IMPACTS OF WILDFIRE ASH LEACHATE ON TRANSPORT OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS THROUGH POROUS MEDIA

A Thesis

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Master of Science

by

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ABSTRACT

Bank filtration sites rely on hydrologic processes of filtration to remove pathogens from drinking water sources. This study investigated the impact of ash leachate generated from fires on the bank filtration removal of parasites including *Cryptosporidium parvum.* The transport of *C. parvum*, surrogate latex microspheres, and *E. coli* through packed sediment columns was examined in the presence of wildfire ash leachate. Increases in leachate concentration resulted in increased retention, despite theoretical predictions of high dissolved organic carbon and phosphate concentrations in leachate, predicting increased transport. In these studies, *E.coli* was retained more than *C. parvum* oocysts, possibly due to the smaller size and elongated shape of the bacteria. When columns were flushed first with ash leachate and parasites were retained, but then washed with river water, at least 10% of the parasites were transported. Ash leachate seems to favor retention of pathogens and suggests that fire may not be problematic for bank filtration systems.

BIOGRAPHICAL SKETCH

Tanya Petach is a MS/PhD student in the department of Biological and Environmental Engineering at Cornell University. Her work with the Soil and Water lab (PI: Todd Walter) and the USGS (PI: Ronald Harvey) has focused predominantly on the transport of subsurface pathogens in post-wildfire regions. She will graduate in May with a master's degree in Biological and Environmental Engineering with a focus on hydrology.

To Mom and Dad, for taking me to the library.

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CHAPTER 1

INTRODUCTION

1.1 Research Goal

This study aims to determine the impact of wildfire ash leachate on the fate and transport of *Cryptosporidium parvum* oocysts and tracer latex microspheres in forest soils by mimicking the natural conditions in a soil column.

1.2 Cryptosporidium in Water Supplies

Cryptosporidium species are parasites that infect hosts predominantly within the orders Artiodactyla, Primates, and Rodentia by transferring environmentally resistant oocysts by the fecal-oral route from host to host (Fayer, 2004). *C. parvum* is of special concern as it is widespread, infectious to humans, and environmentally recalcitrant. Therefore, the transport of *C. parvum* through the subsurface has been a strong focus of many previous studies (Harvey et al., 1989; Harvey et al., 2002; Dai et al., 2002; Dai et al., 2003; Abudalo et al., 2005). However, transport of *C. parvum* in groundwater after wildfires has not been examined, and is of increasing concern as wildfires are shown to be on an increasing frequency and intensity trend (Flanigan et al., 2000). Wildfire ash is known to have impacts on both the physical and chemical properties of soils and may impact the transport of pathogens through soil.

Cryptosporidium outbreaks remain one of the most common waterborne diseases worldwide. In the United Kingdom, 69% of reported waterborne disease cases contracted between 1992-2003 were associated with *Cryptosporidium* (Smith et al.,

2006). Of the 15 known species of *Cryptosporidium*, seven infect human hosts including the most frequently reported zoonotic species, *C. parvum*. *C. parvum* oocysts can remain infective for months in cool, moist conditions (such as soils, rivers, lakes, and ponds with low temperatures that remain above freezing) and are resilient to removal and inactivation through conventional water treatment (coagulation, sedimentation, filtration, and chlorine disinfection) (King et al., 2017). Thus, the role of water as a major vehicle for transmission of Crypotosporidosis is of great concern.

C. parvum is of particular interest due the high prevalence of outbreaks and high mortality rates in immunocompromised individuals. *C. parvum* infection is typically associated with 72 hours of diarrhea, abdominal cramping, and vomiting, and can become lethal for infected individuals who are unable to stay hydrated or are immunocompromised before infection (Fayer, 2004). Perhaps the most notable outbreak in United States occurred in Milwaukee in 1994 where contamination of municipal drinking water led to over 400,000 cases of *C. parvum* and over 100 deaths (MacKenzie, 1994).

Attenuation of *C. parvum* oocysts from drinking water sources often relies on granular-media filtration as it is not susceptible to standard chemical deactivation methods (King et al., 2017). *Cryptosporidium* oocytes are typically 3-7 μm (diameter) and can be strained in small pore-sized granular media. Bank filtration is a sustainable and cost effective method of removing *C. parvum* from surface water during filtration through bottom and aquifer sediments (Ray et al., 2002). The colmation layer (or the biologically active layer of sediments underlying the sediment-water interface) has been

shown to play a critical role in bank filtration (Tufenkji et al., 2002). Oocysts can be removed in the colmation layer through either physical straining or sorptive-filtration processes, the latter of which are impacted by numerous parameters including collector efficiency, Van der Waals interactions, steric repulsion, surface charges of both oocysts and porous media, and diffusion coefficients. This study investigates the role of colmation layers (sediments immediately beneath the sediment-water interface) in the removal of *C. parvum* oocysts.

Pathogen attenuation within the colmation layer is often modeled as a combination of equilibrium adsorption, irreversible adsorption (filtration), and pathogen inactivation (Yates and Ouyang, 1992; Jin et al., 1997); however, colloid filtration theory assumes permanent attachment, which has also been an assumption in previous experiments given that most pathogens are quickly inactivated outside of host organisms relative to the adsorption time (Brush et al., 1999; Dai and Hozalski, 2002). Recent studies indicate that *C. parvum* persists for much longer than previously expected in the environment and may be capable of reproducing outside of host organisms (Ryan et al., 2016), suggesting that reversible adsorption may not retard *C. parvum* transport long enough to inactivate organisms before reaching drinking water sources.

Oocyst removal through colmation layer filtration is predominantly driven by adsorption and inactivation processes (Ryan and Elimelech, 1996). *C. parvum* oocysts adsorb to biofilms and grains within the colmation layer and are trapped in one of two categories of adsorption binding sites: (1) sites where both attachment and detachment

can occur and (2) sites where permanent attachment can occur. The governing equations of solute transport include advection, dispersion, and inactivation (which includes both adsorption processes and inactivation). Constants and variables used in the governing equations of pathogen fate and transport are defined as follows:

C = concentration of free pathogen per aqueous volume [kg m^{-3}]

n = porosity [no units]

S_{kin} = sorbed concentration of pathogen in kinetic sites [kg kg^{-1}]

S_{eq} = sorbed concentration of pathogens at equilibrium [kg kg^{-1}]

ρ_B = bulk density of saturated soil [kg m^{-3}]

D = diffusivity coefficient [m^2 s^{-1}]

v = pore water velocity [m s^{-1}]

k_{eq} = distribution coefficient [s^{-1}]

k_{att} = attachment rate coefficient [s^{-1}]

k_{det} = detachment rate coefficient [s^{-1}]

μ_1 = inactivation rate coefficient, free pathogens [s^{-1}]

$\mu_{s,eq}$ = inactivation rate coefficient, sorbed equilibrium pathogens [s^{-1}]

$\mu_{s,kin}$ = inactivation rate coefficient, sorbed kinetic pathogens (can be re-released) [s^{-1}]

Q = variable parameter (defined in equation [4])

The governing equations for one directional fate and transport of pathogens through the subsurface (adapted from Schijven and Hassanizadeh, 2000) are:

$$n\left(\frac{\partial C}{\partial t}\right) + \left(\frac{\partial \rho_B S_{eq}}{\partial t}\right) + \left(\frac{\partial \rho_B S_{kin}}{\partial t}\right) = \nabla \cdot (nD \cdot \nabla C) - \nabla \cdot (nvC) - Q \qquad (1)$$

$$S_{eq} = k_{eq}C \quad (2)$$

$$\left(\frac{\partial \rho_B S_{kin}}{\partial t}\right) = nk_{att}C - k_{det}\rho_B S_{kin} - \mu_{s,kin}\rho_B S_{kin} \quad (3)$$

$$Q = n\mu_1 C + \mu_{s,eq}\rho_B S_{eq} + \mu_{s,kin}\rho_B S_{kin} \quad (4)$$

However, in sediment columns, the governing equations can be reduced and simplified. Column experiments use uniformly packed tubes of soil and sediment and under these conditions, some parameters of the pathogen adsorption governing equations simplify because:

1. Dispersive effects within the column are small and can therefore be neglected.
2. Flow is predominantly in just one direction and can therefore be studied as a single direction experiment.
3. Test tube experiments are easier to control and therefore not subject to permits and other restrictions from field experiments.
4. Equilibrium adsorption can be realistically achieved.
5. Easier to set boundary conditions.

Thus, column experiments can be characterized by a Langmuir isotherm to model the relative concentration of oocysts on sediment surfaces relative to the concentration of oocysts in suspension. The Langmuir isotherm depends on the following variables:

Γ_{max} = Total number of surface sites per mass of sorbent (determined from analysis of biofilms) [kg^{-1}]

K_l = Sorbate affinity constant [M^{-1}]

C = Concentration of oocysts in water [M]

C_s = Concentration of oocysts on solid [fraction of occupied sites]

And is defined as follows:

$$C_s = \frac{\Gamma_{max} K_l C}{1 + K_l C} \quad (5)$$

Estimated ranges of Langmuir parameters for this study are based on values from Tufenkji (2007).

This latter theoretical description of the removal of oocysts is important since it can be used to determine the concentration of oocysts on the solid media (the fraction of oocysts that are removed in the experiment).

Surface charges on porous media alter the filtration of pathogens. Surface charge attraction is a mechanism to bind pathogens (when the charges on the particles and the pathogen are opposite in sign). Surface charge repulsion would cause the pathogens to be transmitted through the porous media if the charge on the pathogens is the same as the charge on the porous media particles. Surface charges can be altered by changing the pH of the media or adding charged ions (e.g., Ca^{2+}, Al^{3+}) to the filter.

Presence of humic matter in surface water promotes microbial transport into and within the colmation layer and aquifer (Dai and Hozalksi, 2002; Janjaroen et al., 2010; Harvey et al., 2011). Models developed in the 1990s suggest that the presence of organic matter provides competitive binding for sorption binding sites onto soil grains (Swanton, 1995) and may disrupt hydrophobic bonds between oocysts and soil grains (Ryan and Elimelech, 1995). Since natural organic matter concentrations in riverine waters

undergo drastic variation (such as the spike in dissolved organic carbon in rivers following wildfires), it is an important parameter in determining the adsorption process.

1.3 Impact of Fire on Water Quality

Fire is a natural part of forest ecosystems which impacts both physical and chemical properties of the underlying soil and thus also impacts watershed water quality, including sediment loading, chemical quality, and microbial content. When wildfires pass through an ecosystem, the burned organic matter ("ash") initially remains on the surface of the soil. Ash leachates form when water passing through ash is chemically altered by the ash dissolution. Both particulate ash and ash leachates can have dramatic ecological effects. In the ash leachate studies in this project, the sorbate affinity constant (described in Equation [5]) may be altered by the components of the ash leachate.

Ash creates physical changes in the soil which in turn impact infiltration and runoff rates. There is much debate about whether ash increases or decreases surface runoff (Cerdà and Doerr, 2008; Woods and Balfour, 2008). There is some indication that ash may clog water flow and alter the permeability of soils; however, studies in specific soil types indicate that the effects of ash clogging water flow are temporary. Additionally, it is unlikely that ash reduces runoff by clogging pores in the sediments since both the ash and the sediments are negatively charged and should repel each other; instead, the ash may hold water and delay its release (Stoof et al., 2016). Other studies indicate that ash may increase runoff by sealing the soil surface by clogging the macropores or forming a surface crust, which decreases infiltration rates and increase runoff (Woods and

Balfour, 2010), making the soil behave more hydrophobically. Some ash particles are also hydrophobic which may also increase runoff rates (Bodi et al., 2011). However, under some conditions, including non-macroporous soils or thick layers of ash that store water, the runoff rates decrease after wildfires. Thus, the specific details of underlying soil type, ash layer thickness, size and shape of ash particles, and other physical properties influence runoff rates and infiltration in post-wildfire regions (Balfour and Woods, 2013). Runoff rates in turn impact water quality due to the changes in sediment loading in the waters, which have been summarized (Langhans et al., 2016).

Ash leachate has similarly complex, yet less examined, ecological impacts to physical ash particles. While physical ash has dramatic ecological effects in the immediate vicinity of the fire, the effects of ash leachate are much more dispersed and can extend for many kilometers downstream of a fire (Writer and Murphy, 2012). Due to high concentrations of dissolved organic carbon, leachates provide a large carbon source for ecosystems; however, the ramifications of such pulses are varied. The impacts of the wildfire ash leachate depend upon the nature of the soil, type of vegetation coverage, fire severity, and ash production (Maia et al., 2012). Wildfire ash leachates impact water quality by changing the distribution of ions and the altering pathogen binding in the soil. Although the specific chemical changes that occur following wildfires vary greatly and are dependent upon the burn temperature and the vegetation types, typically Ca, Na, Mg, and Mn concentrations are increased as are pH, conductivity and DOC (Costa et al., 2014). Coniferous ash is also known to be associated with higher concentrations of the trace elements Fe, As, Cr, Al, Ba, and Pb in ash leachate (Smith et

al., 2011). Santin et al. (2015) correlated these increase metal concentrations with severity of fire and showed that more severe fires led to higher Al concentrations in the subsurface soil. Al is typically considered a lithogenic element, arising from the minerals in the soil rather than the vegetation, and may also be an indication that soils are burned during wildfires in addition to vegetation (Santin et al., 2015). When wildfires encroach into urban areas, there can also be a multitude of refractory, highly toxic compounds present in leachates.

These ash leachates remain present further away from the site of a fire as the ions are carried downstream. Given the extreme concentrations of organic compounds and polyvalent ions in leachates (DOC levels above 100mg/L and calcium concentrations above 5000mg/L) (Murphy et al., 2015; Periera et al., 2012), leachates can impact ecosystems for tens of kilometers downstream of the fire. For example, Murphy et al. (2015) studied water-quality in response to hydrologic events for three years after a wildfire in the Fourmile Creek Watershed, near Boulder, Colorado, and observed that >6km downstream of a burned area, total suspended sediment, dissolved organic carbon, nitrate, and manganese concentrations were 10-156 times higher than upstream.

1.4 Research Question

This project investigates the role that wildfire ash leachate plays on the transport of *C. parvum* in porous media and natural soils. How does wildfire ash leachate impact the transport of *C. parvum* oocysts and related pathogens through aquifer sediments?

This study uses bench-scale experiments to quantify adsorption, transport, and retention to determine the impact of wildfire ash leachate on the transport of *C. parvum* through sediment columns.

CHAPTER 2

MATERIALS AND METHODS

2.1 Study sites for sediment samples

Sediment samples were collected at two different sites (Boulder Creek, CO and Russian River, CA), representing two different sediment types.

- Boulder Creek sediments are derived predominantly from the Boulder Creek granodiorite and are feldspathic, quartz-rich, with some clay minerals included. Boulder Creek sediment was collected above the Boulder Municipal Waste Water Treatment effluent return site to minimize alterations in grain size or chemistry from wastewater treatment processes. The Boulder Creek sediments were obtained from a USGS collection site on Boulder Creek (Figure 1).
- Russian River sediments are derived from an aluminum and iron-rich batholithic material and are much more varied in mineralogy. Russian River sediment was collected near the Wohler Filtration Site, one of the bank-filtration locations along the river (Figure 2).

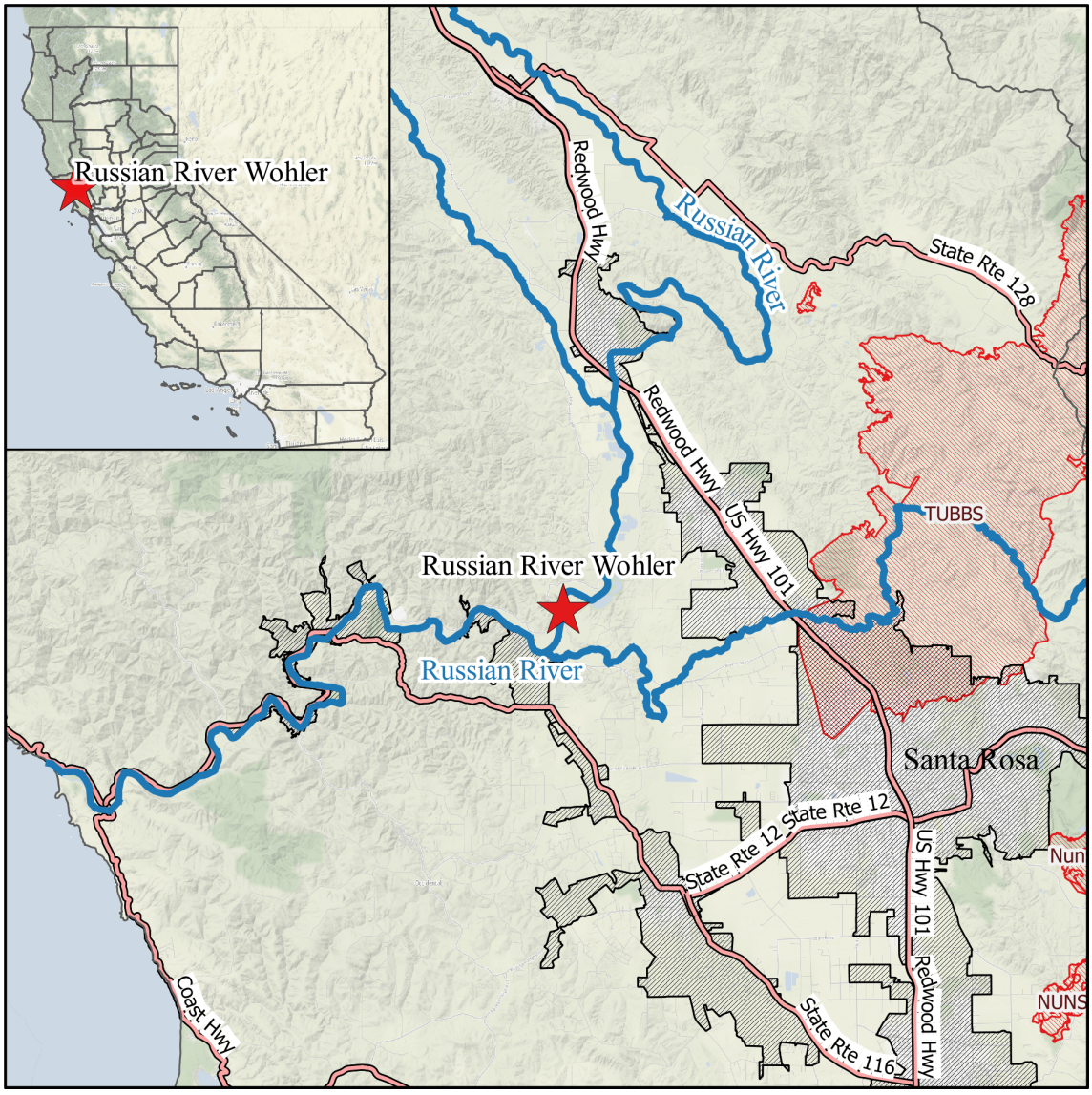

Figure 1. Map of the Sonoma County Water Agency site at which Russian River sediments and water samples were collected. The star indicates the collection site. (Background Layer Source: OpenStreetMap)

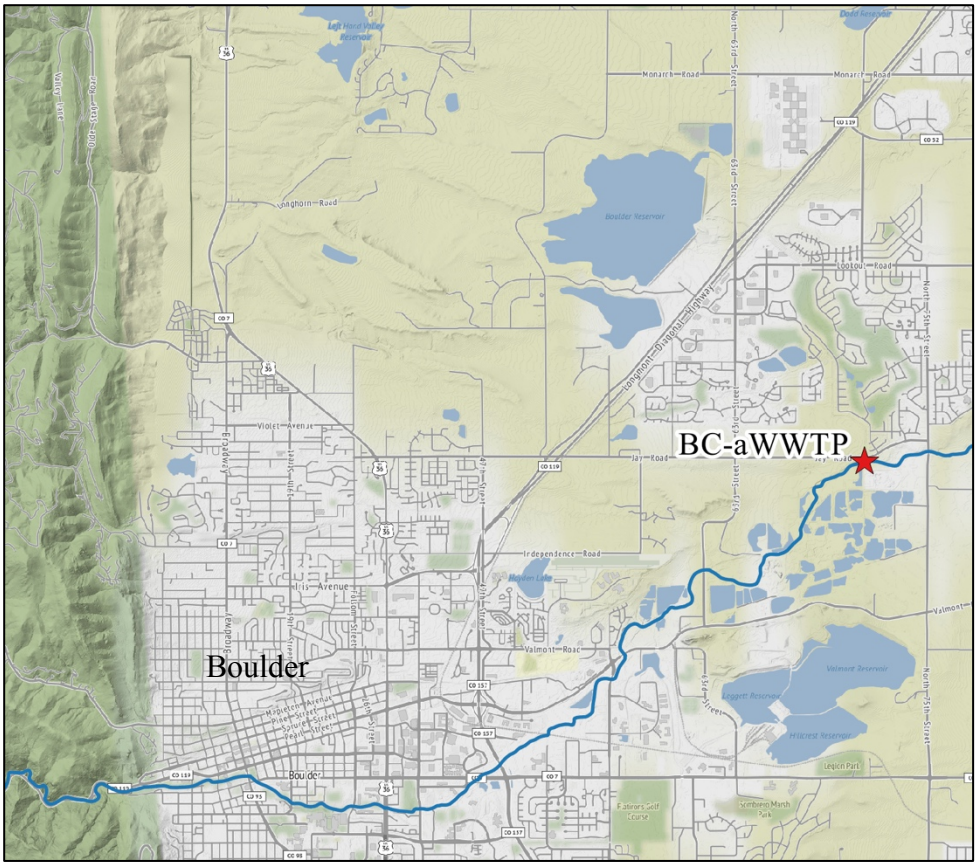

Figure 2. Map of the Boulder Creek site at which sediments and water samples were collected. The star indicates the collection site. (Image: Google Maps)

Both study sites are located in high fire-risk areas. The Fourmile Canyon fire (2010) burned an estimated 2500 hectares (6,181 acres) approximately 7.2 kilometers (4.5 miles) upstream of the Boulder Creek sediment collection site. The Tubbs fire (2017), Pocket fire (2017), and Independence fire (2017) burned a collective 21,924 hectares (54,175 acres) in the Russian River watershed. Ash samples from both the Fourmile Canyon fire and the Tubbs fire sites were collected for analysis.

2.2 Determination of appropriate particle sizes for sediments in the columns

Grain-size frequency distributions for Boulder Creek and Russian River (Figure 3) sediments were determined from sieving experiments. Sieving experiments followed the protocol from Tufenkji et al. (2004) to create grain-size frequency distributions. The results of the sediment fractionation experiments were used to identify the correct sediment size for use in the column experiments.

Cobble or larger sized grains (64mm diameter) were removed before the final determination of the D50 (50% finer than). After removing sediment grains that were cobble sized or larger, the D50 for these two sediment samples was found to be 0.34 mm and 0.88 mm for the Russian River and Boulder Creek samples, respectively. Hence, columns were packed with sediments ranging from 0.25-0.50 mm in size, which more closely mimics the size distribution in the Russian River sediments than those from Boulder Creek. The size distribution was chosen to more accurately represent the Russian River sediments as the objective of this study was the assessment of the susceptibility of the Russian River to a *C. parvum* outbreak after a wildfire; therefore, sediment sizes were chosen to more closely mimic the Russian River sediments.

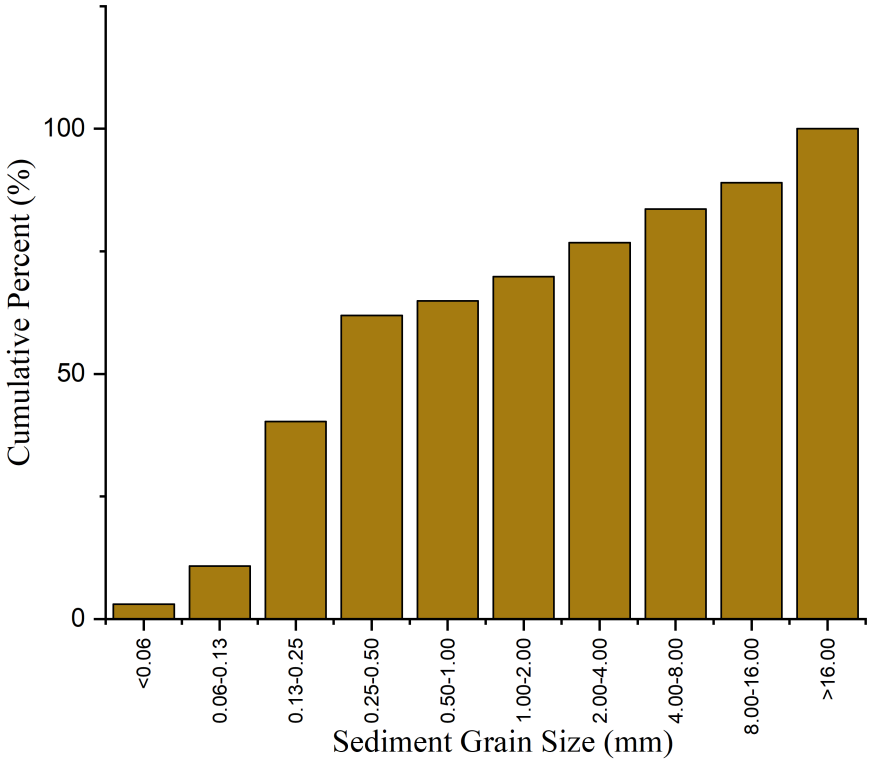

Figure 3. Sediment Size Distribution for Russian River Sediment samples.

2.3 Preparing and characterizing wildfire ash leachates

Ash leachates were prepared using *Pinus ponderosa* ash collected from the 2010 Fourmile Canyon fire, CO. *P. ponderosa* ash was selected for use in this study as it is the primary vegetation type in the area and does not accumulate anomalous concentrations of trace elements as occurs for shrub ash (Hageman et al., 2008).

The major chemical composition for ashes from predominant species found at the two sites examined in this study (*Ribes, Juniperus,* and *Pinus*) was previously determined

(Stoof et al., 2016). That analysis was carried out using an inductively coupled plasma atomic emission spectrometer (ICP-AES) and measured in triplicate (Table 1).

Table 1. Chemical Composition of Wildland Fire Ash (Stoof et al., 2016)

Ash type	**Elemental composition (g/kg)**									
	Al	Ca	Fe	K	Mg	Mn	P	Pb	S	Zn
Ribes[1]	18.2	23.4	22.9	7.7	8.6	1.0	2.5	0.1	2.6	0.2
Juniperus[1]	15.7	28.2	21.7	7.7	8.1	0.8	1.9	0.1	1.2	0.2
Pinus[2]	18.3	20.5	21.0	5.3	4.4	1.0	1.9	0.1	1.9	0.2

[1]Ash from the Fourmile Fire in Colorado (2010)
[2]Ash from the Overland Fire in Colorado (2003)

Ash leachates were prepared using the standard USGS method using a modified method from Hageman et al. (2007). Wildfire ash samples were collected within one month after the September 2010 wildfire at Fourmile Canyon, CO. The ash samples were sieved through a 2-mm stainless steel sieve. For each ash sample, 50 g of sieved ash was poured into a 1000-mL Teflon bottle that contained 1000 g filtered river water. The bottle was shaken horizontally for five minutes. The samples were filtered, preserved, and analyzed with the same methods used for water samples (Murphy, 2006; McCleskey et al., 2012). Final samples were filtered using vacuum-suction through a 0.22 μm pore-sized filter to ensure that bacteria and protozoa and ash particles were removed. This final filtration was important to remove all contributions from the solid particles and only include chemical differences in the ash leachates. Ash leachates and Russian River water were analyzed using an ICP Mass Spectrometer to determine elemental data (Table 2). As expected, elemental compositions are higher for leachate than Russian River water.

Ash leachates were also analyzed for dissolved organic carbon (DOC) using the absorbance method. Leachate samples were stored on ice for less than 24 hours prior to analysis. The samples were filtered through 0.45 µm filters and DOC was determined using an OI Analytical 700 TOC Analyzer. UV-VIS measurements were made on an HP spectrometer, Model 8453 at 254nm wavelength using the methods in Weishaar et al. (2003).

Table 2. ICP Mass Spectrometer Elemental Analysis for Ash Leachate and Water. *

Sample	As (µg/L)	B (µg/L)	Ba (µg/L)	Be (µg/L)	Ca (mg/L)	Co (µg/L)	Cu (µg/L)
***P. ponderosa* Leachate**	40	421	104	0.1	84.9	4	4.5
Russian River Water	<30	227	72.6	0.1	22.7	<2	3.8
	Fe (µg/L)	**K (mg/L)**	**Li (µg/L)**	**Mg (mg/L)**	**Mn (µg/L)**	**Mo (µg/L)**	**Na (mg/L)**
***P. ponderosa* Leachate**	4	29.8	7.69	28.3	132	7	9.1
Russian River Water	<2	1.16	2.68	13.0	<4	<5	8.5
	Ni (µg/L)	**P (µg/L)**	**Rb (µg/L)**	**S (mg/L)**	**SiO2 (mg/L)**	**Sr (µg/L)**	**Zn (µg/L)**
***P. ponderosa* Leachate**	6	1030	13	70.2	15.4	407	28
Russian River Water	<3	<40	<1	5.2	15	223	22

* Al, Cd, Cr, Pb, Sb, Se, U, and V values were all below the detection limits and are not reported here.

To ensure that ash leachates did not change over the timespan of the experiments, the pH and conductivity of solid ash samples, that were stirred on a stir plate with deionized water, were measured over a 22-hour period (Figure 4).

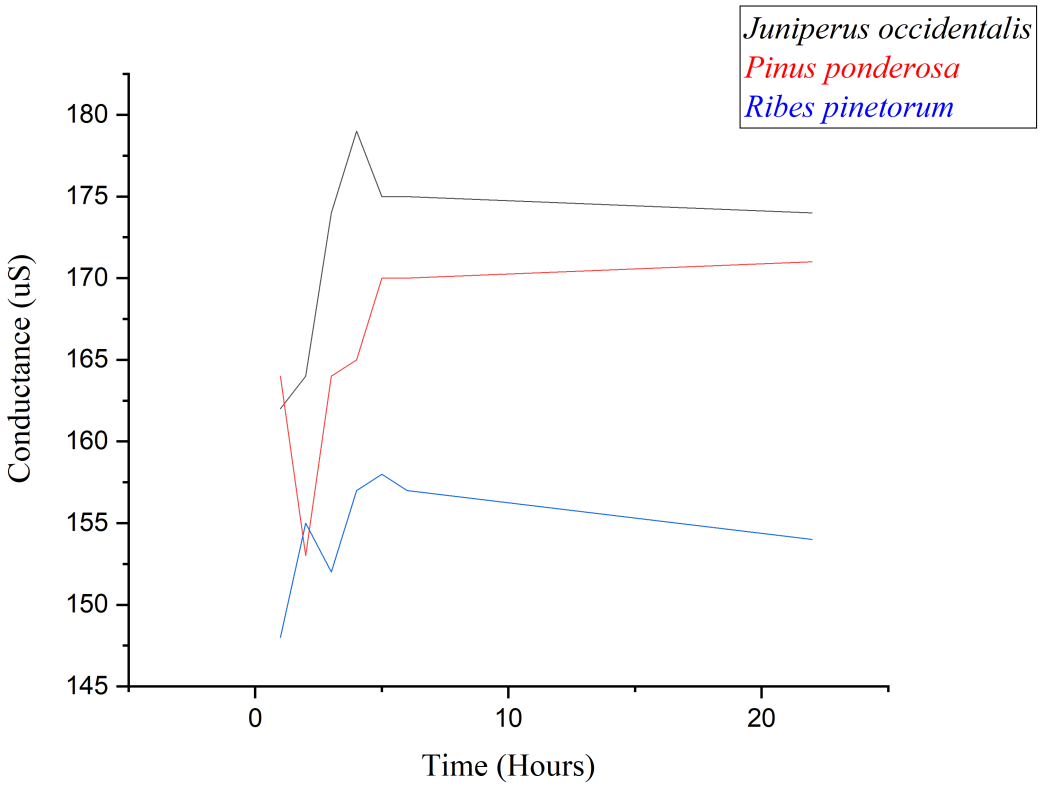

Figure 4. Conductivity-vs-Time for Ash Leachates. Ash leachates were made from three different vegetation sources common in both study site areas: *Juniperus occidentalis*, *Ribes pinetorum*, and *Pinus ponderosa*.

Conductivity of leachates stabilized for all samples after 5 hours; however, since conductivity changed less than 10% from five minutes to 22 hours, the USGS five minute leachate method was used in this study.

2.4 Sourcing and characterizing microspheres and deactivated pathogens

Five types of polystyrene microspheres were obtained from Polysciences (Warrington, PA, USA) for use in these studies labeled as: M673, M674, 1408MS, YG MS, and Blue MS. These microspheres were chosen as their diameters and zeta potential at experimental pH (9.5) cleanly bracket the size and zeta potentials of *E. coli* cells and

C. parvum oocysts (Table 3). The concentration of microspheres used in the column studies was between 2 and 6 x 10^6 mL^{-1}.

Table 3. Measured Zeta Potentials for Microspheres and Pathogens

Microsphere or Pathogen	Diameter (µm)	Measured zeta potential (mV) at pH 9.5
M673	2.0	-25.4
M674	1.8	-37.8
1408 MS	1.0	-73.0
0.2 µm YG MS	0.2	-38.8
1.0 µm Blue MS	1.0	-39.4
***C. parvum* oocycsts**	2-5	-32.6
***E. coli* cells**	0.5 x 2.0 (rod-shaped)	-55.3

Zeta potentials were determined by measuring electrophoretic mobility using laser Doppler microelectrophoresis (Zeta PALS Potential Analyzer, Brookhaven Instruments, NY, USA). The Smoluchowski equation was used to calculate zeta potentials from electrophoretic mobility (Masliyah and Bhattacharjee, 2006). The constants and variables in the Smoluckowski equation and the necessary Debye-Hückel are defined as follows:

U_E = electrophoretic mobility [V/s]

$\varepsilon_r\varepsilon_0$ = permittivity [F m^{-1}]

μ = solution viscosity [kg m^{-1} s^{-1}]

r = particle radius [m]

κ = Debye-Hückel parameter, or length [m^{-1}]

n_0 = bulk ionic concentration [M]

z = valence charge

e = charge of an electron [1.60 × 10^{-19} C]

k_B = Boltzman constant [1.38×10^{-23} m^2 kg s^{-2} K^{-1}]

T = temperature [K]

The governing equation determining the Debye-Hückel parameter is (Masliyah and Bhattacharjee, 2006):

$$\kappa = \left(\frac{2n_0 z^2 e^2}{\varepsilon_r \varepsilon_0 k_B T}\right)^{\frac{1}{2}} \quad (6)$$

Using this Debye-Hückel parameter, the zeta-potential can be calculated using the following equations (Hendricks, 2006):

$$U_E = 4\pi\varepsilon_0\varepsilon_r\zeta 6\pi\mu(1 + kr) \quad (7)$$

A sample of 1 mL of stock solution of approximately 2 x 10^6 microspheres or oocysts or 1.5 x 10^6 *E. coli* was added to 50 mL of buffer and mixed by stirring on a stir plate for 22 hours. A 1.5 mL sample of diluted solution was analyzed with the Zeta PALS instrument for determination of zeta potentials.

C. parvum oocysts (Sterling Parasitology Laboratory, Department of Veterinary Science and Microbiology, University of Arizona, AZ, USA) were collected from the fecal matter of infected Holstein calves and then concentrated, isolated, and formalin-deactivated by the vendor using the method from Abudulo et al. (2005). Deactivated oocysts were used in these experiments to: (i) enable comparisons between these

experiments and other studies examining deactivated oocysts; and, (ii) to ensure laboratory safety by not using live pathogens. Oocysts in these experiments were an average size of 3.6μm and were used in column experiments at concentrations between 2 and 6 x 10^6 mL^{-1}. Oocysts in these experiments were stained using a DAPI stain to be identified on the flow cytometer.

Non-infectious, ampicillin-resistant K-12 strain *Escherichia coli* (from Bio-Rad Laboratories, California) was grown in Luria broth with ampicillin for use in these experiments. The ampicillin-resistant *E. coli* allows for all experiments to be run with ampicillin to limit contamination from other bacteria. *E. coli* were stained using SYBR Green I stain to be identified on the flow cytometer.

Justifications for using microspheres as surrogates for oocysts

Since oocysts are strongly negatively charged, buoyant spheres, carboxylated microspheres are often used as a surrogate for oocysts in laboratory studies (Harvey and Harms, 2003). Criticisms of using microspheres as oocyst mimics are that microspheres are uniformly carboxylated to form negative charges on the outside of the sphere, while negatively charged oocysts likely have a more complex surface chemistry. However, extensive experimental results suggest that microspheres can be used to provide a conservative estimate of oocyst removal in studies of negatively charged media (Dai and Hozalski, 2003). Metge et al. (2007) showed that near the sediment-water interface, the microspheres were good mimcs for oocycsts, but their behavior is less certain in deeper sediments. Recently, a retention study of oocysts and

microsphere surrogates in Russian River sediments showed similar removal rates (Metge et al., 2007), however, the authors cautioned that the microsphere surrogates may underestimate the degree of oocyst transport in some aquifers. This research used microspheres as mimics for oocysts except for studies of reversible adsorption of oocysts on sediments.

The oocysts in my experiments are formalin-deactivated, and some criticisms have been made when using formalin-deactivated oocysts instead of active oocysts. Deactivation of oocysts may change the surface chemistry, and some studies indicate that formalin-deactivated oocysts are subject to more retention than active oocysts (Kuznar et al., 2006; Byrd et al., 2007). However, at neutral pH, other studies have found that surface chemistry of formalin-deactivated and viable oocysts are similar (Butkus et al., 2003; Abudulo et al., 2005). My research experiments use only formalin-deactivated oocysts when oocysts are used in the experiments.

2.5 Building sediment columns

Columns were established to mimic a variety of sediment conditions that would emulate natural bank filtration sites. The columns were packed with river sediments to allow for the assessment of how grain size and ash leachate would alter pathogen or microsphere attachment to the columns (Figure 5). The components of the sediment columns included:

- Eluents for the columns (prepared as described above):

- Ash leachate (filtered) from *P. ponderosa*
- Distilled water
- Boulder Creek (filtered) river water
- Russian River (filtered) river water

- Pathogens inoculated into column (sources described above):
  - *E. coli*
  - *C. parvum*
  - Microspheres (YG MS, Blue MS, M673, M674, 1408MS)

- Sediment from Boulder Creek or Russian River (prepared as described above)

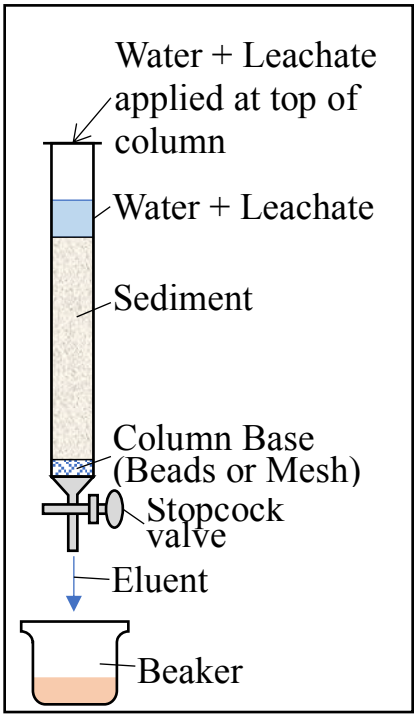

Figure 5. Schematic of static column set up. The experimental design process included determining appropriate volume of sediment, volume of water, and type of column base.

2.6 Establishing sediment column parameters

Sediment volume required to retain pathogens

The utility of the column experiments is to identify conditions that impact pathogen retention in soil/ash leachate systems. As the sediment column is lengthened, eventually, pathogens will tend to be fully retained. Thus, for these column experiments to be useful in the study of pathogen retention, the volume of column sediment must provide for differential retention of pathogens under different conditions. Both 5mL and 10mL sediment volumes were tested. Static columns were wet-packed with sediment and then tested for retention of pathogens (Figure 6).

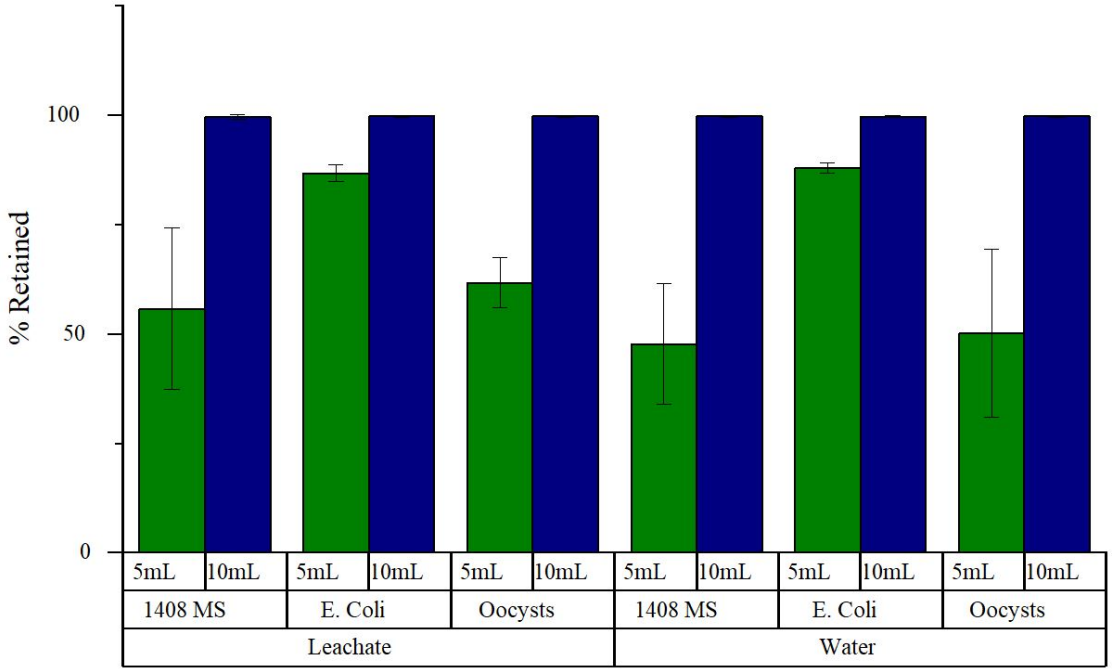

Figure 6. Percent Retained -vs- Volume of Sediment (from Russian River). Static columns were prepared with *E. coli*, *C. parvum* oocysts, 1408MS microspheres, eluted with Russian River leachate or Russian River water to determine the efficacy of 10mL and 5mL of wet-packed sediment for pathogen removal. Green bars indicate columns run with 5mL of sediment; blue bars indicate columns run with 10mL of sediment.

Given that all columns run with 10mL of sediment had near-perfect (>99.5%) retention of all pathogens, the 10mL sediment column creates too much retention and would not yield information to differentiate between different pathogen attachments.

Thus, all columns in this study used the 5mL volume of wet-packed sediment. This approach aligns with previous static column experiments that have tested 5mL and 10mL volumes of sediment in similarly sized static columns (Metge et al., 2001).

Structure of column base to retain sediment

Since previous static column experiments have used either a mesh film or tightly packed glass beads as a column base to retain the sediment in the column (Metge et al., 2001; Harvey et al., 2004), these two column bases were tested to determine which would have less impact on pathogen binding (Figure 7).

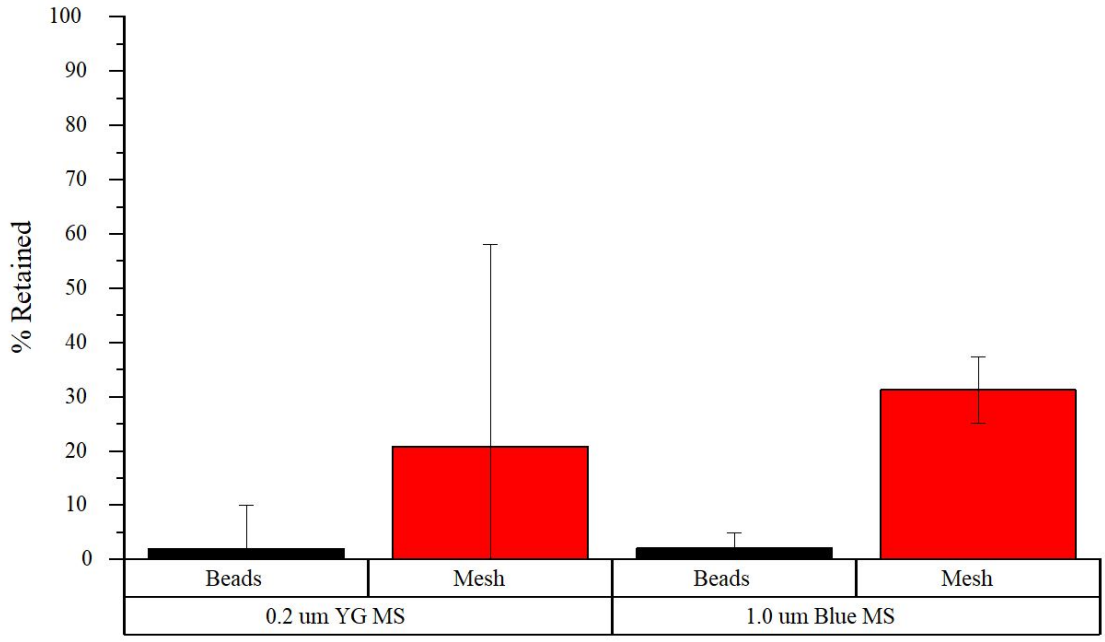

Figure 7. Percent Retained -vs- Column Base. Static columns were set up with either of two different-sized microspheres, Russian River leachate, and Russian River sediment to determine the relative impact of glass beads and mesh as column bases.

Mesh films had a substantial impact (>20%) on the removal of pathogens in static columns. To minimize the impact of the column base on the results of pathogen retention, all transport experiments in this study used tightly packed glass beads as the column base.

2.7 Methods for creating sediment columns

Experiments to study the retention of pathogens were carried out in static sediment columns (Figure 8).

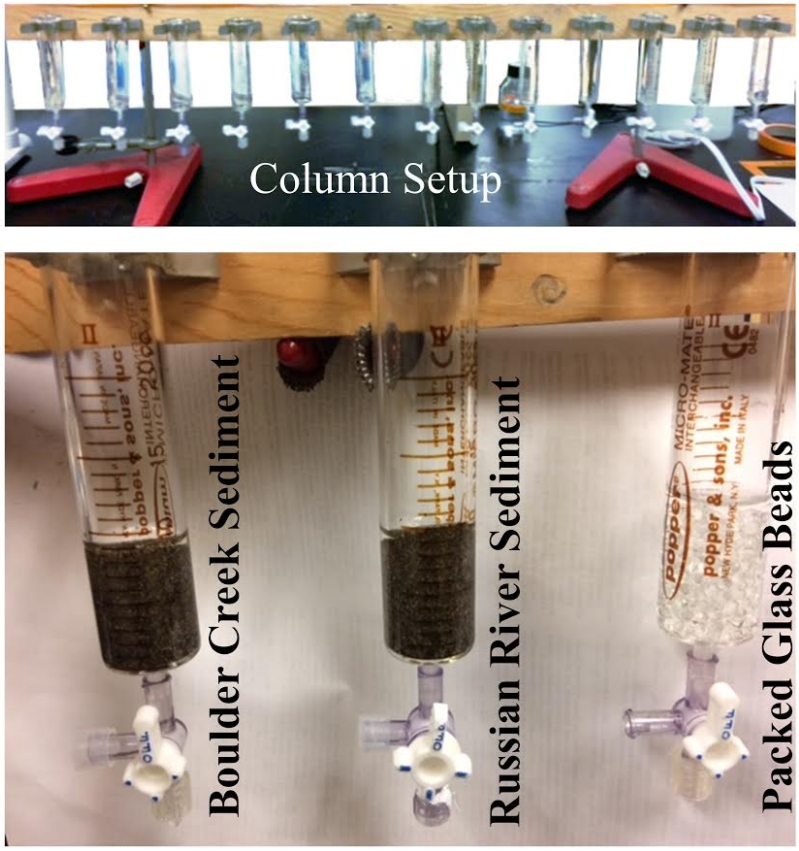

Figure 8. Column Experiment Setup. Columns were packed with 5mL of sediment or glass beads, flushed with 20 pore volumes of eluent, retained with a thin layer of glass beads packed at the bottom of each column, and inoculated with pathogens or microspheres for four hours before flushing.

The column is 25mm diameter, flow-through glass, fitted with a stopcock at the base. Columns were wet-packed with 5mL of Boulder Creek sediments, Russian River sediments, or glass beads (as a control where no pathogen retention should take place). Sediment size ranged from 0.25-0.5mm based on the D50 of the sediments. The sediment columns were flushed with 0.45μm-filtered eluent. Pore volumes for the sediment columns (determined from the difference in mass between the dry sediment and the saturated sediment of the column) were recorded after wet-packing.
The columns were equilibrated by flowing eluent through the columns to reach a stable eluent pH and conductivity, and the volume of eluent used to reach equilibrium was reported in pore volumes. Columns were flushed with the volume of eluent necessary to reach equilibrium (found to be 20 pore volumes as described in Section 3.1 in Results), and then inoculated with 1mL of inoculum diluted in eluent to equal one pore volume of fluid.

Inoculum was prepared using an Apogee Flow Systems flow cytometer to a concentration ~5,000 events/μL of each "pathogen" before dilution to one pore volume. The inoculum and eluent were drawn into the column and left to incubate for 4 hours. The columns were then flushed with 20 pore volumes of eluent and the effluent was analyzed on an Apogee Flow Systems flow cytometer to determine pathogen concentration. Details on the flow cytometry are discussed in Appendix A. The percentage pathogen retained is the ratio between the pathogen concentration remaining on the column and the initial pathogen concentration inoculated.

The eluents, microorganisms, microspheres, and sediment sources used for packing columns in this experiment are summarized in Table 4. Columns were first wet-packed with sediments and equilibrated, and then pathogens were introduced to the columns. Each column experiment was run in triplicate. Error bars shown in figures are standard deviations between the triplicate measurements.

Table 4. Components of the Column Experiments

Eluent (leachate or river water)	Sediment for column packing	Pathogen/Microsphere
P. ponderosa leachate in Boulder Creek Water	Boulder Creek	*E. coli* cells
P. ponderosa leachate in Russian River Water	Russian River	*C. parvum* oocysts
Boulder Creek Water		YG MS
Russian River Water		Blue MS
Deionized Water		M673
		M674
		1408MS

Sediment key:

Boulder Creek is feldspathic, quartz-rich riverine sediment with larger grain size. Russian River is aluminum and iron-rich batholithic sediment with smaller grain size. However, whether the sediments were from the Russian River or Boulder Creek, a standard sediment size range (0.25-0.5 mm) was used.

2.8 Methods for unique pathogen transport experiments

Once the experimental design was optimized for sediment size, equilibration, column volume and base, and the properties of the ash leachate and microspheres and pathogens were understood, the pathogen transport experiments were carried out to identify the environmental parameters that affect the retention of pathogens. Most of the column experiments followed the standard protocol described in the Section 2.7, however, a few experiments required customized protocols.

To determine the reversible and irreversible retention of pathogens with changing eluent, inactivated *C. parvum* oocysts were inoculated onto columns (rather than surrogate microspheres, since the reversibility of pathogen binding is likely to be strongly dependent on surface chemistry). After column equilibration and inoculation with *C. parvum*, the column was eluted with 15 pore volumes ash leachate, followed by 15 pore volumes of Russian River water, and finally 15 pore volumes of deionized (DI) water. Each pore volume was collected and analyzed individually.

To determine the role of size and charge of the microspheres on their retention, two microspheres of similar diameter, but different zeta potential were compared using the same column experiment.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Properties of wildfire ash leachate

The dissolved organic carbon concentration (DOC) of the ash leachates ranged from 73.5-97.1 mg/L depending on the size fraction of ash examined. Note that smaller diameter ash tends to have increased DOC concentrations as the surface area to volume ratio is more favorable to solution, however, if the size fractions are normalized by surface area to volume ratios, then larger ash grains tend to have higher DOC concentrations as they are typically less severely burned than small ash. The average DOC concentration for bulk ash (not size fractionated) was found to be 81.6mg/L for *P. ponderosa*. For comparison, filtered Boulder Creek and Russian River water have DOC concentrations of 2.0mg/L and 2.7mg/L, respectively, suggesting that the ash leachate is high in DOC.

Conductivity of *P. ponderosa* ash leachate after 5 minutes of solution time averaged at 164 μS. Conductivity of leachates did increase slightly (<10%) if exposed to ash for longer than 5 minutes (up to 22 hours). However, since the magnitude of change was less than 10%, the USGS 5 minute leachate technique was considered an adequate time for leaching and is reported to simulate the short expected water runoff times over ash (Woods and Balfour, 2008).

pH of leachates ranged from 9.46 to 9.53 in these experiments.

3.2 Results to ensure equilibration of columns

To ensure that columns would not have soluble contaminants on the sediment surfaces that might impact the studies, the columns were flushed with river water until the column eluent reached equilibrium, as measured by pH and conductivity (<10% change). The number of pore volumes required to reach equilibrium was 20 (Figure 9). Given that after 20 pore volumes of flushing, conductivity measurements were stable to within 10% and pH to within 0.5%, a 20 pore volume flush was deemed adequate for removing readily soluble compounds that impact pH or conductivity. Remaining compounds are presumed to be permanently adsorbed. This number of pore volumes aligns well with previous static column experiments that required either 20 pore volumes or 60 pore volumes of eluent fluid to flush similarly sized static columns (Metge et al., 2001; Harvey et al., 2004).

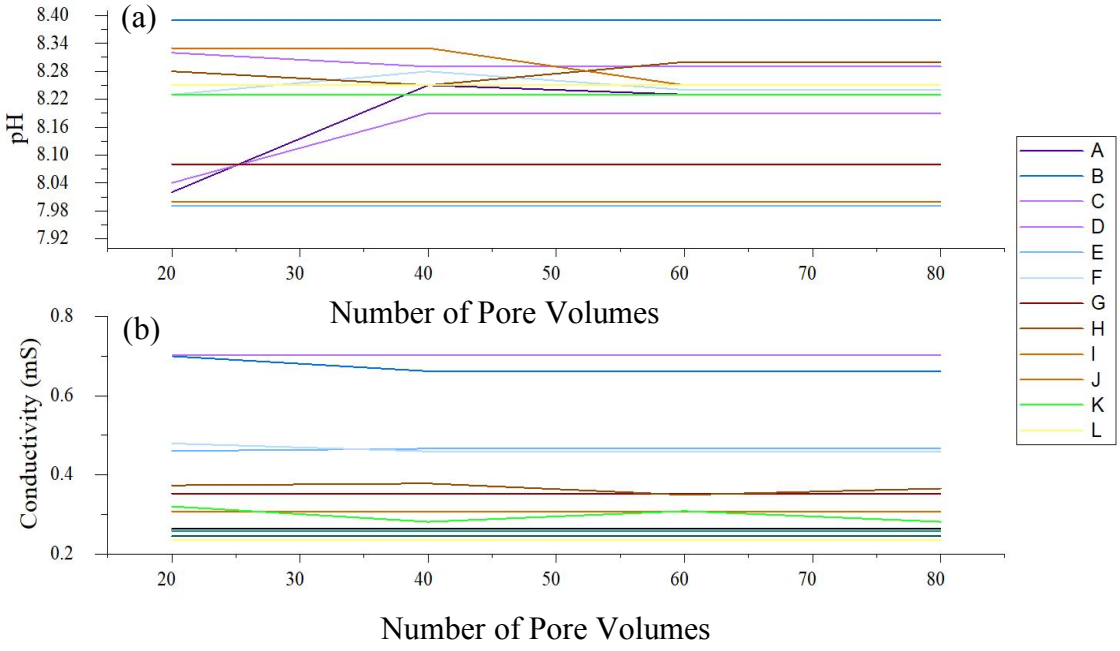

Figure 9. (a) pH and (b) Conductivity -vs- Number of Pore Volumes flushed in static columns. Columns were tested with either quartz sand or Boulder Creek sediment. The eluent was Russian River leachate or Russian River water. Different colored lines in the graph indicate different sediment/eluent combinations.

The legend for the various sediments and eluents is:

Line ID	Sediment	Eluent	Concentration of ash in leachate (mg/L), used as eluent
A	Boulder Creek	Leachate	50
B	Quartz sand	Leachate	50
C	Boulder Creek	Leachate	25
D	Quartz sand	Leachate	25
E	Boulder Creek	Leachate	12.5
F	Quartz sand	Leachate	12.5
G	Boulder Creek	Leachate	6.25
H	Quartz sand	Leachate	6.25
I	Boulder Creek	Russian River Water	NA
J	Quartz sand	Russian River Water	NA
K	Boulder Creek	Russian River Water	NA
L	Quartz sand	Russian River Water	NA

3.3 Results for the impact of ash leachate concentration and sediment type on the transport of pathogens and microspheres

Experiments were carried out to understand how the environment influences the retention of pathogens.

Impact of ash leachate concentration on transport of microspheres

Wildfire ash leachates of *P. ponderosa* (filtered so that no physical pore clogging would occur due to ash particles) at different concentrations were studied to determine how concentration of ash leachate affected the transport of microspheres through the porous sediment (Figure 10).

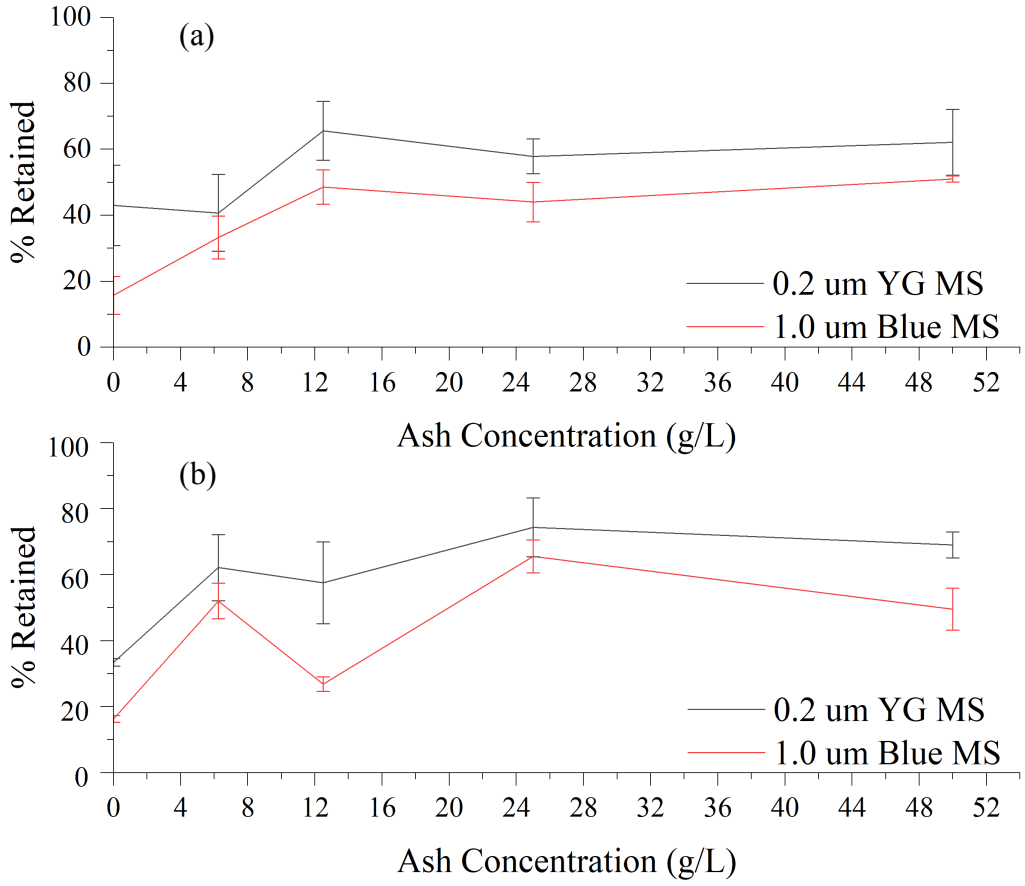

Figure 10. Percent Retained -vs- Ash Concentration (g/L). Microspheres were eluted using *P. ponderosa* ash leachate in different sediments, (a) Boulder Creek sediment and (b) quartz sand. Columns were run using two different types of microspheres as surrogate pathogens (0.2µm YG MS and 1.0µm Blue MS).

Higher ash leachate concentrations increase retention (decreases transport) of microspheres (supported by a Spearman's Correlation Coefficient of 0.63, which indicates a moderate positive correlation between the variables with a $p < 0.05$). In natural watersheds, ash leachate concentrations are typically variable and change as a function of exposure time to ash, quantity of ash, and the dilution from streamwater.

As such, the effects of ash leachate concentration on pathogen retention can be important to water quality.

Role of sediment type on transport of pathogens in the presence or absence of ash leachate

Different geologic sediments were studied to determine whether sediment would affect the transport of pathogens: low solubility calcium aluminosilicate Boulder Creek sediment and aluminum and iron-rich Russian River sediment. Sediment columns were inoculated with *E. coli*, *C. parvum* oocysts, or 1408MS microspheres (which have a similar size, density, and surface charge to the oocysts) and were eluted with either *P. ponderosa* ash leachate (50g/L) or filtered river water (Figure 11). Boulder Creek sediments have higher retention of pathogens than Russian River sediments (paired, two-tailed t-test, $p < 0.05$) which is notable since Russian River sediments have a higher cation exchange capacity, which might be expected to increase attachment and thus increase retention of pathogens. Also, Boulder Creek sediments are less weathered than Russian River sediments (leading to blockier grain structures).

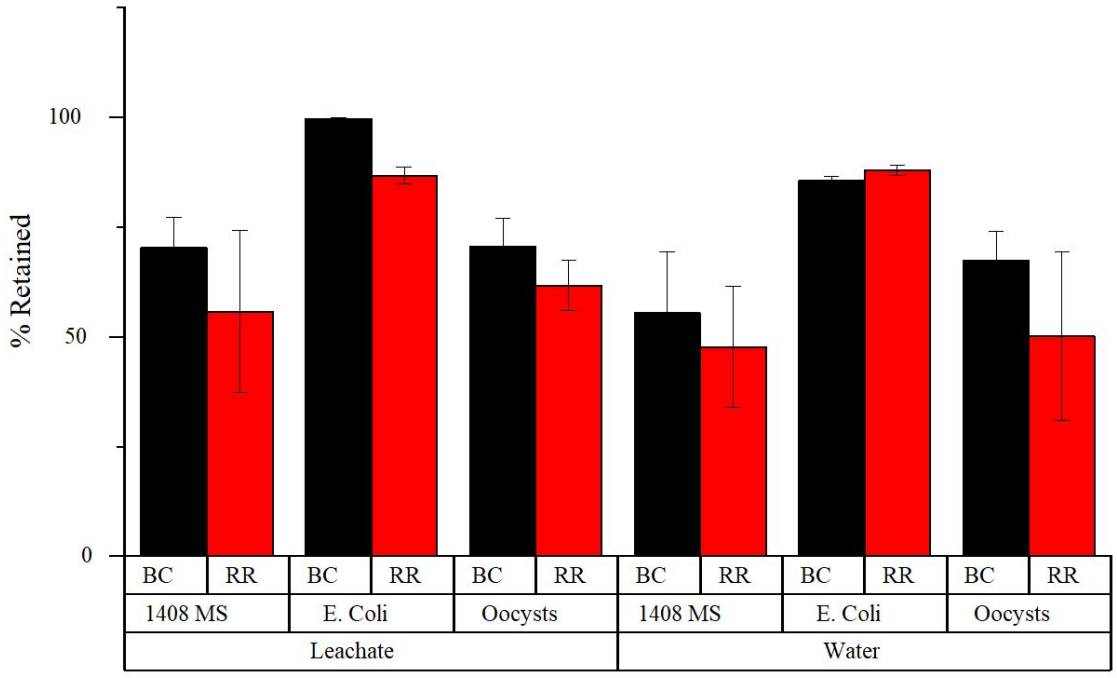

Figure 11. Percent Retained -vs- Sediment Type. Pathogen retention was compared for flow through Boulder Creek (BC) or Russian River (RR) sediments, eluted with ash leachate or river water. Percent retained was calculated after 20 pore volumes.

Role of ash leachate on transport of pathogens in the presence of sediments

Ash leachate eluent leads to higher retention of pathogens than filtered river water (Figure 11) (paired, two-tailed t-test, $p < 0.05$). This result is similar to that observed in Figure 10 for varying concentrations of ash leachate.

3.4 Difference between pathogens in their retention in the presence of ash leachate.

Three different microbial-sized colloids were compared: *E. coli, C. parvum,* and 1408MS microspheres. 1408MS microspheres are ~1μm in diameter, near neutrally buoyant, and have been used in previous studies to mimic *C. parvum* oocysts (Metge et al., 2001; Harvey et al., 2004; Harvey et al., 2011). All three pathogens have a

negative surface charge at the experimental pH of 8.5. The pathogen transport experiments were carried out in either *P. ponderosa* ash leachate or filtered river water and in columns packed with either Boulder Creek or Russian River sediments (Figure 12).

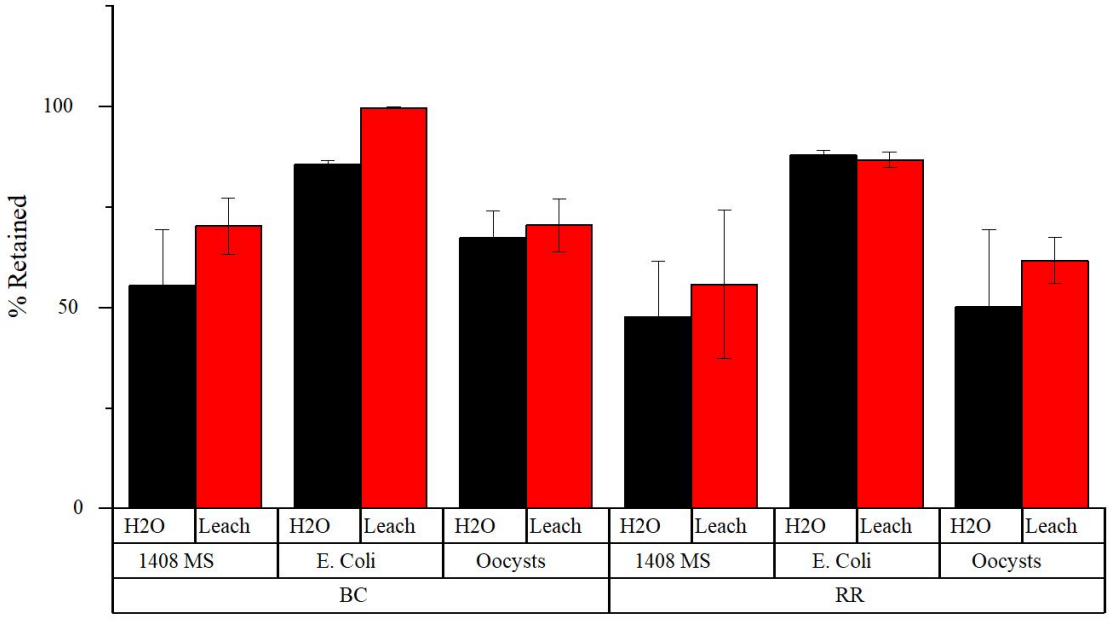

Figure 12. Percent Retained -vs- Pathogen type. Pathogen retention was compared for the *P. ponderosa* ash leachate or filtered river water eluent through Boulder Creek (BC) or Russian River (RR) sediments, using the same data as in Figure 11.

E. coli were retained in the sediments more than *C. parvum* oocysts or 1408MS microspheres (ANOVA test, $p < 0.05$). Retention of the *C. parvum* oocysts and the 1408MS microspheres were statistically indistinguishable from one another. It is possible that the *E. coli* were more efficiently retained because of their different surface chemistry or their rod-like shape. In all cases, columns eluted with ash leachate retained pathogens equally well or better than columns eluted with filtered river water.

3.5 Contributions of reversible and irreversible retention of pathogens

One major concern with the removal of pathogens in post-wildfire regions is the changing water chemistry after large storms. After large storms, the chemical composition of riverine waters is impacted by ash leachate (from surface run-off over burned areas). However, after the pulse of ash leachate from a storm has passed, the chemical composition of riverine waters tends to return to its pre-storm conditions. This experiment was designed to quantify the retention of pathogens under ash leachate conditions that were later released when the system returns to pre-storm conditions (Figure 13).

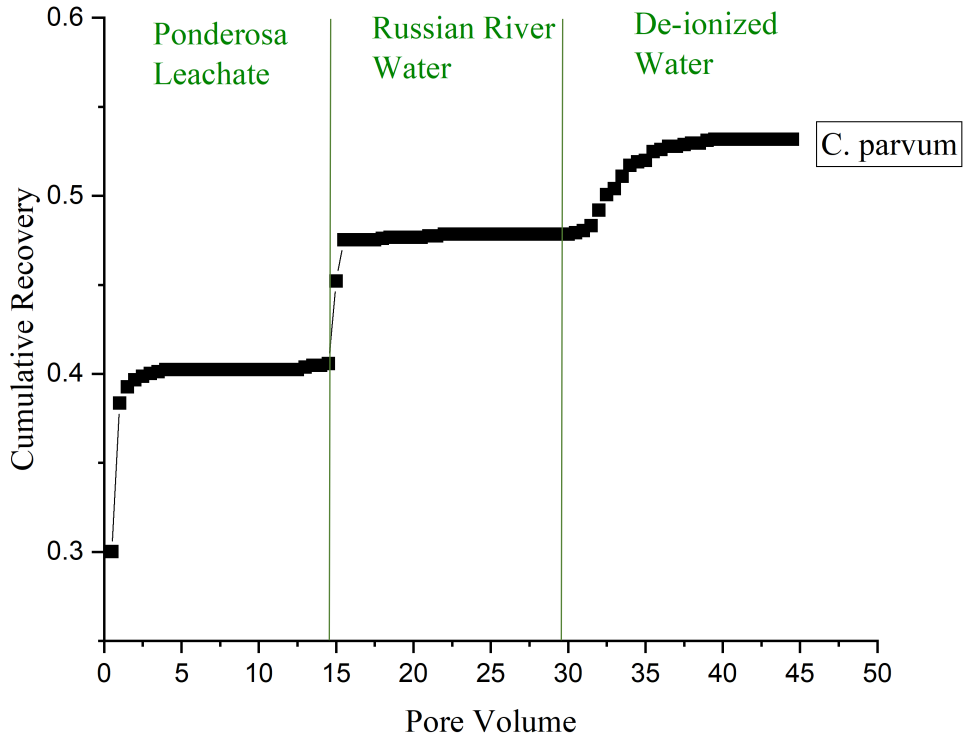

Figure 13. Cumulative recovery of *C. parvum* oocysts. Columns were packed with Russian River sediments and inoculated with *C. parvum*. Since this experiment is strongly dependent on surface chemistries, actual *C. parvum* oocysts were used instead of surrogate microspheres. Recovery data was collected after each pore volume.

A total of 40.6% of oocysts were recovered after flowing ash leachate through the columns for 15 pore volumes (to emulate a high surface runoff storm event). After the eluent was switched to filtered Russian River water (to emulate a return to steady state flow conditions), an additional 7.2% of oocysts were dis-attached and released from the columns. After switching to de-ionized water, an additional 5.4% of oocysts were released from the sediment column. These results suggest that as rivers return to steady state conditions after high runoff storm events, pathogens may be released from the soil system.

3.6 Role of pathogen size and electrostatic charge in their retention

The presence of exceptionally high DOC (>79mg/L) and P (>1mg/L as phosphate) has the potential to neutralize many of the positive charges on grain surface metal oxides as both DOC and P are strongly surface active and readily sorb to Al and Fe oxides. Thus, the presence of DOC-rich ash leachate might diminish the retention of pathogens and microspheres by blocking sorption sites. However, the previous results (Figure 12) indicate that pathogen and microsphere removal in the presence of DOC-rich ash leachate is as good as or better than removal in filtered river water.

This observation that ash leachate does not seem to diminish pathogen retention raises question about whether pathogens are removed through adsorption to the sediment particles or through physical trapping in the sediment pore spaces. If the retention mechanism is physical trapping, then high DOC and P concentrations would not be expected to influence pathogen retention. To test the relative roles of physical

trapping and pathogen adsorption in this system, two different microspheres were tested (Table 5).

Table 5. Size and Zeta Potential of Microspheres M673 and M674

Microsphere	**Zeta Potential (mV)**	**Diameter (µm)**
M673	-25.43	2.02
M674	-37.81	1.80

Thus, if physical trapping is playing a larger role in this system, the slightly larger diameter microsphere (M673) might be removed more effectively. However, if adsorption is the dominant role in pathogen and microsphere retention, the more negatively charged microsphere (M674) would be removed more effectively. The microspheres were inoculated into Russian River water and run with both *P. ponderosa* leachate and filtered Russian river water.

The M673 (slightly larger, but less charged) microsphere is more effectively retained and may suggest that physical trapping is more influential than adsorption onto the sediments (Figure 14). However, M674 (slightly smaller, and more negatively charged) is significantly impacted (t-test, $p=0.05$) by the type of eluent. Thus, it is possible that the influence of ash leachate on retention of microspheres and pathogens is more complex and is exacerbated with more strongly negative pathogens and microspheres.

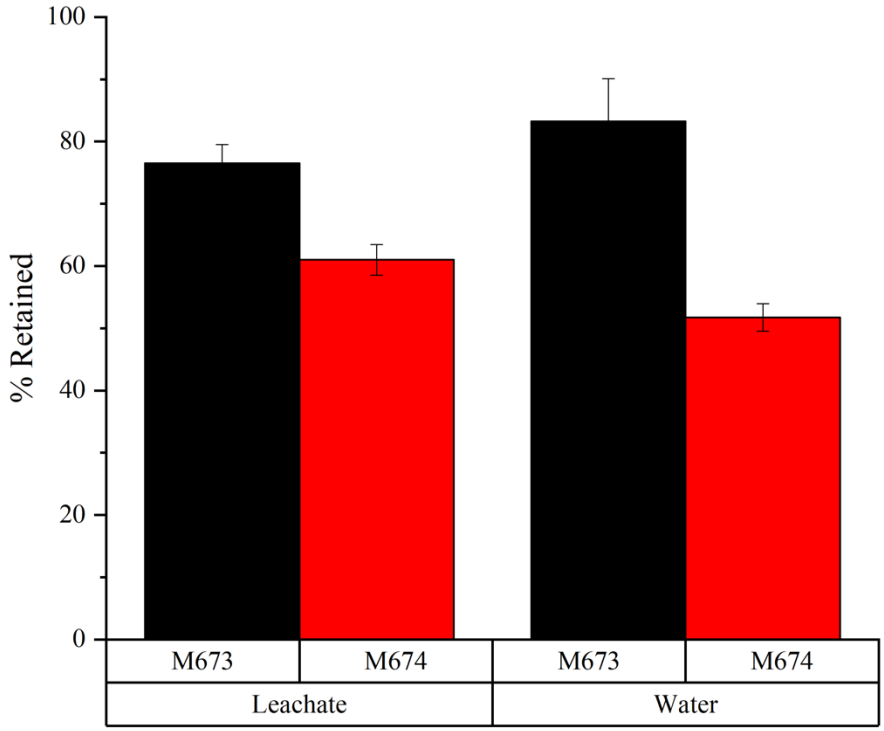

Figure 14. Percent Retained -vs- Microsphere type in the presence of both ash leachate and water. M673 is more effectively removed in both ash leachate and water, which may indicate that physical trapping is the dominant the retention mechanism.

Key findings

Wildfires are cyclic recurrences in many arid ecosystems and due to the chemical and physical properties of the resulting ash, the water quality in these ecosystems is impacted. This study focused on determining how the chemical contributions of ash leachate impact pathogen retention in the watershed, an important ecosystem service of watersheds in improving drinking water quality. The specific attributes of the ash leachate/sediment/pathogen system that were studied for their impact on pathogen removal include:

- Concentration (of ash leachate)
- Sediment type (low solubility calcium aluminosilicate Boulder Creek, aluminum and iron-rich Russian River)
- Pathogen type (*E. coli*, *C. parvum* oocysts, microsphere surrogates)
- Changing eluents (leachate to filtered river water transition)
- Electrostatic charge (size -vs- electrostatic charge)

The key finding is that transport of these pathogens (including *E.coli*, deactivated oocysts from *C. parvum*, and microspheres that behave as oocyst mimics) is not increased by ash leachate. In many cases, the presence of ash leachate retards the transport of pathogens and microspheres and retains the pathogens in the sediments.

Influence of ash leachate concentration on pathogen removal

Increasing ash concentration in the leachate (pre-filtration) tends to increase retention of microspheres, further indicating that ash leachate may retard pathogen transport. It is possible that high concentrations of polyvalent cations (Al, Fe, Ca, Mg, Mn, Sr) in the ash leachate may be neutralizing many of the negative charges in the system and increasing pathogen retention. Increasing ash concentration beyond 25g/L of ash used to prepare leachates seems to have little to no effect.

Influence of sediment type on pathogen removal

The weak trend that showed Boulder Creek sediments (with lower cation exchange capacity) retained higher percentages of pathogens than Russian River sediments

indicates that cation exchange capacity is not the dominating sediment characteristic influencing pathogen and microsphere retention. It is possible that the Boulder Creek sediments, which are geologically more immature, consist of more angular media (blockier grain structures) which tend to strain a wider variety of particle sizes (Barton and Buchberger, 2007). Increased constrictions may then lead to increased removal of pathogens through physical straining, rather than through chemical adsorptive removal.

Influence of pathogen type on pathogen removal

Although the pathogens were all removed at least as well in the presence of ash leachate as in river water, *E. coli* was removed more effectively than *C. parvum* and surrogate microspheres. *E. coli* is a rod-shaped microbe which may lend itself to better physical removal, or may enable *E. coli* to bind in the matrix in spaces that might repel spherically shaped pathogens. Ash leachate tended to increase the retention of pathogens, although the trend was not statistically significant. The retention of 1408MS microspheres and the *C. parvum* oocysts were statistically indistinguishable from one another, indicating that some microspheres may be good indicators of *C. parvum* oocysts transport through the subsurface.

Influence of changing eluents on pathogen removal

Natural systems often experience pulses of ash leachate into riverine systems (for example, after a large storm, high concentrations of ash leachates may contribute to the surface runoff; however, after the storm has subsided, riverine waters return to pre-

storm equilibrium chemical compositions). To emulate these conditions in lab, columns were run first with leachate and then transitioned to river water. These columns experienced a second pulse of transport after the transition to river water. These pathogens may have been stably retained in the presence of leachate, but became mobile under river water conditions.

Influence of electrostatics on pathogen removal

DOC is negatively charged due to its acidity, and it is expected to enhance the passage of negatively charged pathogens through the sediment matrix due to the repulsion of negative charges from the negatively charged sediments. High concentrations of phosphate in the ash leachate exacerbate this problem as phosphate is extremely surface active and readily sorbs to Al and Fe oxides, thus neutralizing many of the negatively charged sediment surfaces.

Since ash leachate is high in DOC and phosphate, pathogen transport is expected to be increased in the presence of ash leachate, and pathogens would be less retained on the column sediments. However, in these studies the ash leachate eluent columns (with high DOC and phosphate content) removed pathogens at least as well as filtered river water and thus the ash leachate did not increase pathogen transport. And, as the concentration of ash leachate increased, the trend continued and the retention of microspheres increased.

This result may suggest that the neutralization of surface charges by DOC and phosphate is not the dominant retention mechanism in these ash leachate treated columns. The mechanism of retention may instead involve: (1) the high polyvalent cation concentrations of the wildfire ash leachate, especially calcium, iron, and aluminum (Pereira et al., 2012) that are counteracting the DOC and increasing adsorption of pathogens through porous media; or, (2) the retention is dominated by physical trapping that overshadows the chemical sorption. While the second hypothesis does account for no increased transport in the presence of leachate, it does not adequately account for the increased retention in the presence of leachate. Other studies have shown that the DOC-mediated effects of pathogen transport can be altered by surfactants and other organic compounds (Mohanram et al., 2011); their results suggest that the impact of DOC on pathogen transport depends on both the surface characteristics of the colloid and the nature of the DOC and the soil minerals.

The effect of DOC on the transport of pathogens through sediments is known to be sensitive to other components in the eluent (Metge et al., 2011). High polyvalent cation concentrations can neutralize negative charges on the sediments and reduce transport of microspheres.

Limitations of Interpretation

Although this study provides trends in the transport of pathogens in the presence of ash leachate, the broader application of these results may be limited by some of the experimental constraints:

1. Microspheres were used as a pathogen surrogate for many of these pathogen transport studies. Microspheres are produced with a variety of surface chemistries and surface charges that are used to mimic *C. parvum* oocysts. However, the complex, heterogeneus surface chemistry of a viable oocyst is difficult to accurately mimic on a homogeneously charged latex sphere, and under conditions where heterogeneity influences retention, then microspheres may not be a good mimic for oocysts.
2. The ash leachate is sourced from actual burn areas, so the content of that ash leachate is highly variable. Although ash leachate was extracted from mapped forests (with known locations of specific tree types), it is still susceptible to variations in the source vegetation. Thus, the conclusions from this study may not be generalizable to all ash from a specified vegetation.

Implications

As wildfires increase in both frequency and intensity (Flanigan et al., 2000), there is increasing concern about pathogen transport in post-wildfire regions. Previous studies have examined the physical changes in pathogen filtration and transport after wildfires based on the physical properties of ash and high temperatures on the porosity of the soil surface (Stoof et al., 2016); however, this study focused on the chemical impacts of the ash leachates in locations downstream of wildfires.

The relevance of these findings was highlighted at the Wohler Water Treatment Plant in Sonoma, California. The Sonoma County Water Agency (SCWA) operates the Wohler Water Treatment Plant along the Russian River in northern California to

provide water for the region of 600,000 people. The SCWA relies solely on bank filtration and chlorine disinfection (no coagulation, flocculation, or sedimentation processes are employed). Water is filtered through 25m of sediment below the Russian River to remove pathogens before it reaches the treatment plant for final chlorination and distribution. If the riverbank sediments fail to remove all oocysts, this process is susceptible to *C. parvum* infection since the final chlorination does not disinfect *C. parvum* oocysts. When wildfires create ash in the soil, water treatment facility managers such as those at the Wohler Water Treatment Plant must decide whether additional water treatment is necessary due to effects of the ash leachates.

In October, 2017, the Wohler site on the Russian River was impacted by three major upstream fires (Tubbs Fire, Pocket Fire, and Nuns Fire). The Wohler site, which relies almost entirely on bank filtration to remove pathogens and pollutants prior to distribution, is under intense scrutiny from both the EPA and the public to examine the durability of these drinking water treatment systems after wildfires. *C. parvum* is a critical organism of interest for the EPA due to the low infectious doses and the high environmental recalcitrance.

It is unclear how well these results, which were all derived using sediment columns, can be scaled to a larger, natural system. However, these results provide optimism regarding the durability of sediment filtration in the presence of wildfire ash leachate.

APPENDIX A

This study relied on flow cytometry to identify and quantify microspheres and pathogens in column experiments based on the particle size and wavelength. The flow cytometry studies are used before and after column experiments to determine the number of particles innoculated onto the columns and the number of particles that are recovered from the columns. All samples are sonicated prior to running in the flow cytometer to ensure that all particles are separate and not clumped together.

The microspheres used in these experiments are fluorescent at a specific wavelength, and the pathogens were stained with SBYR green (excitation at 497 nm; emission at 520 nm) or DAPI stain (excitation at 358 nm; emission at 461 nm) to induce fluorescence. The flow cytometer measures fluorescence intensity at each of these wavelengths as the particles pass the sensor on the instrument (Figure 15).

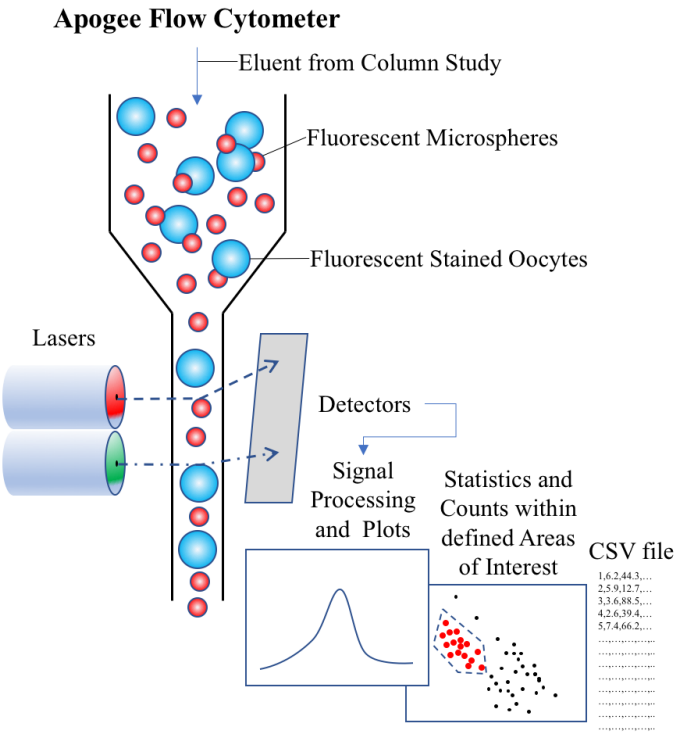

Figure 15. Schematic of the Apogee Flow Cytometer column with fluorescently stained particles that pass lasers that excite the fluorophores and allow detectors to measure the fluorescent emission.

A calibration bead mix was used to test and confirm that the flow cytometer was a suitable method for examining particles in the size range of interest (0.5-2µm).

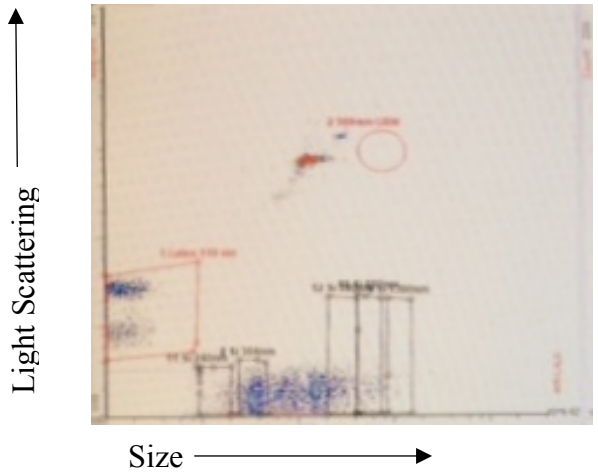

Figure 16. Calibrating the flow cytometer. The cytogram (Light Scattering vs Size) indicates the regions of interest for the calibration beads and confirms that appropriate counts of beads are read in each light scattering and size bin.

To distinguish the pathogens and microspheres used in these experiments, the flow cytometer was used to count particles based on size and fluorescence. Regions of interest (Figure 16) were developed for each microsphere and pathogen. A region of interest is a narrow set of light scattering and size as shown in the cytogram, outlined as a red polygon in Figure 16. That region of interest then represents a single pathogen or microsphere and is counted to quantify the number of particles.

Data for an example column is shown below. “Column 11” was incubated with three different types of microspheres (types 1426, 673, and 674). Each microsphere has a different region of interest based on its size and light scattering. Microspheres 1426 and 673 fluoresce in the green wavelengths and are identified in cytograms in Figure 17 (a) and (c) and are shown in the outlined polygons. Microsphere 674 fluoresces in the red wavelengths and is identified in the cytogram in Figure 17 (b).

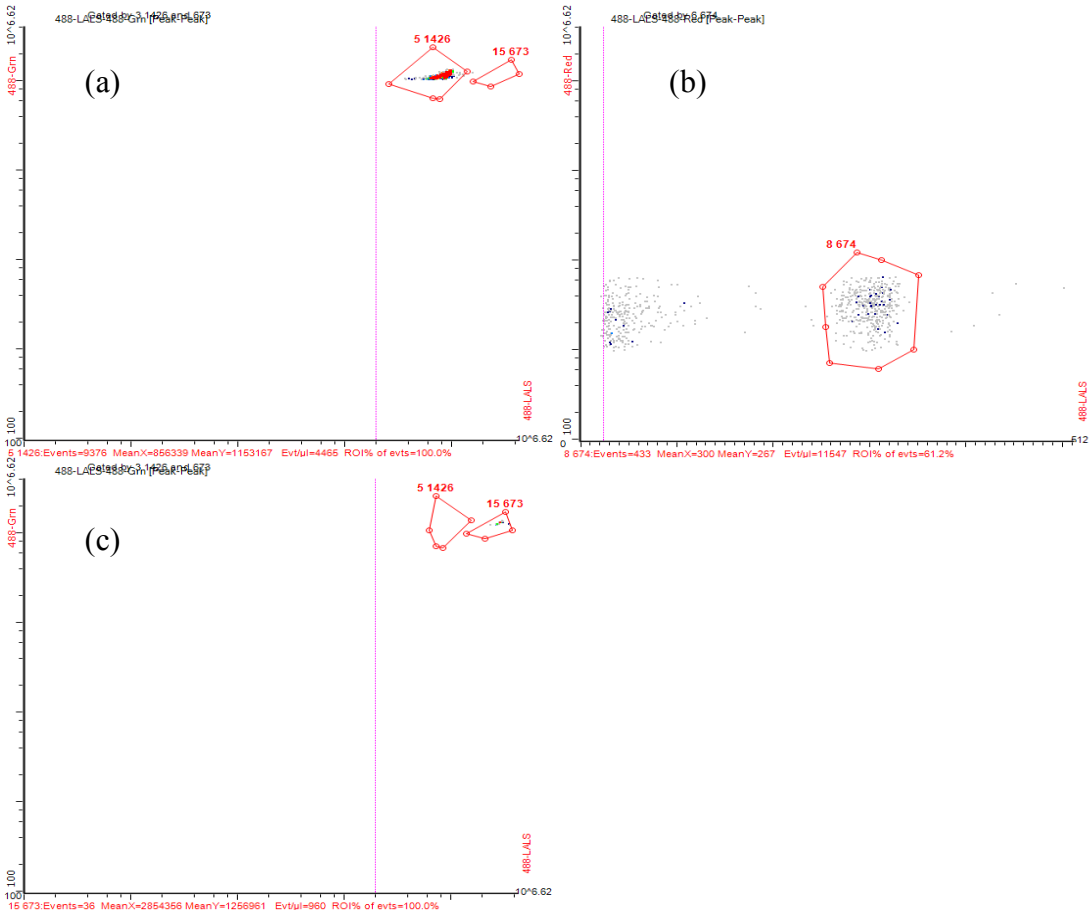

Figure 17. Light scattering vs Size. Regions of interest after running beads through Column 11. Graphs (a), (b), and (c) all represent the same sample with three different microspheres present. Each graph represents a different wavelength for analysis.

The number of events (which indicates the number of beads) is measured in the region of interest for each microsphere to quantify the final number of beads in the sample.

REFERENCES

Abudalo, R. A., Bogatsu, Y. G., Ryan, J. N., Harvey, R. W., Metge, D. W., & Elimelech, M. (2005). Effect of Ferric Oxyhydroxide Grain Coatings on the Transport of Bacteriophage PRD1 and Cryptosporidium parvum Oocysts in Saturated Porous Media. *Environmental Science & Technology*, *39*(17), 6412–6419. https://doi.org/10.1021/es050159h

Balfour, V. N., & Woods, S. W. (2013). The hydrological properties and the effects of hydration on vegetative ash from the Northern Rockies, USA. *Catena*, *111*, 9–24. https://doi.org/10.1016/j.catena.2013.06.014

Balfour, V., Hatley, D., & Woods, S. (2011). Fire induced changes in aggregate stability: the interacting effects of soil heating and ash leachate. *American Geophysical Union*.

Barton, J. M. H., & Buchberger, S. G. (2007) Effect of media grain shape on particle straining during filtration. *Journal of Environmental Engineering, 133*(2), 211-219.

Bition, G., & Harvey, R. W. (1992). Transport of pathogens through soils and aquifers. In Mitchell, R., ed., *Environmental Microbiology*: New York, Wiley, pp. 103–124.

Bodí, M. B., Mataix-Solera, J., Doerr, S. H., & Cerdà, A. (2011). The wettability of ash from burned vegetation and its relationship to Mediterranean plant species type, burn severity and total organic carbon content. *Geoderma*, *160*(3–4), 599–607. https://doi.org/10.1016/j.geoderma.2010.11.009

Bowman, D. M. J. S., Balch, J. K., Artaxo, P., Bond, W. J., Carlson, J. M., Cochrane, M. A., … Pyne, S. J. (2009). Fire in the Earth System. *Science*, *324*(5926), 481–484. https://doi.org/10.1126/science.1163886

Brush, C. F., Ghiorse, W. C., Anguish, L. J., Parlange, J.-Y., & Grimes, H. G. (1999). Transport of Cryptosporidium parvum Oocysts through Saturated Columns. *Journal of Environment Quality*, *28*(3), 809. https://doi.org/10.2134/jeq1999.00472425002800030011x

Butkus, M. A., Bays, J. T., & Labare, M. P. (2003). Influence of Surface Characteristics on the Stability of Cryptosporidium parvum Oocysts. *Applied and Environmental Microbiology*, *69*(7), 3819–3825. https://doi.org/10.1128/AEM.69.7.3819-3825.2003

Byrd, T. L., & Walz, J. Y. (2007). Investigation of the Interaction Force between Cryptosporidium parvum Oocysts and Solid Surfaces. *Langmuir*, *23*(14), 7475–7483. https://doi.org/10.1021/la0701576

Cerdà, A., & Doerr, S. H. (2008) The effect of ash and needle cover on surface runoff and erosion in the immediate post-fire period. *Catena, 74*(3), 256-263.

Costa, M. R., Calvão, A. R., & Aranha, J. (2014). Linking wildfire effects on soil and water chemistry of the Marão River watershed, Portugal, and biomass changes detected from Landsat imagery. *Applied Geochemistry*, *44*, 93–102. https://doi.org/10.1016/j.apgeochem.2013.09.009

Dai, X., & Hozalski, R. M. (2002). Effect of NOM and biofilm on the removal of Cryptosporidium parvum oocysts in rapid filters. *Water Research*, *36*(14), 3523–3532. https://doi.org/10.1016/S0043-1354(02)00045-3

Dai, X., & Hozalski, R. M. (2003). Evaluation of Microspheres as Surrogates for Cryptosporidium parvum Oocysts in Filtration Experiments. *Environmental Science & Technology*, *37*(5), 1037–1042. https://doi.org/10.1021/es025521w

Fayer, R. (2004). Cryptosporidium: a water-borne zoonotic parasite. *Veterinary Parasitology*, *126*(1–2), 37–56. https://doi.org/10.1016/j.vetpar.2004.09.004

Flannigan, M. D., Stocks, B. J., & Wotton, B. M. (2000). Climate change and forest fires. *Science of the total environment*, *262*(3), 221-229.

Gao, X., Metge, D. W., Ray, C., Harvey, R. W., & Chorover, J. (2009). Surface complexation of carboxylate adheres Cryptosporidium parvum oocysts to the hematite-water interface. *Environmental Science and Technology*, *43*(19), 7423–7429. https://doi.org/10.1021/es901346z

Hageman, P. L., Plumlee, G. S., Martin, D. A., Hoefen, T. M., Meeker, G. P., Adams, M., & Anthony, M. W. (2008). *Leachate Geochemical Results for Ash and Burned Soil Samples from the October 2007 Southern California Wildfires* (Version 1.0). *Open-File Report*. Retrieved from http://pubs.er.usgs.gov/publication/ofr20081139

Harvey, R. W., & Harms, H. (2003). Tracers in Groundwater: Use of Microorganisms and Microspheres. *Encyclopedia of Environmental Microbiology*, 3194–3201. https://doi.org/10.1002/0471263397.env157

Harvey, R. W., George, L. H., Smith, R. L., & LeBlanc, D. R. (1989). Transport of Microspheres and Indigenous Bacteria through a Sandy Aquifer: Results of Natural and Forced-Gradient Tracer Experiments. *Environmental Science and Technology*, *23*(1), 51–56. https://doi.org/10.1021/es00178a005

Harvey, R. W., Mayberry, N., Kinner, N. E., Metge, D. W., & Novarino, F. (2002). Effect of Growth Conditions and Staining Procedure upon the Subsurface Transport and Attachment Behaviors of a Groundwater Protist. *Applied and Environmental Microbiology*, *68*(4), 1872–1881. https://doi.org/10.1128/AEM.68.4.1872-1881.2002

Harvey, R. W., Metge, D. W., Barber, L. B., & Aiken, G. R. (2010). Effects of altered groundwater chemistry upon the pH-dependency and magnitude of bacterial attachment during transport within an organically contaminated sandy aquifer. *Water Research*, *44*(4), 1062–1071. https://doi.org/10.1016/j.watres.2009.09.008

Harvey, R. W., Metge, D. W., LeBlanc, D. R., Underwood, J., Aiken, G. R., Butler, K., … Jasperse, J. (2015). Importance of the Colmation Layer in the Transport and Removal of Cyanobacteria, Viruses, and Dissolved Organic Carbon during Natural Lake-Bank Filtration. *Journal of Environment Quality*, *44*(5), 1413. https://doi.org/10.2134/jeq2015.03.0151

Harvey, R., Metge, D., Sheets, R., & Jasperse, J. (2011). Fluorescent Microspheres as Surrogates in Evaluating the Efficacy of Riverbank Filtration for Removing Cryptosporidium parvum Oocysts and Other Pathogens (pp. 81–96). https://doi.org/10.1007/978-94-007-0026-0_6

Hendricks, D. W.(2006). *Water Treatment Unit Processes: Physical and Chemical.* CRC Press: Florida. (References for Smoluchowski, 1916)

Janjaroen, D., Liu, Y., Kuhlenschmidt, M. S., Kuhlenschmidt, T. B., & Nguyen, T. H. (2010). Role of Divalent Cations on Deposition of Cryptosporidium parvum Oocysts on Natural Organic Matter Surfaces. *Environmental Science & Technology*, *44*(12), 4519–4524. https://doi.org/10.1021/es9038566

Jin, Y., Yates, M. V., Thompson, S. S., & Jury, W. A. (1997). Sorption of viruses during flow through saturated sand columns. *Environ. Sci. Technol. 31*, 548–555.

Kile, D. E., & Eberl, D. D. (2003). Quantitative mineralogy and particle-size distribution of bed sediments in the Boulder CreekWatershed, Colorado, Chapter 7. *Comprehensive Water Quality of the Boulder Creek Watershed, Colorado, during High-Flow and Low-Flow Conditions, 2000*, 173–184.

King, P., Robinson, G., Elwin, K., Tyler, K. M., Hunter, P. R., & Chalmers, R. M. (2017). Prevalence and epidemiology of human Cryptosporidium parvum IIc infections in England and Wales. *The Lancet*, *389*, S56. https://doi.org/10.1016/S0140-6736(17)30452-X

Kuznar, Z. A., & Elimelech, M. (2006). Cryptosporidium Oocyst Surface Macromolecules Significantly Hinder Oocyst Attachment. *Environmental Science & Technology*, *40*(6), 1837–1842. https://doi.org/10.1021/es051859p

Langhans, C., Smith, H. G., Chong, D. M. O., Nyman, P., Lane, P. N. J., & Sheridan, G. J. (2016). A model for assessing water quality risk in catchments prone to wildfire. *Journal of Hydrology*, *534*, 407–426. https://doi.org/10.1016/j.jhydrol.2015.12.048

Mac Kenzie, W. R., Hoxie, N. J., Proctor, M. E., Gradus, M. S., Blair, K. A., Peterson, D. E., … Davis, J. P. (1994). A Massive Outbreak in Milwaukee of Cryptosporidium Infection Transmitted through the Public Water Supply. *New England Journal of Medicine*, *331*(3), 161–167. https://doi.org/10.1056/NEJM199407213310304

Maia, P., Pausas, J. G., Arcenegui, V., Guerrero, C., Pérez-Bejarano, A., Mataix-Solera, J., … Keizer, J. J. (2012). Wildfire effects on the soil seed bank of a maritime pine stand — The importance of fire severity. *Geoderma*, *191*, 80–88. https://doi.org/10.1016/j.geoderma.2012.02.001

Masliyah, J. H., & Bhattacharjee, S. (2006). Electrokinetic Phenomena. In *Electrokinetic and Colloid Transport Phenomena* (pp. 221–227). Hoboken, NJ, USA: John Wiley & Sons, Inc. https://doi.org/10.1002/0471799742.ch7

Metge, D. W., & Harvey, R. W. (2001). Potential for Cryptosporidium oocyst migration within rierbank filtration systems. In *American Water Works Association Water Quality Technology Conference*.

Metge, D. W., Harvey, R. W., Aiken, G. R., Anders, R., Lincoln, G., Jasperse, J., & Hill, M. C. (2011). Effects of Sediment-Associated Extractable Metals, Degree of Sediment Grain Sorting, and Dissolved Organic Carbon upon Cryptosporidium parvum Removal and Transport within Riverbank Filtration Sediments, Sonoma County, California. *Environmental Science & Technology*, *45*(13), 5587–5595. https://doi.org/10.1021/es200544p

Metge, D. W., Harvey, R. W., Anders, R., Rosenberry, D. O., Seymour, D., & Jasperse, J. (2007). Use of Carboxylated Microspheres to Assess Transport Potential of Cryptosporidium parvum Oocysts at the Russian River Water Supply Facility, Sonoma County, California. *Geomicrobiology Journal*, *24*(3–4), 231–245. https://doi.org/10.1080/01490450701456867

Mohanram, A., Ray, C., Harvey, R. W., Metge, D. W., Ryan, J. N., Chorover, J., & Eberl, D. D. (2010). Comparison of transport and attachment behaviors of Cryptosporidium parvum oocysts and oocyst-sized microspheres being advected through three minerologically different granular porous media. *Water Research*, *44*(18), 5334–5344. https://doi.org/10.1016/j.watres.2010.06.015

Mohanram, A., Ray, C., Metge, D. W., Barber, L. B., Ryan, J. N., & Harvey, R. W. (2012). Effect of Dissolved Organic Carbon on the Transport and Attachment Behaviors of Cryptosporidium parvum oocysts and Carboxylate-Modified Microspheres Advected through Temperate Humic and Tropical Volcanic Agricultural soil. *Environmental Science & Technology*, *46*(4), 2088–2094. https://doi.org/10.1021/es2003342

Murphy, S. F. (2006). State of the watershed: water quality of Boulder Creek Colorado *US Geological Survey Circular 1284* (http://pubs.usgs.gov/circ/circ1284/)

Murphy, S., Writer, J., Mccleskey, R., Martin, D. (2015). The role of precipitation type, intensity, and spatial distribution in source water quality after wildfire. *Environmental Research Letters*, *10*(8), 84007.

Pereira, P., Úbeda, X., & Martin, D. A. (2012). Fire severity effects on ash chemical composition and water-extractable elements. *Geoderma*, *191*, 105–114. https://doi.org/10.1016/j.geoderma.2012.02.005

Ray, C., Grischek, T., Schubert, J., Wang, J. Z., & Speth, T. F. (2002). A perspective of riverbank filtration. *American Water Works Association Journal*, *94*(4), 149.

Ryan, J. N., & Elimelech, M. (1996). Colloid mobilization and transport in groundwater. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *107*, 1–56. https://doi.org/10.1016/0927-7757(95)03384-X

Ryan, U., Paparini, A., Monis, P., & Hijjawi, N. (2016). It's official – Cryptosporidium is a gregarine: What are the implications for the water industry? *Water Research*, *105*, 305–313. https://doi.org/10.1016/j.watres.2016.09.013

Santín, C., Doerr, S. H., Otero, X. L., & Chafer, C. J. (2015). Quantity , composition and water contamination potential of ash produced under different wild fi re severities. *Environmental Research*, *142*, 297–308. https://doi.org/10.1016/j.envres.2015.06.041

Schijven, J. F., & Massanizadeh, S. M. (2000). Virus removal by soil passage at field scale and ground-water protection of sandy aquifers. *Water Science and Technology*, *46*(3), 123–129.

Scholl, M. A., & Harvey, R. W. (1992). Laboratory investigations on the role of sedminet surface and groundwater chemistry in transport of bacteria through a contaminated sandy aquifer. *Environmental Science & Technology*, *26*(7), 1410–1417.

Sharma, U., Sharma, J. C., & Devi, M. (2017). Effect of Forest fire on soil nitrogen mineralization and microbial biomass: A review, *Journal of Pharmacognosy and Phytochemistry, 6*(3), 682-685.

Smith, H. G., Sheridan, G. J., Lane, P. N. J., Nyman, P., & Haydon, S. (2011). Wildfire effects on water quality in forest catchments: A review with implications for water supply. *Journal of Hydrology*, *396*(1–2), 170–192. https://doi.org/10.1016/j.jhydrol.2010.10.043

Smith, H. V., Cacciò, S. M., Tait, A., McLauchlin, J., & Thompson, R. C. A. (2006). Tools for investigating the environmental transmission of Cryptosporidium and Giardia infections in humans. *Trends in Parasitology*, *22*(4), 160–167. https://doi.org/10.1016/j.pt.2006.02.009

Stoof, C. R., Gevaert, A. I., Baver, C., Hassanpour, B., Morales, V. L., Zhang, W., … Steenhuis, T. S. (2016). Can pore-clogging by ash explain post-fire runoff? *International Journal of Wildland Fire*, *25*(3), 294–305. https://doi.org/10.1071/WF15037

Stoof, C. R., Gevaert, A. I., Baver, C., Hassanpour, B., Morales, V. L., Zhang, W., … Steenhuis, T. S. (2016). Can pore-clogging by ash explain post-fire runoff? *International Journal of Wildland Fire*, *25*(3), 294–305. https://doi.org/10.1071/WF15037

Swanton, S. W. (1995). Modelling colloid transport in groundwater; the prediction of colloid stability and retention behaviour. *Advances in Colloid and Interface Science*, *54*, 129–208. https://doi.org/10.1016/0001-8686(95)90146-6

Tufenkji, N. (2007). Modeling microbial transport in porous media: Traditional approaches and recent developments. *Advances in Water Resources*, *30*(6–7), 1455–1469. https://doi.org/10.1016/j.advwatres.2006.05.014

Tufenkji, N., Miller, G. F., Ryan, J. N., Harvey, R. W., & Elimelech, M. (2004). Transport of Cryptosporidium Oocysts in Porous Media: Role of Straining and Physicochemical Filtration. *Environmental Science & Technology*, *38*(22), 5932–5938. https://doi.org/10.1021/es049789u

Tufenkji, N., Miller, G. F., Ryan, J. N., Harvey, R. W., & Elimelech, M. (2004). Transport of Cryptosporidium oocysts in porous media: Role of straining and physicochemical filtration. *Environmental Science and Technology*, *38*(22), 5932–5938. https://doi.org/10.1021/es049789u

Tufenkji, N., Ryan, J. N., & Elimelech, M. (2002). Peer Reviewed: The Promise of Bank Filtration. *Environmental Science & Technology*, *36*(21), 422A–428A. https://doi.org/10.1021/es022441j

Turner, N. B., Ryan, J. N., & Saiers, J. E. (2006). Effect of desorption kinetics on colloid-facilitated transport of contaminants: Cesium, strontium, and illite colloids. *Water Resources Research*, *42*, W12S09. https://doi.org/10.1029/2006WR004972

Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., & Mopper, K. (2003). Evaluation of Specific Ultraviolet Absorbance as an Indicator of the Chemical Composition and Reactivity of Dissolved Organic Carbon. *Environmental Science & Technology*, *37*(20), 4702–4708. https://doi.org/10.1021/es030360x

Woods, S. W., & Balfour V. N. (2008) The effect of ash on runoff and erosion after a severe forest wildfire, Montana, USA. *International Journal of Wildland Fire* 17(5), 535-5.

Woods, S. W., & Balfour, V. N. (2010). The effects of soil texture and ash thickness on the post-fire hydrological response from ash-covered soils. *Journal of Hydrology*, *393*(3–4), 274–286. https://doi.org/10.1016/j.jhydrol.2010.08.025

Writer, J. H. & Murphy, S. (2012) *Wildfire effects on source-water quality--Lessons from Fourmile Canyon fire, Colorado, and implications for drinking-water treatment*. No. 2012-3095. US Geological Survey.

Yates, M. V, & Ouyang, Y. (1992). VIRTUS, a model of virus transport in unsaturated soils. *Applied and Environmental Microbiology*, *58*(5), 1609–1616. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1622