocarpus spinulosus	Dwarf Plum Pine or Spiny-leaf Podocarp	Australia	New South Wales and Queensland				
Podocarpaceae	Podocarpus totara	Totara	Australia	New Zealand				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Podocarpaceae	Prumnopitys ladei	Mount Spurgeon black pine	Australia	Queensland				
Podocarpaceae	Prumnopytis ferruginea	Miro	Australia	New Zealand, Stewart Islands				
Podocarpaceae	Retrophyllum comptonii	-	Australia	New Caledonia				
Podocarpaceae	Retrophyllum minor	Bois bouchon	Australia	New Caledonia				
Podocarpaceae	Saxegothaea conspicua	Manio (`https://www.conifers.org/po/Saxegothaea.php`)	South America	Chile, Argentina				
Podocarpaceae	Sundacarpus amarus	Black pine	Asia, Australia	Australia (Queensland), New Guinea (incl. New Britain & New Ireland), Moluccas (Buru, Halmaheira, Morotai), Lesser Sunda Islands (Timor, Flores, West Sumbawa, Lombok), Java, Central and SW Celebes (Bonthain), Philippines (Mindanao, Luzon), Borneo (only in Sabah), Sumatra (Central-N., Batak region, rare in S. Palembang)				
Sciadopityaceae	Sciadopitys verticillata	Japanese umbrella tree	Asia	Japan (S Honshu, Kyushu and Shikoku)				
Taxaceae	Taxus baccata	Common yew, English yew, or European yew	Asia, Europe, North America	Britain, Slovenia, Croatia, Poland, Iran, USA (Washington)				
Taxaceae	Taxus baccata	Common yew, English yew, or European yew	Asia, Europe, North America	Britain, Slovenia, Croatia, Poland, Iran, USA (Washington)	Sapwood is usually a thin band of pale yellow or tan color	the heartwood is an orangish brown, sometimes with a darker brown or purplish hue. Color tends to darken with age.	Yew is relatively uncommon, and larger tree trunks are usually hollow. Selection and sizes are somewhat limited, especially since most trunks are also full of knots, resulting in a high waste factor for many projects. Though sections of wood can sometimes be obtained for moderate prices, the overall cost of usable wood tends to be high.	https://www.wood-database.com/wood-finder/
Taxaceae	Taxus brevifolia	Pacific yew, Western yew, Oregon yew	North America	USA (Alaska, Montana, Idaho, Oregon, Washington and California), Canada (British Columbia, Alberta)				

Family	Species	Common name	Continent of availability	Specific geographic availability	Sapwood appearance	Heartwood appearance	Availability and pricing	Reference
Taxaceae	Taxus brevifolia	Pacific yew, Western yew, Oregon yew	North America	USA (Alaska, Montana, Idaho, Oregon, Washington and California), Canada (British Columbia, Alberta)	Sapwood is usually a thin band of pale yellow or tan color	the heartwood is an orangish brown, sometimes with a darker brown or purplish hue.	Yew is relatively uncommon, and larger tree trunks are usually hollow. Selection and sizes are somewhat limited, especially since most trunks are also full of knots, resulting in a high waste factor for many projects. Though sections of wood can sometimes be obtained for moderate prices, the overall cost of usable wood tends to be high.	https://www.wood-database.com/wood-finder/
Taxaceae	Torreya californica	California nutmeg or California torreya	North America	USA (California)				
Taxaceae	Torreya grandis (fortune)	Chinese nutmeg yew	Asia	China (Anhui to Guizhou)				
Taxaceae	Torreya nucifera	kaya Japanese torreya or Japanese nutmeg-yew	Asia	Japan				
Zamiaceae	Dioon spinulosum	Giant dioon, Gum palm	Central America	Mexico, south of Nicaragua				
Zamiaceae	Microcycas calocoma	-	Central America	Cuba (highlands of Pinar del Rio)				

Appendix C

Structural and degradation characteristics of gymnosperms

References

- Information on family, species names, common name, continent of availability and specific geographic availability has been obtained from https://www.wikipedia.org/ and https://www.conifers.org/.

- References for in the information in other columns have been provided in the last column. These include the following papers:

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Family	Species	Common name	Tracheid length (mm)	Trachied diameter (microns)	Tracheid area resistivity ((MPa-s/m^2))	P50 (MPa)	Pit membrane pore size (nm)	Presence of resin canals	Decay resistance	Plant organ tested	Reference
Araucariaceae	Agathis atropurpurea	Black kauri or blue kauri				-2.89 ± 0.27				Young branches	Bouche et al. (2014)
Araucariaceae	Agathis australis	Kauri	1.4	13	1160	2.58				Stem and root	Pittermann et al. (2006)
Araucariaceae	Agathis australis	Kauri				-2.13				Young branches	Bouche et al. (2014)
Araucariaceae	Agathis microstachya	Bull kauri				-2.55 ± 0.07				Young branches	Bouche et al. (2014)
Araucariaceae	Agathis ovata	Mountain kauri	1.93	17.2	1010	1.77				Stem and root	Pittermann et al. (2006)
Araucariaceae	Agathis ovata	Mountain Kauri		17.2 (Pittermann et al., 2006)							https://www.wood-database.com/wood-finder/
Araucariaceae	Agathis robusta	Queensland kauri pine or smooth-barked kauri				-2.90 ± 0.06				Young branches	Bouche et al. (2014)
Araucariaceae	Agathis spp. (A. australis, A. alba, and A. vitiensis)	Kauri, Ancient Kauri		35-50; contains resinous tracheids				Absent	Varies with species, generally non-durable to moderately durable. Vulnerable to insect attack.		https://www.wood-database.com/wood-finder/
Araucariaceae	Araucaria angustifolia	Parana Pine		35-60				Absent	Rated as non-durable to perishable; poor insect resistance.		https://www.wood-database.com/wood-finder/
Araucariaceae	Araucaria araucana	Monkey Puzzle, Chilean Pine		35-60				Absent	Rated as non-durable to perishable; poor insect resistance. Also susceptible to fungal staining.		https://www.wood-database.com/wood-finder/
Araucariaceae	Araucaria araucana	Monkey puzzle tree or Chilian pine				-3.06 ± 0.81				Young branches	Bouche et al. (2014)
Araucariaceae	Araucaria bidwillii	Bunya pine				-3.01 ± 0.08				Young branches	Bouche et al. (2014)
Araucariaceae	Araucaria cunninghamii	Hoop pine, Colonial pine, Queensland pine, Dorrigo pine, Moreton Bay pine and Richmond River pine		35-60				Resin canals absent	Rated as non-durable to perishable; poor insect resistance. Also susceptible to fungal staining.		https://www.wood-database.com/wood-finder/
Araucariaceae	Araucaria cunninghamii	Hoop pine, Colonial pine, Queensland pine, Dorrigo pine, Moreton Bay pine and Richmond River pine				-2.64 ± 0.08				Young branches	Bouche et al. (2014)

Family	Species	Common name	Tracheid length (mm)	Trachied diameter (microns)	Tracheid area resistivity (MPa-s/m^2)	P50 (MPa)	Pit membrane pore size (nm)	Presence of resin canals	Decay resistance	Plant organ tested	Reference
Araucariaceae	Araucaria heterophylla	Norfolk Island Pine		35-60				Absent	Rated as non-durable to perishable; poor insect resistance. Also susceptible to fungal staining.		https://www.wood-database.com/wood-finder/
Araucariaceae	Araucaria heterophylla	Norfolk Island pine				-2.96 ± 0.07				Young branches	Bouche et al. (2014)
Araucariaceae	Araucaria hunsteinii	Klinki or Klinkii				-2.43 ± 0.08				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Araucariaceae	Araucaria laubenfelsii	De Laubenfels' araucaria	1.45	11.5	3590					Stem and root	Pittermann et al. (2006)
Araucariaceae	Wollemia nobilis	Wollemi pine				-3.32 ± 0.15				Young branches	Bouche et al. (2014)
Callistophytaceae	Callistophyton poroxyloides	Family of seed ferns, which formed an important component of Late Pennsylvanian vegetation	10	100			40				Rothwell (1975); Referenced by Wilson and Knoll (2010)
Cephalotaxaceae	Cephalotaxus fortunei	Chinese plum yew				-7.26 ± 0.48				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Cephalotaxaceae	Cephalotaxus harringtonia	Korean plum yew, Japanese plum-yew, Harrington's cephalotaxus, or Cowtail pine				-7.21 ± 0.47				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Cephalotaxaceae	Cephalotaxus wilsoniana	Taiwan plum yew, Taiwan cow's-tail pine, and Wilson plum yew				-7.92 ± 0.29				Young branches	Bouche et al. (2014)
Cordaitaceae	Cordaites sp.	No common names, Genus of extinct gymnosperms	4.25	25			40				Wilson and Knoll (2010)
Cordaitaceae	Cordaites sp.	No common names, Genus of extinct gymnosperms	3.3	30			40				Wilson and Knoll (2010)
Cordaitaceae	Cordaites sp.	No common names, Genus of extinct gymnosperms	4.25	42.4			40				Wilson and Knoll (2010)
Cordaitaceae	Cordaites sp.	No common names, Genus of extinct gymnosperms	2.25	18.55			40				Wilson and Knoll (2010)
Cordaitaceae	Cordaites sp.	No common names, Genus of extinct gymnosperms	5	30.21			40				Wilson and Knoll (2010)

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Cupressaceae	Actinostrobus pyramidalis	Swamp cypress, Swan River cypress and King George's cypress pine				-10.72 ± 0.57				Young branches	Delzon et al. (2010)
Cupressaceae	Athrotaxis cupressoides	Pencil pine				-3.16 ± 0.34				Young branches	Bouche et al. (2014)
Cupressaceae	Athrotaxis laxifolia	Tasmanian Pencil Pine, Summit Cedar (https://landscapeplants.oregonstate.edu/plants/athrotaxis-laxifolia)				-2.47 ± 0.19				Young branches	Bouche et al. (2014)
Cupressaceae	Austrocedrus chilensis	Chilean cedar (https://www.iucnredlist.org/species/31359/2805519)				-4.96 ± 0.19				Young branches	Bouche et al. (2014)
Cupressaceae	Callitris columellaris	Coast cypress pine, (https://www.conifers.org/cu/Callitris_columellaris.php)				-15.79 ± 0.18				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Cupressaceae	Callitris columellaris (= C. glaucophylla)	Australian Cypress, White Cypress Pine		35-60				Absent	Reported to be very durable regarding decay resistance, and is also resistant to insect attack.		https://www.wood-database.com/wood-finder/
Cupressaceae	Callitris endlicheri	Black cypress pine				-12.94 ± 0.70				Young branches	Bouche et al. (2014)
Cupressaceae	Callitris glaucophylla	Australia's 'White Cypress Pine'				-15.30 ± 0.36				Young branches	Bouche et al. (2014)
Cupressaceae	Callitris gracilis	Southern cypress pine (https://www.stateflora.sa.gov.au/about-us/latest-articles/cypress-pine)				-12.26 ± 0.59				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Cupressaceae	Callitris intratropica	Blue cypress				-12.81 ± 0.73				Young branches	Bouche et al. (2014)
Cupressaceae	Callitris oblonga	South Esk pine, pigmy cypress pine, river pine, or Tasmanian cypress pine				-10.88 ± 0.85				Young branches	Bouche et al. (2014)
Cupressaceae	Callitris preissii	Rottnest Island pine, Murray pine, maroong, slender cypress pine				-14.97 ± 0.50				Young branches	Bouche et al. (2014), Jansen et al. (2012)

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Cupressaceae	Callitris rhomboidea	Oyster Bay pine				-10.32 ± 0.53				Young branches	Bouche et al. (2014), Jansen et al. (2012), Delzon et al. (2010)
Cupressaceae	Calocedrus decurrens (syn. Libocedrus decurrens)	Incense Cedar, California White Cedar		35-50				Absent	Despite the commonness of pockets of fungal decay (sometimes referred to as "pecky cedar"), dried wood is rated as durable to very durable in regards to decay resistance		https://www.wood-database.com/wood-finder/
Cupressaceae	Calocedrus formosana	Taiwanese incense cedar (`https://conifersociety.org/conifers/calocedrus-formosana`)				-4.92 ± 0.65				Young branches	Bouche et al. (2014)
Cupressaceae	Chamaecyparis lawsoniana	Port Orford Cedar, Lawson's Cypress		35-60				Absent	Reported to be durable to very durable regarding decay resistance, and also resistant to most insect attacks.		https://www.wood-database.com/wood-finder/
Cupressaceae	Chamaecyparis obtusa	Japanese cypress, hinoki cypress or hinoki				-3.71 ± 0.12				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Cupressaceae	Chamaecyparis pisifera	Sawara cypress or Sawara				-3.46 ± 0.21				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Cupressaceae	Chamaecyparis thyoides	Atlantic White Cedar, Southern White Cedar		25-50				Absent	Reported to be durable to very durable regarding decay resistance.		https://www.wood-database.com/wood-finder/
Cupressaceae	Cryptomeria japonica	Sugi, Japanese Cedar		25-50				Absent	Rated as moderately durable to durable; moderate insect/borer resistance.		https://www.wood-database.com/wood-finder/
Cupressaceae	Cryptomeria japonica	Sugi, Japanese Cedar		25-50				Absent	Rated as moderately durable to durable; moderate insect/borer resistance.		https://www.wood-database.com/wood-finder/
Cupressaceae	Cryptomeria japonica	Japanese cedar or sugi				-3.66 ± 0.16				Young branches	Bouche et al. (2014)
Cupressaceae	Cunninghamia lanceolata	China fir (`https://www.conifers.org/cu/Cunninghamia.php`)				-3.50 ± 0.17				Young branches	Bouche et al. (2014)
Cupressaceae	Cupressus dupreziana	Saharan cypress, Moroccan cypress, or tarout				-10.29 ± 0.06				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Cupressaceae	Cupressus funebris	Chinese weeping cypress				-10.63 ± 0.61				Young branches	Bouche et al. (2014)

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Cupressaceae	Cupressus glabra	Arizona smooth bark cypress or smooth Arizona cypress				-11.32 ± 1.03				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Cupressaceae	Cupressus goveniana	Gowen Cypress		25-50				Absent	Rated as moderately durable; mixed resistance to insect attack.		https://www.wood-database.com/wood-finder/
Cupressaceae	Cupressus lusitanica	Mexican Cypress, Cedar of Goa		25-50				Absent	Conflicting reports on durability: from non-durable to moderately durable; mixed resistance to insect attack.		https://www.wood-database.com/wood-finder/
Cupressaceae	Cupressus macrocarpa	Monterey Cypress		25-50				Absent	Rated as moderately durable; mixed resistance to insect attack.		https://www.wood-database.com/wood-finder/
Cupressaceae	Cupressus macrocarpa	Monterey cypress				-6.73 ± 0.38				Young branches	Bouche et al. (2014)
Cupressaceae	Cupressus nootkatensis	Alaskan Yellow Cedar, Nootka Cypress		25-50				Absent	Reported to be durable to very durable regarding decay resistance, and also resistant to most insect attacks.		https://www.wood-database.com/wood-finder/
Cupressaceae	Cupressus sargentii	Sargent's cypress	2.66	29.2			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Cupressaceae	Cupressus sargentii	Sargent's cypress	2.91	31.8			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Cupressaceae	Cupressus sempervirens	Mediterranean Cypress, Italian Cypress		25-50				Resin canals absent	Rated as durable; mixed resistance to insect attack.		https://www.wood-database.com/wood-finder/
Cupressaceae	Cupressus sempervirens	Mediterranean Cypress, Italian Cypress				-10.39 ± 1.10				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Cupressaceae	Cupressus torulosa	Himalayan cypress or Bhutan cypress				-8.35 ± 0.59				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Cupressaceae	Cupressus x leylandii	Leyland Cypress		25-50				Resin canals absent	Rated as moderately durable; mixed resistance to insect attack.		https://www.wood-database.com/wood-finder/
Cupressaceae	Diselma archeri	Chestnut pine				-8.72 ± 0.54				Young branches	Bouche et al. (2014)
Cupressaceae	Fitzroya cupressoides	Alerce, Lahuan, Patangonian cypress				-5.00 ± 0.37				Young branches	Bouche et al. (2014)
Cupressaceae	Juniperus chinensis	Chinese cypress				-10.88 ± 0.54				Young branches	Bouche et al. (2014)

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Cupressaceae	Juniperus communis	Common juniper				-6.37 ± 0.22				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Cupressaceae	Juniperus deppeana	Alligator Juniper									https://www.wood-database.com/wood-finder/
Cupressaceae	Juniperus monosperma	One-seed juniper				11.8				Stem	
Cupressaceae	Juniperus osteosperma	Utah juniper	771.29	9.77	7710.75	7.81				Stem and root	Pittermann et al. (2006)
Cupressaceae	Juniperus osteosperma	Utah juniper				-8.68 ± 0.35				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Cupressaceae	Juniperus osteosperma	Utah juniper				7.2				Stem	
Cupressaceae	Juniperus scopulorum	Rocky Mountain juniper	784.87	9.81	2062.84	7.2				Stem and root	Pittermann et al. (2010)
Cupressaceae	Juniperus scopulorum	Rocky Mountain juniper				7.3				Stem	
Cupressaceae	Juniperus scopulorum	Rocky Mountain juniper				-9.83 ± 0.30				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Cupressaceae	Juniperus scopulorum Sarg.	Rocky Mountain juniper		Mean: 11 ± 0.3; Max: 19 ± 0.3						Branch	Pittermann and Sperry (2003)
Cupressaceae	Juniperus silicicola (Juniperus virginiana var. silicicola)	Southern Redcedar									https://www.wood-database.com/wood-finder/
Cupressaceae	Juniperus virginiana	Aromatic Red Cedar, Eastern Redcedar		<35				Absent	Regarded as excellent in resistance to both decay and insect attack		https://www.wood-database.com/wood-finder/
Cupressaceae	Juniperus virginiana	Red cedar, Eastern red cedar, Virginian juniper, Eastern juniper, Red juniper, Pencil cedar, and Aromatic cedar	1.98	25.1			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Cupressaceae	Metasequoia glyptostroboides	Dawn redwood	2	33			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Cupressaceae	Metasequoia glyptostroboides	Dawn redwood	4.5	48			400				Bannan (1965); Referenced by Wilson and Knoll (2010)

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Cupressaceae	Metasequoia glyptostroboides	Dawn redwood				-2.91 ± 0.13				Young branches	Delzon et al. (2010), Jansen et al. (2012)
Cupressaceae	Papuacedrus papuana	-				-4.69 ± 0.23				Young branches	Bouche et al. (2014)
Cupressaceae	Platycladus orientalis	Oriental thuja or Oriental arborvitae				-9.04 ± 0.45				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Cupressaceae	Sequoia sempervirens	Coast redwood, coastal redwood and California redwood	1	15.78	552.72					Stem and root	Pittermann et al. (2006)
Cupressaceae	Sequoia sempervirens	Redwood, Sequoia, Coast Redwood, California Redwood, Vavona (burl)		>50				Resin canals absent	Rated as moderately durable to very durable regarding decay resistance. Lumber from old-growth trees tends to be more durable than that from younger second-growth trees.		https://www.wood-database.com/wood-finder/
Cupressaceae	Sequoia sempervirens	Coast redwood, coastal redwood and California redwood	4.12	43.7			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Cupressaceae	Sequoia sempervirens	Coast redwood, coastal redwood and California redwood				-4.38 ± 0.17				Young branches	Bouche et al. (2014), Jansen et al. (2012), Delzon et al. (2010)
Cupressaceae	Sequoia sempervirens (D. Don) Endl./	Coast redwood, coastal redwood and California redwood		Mean: 26 ± 1.2; Max: 48 ± 2.8						Branch (large)	Pittermann and Sperry (2003)
Cupressaceae	Sequoia sempervirens (D. Don) Endl./ Cupressaceae	Coast redwood, coastal redwood and California redwood		Mean: 20 ± 0.8; Max: 42 ± 2.5						Branch (small)	Pittermann and Sperry (2003)
Cupressaceae	Sequoiadendron giganteum	Giant sequoia; also known as giant redwood, Sierra redwood, Sierran redwood, Wellingtonia or simply big tree				-3.78 ± 0.06				Young branches	Bouche et al. (2014), Jansen et al. (2012), Delzon et al. (2010)
Cupressaceae	Taiwania cryptomerioides	Taiwania				-3.38 ± 0.29				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Cupressaceae	Taxodium distichum	Bald cypress	1.64	22.68	139.11					Stem and root	Pittermann et al. (2006)

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Cupressaceae	Taxodium distichum	Cypress, Baldcypress		>50 um				Absent	Old-growth Cypress is rated as being durable to very durable in regards to decay resistance, while wood from younger trees is only rated as moderately durable.		https://www.wood-database.com/wood-finder/
Cupressaceae	Taxodium distichum	Bald cypress				-2.29 ± 0.07				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Cupressaceae	Taxodium distichum (L.)	Bald cypress		Mean: 40 ± 2.7; Max: 76 ± 7.6						Branch	Pittermann and Sperry (2003)
Cupressaceae	Taxodium mucronatum	Montezuma bald cypress, Montezuma cypress, sabino, or ahuehuete				-2.23 ± 0.11				Young branches	Bouche et al. (2014)
Cupressaceae	Tetraclinis articulata	Arartree, alerce, sandarac gum tree, gharghar [Maltese], thuya d'Algérie, thuya de Barbarie, bois de titre, Barbary arbor-vitae, alerce; Mediterranean alerce; citron-wood tree; and African juniper							Rated as durable; good insect/borer resistance.		https://www.wood-database.com/wood-finder/
Cupressaceae	Tetraclinis articulata	Arartree, alerce, sandarac gum tree, gharghar [Maltese], thuya d'Algérie, thuya de Barbarie, bois de titre, Barbary arbor-vitae, alerce; Mediterranean alerce; citron-wood tree; and African juniper				-13.21 ± 0.75				Young branches	Bouche et al. (2014)
Cupressaceae	Thuja occidentalis	Northern White Cedar, Eastern Arborvitae		<35				Absent	Rated as durable to very durable regarding decay resistance; also resistant to termites and powder post beetles.		https://www.wood-database.com/wood-finder/

Family	Species	Common name	Tracheid length (mm)	Trachied diameter (microns)	Tracheid area resistivity (MPa-s/m^2)	P50 (MPa)	Pit membrane pore size (nm)	Presence of resin canals	Decay resistance	Plant organ tested	Reference
Cupressaceae	Thuja occidentalis	Northern white-cedar or Eastern arborvitae	2.63	28.6			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Cupressaceae	Thuja plicata	Western Redcedar, Western Red Cedar		35-60				Absent	Western Redcedar has been rated as durable to very durable in regard to decay resistance, though it has a mixed resistance to insect attack.		https://www.wood-database.com/wood-finder/
Cupressaceae	Thuja plicata	Western redcedar or Pacific redcedar, Giant arborvitae or Western arborvitae, giant cedar, or shinglewood				-4.20 ± 0.13				Young branches	Bouche et al. (2014), Jansen et al. (2012), Delzon et al. (2010)
Cupressaceae	Thujopsis dolabrata	Hiba arborvitae				-4.15 ± 0.38				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Cupressaceae	Widdringtonia nodiflora	Mountain cypress				-7.87 ± 0.49				Young branches	Bouche et al. (2014)
Cupressaceae	x Cupressocyparis leylandii	Leyland cypress				-8.58 ± 0.17				Young branches	Bouche et al. (2014)
Cupressaceae	Xanthocyparis nootkatensis	Nootka cypress, Alaska-cedar or yellow cypress				-5.13 ± 0.25				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Cycadaceae	Cycas thuarsii	Madagascar cycad	2.8	33			40				Terrazas (1991); rare scalariform, Referenced by Wilson and Knoll (2010)
Cycadeoidaceae	Cycadeoidea spp.	Extinct species	2.3	55.5			80				Genus average from Ryberg et al. (2007); Referenced by Wilson and Knoll (2010)
Ginkgoaceae	Ginkgo beckii	-	2.9	48			400				Scott et al. (1902); Referenced by Wilson and Knoll (2010)
Ginkgoaceae	Gingko biloba L	Ginkgo, Maidenhair tree		Mean: 16 ± 2.7; Max: 28 ± 2.1						Branch	Pittermann and Sperry (2003)

Family	**Species**	**Common name**	**Tracheid length (mm)**	**Trachied diameter (microns)**	**Tracheid area resistivity (MPa-s/m^2)**	**P50 (MPa)**	**Pit membrane pore size (nm)**	**Presence of resin canals**	**Decay resistance**	**Plant organ tested**	**Reference**
Ginkgoaceae	Ginkgo biloba	Ginkgo, Maidenhair tree	2.8	40			400				Bailey and Tupper (1918); Scott et al. (1902); Referenced by Wilson and Knoll (2010)
Ginkgoaceae	Ginkgo biloba	Ginkgo, Maidenhair tree	7	80			400				Scott et al. (1902); Referenced by Wilson and Knoll (2010)
Ginkgoaceae	Ginkgo biloba	Ginkgo, Maidenhair tree				3.1				Stem	
Pinaceae	Abies alba	European silver fir or silver fir				-4.00 ± 0.11				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Pinaceae	Abies alba	European silver fir		35-50				absent (traumatic resin canals occasionally present)	Rated as non-durable to perishable regarding decay resistance, with little resistance to insect attacks.		https://www.wood-database.com/wood-finder/
Pinaceae	Abies amabilis	Pacific silver fir		35-50				absent (traumatic resin canals occasionally present)	Rated as non-durable to perishable regarding decay resistance, with little resistance to insect attacks.		https://www.wood-database.com/wood-finder/
Pinaceae	Abies balsamea	Balsam fir		35-50				absent (traumatic resin canals occasionally present)	Rated as non-durable to perishable regarding decay resistance, with little resistance to insect attacks.		https://www.wood-database.com/wood-finder/
Pinaceae	Abies balsamea	Balsam fir				-3.64 ± 0.34				Young branches	Jansen et al. (2012)
Pinaceae	Abies bracteata	Bristleccone fir, Santa Lucia fir									https://www.wood-database.com/wood-finder/
Pinaceae	Abies cephalonica	Greek fir									https://www.wood-database.com/wood-finder/
Pinaceae	Abies concolor	White fir, Concolor fir		35-60				absent (traumatic resin canals occasionally present)	Rated as non-durable to perishable regarding decay resistance, with little resistance to insect attacks.		https://www.wood-database.com/wood-finder/
Pinaceae	Abies concolor	White fir, Concolor fir				8.4				Stem	
Pinaceae	Abies forrestii	Forrest fir				-3.42 ± 0.07				Young branches	Bouche et al. (2014)
Pinaceae	Abies fraseri	Fraser fir									https://www.wood-database.com/wood-finder/

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Pinaceae	Abies grandis	Grand fir, giant fir, lowland white fir, great silver fir, western white fir, Vancouver fir, or Oregon fir		35-60				absent (traumatic resin canals occasionally present)	Rated as non-durable to perishable regarding decay resistance, with little resistance to insect attacks.		https://www.wood-database.com/wood-finder/
Pinaceae	Abies grandis	Grand fir, giant fir, lowland white fir, great silver fir, western white fir, Vancouver fir, or Oregon fir				-3.65 ± 0.06				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Pinaceae	Abies lasiocarpa	Subalpine fir, alpine fir, balsam fir, white fir, mountain balsam fir, white balsam, western balsam fir, Rocky Mountain fir	0.94	12.81	1582.98					Stem and root	Pittermann et al. (2006)
Pinaceae	Abies lasiocarpa	Subalpine fir, alpine fir, balsam fir, white fir, mountain balsam fir, white balsam, western balsam fir, Rocky Mountain fir		35-50				absent (traumatic resin canals occasionally present)	Rated as non-durable to perishable regarding decay resistance, with little resistance to insect attacks.		https://www.wood-database.com/wood-finder/
Pinaceae	Abies lasiocarpa	Subalpine fir, alpine fir, balsam fir, white fir, mountain balsam fir, white balsam, western balsam fir, Rocky Mountain fir				-3.62 ± 0.07				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Pinaceae	Abies magnifica	California red fir, silvertip fir, red fir		35-60				absent (traumatic resin canals occasionally present)	Rated as non-durable to perishable regarding decay resistance, with little resistance to insect attacks.		https://www.wood-database.com/wood-finder/
Pinaceae	Abies pinsapo	Spanish fir				-4.15 ± 0.14				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Pinaceae	Abies procera	Noble fir		35-60				absent (traumatic resin canals occasionally present)	Rated as non-durable to perishable regarding decay resistance, with little resistance to insect attacks.		https://www.wood-database.com/wood-finder/
Pinaceae	Abies religiosa	Sacred fir, oyamel									https://www.wood-database.com/wood-finder/

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Pinaceae	Abies sachalinensis	Sakhalin fir				-3.23 ± 0.07				Young branches	Bouche et al. (2014)
Pinaceae	Cedrus atlantica	Atlas cedar				-5.13 ± 0.08				Young branches	Bouche et al. (2014), Jansen et al. (2012), Delzon et al. (2010)
Pinaceae	Cedrus deodara	Deodar cedar, Himalayan cedar, or deodar/devdar/devadar/devadaru				-6.69 ± 0.36				Young branches	Jansen et al. (2012), Delzon et al. (2010)
Pinaceae	Cedrus libani	Cedar of Lebanon		25-35				Resin canals absent (though sometimes present due to injury)	Rated as very durable, and generally resistant to insect attack.		https://www.wood-database.com/wood-finder/
Pinaceae	Larix decidua	European Larch, Common Larch							Moderately durable regarding decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Larix decidua	European Larch, Common Larch				-4.11 ± 0.27				Young branches	Bouche et al. (2014), Jansen et al. (2012), Delzon et al. (2010)
Pinaceae	Larix gmelinii	Dahurian larch				-3.13 ± 0.18				Young branches	Bouche et al. (2014)
Pinaceae	Larix kaempferi (syn. L. leptolepis)	Japanese Larch							Moderately durable regarding decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Larix larcina	Tamarack, American Larch, Eastern Larch		25-50				Small resin canals, infrequent and variable in distribution; solitary or in tangential groups of several	Moderately durable regarding decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Larix occidentalis	Western Larch		35-60				Small resin canals, infrequent and variable in distribution; solitary or in tangential groups of several	Moderately durable regarding decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Larix occidentalis	Western larch				-4.21 ± 0.14				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Pinaceae	Picea abies	Norway spruce or European spruce				-3.66 ± 0.09				Young branches	Delzon et al. (2010)
Pinaceae	Picea abies	Norway Spruce, European Spruce, German Spruce							Heartwood is rated as being slightly resistant to non-resistant to decay.		https://www.wood-database.com/wood-finder/

Family	Species	Common name	Tracheid length (mm)	Trachied diameter (microns)	Tracheid area resistivity (MPa-s/m^2)	P50 (MPa)	Pit membrane pore size (nm)	Presence of resin canals	Decay resistance	Plant organ tested	Reference
Pinaceae	Picea engelmanni	Engelmann spruce,white spruce,mountain spruce,or silver spruce	1.16	12.83	854	4.91				Stem and root	Pittermann et al. (2014)
Pinaceae	Picea engelmannii	Engelmann spruce, white spruce, mountain spruce, or silver spruce	2.9	35.4			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Pinaceae	Picea engelmannii	Engelmann spruce, white spruce, mountain spruce, or silver spruce	2.91	36.7			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Pinaceae	Picea engelmannii	Engelmann Spruce							Heartwood is rated as being slightly resistant to non-resistant to decay.		https://www.wood-database.com/wood-finder/
Pinaceae	Picea engelmannii	Engelmann spruce, white spruce, mountain spruce, or silver spruce				8.7				Stem	
Pinaceae	Picea engelmannii	Engelmann spruce, white spruce, mountain spruce, or silver spruce				-4.18 ± 0.09				Young branches	Delzon et al. (2010)
Pinaceae	Picea glauca	White spruce	3.33	37.6			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Pinaceae	Picea glauca	White spruce	3.01	37.3			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Pinaceae	Picea glauca	White Spruce							Heartwood is rated as being slightly resistant to non-resistant to decay.		https://www.wood-database.com/wood-finder/
Pinaceae	Picea glauca	White spruce				-4.35 ± 0.26				Young branches	Jansen et al. (2012)
Pinaceae	Picea likiangensis	Luiang spruce (https://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?taxonid=291750&isprofile=0&)				-3.86 ± 0.13				Young branches	Bouche et al. (2014)
Pinaceae	Picea mariana	Black spruce	1.18	14.42	966.76					Stem and root	Pittermann et al. (2006)

Family	Species	Common name	Tracheid length (mm)	Trachied diameter (microns)	Tracheid area resistivity (MPa-s/m^2)	P50 (MPa)	Pit membrane pore size (nm)	Presence of resin canals	Decay resistance	Plant organ tested	Reference
Pinaceae	Picea mariana	Black Spruce							Heartwood is rated as being slightly resistant to non-resistant to decay.		https://www.wood-database.com/wood-finder/
Pinaceae	Picea mariana	Black spruce	3.02	29.7			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Pinaceae	Picea mariana	Black spruce	3.44	32.5			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Pinaceae	Picea mariana	Black spruce				-5.21 ± 0.19				Young branches	Jansen et al. (2012)
Pinaceae	Picea rubens	Red Spruce, Adirondack Spruce							Heartwood is rated as being slightly resistant to non-resistant to decay.		https://www.wood-database.com/wood-finder/
Pinaceae	Picea sitchensis	Sitka Spruce		35-60				Medium sized resin canals (larger than other spruce), sparse to numerous and variable in distribution; solitary or in tangential groups of several	Heartwood is rated as being slightly resistant to non-resistant to decay.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus albicaulis	Whitebark pine, White pine, Pitch pine, Scrub pine, and Creeping pine				-3.19 ± 0.10				Young branches	Bouch et al. (2014), Delzon et al., (2010)
Pinaceae	Pinus banksiana	Jack Pine		35-50				Medium-sized resin canals, numerous and evenly distributed, mostly solitary	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus banksiana	Jack pine	3.14	34.9			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Pinaceae	Pinus banksiana	Jack pine	3.23	35.4			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Pinaceae	Pinus caribaea	Caribbean pine	2.23	18.2	187					Stem and root	Pittermann et al. (2006)
Pinaceae	Pinus caribaea	Caribbean Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary;	The heartwood is rated as moderately resistant to decay.		https://www.wood-database.com/wood-finder/

Family	Species	Common name	Tracheid length (mm)	Trachied diameter (microns)	Tracheid area resistivity (MPa-s/m^2)	P50 (MPa)	Pit membrane pore size (nm)	Presence of resin canals	Decay resistance	Plant organ tested	Reference
Pinaceae	Pinus cembra	Swiss stone pine or arolla pine				-3.02 ± 0.17				Young branches	Bouch et al. (2014), Delzon et al. (2010)
Pinaceae	Pinus clausa	Sand Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary;	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus contorta	Lodgepole pine and Shore pine	0.9	14.26	1377.55					Stem and root	Pittermann et al., (2006)
Pinaceae	Pinus contorta	Lodgepole pine, Shore pine, Twisted pine, Contorta pine	2.9	35.4			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Pinaceae	Pinus contorta	Lodgepole Pine, Shore Pine		35-60				Medium-sized resin canals, numerous and evenly distributed, mostly solitary	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus contorta	Lodgepole pine, Shore pine, Twisted pine, Contorta pine		Mean: 14 ± 0.6; Max: 23 ± 1.4 "						Branch	Pittermann and Sperry (2003)
Pinaceae	Pinus contorta	Lodgepole pine, Shore pine, Twisted pine, Contorta pine		Mean: 27 ± 0.4; Max: 52 ± 0.8						Trunk	Pittermann and Sperry (2003)
Pinaceae	Pinus contorta	Lodgepole pine, Shore pine, Twisted pine, Contorta pine	3.01	33.3			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Pinaceae	Pinus contorta	Lodgepole pine, Shore pine, Twisted pine, Contorta pine				-3.90 ± 0.18				Young branches	Delzon et al. (2010)
Pinaceae	Pinus echinata	Shortleaf Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus edulis	Pinyon Pine, Two-needle Pinyon, Colorado Pinyon		25-50				Large resin canals, numerous and evenly distributed, mostly solitary;	Since Pinyon Pine isn't generally harvested as lumber, no known durability tests are available; however, a study done on standing dead treesindicates mediocre durability for the species.		https://www.wood-database.com/wood-finder/

Family	Species	Common name	Tracheid length (mm)	Trachied diameter (microns)	Tracheid area resistivity (MPa-s/m^2)	P50 (MPa)	Pit membrane pore size (nm)	Presence of resin canals	Decay resistance	Plant organ tested	Reference
Pinaceae	Pinus edulis	Colorado pinyon, two-needle piñon, pinyon pine, or simply piñon				-4.03 ± 0.06				Young branches	Delzon et al. (2010)
Pinaceae	Pinus elliottii	Slash Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary	The heartwood is rated as moderately resistant to decay.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus flexilis	Limber Pine, Rocky Mountain White Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus flexilis	Limber Pine, Rocky Mountain White Pine				-3.71 ± 0.18				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Pinaceae	Pinus glabra	Spruce Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus halepensis	Aleppo pine, Jerusalem's oren				-4.67 ± 0.05				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Pinaceae	Pinus hartwegii	Hartweg's pine				-3.42 ± 0.05				Young branches	Jansen et al. (2012)
Pinaceae	Pinus jeffreyi	Jeffrey Pine		35-60				Medium-large resin canals, numerous and evenly distributed, mostly solitary; earlywood	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus kesiya (syn. P. insularis)	Khasi Pine, Benguet Pine							The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus lambertiana	Sugar Pine		>35 um, could be more than 60 um also				Very large resin canals, numerous and evenly distributed, mostly solitary;	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus merkusii	Sumatran Pine, Merkus Pine							Heartwood is rated as non-durable to perishable regarding decay resistance. Sumatran Pine is also susceptible to termite/insect attack.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus monophylla	Single-leaf pinyon	0.99	13.35	4348.21					Stem and root	Pittermann et al. (2006)

Family	Species	Common name	Tracheid length (mm)	Trachied diameter (microns)	Tracheid area resistivity (MPa-s/m^2)	P50 (MPa)	Pit membrane pore size (nm)	Presence of resin canals	Decay resistance	Plant organ tested	Reference
Pinaceae	Pinus monticola	Western White Pine, Idaho White Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus mugo	Mugo pine or Swiss mountain pine				-3.74 ± 0.07				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Pinaceae	Pinus nigra	Austrian pine or black pine		35-60				Medium sized resin canals, numerous and evenly distributed, mostly solitary;	Heartwood is rated as moderately durable to non-durable regarding decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus nigra	Austrian pine or black pine				-3.52				Young branches	Jansen et al. (2012)
Pinaceae	Pinus oocarpa	Ocote Pine, Mexican Yellow Pine							The heartwood is rated as moderately durable to non-durable in regards to decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus palustris	Longleaf Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary	The heartwood is rated as moderately resistant to decay.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus patula	Patula Pine							The heartwood is rated as non-durable to perishable in regards to decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus pinaster	Maritime pine or Cluster pine							The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus pinaster	Maritime pine or Cluster pine				-3.72 ± 0.07				Young branches	Bouche et al., (2014), Jansen et al. (2012), Delzon et al. (2010)
Pinaceae	Pinus pinea	Italian stone pine, umbrella pine and parasol pine				-4.34 ± 0.16				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Pinaceae	Pinus ponderosa	Ponderosa pine, Bull pine, Blackjack pine, or Western yellow-pine		35-60				Medium-large resin canals, numerous and evenly distributed, mostly solitary;	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus ponderosa	Ponderosa pine, Bull pine, Blackjack pine, or Western yellow-pine	3.33	38.6			400				Bannan (1965); Referenced by Wilson and Knoll (2010)

Family	Species	Common name	Tracheid length (mm)	Trachied diameter (microns)	Tracheid area resistivity (MPa-s/m^2)	P50 (MPa)	Pit membrane pore size (nm)	Presence of resin canals	Decay resistance	Plant organ tested	Reference
Pinaceae	Pinus ponderosa	Ponderosa pine, Bull pine, Blackjack pine, or Western yellow-pine				-3.86 ± 0.05				Young branches	Bouch et al. (2014), Delzon et al. (2010)
Pinaceae	Pinus pungens	Table Mountain Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus radiata	Radiata Pine, Monterey Pine, Insignis Pine		35-60				Medium-large resin canals, very numerous and evenly distributed, mostly solitary;	The heartwood is rated as non-durable to perishable in regards to decay resistance. The sapwood is readily treated with preservatives and is used in exterior applications.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus radiata	Radiata Pine, Monterey Pine, Insignis Pine				-4.37 ± 0.14				Young branches	Jansen et al. (2012)
Pinaceae	Pinus resinosa	Red Pine, Norway Pine		35-60				Medium sized resin canals, numerous and evenly distributed, mostly solitary;	Heartwood is rated as moderately durable to non-durable regarding decay resistance. Red Pine is readily treated with preservatives and can thereafter be used in exterior applications such as posts or utility poles.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus resinosa	Red Pine, Norway Pine	3.36	37.4			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Pinaceae	Pinus rigida	Pitch Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary;	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus serotina	Pond Pine, Marsh Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary;	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus strobus	Eastern white pine, Northern white pine, White pine, Weymouth pine, and Soft pine	4.04	41			400				Bannan (1965); Referenced by Wilson and Knoll (2010)

Family	Species	Common name	Tracheid length (mm)	Trachied diameter (microns)	Tracheid area resistivity (MPa-s/m^2)	P50 (MPa)	Pit membrane pore size (nm)	Presence of resin canals	Decay resistance	Plant organ tested	Reference
Pinaceae	Pinus strobus	Eastern white pine, Northern white pine, White pine, Weymouth pine, and Soft pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus strobus	Eastern white pine, Northern white pine, White pine, Weymouth pine, and Soft pine	3.72	40.3			400				Bannan (1965); Referenced by Wilson and Knoll (2010)
Pinaceae	Pinus sylvestris	Scots pine				-3.20 ± 0.02				Young branches	Delzon et al. (2010)
Pinaceae	Pinus sylvestris	Scots Pine		35-60				Medium sized resin canals, numerous and evenly distributed, mostly solitary	Heartwood is rated as moderately durable to non-durable regarding decay resistance. Scots Pine is readily treated with preservatives and can thereafter be used in exterior applications such as posts or utility poles.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus taeda	Loblolly Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus uncinata	Mountain pine				-0.17 ± 0.17				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Pinaceae	Pinus virginiana	Virginia Pine, Scrub Pine		35-60				Large resin canals, numerous and evenly distributed, mostly solitary	The heartwood is rated as moderate to low in decay resistance.		https://www.wood-database.com/wood-finder/
Pinaceae	Pinus wallichiana	Himalayan pine or Himalayan white pine				-2.83 ± 0.11				Young branches	Jansen et al. (2012)
Pinaceae	Pseudolarix amabilis	Golden larch				-4.16 ± 0.15				Young branches	Bouche et al. (2014)
Pinaceae	Pseudotsuga menziesii	Douglas fir, Douglas-fir, Oregon pine, and Columbian pine		35-60				Small to medium sized resin canals, infrequent and variable in distribution; solitary or in tangential groups of several;	Douglas-Fir heartwood is rated to be moderately durable in regard to decay, but is susceptible to insect attack.		https://www.wood-database.com/wood-finder/
Pinaceae	Pseudotsuga menziesii	Douglas fir, Douglas-fir, Oregon pine, and Columbian pine	3.39	40.4			400				Bannan (1965); Referenced by Wilson and Knoll (2010)

Family	Species	Common name	Tracheid length (mm)	Trachied diameter (microns)	Tracheid area resistivity (MPa-s/m^2)	P50 (MPa)	Pit membrane pore size (nm)	Presence of resin canals	Decay resistance	Plant organ tested	Reference
Pinaceae	Pseudotsuga menziesii	Douglas fir, Douglas-fir, Oregon pine, and Columbian pine				-3.68 ± 0.15				Young branches	Bouch et al. (2014), Delzon et al. (2010)
Pinaceae	Tsuga canadensis	Eastern Hemlock, Canadian Hemlock		35-60				Absent	Rated as non-durable regarding decay resistance, and also susceptible to insect attack.		https://www.wood-database.com/wood-finder/
Pinaceae	Tsuga heterophylla	Western Hemlock		35-60				Absent	Rated as non-durable regarding decay resistance, and also susceptible to insect attack.		https://www.wood-database.com/wood-finder/
Pinaceae	Tsuga mertensiana	Mountain Hemlock		35-60				Absent	Rated as non-durable regarding decay resistance, and also susceptible to insect attack.		https://www.wood-database.com/wood-finder/
Podocarpaceae	Acmopyle pancheri	-				-3.62 ± 0.07				Young branches	Bouche et al. (2014)
Podocarpaceae	Afrocarpus gracilior	East African yellow wood				-6.36 ± 0.10				Young branches	Bouche et al. (2014)
Podocarpaceae	Dacrycarpus dacrydioides	kahikatea				-2.51 ± 0.16				Young branches	Delzon et al. (2010)
Podocarpaceae	Dacrydium araucarioides	-				-3.78 ± 0.39				Young branches	Bouch et al. (2014)
Podocarpaceae	Dacrydium cupressinum	Rimu	1.65	10.9	1950					Stem and root	Pittermann et al. (2006)
Podocarpaceae	Falcatifolium taxoides	New Caledonian sickle pine				-5.55 ± 0.53				Young branches	Bouche et al. (2014)
Podocarpaceae	Halocarpus bidwillii	Bog pine or Mountain pine				-5.35 ± 0.21				Young branches	Bouche et al. (2014)
Podocarpaceae	Lagarostrobos franklinii	Huon pine or Macquarie pine		35-60				Absent	Varies depending on species and application. Generally regarded as having good durability in marine applications, though Dacrydium spp. are rated as non-durable in applications of direct ground contact.		https://www.wood-database.com/wood-finder/
Podocarpaceae	Lagarostrobos franklinii	Huon pine or Macquarie pine				-4.35 ± 0.36				Young branches	Bouche et al. (2014)
Podocarpaceae	Manoao colensoi	Manoao, Silver pine, Westland pine, or White silver pine				-2.88 ± 0.21				Young branches	Bouche et al. (2014)
Podocarpaceae	Phyllocladus trichomanoides	Tanekaha	1.68	11.1	1410					Stem and root	Pittermann et al. (2006)

Family	**Species**	**Common name**	**Tracheid length (mm)**	**Trachied diameter (microns)**	**Tracheid area resistivity (MPa-s/m^2)**	**P50 (MPa)**	**Pit membrane pore size (nm)**	**Presence of resin canals**	**Decay resistance**	**Plant organ tested**	**Reference**
Podocarpaceae	Phyllocladus trichomanoides	Tanekaha				-7.10 ± 0.25				Young branches	Bouche et al. (2014)
Podocarpaceae	Podocarpus cunninghamii	Hall's tōtara, Mountain tōtara or Thin-barked tōtara	1.34	10.8	1480					Stem and root	Pittermann et al. (2006)
Podocarpaceae	Podocarpus elatus	Plum pine, Brown pine or the Illawarra plum				-6.74 ± 0.39				Young branches	Bouche et al. (2014)
Podocarpaceae	Podocarpus lawrencei	Alpine plum pine, mountain plum pine (https://www.conifers.org/po/Podocarpus_lawrencei.php)				-3.82 ± 0.21				Young branches	Bouche et al. (2014)
Podocarpaceae	Podocarpus rubens	-				-3.73 ± 0.04				Young branches	Bouche et al. (2014)
Podocarpaceae	Podocarpus salignus	Willow-leaf podocarp				-4.28 ± 0.16				Young branches	Bouche et al. (2014)
Podocarpaceae	Podocarpus spinulosus	Dwarf Plum Pine or Spiny-leaf Podocarp				-6.84 ± 0.34				Young branches	Bouche et al. (2014)
Podocarpaceae	Podocarpus totara	Totara				-4.95 ± 0.16				Young branches	Bouche et al. (2014)
Podocarpaceae	Prumnopitys ladei	Mount Spurgeon black pine				-6.68 ± 0.23				Young branches	Bouche et al. (2014)
Podocarpaceae	Prumnopytis ferruginea	Miro	1.63	11.5	2120					Stem and root	Pittermann et al. (2006)
Podocarpaceae	Retrophyllum comptonii	-				-2.54 ± 0.09				Young branches	Bouche et al. (2014)
Podocarpaceae	Retrophyllum minor	Bois bouchon	1.72	17.7	808					Stem and root	Pittermann et al. (2006)
Podocarpaceae	Saxegothaea conspicua	Manio (https://www.conifers.org/po/Saxegothaea.php)				-3.39 ± 0.23				Young branches	Bouche et al. (2014)
Podocarpaceae	Sundacarpus amarus	Black pine				-2.83 ± 0.14				Young branches	Bouche et al. (2014)
Sciadopityaceae	Sciadopitys verticillata	Japanese umbrella tree				-4.07 ± 0.10				Young branches	Bouche et al. (2014), Jansen et al. (2012)
Taxaceae	Taxus baccata	Common yew, English yew, or European yew				-6.49 ± 0.31				Young branches	Bouche et al. (2014), Delzon et al., (2010)
Taxaceae	Taxus baccata	Common yew, English yew, or European yew		<25				Resin canals absent;	European Yew ranges from durable to very durable in regard to decay resistance, and is also resistant to most insect attack.		https://www.wood-database.com/wood-finder/

Family	Species	Common name	Tracheid length (mm)	Trachied diameter (microns)	Tracheid area resistivity (MPa-s/m^2)	P50 (MPa)	Pit membrane pore size (nm)	Presence of resin canals	Decay resistance	Plant organ tested	Reference
Taxaceae	Taxus brevifolia	Pacific yew, Western yew, Oregon yew				-6.44 ± 0.30				Young branches	Bouche et al. (2014), Delzon et al. (2010)
Taxaceae	Taxus brevifolia	Pacific yew, Western yew, Oregon yew		<25				Resin canals absent	Pacific Yew is very durable in regard to decay resistance, and is also resistant to most insect attack.		https://www.wood-database.com/wood-finder/
Taxaceae	Torreya californica	California nutmeg or California torreya				-6.39 ± 0.30				Young branches	Bouch et al. (2014), Jansen et al. (2012)
Taxaceae	Torreya grandis (fortune)	Chinese nutmeg yew				-4.69 ± 0.25				Young branches	Bouch et al. (2014), Jansen et al. (2012)
Taxaceae	Torreya nucifera	kaya Japanese torreya or Japanese nutmeg-yew				-5.95 ± 0.30				Young branches	Bouch et al. (2014), Jansen et al. (2012)
Zamiaceae	Dioon spinulosum	Giant dioon, Gum palm	6.8	32			40				Bailey and Tupper (1918), Langdon (1920), Greguss (1968); Referenced by Wilson and Knoll, 2010
Zamiaceae	Microcycas calocoma	-	6	45			40				Half scalariform, Chrysler (1926) , Referenced by Wilson and Knoll (2010)

Appendix D

Development of graphene membrane-based device for analytical sample preparation for biotherapeutic applications

D.1 Introduction

Therapeutic antibodies are increasingly being used as a treatment modality for several diseases [190]. The ability to characterize and quantify antibodies and antibody mixtures is critical for the development of biotherapeutics. Liquid Chromatography/Mass Spectrometry (LC/MS) is a widely used tool for the qualitative and quantitative assessment of antibodies and antibody mixtures [191, 192]. The LC separates out the components in the sample and introduces them into the MS, which creates and detects charged ions [193]. The separation in the LC is accomplished by injecting the analyte into a solvent (referred to as mobile phase) that passes through a column packed with chromatographic column material (referred to as stationary phase). The separation occurs on the basis of the interaction of the sample components with the mobile and stationary phase [194]. Once the analyte is injected into the mass spectrometer, it is ionized. The ions are then accelerated and deflected based on their mass to charge ratio and subsequently sent to a detector [195]. While the LC/MS system offers a reliable method for detecting biological compounds, the mobile phase in the LC often has high concentrations of salts, which affect the sensitivity and accuracy of MS measurements [196]. Issues associated with presence of salts include high signal to noise ratio, inability to detect target molecules at low concentrations, poor resolution, lower peak intensity, and clogging of ion tips and corrosion [196, 3, 197]. A comparison between the mass spectrum of an antibody in the presence and absence of salts is shown in Figure D-1.

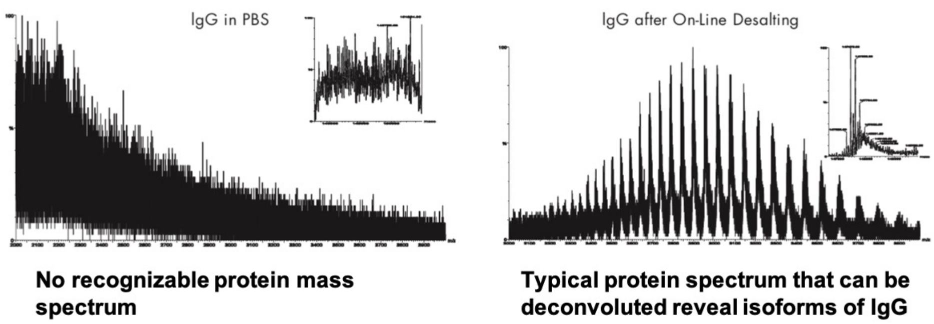

Figure D-1: Effect of presence of salts on MS spectrum of IgG [3]

Therefore, it is essential to remove salts from the analyte prior to its injection into the MS. Desalting of the analyte can be performed via a variety of methods, which could be online or offline. Some of the common principles which are employed for desalting are described below [198, 199, 200, 201]:

- Size exclusion chromatography: Different sample components are separated on the basis of their size. The column is packed with beads containing pores. Smaller molecules diffuse through the pores, experiencing slower movement as opposed to larger molecules, which move through the void space between the beads and elute first.
- Solid phase extraction: Components in the sample are separated through selective adsorption and elution based on their affinity for the stationary and mobile phase.
- Filtration: Components are based on size-based physical sieving.
- Dialysis: Components are separated based on their diffusivity across a semi-permeable membrane.

While several methods exist for desalting, they often require sophisticated process or device design, impose long wait times, or involve multiple steps which can impede automation and/or cause user inconvenience. For example, many offline methods based on gel filtration require 10-15 user steps, solvents used for eluting analytes from columns in solid phase extraction are often incompatible with the MS, and dialysis-based methods typically involve long wait times [202, 196].

Nanoporous graphene membranes made of a single layer of atomically thin graphene with nanoscale pores engineered into its lattice offer potential for creation of de-salting devices which could address some of these challenges. The atomic thickness and low surface area of the membrane could help achieve high permeability (resulting in faster sample processing rates) and low adsorptive sample loss respectively.

D.2 Objective

This thesis aims to design and build a functional, graphene-membrane based device prototype for desalting antibody samples. Towards this goal, this thesis focuses on the following sub-aims:

1. Identification of key design considerations and performance targets
2. Design and fabrication of device to meet performance targets
3. Characterization of device performance

D.3 Device design criteria and requirements

A variety of factors govern the choice of desalting method used for sample preparation. A list of important performance metrics and their relevance for different applications is provided in Table D.1.

The design targets for a proof-of-concept, graphene-membrane based device were formulated in consultation with Agilent Technologies, an industry leader in developing solutions for analytical sample preparation. The design targets are listed below:

1. Sample volume: 10-1000 μL
2. Desalting efficiency: 90% with an initial concentration of 100 mM

Table D.1: Performance metrics for a desalting device

Performance metrics	**Application notes**
Sample loss	Important for small scale applications where sample is available in limited quantities.
Sample volume	
Time for desalting	Important for large scale production, where high throughput is required
Desalting efficiency and final salt concentration	Might vary with type of ionization technique and sample type
User friendliness/number of user steps	Particularly relevant for offline desalting systems
Need for auxiliary instruments	Includes pumps, centrifuge, etc.
Sample carryover/dispersion	Relevant for continuous desalting systems
Lifetime	Number of injections for online methods Single or multi-use for offline methods
Non-specific interactions	To be minimized
Contamination and modification of sample	
Compatibility with organic solvents	Important for exchanging analyte into MS-compatible solvent

3. Desalting rate: 15-20 minutes
4. Sample loss: $< 20\%$
5. Device operation (batch/continuous): Batch for initial proof-of-concept, to be adapted for continuous operation later
6. Other requirements: The antibody should be finally transferred to an MS-compatible solvent, such as 80% acetonitrile or methanol

D.4 Device development

A design schematic for a device that can house graphene membranes and facilitate removal of salts from antibodies was developed (Figure D-2). The device consists of an upper reservoir for introducing the feed solution and a lower reservoir for buffer flow. The graphene membrane placed on a support membrane is mounted between the two reservoirs and sealed from the lower end using an O-ring. Both the upper and lower reservoirs have magnetic stirrers for improving mixing. The entire device can be placed on a magnetic stir plate for operation.

The key design parameters for the device include the reservoir volume on the feed side, buffer flow rate, membrane area, and stirring speed. The reservoir volume will depend on the volume of sample the device is expected to handle. The membrane area, stirring speed, and buffer flow rate would govern the transport kinetics of the device and would depend on the desired rate of desalting. Further, the membrane area would also be limited by the

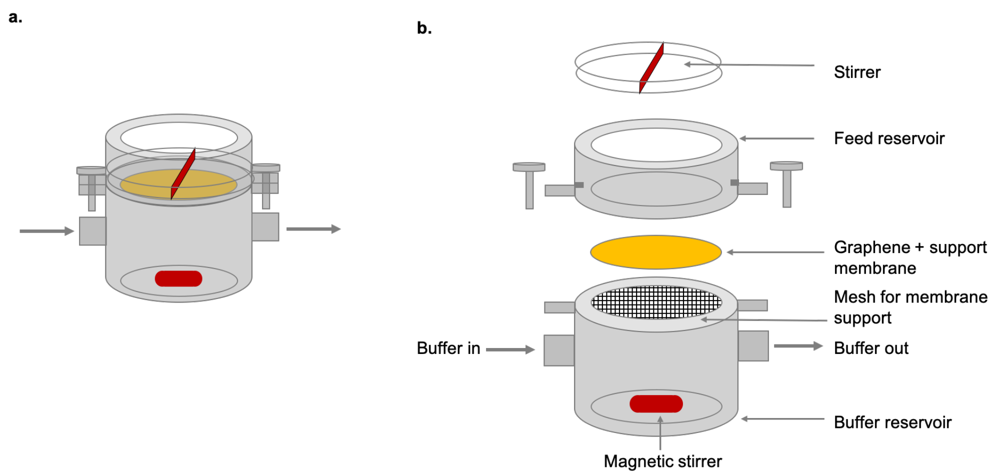

Figure D-2: Schematic of graphene-membrane based device for desalting

scalability of the manufacturing process for producing large area graphene membranes.

As the device was expected to handle 10-1000 μL of sample, the top reservoir volume was designed to be 2 mL, while accounting for the volume occupied by the stirrer. To estimate the membrane area, knowledge of the mass transport rate of salts from the feed to buffer reservoir is essential. The transport of salts was expected to encounter two sources of resistance: one that arises from the boundary layer on either sides (R_b) and the other that arises from the nanoporous graphene and porous support (R_m). While R_b for a given solute is affected by the boundary layer thickness, R_m is affected by membrane properties, such as porosity, pore thickness, etc.. It was anticipated that the majority of membrane resistance, R_m, would arise from the porous support as opposed to nanoporous graphene. An estimate of total mass transfer resistance ($R_t = R_m + 2R_b$; factor of 2 arises because of the presence of boundary layer on both, the feed and permeate side) and boundary layer resistance (R_b) was developed based on literature reported values (Figure D-3) [203]. The measurements in Figure D-3 correspond to the diffusion of urea across a polycarbonate track-etched membrane mounted in a stirred diffusion cell.

Figure D-3 shows that increase in stirring speeds reduces R_b and thus the overall mass transfer resistance. However, increasing the stirring speed above $\sim$350 rev/min results in marginal decrement in R_b, indicating that the mass transport here is limited by R_m.

Assuming that the device can be operated at high enough stirring speeds, the boundary layer thickness was estimated to be $\sim$55 μm, which corresponds to a boundary layer resistance

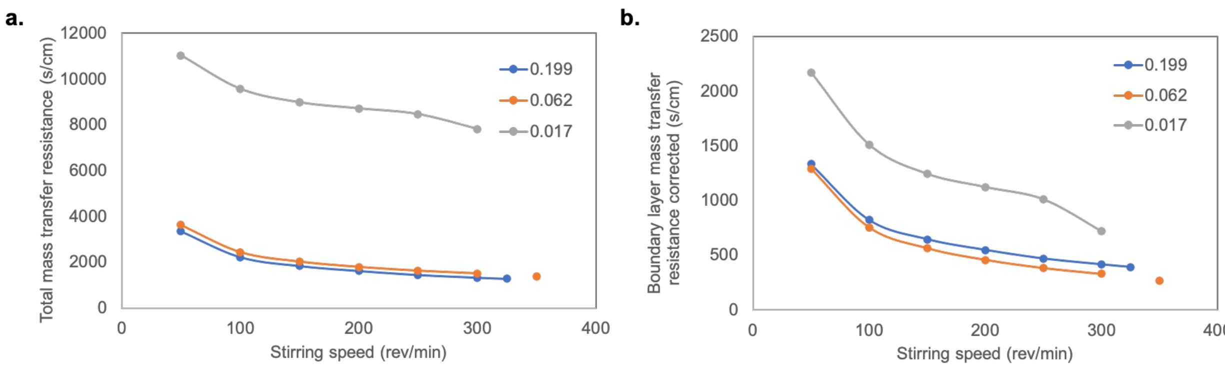

Figure D-3: Estimates of total mass transfer resistance and boundary layer resistance across support membranes for different porosities and stirring speeds

of 550 s/cm for NaCl (diffusion coefficient of NaCl is $\sim 10^{-5}$ cm^2/s. The estimate was based on Figure D-3, where a boundary layer resistance of ∼400 s/cm was reported for stirring speeds >350 rev/min; the boundary layer resistance was normalized by the diffusion coefficient (1.38×10^{-5} cm^2/s) for urea to obtain the boundary layer thickness [204].

Membrane resistance was estimated based on structural characeristics of the support membrane to be used for mounting graphene, assuming that the resistance from graphene with pores created for salt diffusion would be minimal. Polyimide (PI) membranes with pore size of 50 nm, pore thickness of 8 μm, and porosity of 0.11 was chosen as the porous support. The pore size was decided based on its effect on defect control and transport rate; smaller pore sizes are associated with fewer defects during transfer of graphene onto the support membrane, but they also reduce the transport rate. The choice of the material for support membrane was decided based on its availability and compatibility with organic solvents. The corresponding mass transfer resistance was estimated using the following equation:

$$R_m = \frac{\tau L}{\phi D} \approx 800 s/cm$$

where, τ is the tortuosity (1.2), L is the pore thickness (8 μm), ϕ is the porsosity (0.1175), and D is the diffusivity of the solute ($\sim 10^{-5}$ cm^2/s for NaCl).

$$R_t = R_m + 2R_b = 1600$$

s/cm.

Knowing R_t, the required membrane area can be estimated as follows:

$$J = -\frac{C_{feed} - C_{buffer}}{R_t}$$

where J is the flux, C_{feed} if the solute concentration on the feed side and C_{buffer} is the concentration on the buffer side.

$$\frac{V}{A}\frac{dC_{feed}}{dt} = -\frac{C_{feed} - C_{buffer}}{R_t}$$

where V is the volume of feed reservoir and A is the membrane area.

$C_{buffer} \approx 0$ if fresh buffer is constantly flowed through the buffer reservoir.

$$\frac{V}{A}\frac{dC_{feed}}{dt} = -\frac{C_{feed}}{R_t}$$

$$\frac{dC_{feed}}{C_feed} = -\frac{A}{VR_t}dt$$

$$C_{feed} = C_{feed,t=0}e^{-\frac{t}{\tau}}$$

where the time constant, $\tau = \frac{VR_t}{A}$.

To achieve 90% desalting in 20 min, $\tau = 8.7$ min. Assuming the device handles a 0.5 mL solution, area required for obtaining the calculated τ is ~1.15 cm^2.

Based on the aforementioned estimates, a device prototype was developed (CAD drawings and photographs of the device prototype are shown in Figure D-5). The device prototype was fabricated using teflon due to the material's compatibility with acetonitrile and methanol (solvents in which desalted antibody has to be transferred for MS analysis).

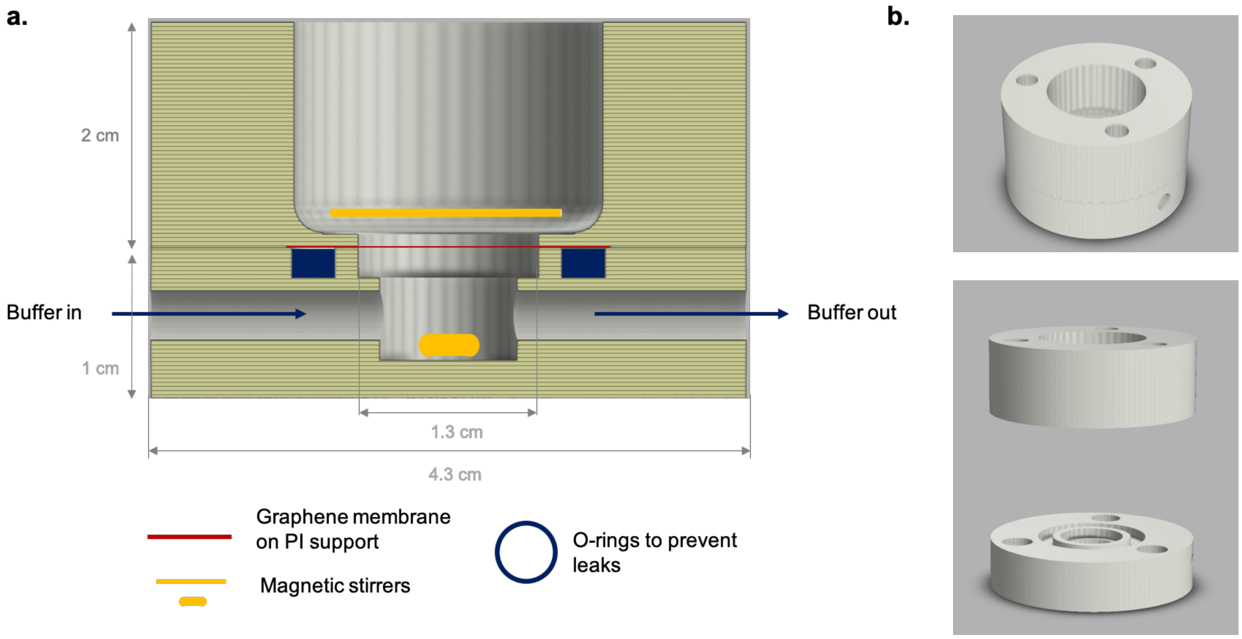

Figure D-4: CAD drawings of device prototype. a) Device components and dimensions. b) Device assembly

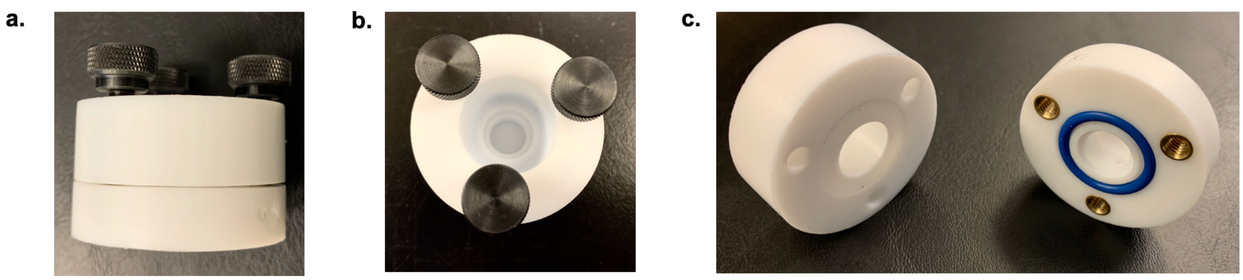

Figure D-5: Photographs of device prototype. a) Side view of assembled device. b) Top view of assembled device. c) Buffer and feed reservoir (shown on left and right respectively).

D.5 Performance testing

The performance testing of the device was planned in three stages:

1. Characterization of salt diffusion across the membrane
2. Characterization of salt diffusion in conjunction with solvent exchange (exchange of the de-salted antibody from an aqeuous solution into an MS-compatible solvent such as 80% acetonitrile or methanol is necessary for subsequent analysis; the solvent exchange can be performed sequentially or in parallel with the desalting step although the former might be preferred to mitigate issues associated with salt precipitation in acetonitrile, which has a low salt solubility).

3. Demonstration of antibody desalting in accordance with target performance metrics

The salt diffusion characteristics of the device were measured using phosphate buffered saline (PBS) and deionized (DI) water on the feed and buffer side respectively. Buffer flow rate was maintained at 1 mL/min using a syringe pump. Magnetic stirrers were operated at a speed of 500 rev/min. The diffusion of salts was measured by monitoring the conductivity of the solution on the feed side. The change in conductivity as a function of time for bare support or polyimide (PI) and PI with graphene (without pore creation) mounted on it is shown in Figure D-6.

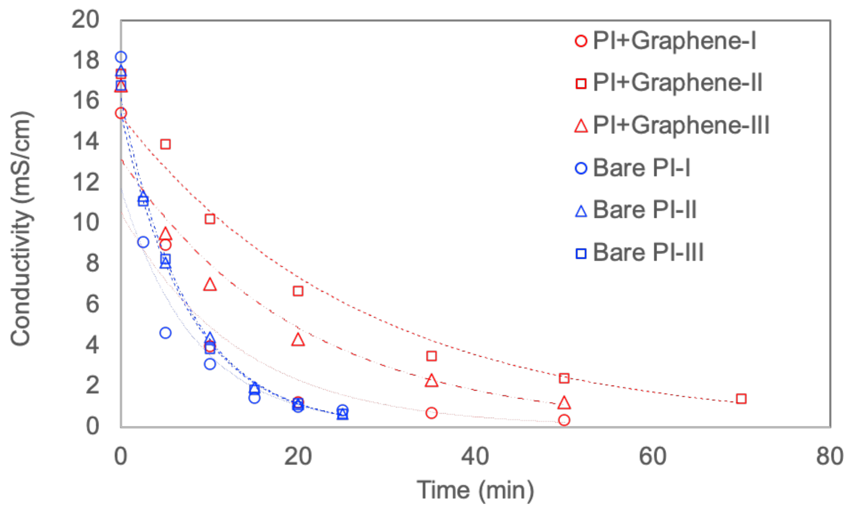

Figure D-6: Change in conductivity as a function of time. PI stands for polyimide.

The time constant for bare PI membranes and those with graphene mounted on them was 7.84 ± 0.46 min and 20.31 ± 7.31 min, respectively. The time constant with bare PI closely matched the predicted value of time constant required for obtaining 90% de-salting within 20 min. This time constant can be reduced substantially with addition of pores.

Solvent exchange and demonstration of antibody de-salting constitute ongoing work and are expected to completed in the future.

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