TRANSPORT OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS THROUGH DISPARATE AGRICULTURAL SOILS

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DEDICATIONS

"…who is your advisor?"

"Ron Harvey"

"You are in safe hands..."

-- *Charles O' Melia, I st International conference on Microbial Transport and Survival in the Subsurfaces, Summer 2009, Niagra-on-the-Lake, Ontario, Canada.*

This is for you Ron for being such a great mentor.

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ABSTRACT

The presence of *Cryptosporidium parvum* oocysts in source waters is a cause of major public health concern for drinking-water treatment plants. The oocysts occurring in the feces of livestock can be disseminated horizontally via runoff during storm events and contaminate surface waters. Soils are often the initial barrier to subsurface transport of *Cryptosporidium parvum* oocysts which receive oocyst-laden manure from grazing livestock. However, rainfall events can cause rapid vertical movement of oocysts through preferential flow paths in the soils leading to ground water contamination. A systematic study was undertaken to assess the efficacy of three disparate agricultural soils (two tropical, variable-charge soils of volcanic origin from Hawaii and a humic, quartz-rich temperate soil from Illinois) to remove *Cryptosporidium parvum* oocysts and oocyst-sized microspheres in batch and flow-through columns.

To test the effect of soil mineralogy on oocysts transport, saturated flow-through column experiments were conducted by packing the three agricultural soils and injecting oocysts and microspheres. The results showed that oocysts were transported through preferential flow paths in high-clay, high-iron tropical soil from Hawaii and humic-rich quartz dominated temperate soil from Illinois. Transport through volcanic-ash soil collected from the island of Hawaii was highly reversible because of high soil organic matter content.

The effects of dissolved organic carbon (DOC) on oocysts and microspheres transport through these soils were assessed in a subsequent phase of this study. DOC in

form of natural organic matter enhanced the removal of oocyst-sized colloids (microspheres and oocysts), whereas surfactants lowered the removal efficiency of oocysts and microspheres. The transport potentials of oocysts and microspheres were affected differentially by the physicochemical properties of the soils. Whereas oocysts transport was more strongly affected by soil mineralogy, microspheres transport was much more sensitive to the nature of DOC.

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CHAPTER 1

INTRODUCTION

PROBLEM DESCRIPTION AND MOTIVATION

Groundwater contamination by pathogenic bacteria, viruses and protists is a major source of concern for water-treatment plants because of the growing dependence on aquifers as source of potable clean water. The latest World Health Organization (WHO) report shows that diarrheal disease are the primary cause of deaths of 2.2 million children across the world every year, 90% of which are caused by waterborne pathogens (WHO 2010). *Cryptosporidium parvum* oocysts cause a gastro-intestinal infection called Cryptosporidiosis in humans and in a variety of domestic and wild mammals. The feces of mammalian hosts, particularly calves, are a major source of oocysts which is highly resistant to chemical treatment methods such as chlorination (Rose, 1997). The EPA requires 3 log removal (99.9 %) of oocysts from source waters. Aging water treatment plant utilities that use traditional filtration techniques such as deep-bed granular filtration, slow sand filtration are facing a great challenge for removal of this pathogen from source water.

In the past couple of decades, a growing body of research has facilitated our understanding of the transport characteristics of *Cryptosporidium* oocysts, allowing us to better predict their movement in subsurface environments. Bench-scale studies on the movement of oocysts in porous media such as quartz fragments (Tufenkji et al., 2004) and glass beads (Brush et al., 1999) have allowed a better understanding of the basic mechanisms underlying oocysts deposition and how they relate to colloid filtration theory (CFT). However, findings deriving from studies that employ model porous media are not always helpful in understand oocysts transport in heterogeneous natural granular media.

The few studies that have been conducted with natural soils have shown that oocysts can be readily transported through the preferential flow paths (Darnault et al., 2004). Most of these findings have been done with soils from temperate regions that are classified as permanently charged soils (PCS). Such soils are dominated by clay minerals such as smectites and vermiculites that impart an overall negative charge, irrespective of solution chemistry (Sposito 2008).

On the other hand, soils from tropical regions are highly weathered and consequently have metal oxide coating on their surfaces. These metal oxide edges on the soils impart a pH-dependent charge. Thus, soils in tropical regions are called variably charge soils (VCS). Although oocysts contamination of waters in regions characterized by VCS, e.g., Southeastern United States and parts of Brazil, have been reported (Xiao et al., 2007, Souza et al., 2008), there has been no systematic study to assess the filtration efficiency of oocysts in tropical soils. Agricultural and farming regions in tropical regions carry high dissolved organic matter loading because of the abundance of vegetation and due to the application of manure as fertilizer during irrigation practices. It has been reported in literature that dissolved organic matter (DOM) can reduce the filtration capacity of the soils and promote oocysts transport (Dai et al., 2003). However, there is a dearth of information on the effects of DOM coating on agricultural soils in Hawaii dominated by VCS. In addition, because the surface chemistry of VCS is rich in metal oxides and show strong dependence on solution chemistry and pH, oocysts attachment is likely to be very different from that of PCS. Nevertheless, there has been no attention studying the concerted effects of soil mineralogy of VCS as well as DOM on *Cryptosporidium* transport. Hence, there is an immediate need to study the efficacy of

removal of *C. parvum* oocysts in soils from tropical regions such as the Hawaiian Islands. Understanding *Cryptosporidium* transport in VCS is necessary because they are agricultural soil used for irrigation of various crops in the Islands and they also receive the manure laden oocysts from livestock and farm animals.

We performed transport studies in laboratory experiments by collecting two different tropical soils in Hawaii. A PCS from the mainland US was used as reference.

USDA GRANT AND WATERSHED PROGRAM

Since 2005, the United States Department of Agriculture (USDA) funded research on pathogen and water supply at improving and maintaining healthy watershed habitat and water supplies. The grant seeks to reduce pathogenic bacteria, viruses and protozoa in water derived from agriculture. These studies were founded by the Cooperative State Research, Education and Extension Service (CSREES) as National Research Initiative (NRI) grants and came under National Institute of Food and Agriculture (NIFA), and the Food Research Initiative (FRI). Its purpose is to support research, extension and education grants that address key problems of national, regional and multi-state importance in sustaining all components of agriculture. The findings reported in this PhD thesis were made possible by a USDA grant to University of Hawaii, the United States Geological Survey, and the University of Arizona in Tucson.

OBJECTIVES AND THESIS ORGANIZATION

The basic aim of this work is to study the filtration capacity of agricultural soils for removing *Cryptosporidium parvum* oocysts and oocyst-sized microspheres. Fluorescent carboxylated microspheres of different sizes were used as surrogates for oocysts. Flow-through column and batch studies were undertaken by co-injecting *C. parvum* oocysts and microspheres through the soil columns packed with three agricultural soils with disparate mineralogies from Hawaii and Illinois. The specific objectives were:

1. Determine the efficacy of two variably charged agricultural soils from Oahu and Hawaii and one permanently charged soil from Illinois to remove *C. parvum* oocysts and microspheres.

2. Determine the influence of dissolved organic carbon, including natural organic matter and anionic surfactant, on the transport behaviors of oocysts and microspheres in these soils.

3. Determine the interaction of soil fines and natural organic matter on the surface characteristics of oocysts and microspheres

The dissertation has been written in the order in which the experiments were conducted. Chapter one states the problems and motivation for the current work. Chapter two gives the background information and a literature review on the various studies conducted with regard to *C. parvum* transport. The following chapters (3 and 4) were written as journal manuscripts for consideration as contributions in the environmental science journals *Water Research* and *Environmental Science & Technology*. Chapter

three gives the preliminary results of transport study of oocysts and microspheres transport in the three agricultural soils. In chapter four, oocysts and microspheres transport through the three agricultural soils coated with dissolved organic carbon has been evaluated. In addition, the effect of soil fines and organic matter on the adhesion properties of oocysts and microsphere surfaces was also evaluated. Chapter five summarizes the findings in this study and suggests some areas of future research in understanding the fate and transport of *C. parvum* oocysts in natural soil and granular porous media.

CONTRIBUTIONS

The work summarized in this thesis contributes to the general understanding of the fate and transport of *Cryptosporidium parvum* oocysts and oocysts surrogates through agricultural soils from two different climate zones – tropical and temperate regions of the US. Stakeholders that can make use of the information gained from this study are water utilities, local health departments, agricultural extension agents, and water treatment plant operators.

The work described herein has been published or have been submitted for publication. Here is the list of following recently published or anticipated papers:

1. Mohanram, A., Ray, C., Harvey, R.W., Metge, D.W., Ryan, J.N., Chorover, J. (2010) Comparison of transport and attachment behaviors of *Cryptosporidium parvum* oocysts and oocyst-sized microspheres being advected through three mineralogically different granular porous media. *Water Research*, 44, 5334-5344.
2. Mohanram, A., Ray, C., Harvey, R.W., Metge, D.W. (2010) Effect of anionic surfactant, SDS, on the transport and attachment behaviors of *Cryptosporidium*

parvum oocysts and oocyst-sized microspheres being advected through granular porous media. *Proceedings at India 2010: 3rd International Perspective on Current & Future State of Water Resources & the Environment* at Indian Institute of Technology (IIT), Madras, India.

3. Mohanram, A., Ray, C., Metge, D.W., Barber L.B.B., Ryan, J.N., Harvey, R.W. (2011) Effect of dissolved organic carbon on the transport and attachment behaviors of *Cryptosporidium parvum* oocysts and carboxylate-modified microspheres advected through agricultural soils. *Environmental Science and Technology*, *submitted.*

4. Harvey, R.W., Metge, D.W., Mohanram, A., Gao X., Chorover, J. (2011) Differential effects of dissolved organic carbon upon re-entrainment of groundwater bacteria and bacteria-sized microspheres during transport through a contaminated, sandy aquifer. *Environmental Science and Technology*, 45, 3252-3259.

5. Mohanram, A., Ray, C., Harvey, R.W., Metge, D.W. (2011) Effects of clay type upon surface properties and aggregation of *Cryptosporidium parvum* oocysts. *Environmental Science and Technology*, *in preparation.*

6. Bradford, S., Harvey, R.W., Mohanram, A., Morales,V., Zhang,W., Packman, A., Welty, C. (2011) Transport and fate of pathogens in agricultural settings. *Critical Reviews in Environmental Science and Technology*, *in preparation*.

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Souza, V.S., Vidal-Torrado, P., Garcia-Gonzalez, M.T., Otero, X.L., Macias, F. (2008) Soil mineralogy of mangrove forests from the state of Sao Paulo, Southeastern Brazil. Soil Science Society of America Journal, 72(3), 848-857.

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W.H.O. (2010) World Health Organization : UN-water global annual assessment of sanitation and drinking-water (GLAAS) 2010: targeting resources for better results.

Xiao, L., Zhou, L., Santin, M., Yang, W. and Fayer, R. (2007) Distribution of *Cryptosporidium parvum* subtypes in calves in eastern United States. Parasitology Research, 100(4), 701-706.

CHAPTER II

BACKGROUND

CRYPTOSPORIDIUM PARVUM OOCYSTS AND PUBLIC HEALTH

Cryptosporidium oocysts are protozoan pathogens that infect the small intestine of humans and certain mammals. The oocysts range in size from 2.5-7μm (diameter). Ingestion of as little as five oocysts, has shown to cause Cryptosporidiosis for people with severely compromised immune systems (e.g., cancer patients undergoing chemotherapy and AIDS patients) (Casemore et al., 1997). In a recent report reviewing 325 documented outbreaks caused by parasitic protozoa, *Cryptosporidium parvum* was responsible for 51% of the outbreaks (Rosa et al., 2010). Cryptosporidiosis affects the small intestine and respiratory tract and is often accompanied by diarrhea, fever, stomach pain and muscle cramps. Of the 14 species of *Cryptosporidium* that have been reported, *C. hominis* and *C. parvum* have been associated with human infection (Fayer et al., 1997) The feces of farm animals have been identified as a major source of oocysts in the environment. Outside the host, the parasite is incapable of replication, but has been reported to remain viable for up to six months in moist environments (Kato et al., 2004). The thick walled oocysts contain four sporozoites that are the cause of the infection. The fecal-oral route has been identified as the primary route of transmission. Whereas infection may cause healthy adults to become sick, the disease can more severely affect people with weak or developing immune systems such as the elderly or children younger than five. It has proven to be fatal in people with compromised immune systems.

The first case of documented outbreak relating to *Cryptosporidium* occurred in 1984 in Braun Station, TX resulted in 200 people being infected and was caused by sewage contamination of the public water supply (D'Antonio et al., 1985). In 1989,

another outbreak occurred in Carrollton, GA even though the water system met all regulatory-agency quality standards. These events led to widespread monitoring of surface and ground water sources for the presence of *Cryptosporidium* oocysts. One of the first comprehensive surveys of surface water sources was done across seventeen states in the US. This study found oocyst concentrations between 0.001 to 44 oocysts L^{-1} in pristine watersheds (Rose et al., 1991). Hancock et al. (Hancock et al., 1998) sampled groundwater from 23 states in the US and found that 11% of 463 samples contained *C. parvum* oocysts.

The largest recorded outbreak of Cryptosporidiosis occurred in 1993 in Milwaukee, WI where over 400,000 people were infected and 100 people died following consumption of contaminated water (Mackenzie 1994). This outbreak caused heightened interest in this pathogen and it became well-recognized that *Cryptosporidium* oocysts were highly resistant to chlorine disinfection. Consequently, the USEPA promulgated the Interim Enhanced Surface Water Treatment Rule prescribing two-log removal of *Cryptosporidium* oocysts from source waters. In 2006, the Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR) required stricter controls on source water under the direct influence of ground water. (USEPA 2006). More recently, there has been an increasing shift towards using advanced treatment processes such as UV-photolysis or ozonation in addition to conventional filtration processes (coagulation, flocculation and sedimentation and slow sand filtration) to destroy oocysts in source water.

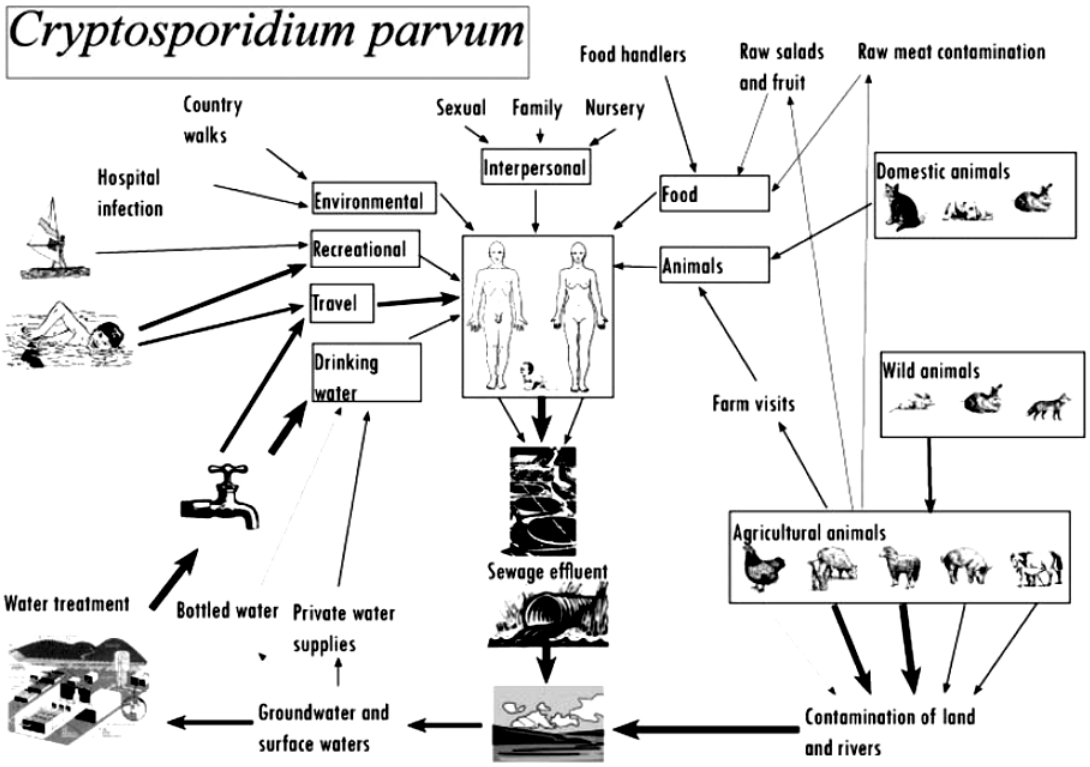

Figure 2.2. The different sources and pathways by which the oocysts can infect humans (Fayer et al., 1997).

BIOLOGY OF *CRYPTOSPORIDIUM*

Cryptosporidium is a genus of protist infects the gastrointestinal tract of vertebrates (Fayer et al., 1997). There have been fifteen genotypes identified. Among these, *Cryptosporidium parvum* is the most studied species by public health microbiologists, molecular biologists, and water-quality engineers because of its widespread occurrence across the world and affecting a broad range of organisms. The encysted environmental form of *Cryptosporidium* is, as the oocyst, which contains four sporozoites. The organism completes its complex life cycle within a host and because it is an obligate parasite, it replicates only within its host. Figure 2.2 depicts the life cycle of *Cryptosporidium*. After ingestion of the oocysts by the host, excystation of the

sporozoites is triggered by the presence of a warm bile solution within the mammalian intestinal tract. During excystation the oocysts rupture along a zipper-like structure and release the sporozoites. The sporozoites cause infection of the epithelial cells of the intestinal mucosa. They then differentiate into trophozoites which undergo asexual multiplication to form Type 1 meronts, a stage in the life cycle of protist where multiple asexual fission occurs resulting in the production of merozoite. The merozoites can infect another host cell. The type II meronts undergo sexual reproduction by differentiating into microgamont (male) or macroganont (female) stage. The meronts finally transform into oocysts that are excreted in the feces. The oocysts sporulate within the gastrointestinal tract, which are then excreted in the feces, whereas those in the respiratory tract are excreted in nasal secretions. Some reports suggest that the thin walled oocysts further infect the host whereas thick walled oocysts leave the body to infect other hosts. Auto infection occurs when the asexual and sexual phase of reproduction are repeated in the same host.

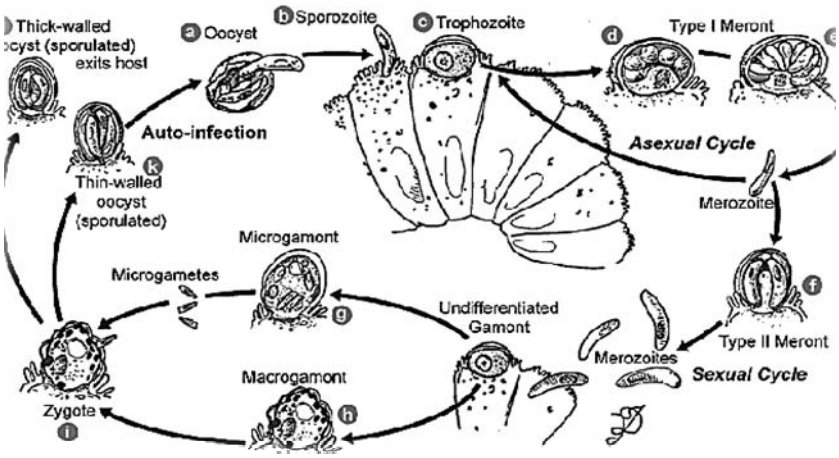

Figure 2.2. Life cycle of *Cryptosporidium* oocysts [http://www.dpd.cdc.gov/].

SURFACE CHARACTERISTICS OF *CRYPTOSPORIDIUM PARVUM*

The extremely tough shell of oocysts shows great resistance to stresses during water treatment and are resistant to chemical disinfection, ultra sonication and even grinding using a mortar and pestle (Rose 1997, Harris et al., 1999).

Their surface consists of weakly charged carbohydrates mixed with thin layer of charged proteins that protonate and deprotonate with changing pH and hence give it a pH-dependent charge (Kuznar et al., 2006). The point of zero charge (PZC) of oocysts has been found to be between pH 2-3 (Lytle et al., 2002). Considine et al., (Considine et al., 2002) studied the interaction between oocysts on silica surfaces using AFM and found that oocysts surfaces contain proteins moieties having ionizable functional groups in the form of charged brush-like structures. Kuznar and Elimelech (Kuznar et al., 2006) studied the attachment of oocysts in presence and absence of surface macromolecules (consisting of proteins, lipids and carbohydrates) and concluded that the brush-like appendages hindered attachment to quartz surfaces. More recent studies (Jenkins et al., 2010) have looked at the ultrastructure of oocysts under transmission electron microscope and proposed a model (Figure 2.3) of oocysts cell structure consisting of an outer brush-like glycocalyx layer. The glycocalyx is made of polysaccharides that give it a pH dependent behavior. They found that this outer brush layer can be removed when the oocysts are subject to environmental stresses. However, removal can modify the surface charge of the oocysts and hence its attachment and transport characteristics. The thinner inner layer consists of high molecular weight (approximately 15-60 kDa) long-chain fatty acids, proteins, and a thick layer of polysaccharide. The lipid layer underlying the

glyocalyx, because of its hydrophobic properties, has been shown to have a role in oocysts attachment, viability and infectivity.

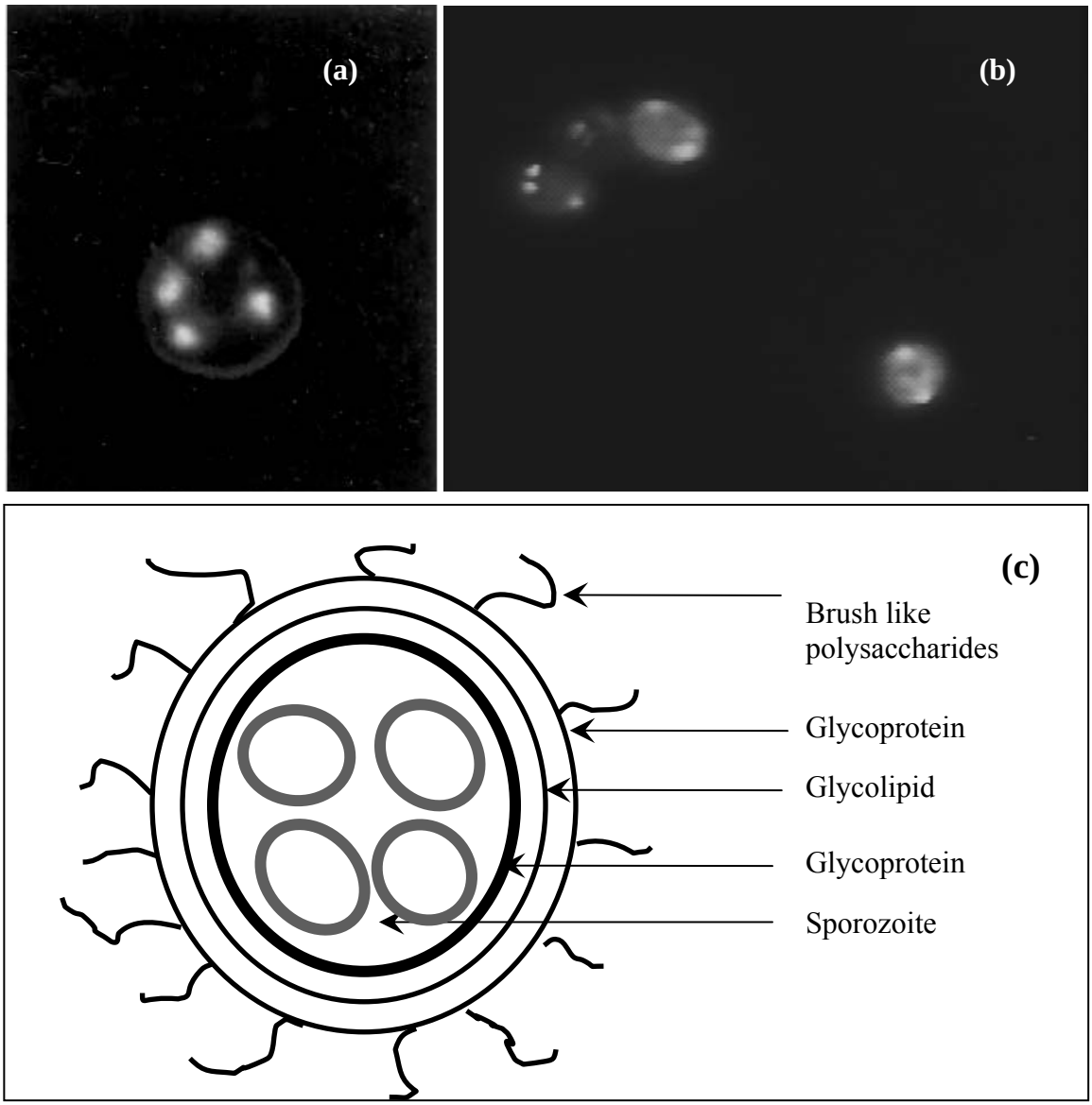

Figure 2.3. (a) DAPI stained *Cryptosporidium* oocysts with sporozoites (Johnson et al., 1996) (b) Aggregated oocysts (c) Model representation of oocysts surface.

ELECTRICAL PROPERTIES OF OOCYSTS

The surface charge of a microorganism that is of colloidal dimensions is an important factor in its fate and transport characteristics in the natural environment. The interaction between oocysts and collector surfaces is determined, in part, by the surface charge on the oocysts. Consequently, surface charge is important in understanding removal of oocysts during water treatment processes as well as in the subsurface. The surface charge is determined using electrophoretic mobility measurement which generally vary as a function of solution chemistry, particularly pH, ionic strength, divalent cations, and DOC). Electrophoretic mobility is converted to zeta potential using the Smoluchowski equation (Masliyah et al., 2006). The point of zero charge of the oocysts is approximately pH 2. The zeta potential of oocysts increases (i.e. becomes more negative) with increasing pH (Lytle et al., 2002). It has been reported that the purification, preservation and inactivation methods can significantly impact the zeta potential of the oocysts and hence its attachment to grain surfaces (Tufenkji et al., 2006). For example, (Kuznar et al., 2006) observed that heat treatment significantly altered the carbohydrate layer of the oocysts and formalin inactivation changes the surfaces of oocysts by cross-linking the proteins. All the methods of inactivations, atleast to some degree result in surface modification of oocysts.

MATHEMATICAL FRAMEWORK FOR DESCRIBING MICROBIAL TRANSPORT IN GRANULAR MEDIA

This section lays the foundation for understanding the mechanism of colloid transport by describing the various theories associated to predict microbial fate and transport.

Because of their size, the transport of *Cryptosporidium parvum* oocysts through soils can be considered within the mathematical framework of colloid filtration theory. Because the oocysts fall in the colloidal size range (Figure 2.4), their behavior resembles that of colloid particles.

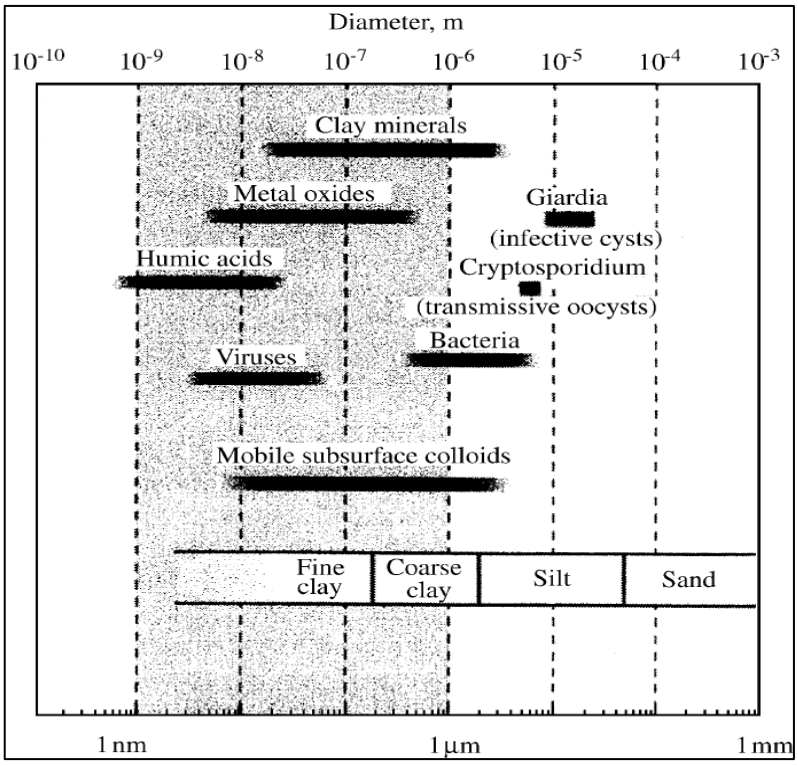

Figure 2.4. Size range of pathogens and soil colloids (Kretzschmar et al., 1999).

The transport of colloids under one dimensional homogenous saturated flow conditions is described using an advective-diffusion transport equation including terms for colloid deposition and release. :

$$\frac{\partial C}{\partial t} = D_p \frac{\partial^2 C}{\partial x^2} - v_p \frac{\partial^2 C}{\partial x} - \frac{\rho_b}{\varepsilon} \frac{\partial S}{\partial t}$$

$$\frac{\rho_b}{\varepsilon} \frac{\partial S}{\partial t} = k_d C - \frac{\rho_b}{\varepsilon} k_r S$$

Here C is the microbe concentration in the aqueous phase at distance x and time t. S is the attached microbe concentration, D is the hydrodynamic dispersion coefficient and v_p is the average interstitial microbe velocity, ρ_b is the bulk density of the porous media, ϵ is the porosity, k_d and k_r are the colloid deposition and release rate coefficients, respectively.

As microorganisms are transported in porous media they are removed by sorptive filtration. The removal can be an irreversible or reversible process. If reversible, then attachment can be modeled as equilibrium sorption or a time-dependent kinetic process. If irreversible, its removal can be described using clean-bed colloid filtration theory. According to this theory, the porous media is likened to that of packed spheres having an infinite capacity for the removal of colloids. There is negligible release of microbes after attachment. The mass transfer process for the removal of colloids is described using an estimated single-collector removal efficiency, $\eta_{0,}$ defined as the ratio of the number of collisions to the total number of colloids approaching the collector in the projected area

of the grains, and an empirically determined collision efficiency α, is the fraction of the colloid-collector collisions that result in attachment of colloid particles. For laboratory studies, the influence of hydrodynamic dispersion is considered negligible compared to the advection term or if the Peclet number ($N_{Pe}= vx/D$) denoted by the ratio of advective to diffusive transport is greater than five. For continuous injection of particles at concentration C_o and time t_o the solution to above equations is described using first-order kinetics:

$$C(x) = C_o \exp\left[\frac{k_a}{v} x\right]$$

$$S(x) = \frac{t_0 \varepsilon k_a C_0}{\rho_b} \exp\left[-\frac{k_a}{v} x\right]$$

Where the attachment rate coefficient (k_a) is related to η_0 and α by:

$$k_a = \frac{3(1-\varepsilon)v}{2d_c} \eta_0 \alpha$$

where d_c is the average grain size of the collector surface, η is the single-collector removal efficiency, α is the collision efficiency and v is the pore water velocity. If the grains are not spherical or uniform shape the grain size is characterized by arithmetic mean diameter, the geometric mean diameter or the diameter which 10% of the grains are smaller (d_{10}).

The single collector contact efficiency has been calculated by (Tufenkji and Elimelech, 2004), as:

$$\eta_0 = 2.4 A_s^{1/3} N_R^{-0.081} N P_e^{-0.715} N_{vdW}^{-0.052} + 0.55 A_s N_R^{1.675} N_A^{0.125} + 0.22 N_R^{-0.24} N_G^{1.11} N_{vdW}^{-0.053}$$

A_s is the Happel correction factor, a porosity-dependent parameter given as:

$$A_s = \frac{2(1-\gamma)^5}{2-3\gamma+3\gamma^5-2\gamma^6}$$

where N_R is the ratio of colloid to grain diameter (aspect ratio), N_{Pe} is the Peclet number, ratio of convective to diffusive transport, N_{vdW} is the van der Waals number, the ratio of van der Waals interaction energy to particle thermal energy, N_A is the attraction number, a representation of the influence of London-van der Waals attractive forces and fluid velocity on colloid deposition by interception, and N_G is the gravity number, ratio of the colloid settling velocity to the approach velocity of the fluid.

Colloidal particles usually have unequal distribution of charge density on their molecules. This causes excesses and deficits of electron clouds on their surfaces. This uneven distribution creates a dipole moment. A similar molecule with opposite dipole can result in attraction of the electron clouds. This weak attractive interaction is called the LVDW force. These forces are used to describe the attractive interaction between the collector surface and colloids separated by a small distance.

The DLVO (named after Derjaguin, Landau, Verwey and Overbeek) theory explains stability of interaction between particles or between particle and grain surface. According to this theory, the net effect of colloidal stability is the interplay of two types of forces: the repulsive electrical double layered (EDL) force and attractive London-van der Waals (LVDW) force. The total interaction expressed as potential energy (V_T) is calculated as sum of LVDW (V_A) and EDL (V_R):

$$V_T = V_A + V_R$$

The origin of EDL is due to the innate nature of any solid surface to carry surface charge. The surface charge may be due to polarization of ions on its surface or by sorption of charged moieties from solutions. The charged collector surface attracts oppositely charged ions from the solution. The accumulation of the oppositely charged ions on the surface results in the formation of an electrical-double layer. The double layer concentration of co-ions and counter-ions decreases exponentially into the solution. The colloid and collector surface interaction results in the overlap of double layer clouds which can be attractive (opposite charge) or repulsive (like charges). The magnitude of this force depends on particle size, the nature of the surfaces, separation distance between the colloid and collector, pH, solution chemistry and ionic strength.

The DLVO profiles of interaction of particles are shown in Figure 2.5. If there is a peak of net repulsive energy, then the point of maximum repulsive energy is called the energy barrier. The height of the barrier indicates the stability of the system. If there are attractive forces (colloid and collector are oppositely charged or at high ionic strength)

between the colloidal and collector surfaces there is no energy barrier to deposition. The colloids are deposited irreversibly in the primary minimum. The height of the energy barrier can be controlled by changes in ionic strength of the solution and zeta potential. As total dissolved solids concentrations increase (high ionic strength, e.g. > 10^{-1} M), the height of the energy barrier is reduced and hence the conditions are deemed 'favorable' for deposition of the colloid on the collector surface (Figure 2.5). If the ionic strength is low (< 10^{-3} M), the double layer is diffuse and there is a significant energy barrier to deposition, and the condition is called as 'unfavorable' for deposition (Tufenkji 2007). Recent research indicates that while deposition in primary minima is irreversible, the deposition on secondary minima is reversible and can be more significant than deposition in primary minima (Tufenkji et al., 2005). The depth of the secondary minima increases with particle size and Hamaker constant. The quantitative effect of LDVW force is described using the Hamaker constant. Hamaker described the interaction between two macroscopic bodies by the pairwise summation of all the intermolecular interactions. Hamaker constant is a function of the number of atoms per unit volume of particles. The constant is related to the properties of the surfaces interacting and the solution.

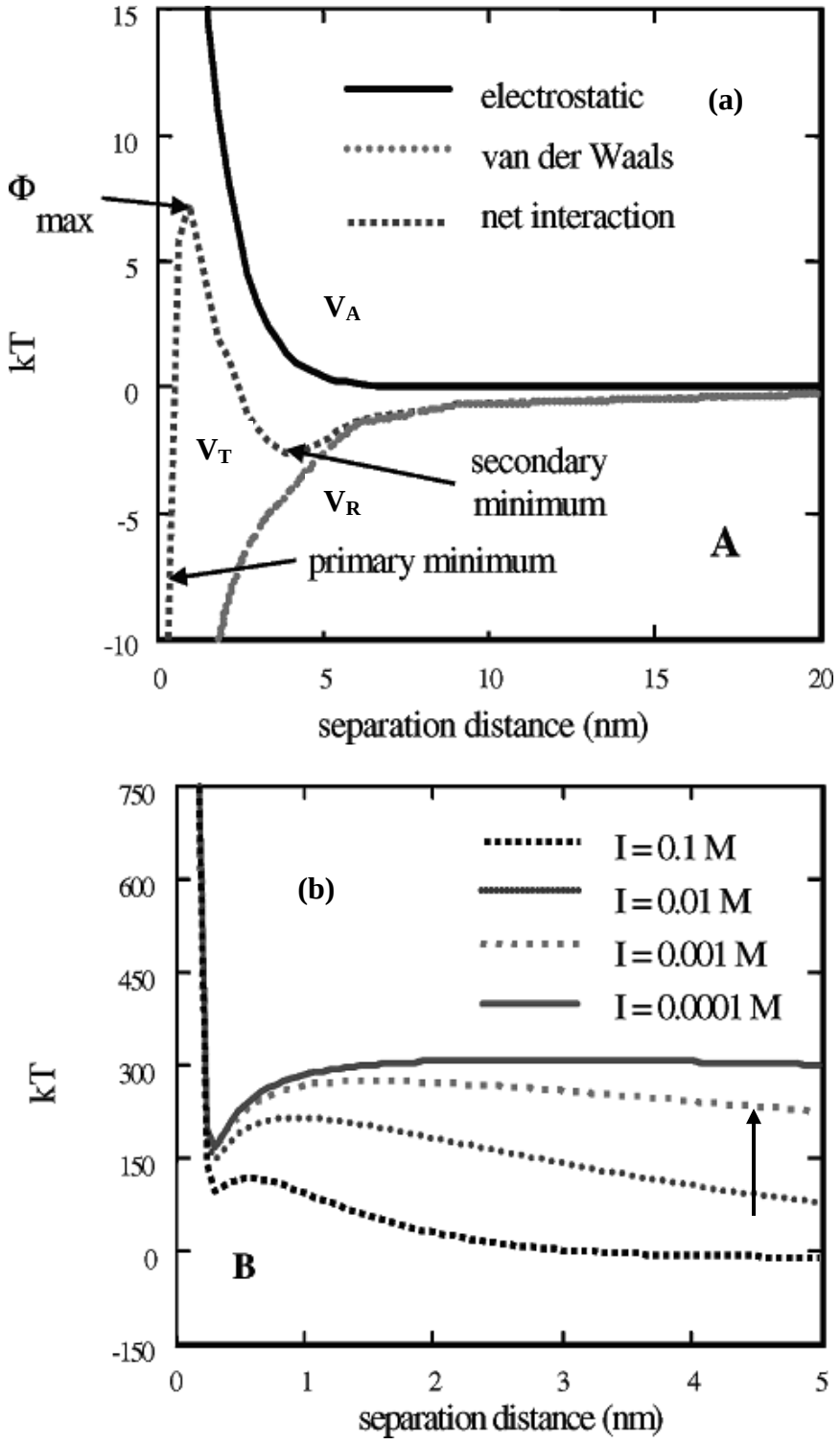

Figure 2.5. (a) Schematic representation of total energy of interaction versus surface to surface separation distance profiles. V_A is van der Waals attractive force. V_R is double layer repulsive force and V_T is the total interactive force. (b) Interaction energy at small separation distance showing the increase in depth of the primary minima with decrease in ionic strength (Hahn et al., 2004).

SOIL PROPERTIES AFFECTING PATHOGEN TRANSPORT

Transport of colloids depends on the physicochemical parameters of the porous media – physical and geochemical heterogeneity, clay minerals, and on the nature of the particle – size, shape, surface charge, hydrophobicity. In addition, solution chemistry and the presence or absence of dissolved organic carbon affects the way particles interact with the collector surface. In this section the various soil properties that affect transport are discussed.

HETEROGENEITY

The transport of pathogens is determined, at least in part, by how they interact with the grain surface. Hence, the grain properties play a key role in the removal of the pathogen in the aquifers. One of the key assumptions in classical colloid filtration theory is that the collector surface within geological media are uniform, smooth and spherical (Yao et al., 1971). However, using filtration theory to predict actual movement of pathogens in aquifers can lead to erroneous results because of physical, chemical and biological heterogeneities (differences in organism type, cell size, cell shape and their surface composition) (Tufenkji 2007).

Physical heterogeneity is the outcome of structured or random distribution of hydraulic conductivity in the porous media. Aquifers, even within relatively short distance can exhibit high variability in the grain size distribution and hence hydraulic conductivity. This leads to the formation of preferential flow paths (fractures and macropores) in the porous media. Geochemical heterogeneity is variability in the

structural composition of grains in the porous media. This is usually portrayed as patches of positively charged metal oxyhydroxide coatings on grain surfaces.

The role of physical heterogeneity on the transport of microspheres was studied by (Harvey et al., 1993, Bradford et al., 2005a, Bradford et al., 2005b). The physically heterogeneous systems used in their studies consisted of various combinations of a cylindrical sand lens embedded in the center of a larger cylinder of matrix sand. They observed higher straining when the microspheres travelled from coarser to finer grains than when the microspheres travelled from finer to coarser grains. When the grains are arranged from coarse to fine, smaller pore throat is available for the movement of microspheres and hence higher straining is observed.

Geochemical heterogeneity occurs due to patchy distribution of surface charges of iron and aluminum oxide on the silica surfaces. Coating with metal oxides changes the surface characteristics of soils and affects the movement of pathogens in subsurfaces (Harvey et al., 2004). An early study performed to assess the effect of aquifer heterogeneity on bacterial transport and bacteria sized microspheres was conducted by (Harvey et al., 1993) in a sandy unconfined glaciofluvial aquifer located in Cape Cod, MA. Bacteria breakthrough was found to be retarded due to deposition in layers or lenses of fine-grain sediments and/or due to sorption on the metal oxide coated grains within the aquifer sediments. Consequently, the extent of deposition varied with depth of the aquifer.

Recent studies have shown a positive correlation between the charge density on iron oxide coated sand and their filtration capacity for oocysts and viruses (Abudalo et al., 2005). Thus the presence of iron oxide coatings provides sorption sites for favorable deposition of *C. parvum* oocysts, viruses such as PRD1, and bacteria (Pieper et al., 1997, Abudalo et al., 2005, Foppen et al., 2008). Soils from a highly weathered landscape such as "variably charged" soils in tropical regions have high clay content and contain Fe and Al oxide composition that may serve as barriers against pathogen movement (Chu et al., 2003, Zhang et al., 2010).

Geological media such as soil consists of particles varying from colloidal dimension to large pebbles, gravel, boulders and stones. However, it is the soil fines (<2-μm) that holds 70-80% of the surface charge of the soil (Mattson 1931). In addition, the surface area per unit mass increases logarithmically as particle diameter decreases (Hattori et al., 1976). Consequently, the soil fines, which consists primarily of clay minerals and hydrous oxides of Al and Fe are very important in controlling the transport properties of pathogen in granular porous media.

Clay minerals have two basic structures; a silica tetrahedron surrounded by oxygen atoms and aluminum or a magnesium octahedron surrounded by hydroxyl ions. The geometry is largely dominated by the oxygen (radius – 0.14nm) or hydroxide (0.15nm), compared to the relatively small cations (Si- 0.04 nm, Al -0.05 nm, Mg- 0.06 nm) (Stotzky 1985). This is depicted in Figure 2.7. The identical symmetry of tetrahedron and octahedron sheets permits the sharing of oxygen atoms between the sheets. Clays are found in either a 1:1 (silica-alumina) ratio or 2:1(silica-alumina-silica)

ratio stacked together to form a three dimensional structure. The 1:1 layers are held together by hydrogen bonding between OH group of octahedron and the O of the tetrahedron on another layer, so these clay minerals normally are non expanding upon wetting. E.g. kaolinite.

For 2:1 clays, the octahedron sheets are sandwiched between two tetrahedrons, so hydrogen bonding is not possible and hence they are held together by weak van der Waals attractive force. Because of the weak nature of these forces, water molecules can enter the basal plates. These clays are called expanding clays, e.g. the smectite group of clays such as montmorillionite. During weathering processes such as crystallization of layer silicate minerals in magmas and in soils, some of the cation, Si^{4+} is replaced by lower valance Al^{3+} or Fe^{3+} and some Al^{3+} in octahedrons by Mg^{2+} or Fe^{2+}. This phenomenon is called isomorphic substitution which leaves one unfulfilled negative charge on the basal plane of the clay minerals. The deficient negative charge on the crystal is replaced by substitution with other cations or variable valances in the soil solution. Because the charge on these types of clays is independent of pH as well as the solution chemistry of the soil solution, soils with 2:1 clay minerals are referred to as permanently charged soils. Most of the poorly weathered, young soils occurring in the temperate regions of the globe have permanently charged soils.

Because the 1:1 clays are non-expanding and are held by strong hydrogen bonds, these clays are inflexible and allow no isomorphic substitution. Consequently, the majority of the charge on these clays is due to the protonation and deprotonation of the OH group on their surfaces and edges. The charge in these soils largely depends on pH

as well as solution chemistry of ions in soil solution, and hence soils dominated by 1:1 clay minerals and hydrous metal oxides are referred to as variably charged soils. These types of clay minerals are seen in highly weathered and volcanic soils which also contain high concentrations of Fe and Al oxides and occur in tropical regions. The Fe and Al oxides sometimes tend to form a thin coating in 'patches' on the edges of clay minerals such as kaolinite (Figure 2.6). This modifies the surface characteristics of clays and increases the reactivity of the clay minerals because of the higher surface area of the metal oxides. Because these oxides carry colloidal charges, even small quantities of surface coating have high surface charge density which results in favorable regions of attachment for negatively charged pathogens such as *C. parvum* oocysts (Abudalo et al., 2010).

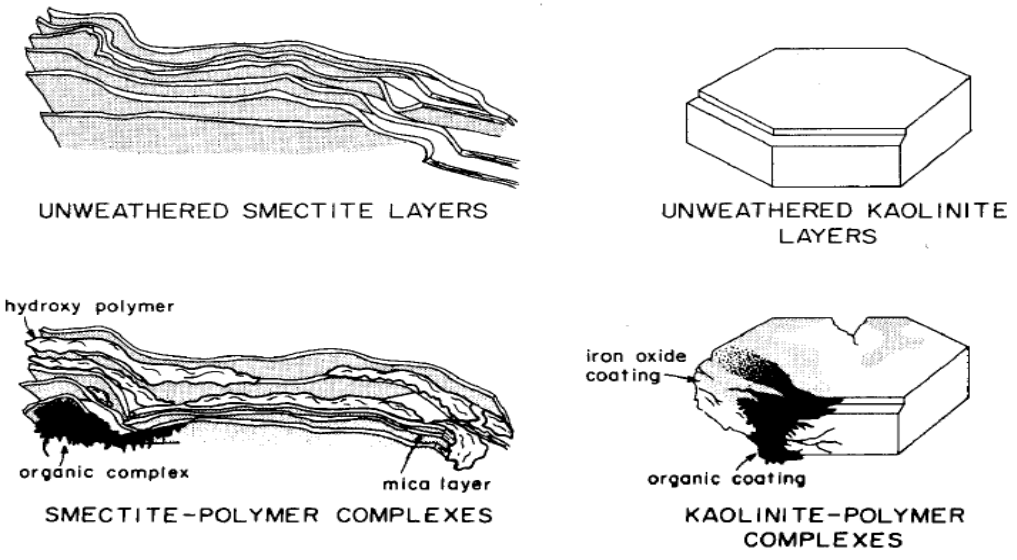

Figure 2.6. Effect of mineral and organic coating on smectite (2:1) clays and kaolinite (1:1) clays. (Sposito, 1984).

The transport of pathogens in soils rich clay minerals and iron oxide minerals have been studied by (Mohanram et al., 2010). The two agricultural soils from tropical regions used in our experiments were characterized by intense weathering, high Al and Fe oxide content, and, pH dependent charge behavior. The soil taken from island of Oahu (Poamoho soil) is rich in clay minerals (68-76%) as well as metal oxides (24-32%), whereas the volcanic ash soils from the Big Island (Lalamilo soil) has lower clay (16-19%) and metal oxide (16-19%) content, but substantially greater organic matter content (45% w/w). The temperate-zone permanently charged agricultural soil (Drummer soil) that we used in our study is rich in quartz (40%), clay minerals (27%) and organic matter (13%) and was used as a reference to distinguish the role of metal oxides in the attachment behavior of oocysts and microspheres.

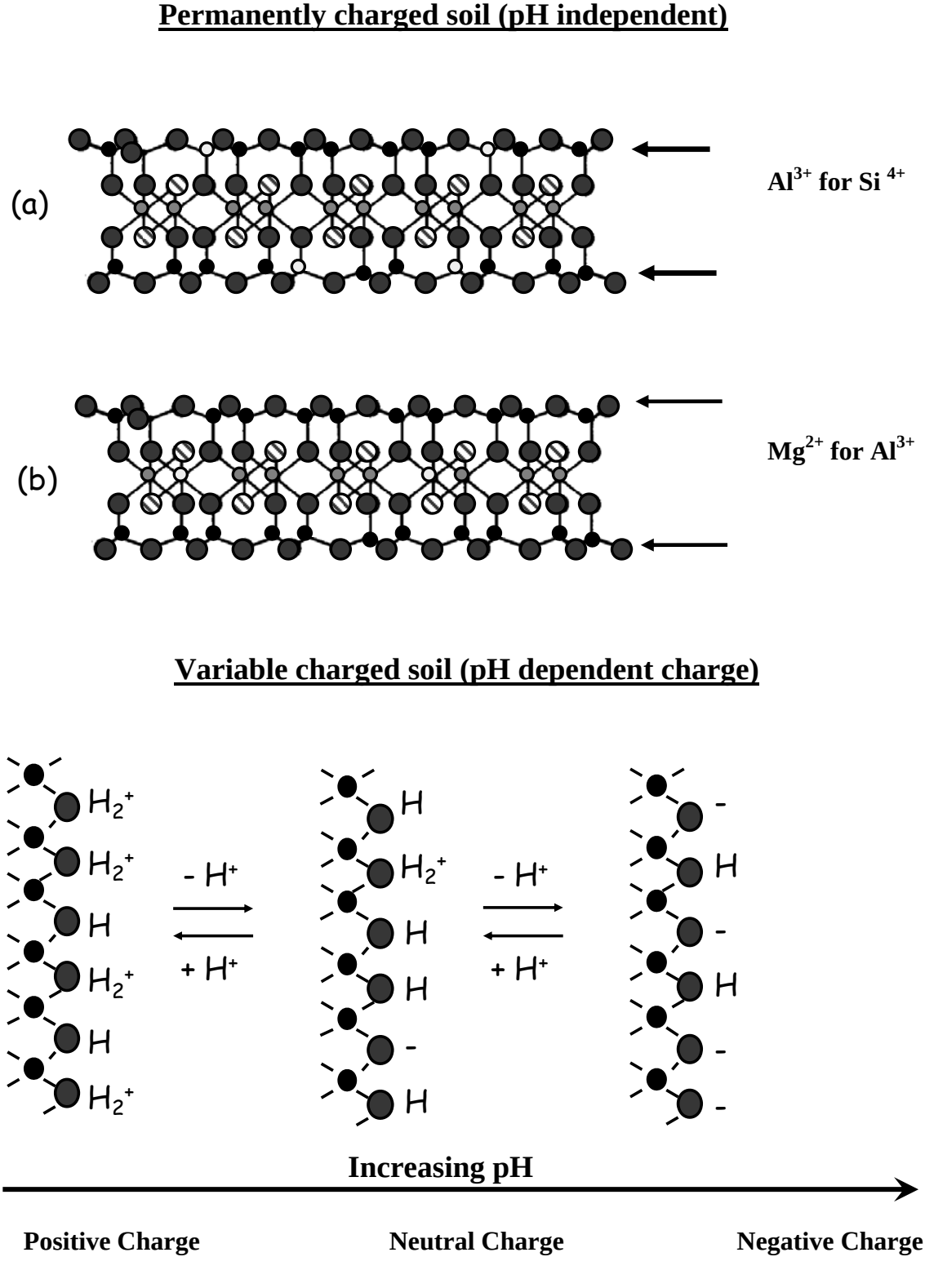

Figure 2.7. Difference between permanently charged and variably charged soils (Figure credits: Jon Chorover, U Arizona, Tucson).

The pH-dependency of oocyst attachment in the three agricultural soils is shown in Figure 2.8. Although the volcanic ash soil from Big Island had significant metal oxide content (16-19%) oocyst attachment displayed a lack of pH dependence. The high SOM (45%) content in this soil was expected to play a dominant role in controlling particle transport behavior. Only the clay-rich variably charged (Poamoho) soil showed pH dependent attachment of oocysts (negative correlation between attachment and pH), due to the protonation and deprotonation of the surface active charges on Fe and Al oxides and clay minerals. Surprisingly, the Drummer soil showed greater pH dependence in the attachment of oocysts than did the clayey soils. Unlike variably charge soils, permanently charged soils display repulsive behavior towards oocysts at all pH levels due to the dominance of quartz mineral in their composition. Thus a consistently lower attachment was observed in Drummer soils than in Poamoho soils. Because oocyst surfaces charge are also pH dependent, the effect of charge repulsion between oocysts and soil grains was more apparent at higher pH values (~30% attachment of oocysts at pH 9) than at low pH's.

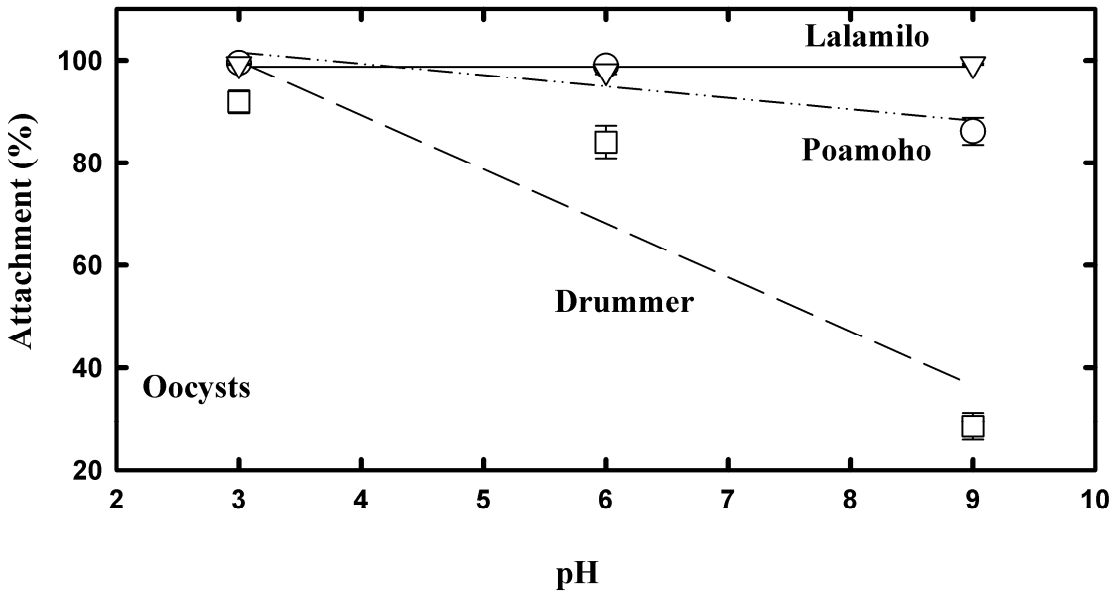

Figure 2.8. pH-dependency of oocyst attachment in agricultural soils.

GRAIN PROPERTIES

Straining is the process of trapping particles in pore throats that are too small to allow their passage (McDowell-Boyer et al., 1986). Straining is a physical mechanism that depends on the size of the particle, collector surface diameter, diameter of the pores, and as has been discovered more recently, solution chemistry (Bradford et al., 2007). Early researchers' (Sakthivadivel 1967) conceptual model of straining assumed grains to be uniform and spherical. However, aquifer sediments vary widely in terms of grain size distribution, and bacteria being advected through those sediments can be rod-, ellipsoid- or spiral- shaped (Weiss et al., 1995). Many researchers recently have studied retention of colloids in various zones between the grains and this is represented in Figure 2.9.

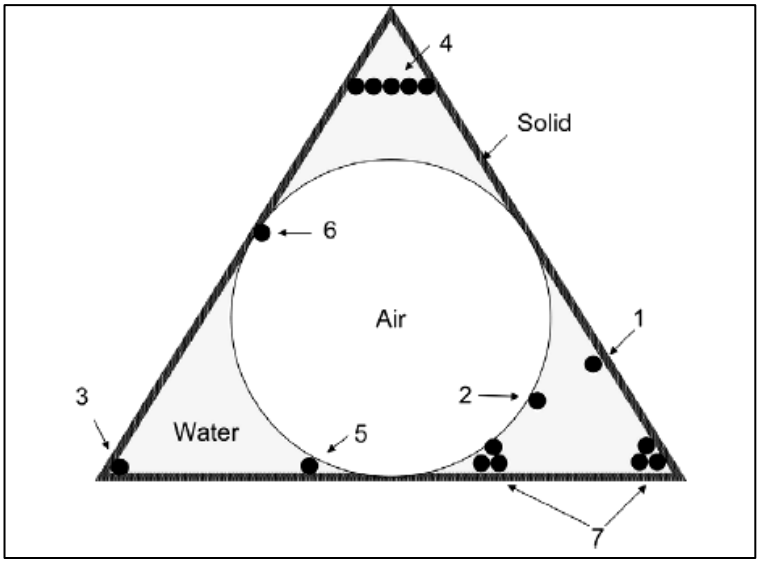

Figure 2.9. Schematic representation of various pore scale processes: 1,2-represent attachment to solid (S)-water (W) and air (A)-water interface. 3,4,5,6,7-different types of Straining mechanisms such as Wedging (3), Bridging (4),SWA retention (5), Film Straining (6).

The role of soil pores in contributing to straining was studied by Carsel and Parrish (Carsel et al., 1988) who simulated the soil pore diameter to be equivalent to that of capillary pressure (P_c), according to Laplace equation. The fraction of pore space where straining would occur (Υ) can be found from measuring capillary pressure curves and residual saturation. Soil water retention was parameterized using the Van Genuchten model (Vangenuchten 1980):

$$\gamma = (1 - S_{rw}) * \left[1 + \left(\frac{2\sigma\alpha_p}{\rho g R} \right)^n \right]^{-m} + S_{rw}$$

S_{rw} (dimensionless) is residual water saturation, α_p is the reciprocal of the air entry pressure head, ρ is the density of the liquid phase, g, is the acceleration due to gravity, σ is the surface tension, R is the pore radius that corresponds to a given colloid size, and m and n are fitting parameters.

Table 2.1. Role of soil pores in contributing to Straining. Average Parameter Values for the Capillary Pressure– Saturation Model for twelve major soil textural groups according to Carsel and Parrish (1988), as well as the calculated percentage of the pore space smaller than critical straining pore diameter for virus, bacteria, and protozoa.

				Percent Excluded Pore Space		
Soil Texture	S_{rw}	α, cm^{-1}	n (m = 1−1/n)	Virus	Bacteria	Protozoa
Sand	0.10	0.15	2.68	10.5	10.5	10.5
Loamy sand	0.14	0.12	2.28	13.9	14.0	14.4
Sandy loam	0.16	0.08	1.89	15.9	17.1	19.3
Loam	0.18	0.04	1.56	19.8	27.0	34.5
Silt	0.07	0.02	1.37	16.9	36.1	50.0
Silt loam	0.15	0.02	1.41	21.1	36.1	47.9
Sandy clay loam	0.26	0.06	1.48	27.7	34.4	40.4
Clay loam	0.23	0.02	1.31	34.0	50.5	61.2
Silty clay loam	0.21	0.01	1.23	42.2	63.2	74.4
Sandy clay	0.26	0.03	1.23	42.2	57.9	66.7
Silty clay	0.19	0.01	1.09	70.8	86.2	91.8
Clay	0.18	0.01	1.09	68.1	83.3	89.3

Table 2.1 represents the average capillary pressure saturation parameters of the twelve major soil textural groups according to (Carsel et al., 1988) as well as the percentage of the pore space where straining would occur according to the above equation. Depending on soil texture the probability of straining was highest in silty clay and clayey soils and lowest in sandy soils for all the biocolloids studied. The virus, bacteria and protist sizes were assumed to be 0.1-µm, 2-µm and 6-µm respectively.

Bradford et al. (Bradford et al., 2002, Bradford et al., 2005a,b, Bradford et al., 2006) did much work elucidating the various factors and conditions that affect straining. In a systematic study using quartz sand as the collector surface ,the authors found that decreasing the grain diameter resulted in a higher percentage of microspheres, oocysts and bacteria being strained out due to the lowering of the pore space available for the movement of microspheres.

The lower attachment of pathogens to larger grain sizes was found to be true with aquifer sediments obtained from a sandy aquifer (Dong et al., 2002). (Hijnen et al., 2005) studied the transport of different sized pathogens such as MS2 phage, *E. coli*, *Clostridium perfringens*, *C. parvum* and *Giardia* in columns packed with aquifer sediment. While there were several log removals for all these pathogens, straining was an important removal mechanism for only bacteria and protists. (Mohanram et al., 2010) similarly observed that in fine-grained (0.21mm) volcanic ash soils *C. parvum* oocysts and larger microspheres (4.9-µm) were found to be strained out because flow reversal caused a significant re-entrainment of the surface attached colloids. From the previous observations, it may appear that straining is purely a physical phenomenon. However, recent evidence indicates that it can be coupled to solution chemistry (Bradford et al., 2007, Kim et al., 2010).

The Figure 2.10 shows that hydrodynamic and adhesive forces have a significant impact on the attachment process and retention at the interfaces ("grain-to-grain" contact area) decreases with increases in flow velocity and colloid concentration. At very high colloid concentrations, the particles tend to aggregate. Aggregation of bacteria has also

been shown to contribute to straining and is often described as being due to hydrophobic interactions (Bradford et al., 2006).

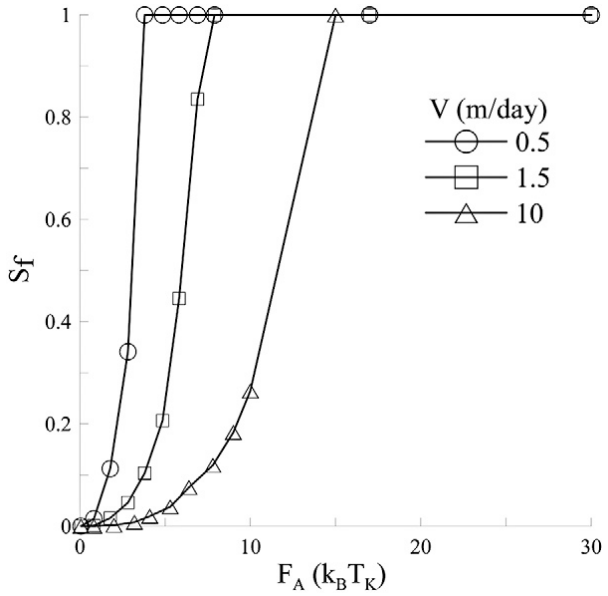

Figure 2.10. Effect of hydrodynamics in the attachment of 1-µm colloids. V is the flow rrate and Sf is fraction of grain surface area favorable for attachment for 1-µm colloids. F_A is the adhesive force at different flow rates (Bradford et al., 2007).

(Tufenkji et al., 2004) studied the removal efficiencies of *C. parvum* oocysts in quartz sand and glass beads. It was found that while 40% of oocysts were strained out by quartz sand, there was no removal of oocysts by the glass beads of same diameter. The authors concluded that the shape of the grains contributed to differences in straining and that removal was due to a combination of both straining and sorptive filtration. (Tong et al., 2006) found that the angularity of grains determined colloidal deposition more than surface roughness. (Saiers et al., 2005) studied the effects of different mineral-grain shapes and surface roughness upon the single-collector contact efficiency $\acute{\eta}$. In their simulation they found $\acute{\eta}$ to be sensitive to colloid size. For colloids less than 1-µm in

diameter, surface roughness did not affect prediction of $\acute{\eta}$, but for particles greater than 1-μm and when particle shape was nonideal (e.g. non-spherical collector surface), there was a significant difference between observed and predicted values of $\acute{\eta}$. They suggested that surface roughness may be a more important parameter for determining the collision efficiency than $\acute{\eta}$. Some studies have found grain surface roughness to be positively correlated to soil texture as well as particle removal efficiency (Bradford et al., 2005a, Morales et al., 2009).

CLAY MINERALS

Unlike model systems, soils contain mineral phases that affect the fate and transport of pathogens in a complex manner. The soil colloids primarily consist of inorganic constituents such as layered silicates, iron and aluminum oxide as well as organic matter. The layered silicates are sometimes coated with varying extents of organic matter and amorphous Fe and Al oxides. Soils rich in clay minerals and metal oxide have a high capacity for the sorption of virus (Chu et al., 2003, Zhao et al., 2008, Zhang et al., 2010) and oocysts (Mohanram et al., 2010). Viruses and bacteria retention in clay- rich soils have been positively correlated to clay content (Huysman et al., 1993b, Lipson et al., 1983).

The nonexpanding 1:1, kaolinite clays tend to have low aggregate stability compared to 2:1 clays which generally have higher cation exchange capacity surface area and high Soil Organic Matter. Consequently, 2:1 clays have higher tendency to flocculate than 1:1 clays (Bronick et al., 2005). Aggregation characteristics of soils have been linearly correlated with clay content and are largely responsible for the formation of

preferential flow paths in soil columns (Kjaergaard et al., 2004). The macropores created during soil wetting become zones of high hydraulic conductivity and hence faster movement of small colloids. Many researchers have hypothesized pathogen contamination of wells and groundwater sources through naturally occurring preferential flow paths for bacteria, viruses and oocysts (Powelson et al., 1993, Darnault et al., 2004, Pang et al., 2008).

The thermodynamics involving sorption of bacteriophages T-2, MS-2 and ΦX-174 on four different types of clays (hectorite, saponite, kaolinite and unknown clay isolated from landfill site, at Norman, OK) were studied by (Chattopadhyay et al., 1999). They used "sphere-sphere", "plate-plate" and "plate-sphere" models to describe the magnitude of double layer forces (ΔG^{EL}) forces. In "sphere-sphere" model, all the particles interacting are considered as spheres while the "plate-plate" model assumes the clays and bacteriophages behave as parallel plates with respect to each other and "plate-sphere" regards that one of the interacting particles behave as sphere while the other behaves as a flat plate. The "plate-sphere" model was found to closely match their experimental data and observations. According to their findings, the clay particles were, on an average, 29 times larger than the bacteriophages and hence depicting the clay particles as flat plates for modeling purposes was reasonable. Their attachment studies with clay minerals showed that all the viruses largely attached via hydrophobic interactions. Attachment by electrostatic forces contributed to a maximum of only 6.81% of the total energy of interaction. Moreover, the sorption of viruses correlated linearly to the surface area of the clay mineral and to the surface hydrophobicity. Consequently,

maximum sorption of all viruses was observed on hectorite (surface area (sa) = 93.4 m^2g^{-1}), followed by the Norman clay fraction (sa = 92.34 m^2g^{-1}). The hectorite clays were the most hydrophobic followed by Norman, kaolinite and saponite clays. Further studies have found that kaolinite clays, due to their high aggregate stability, showed greater attachment of *E.coli* and *Pseudomonas* than montmorillionite clays (Huysman et al., 1993a, Jiang et al., 2007). Other studies have found that oocyst sedimentation velocity increased by 50 times in the presence of kaolinite clays (Searcy et al., 2005).

The effectiveness of kaolinite and bentonite clays to remove viruses (MS2 and ΦX174) by adsorption from dilute aqueous solutions was studied by (Syngouna et al., 2010). Deposition in the primary minima occurs whereby particles overcome an energy barrier and undergo irreversible deposition in the primary energy well. Particles may also be deposited at a larger distance from the energy barrier in the secondary minimum under repulsive electrostatic condition. The depth of the secondary minimum is proportional to the particle size and deposition is reversible upon changes in ionic strength of solution. DLVO calculations showing the total interaction energy profiles (Figure 2.11) were extremely unfavorable for deposition in the primary minima and the majority of the viruses remained in the secondary minima (inset, Figure 2.11). The repulsive peaks under the unfavorable conditions were higher for MS2 (32.7 k_B T) than ΦX174 (23.4 k_B T) for kaolinite. For bentonite, lower energy barriers are observed for both viruses (17.4 k_BT for MS2 and 14.5 k_BT for ΦX174).

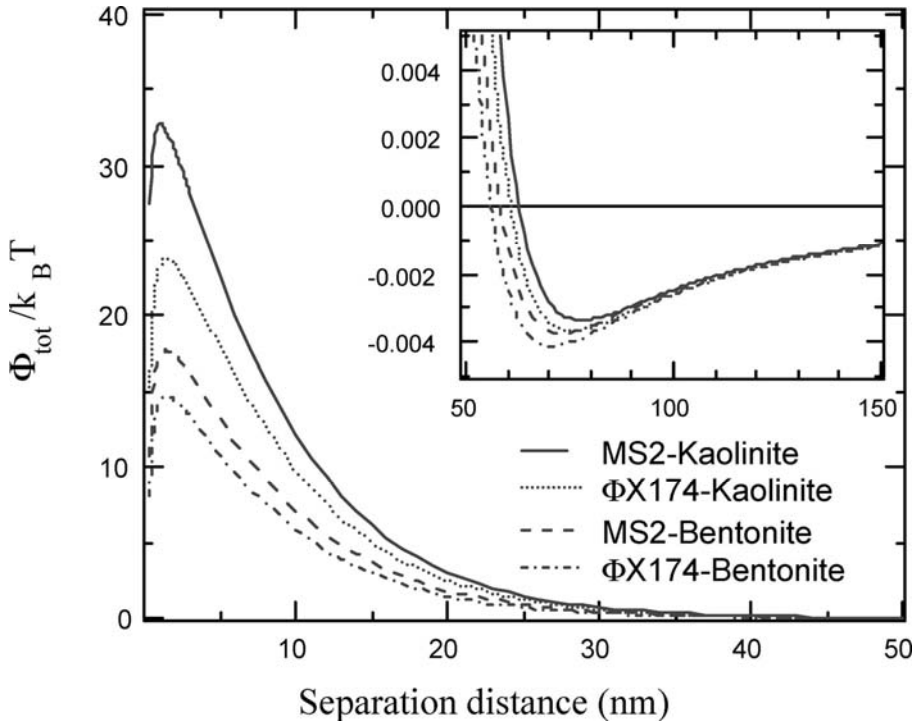

Figure 2.11. Calculated total interaction energy profiles for MS2 and ΦX174 with kaolinite and bentonite as a function of separation distance for the experimental conditions (pH 7, 2 $\times 10^{-3}$M, ionic strength). The inset highlights the corresponding secondary energy minima.

These findings give new insight into the interactions between viruses and clays which were largely unknown. Moreover they signify the importance of secondary minimum deposition. Deposition in secondary minima is often invoked to explain re-entraiment of pathogens from grain surfaces in the laboratory (Scholl et al., 1992, Loveland et al., 1996, Harter et al., 2000, Johnson et al., 2007) and field studies (Schijven et al., 1999, Zhang et al., 2001)

ORGANIC MATTER

The primary role of soil organic matter is to provide aggregate stability to soils. In fact there is a positive correlation between aggregate stability and the presence of soil organic matter (Bronick et al., 2005). Greater aggregate stability of soils results when the hydrophilic fraction of organic matter is oriented towards the interior of the aggregates and the hydrophobic component towards the outside. Consequently, this leads to the formation of a water-repelling coating on soils. In fact, it has been reported that a strong hydrophobic organic coating can prevent water from entering these aggregates and causes the soil to exhibit a hydrophobic character (Wallis et al., 1992) which mask the underlying iron oxide minerals that provide favorable sites for irreversible sorption of pathogens.

Transport experiments conducted with 1.8-µm microspheres showed that the attachment of these particles in the volcanic ash soil was weak and highly reversible (Figure 2.12). After initial attachment, there was steady re-entraiment of low concentrations of microspheres from this soil. Only 0.26% of the microspheres put in this column were recovered in the effluent. Statistical analysis indicated that the remobilization would have continued for over 4000 pore volumes for transport of 99% of the microspheres across this soil. Consequently, it can be hypothesized that rainfall events could promote slow release of pathogens in low concentration from these soils and lead to their long distance transport in subsurfaces environment. However, removal of SOM from this soil by chemical oxidation caused the microspheres to undergo irreversible sorption, characterized by the difference in the shape of the breakthrough

curve and absence of tailing. Higher quantities (5%) of microspheres were transported through columns filled with this SOM-less soil. The authors hypothesized that SOM removal opened up the preferential flow paths causing an increase in microspheres transport.

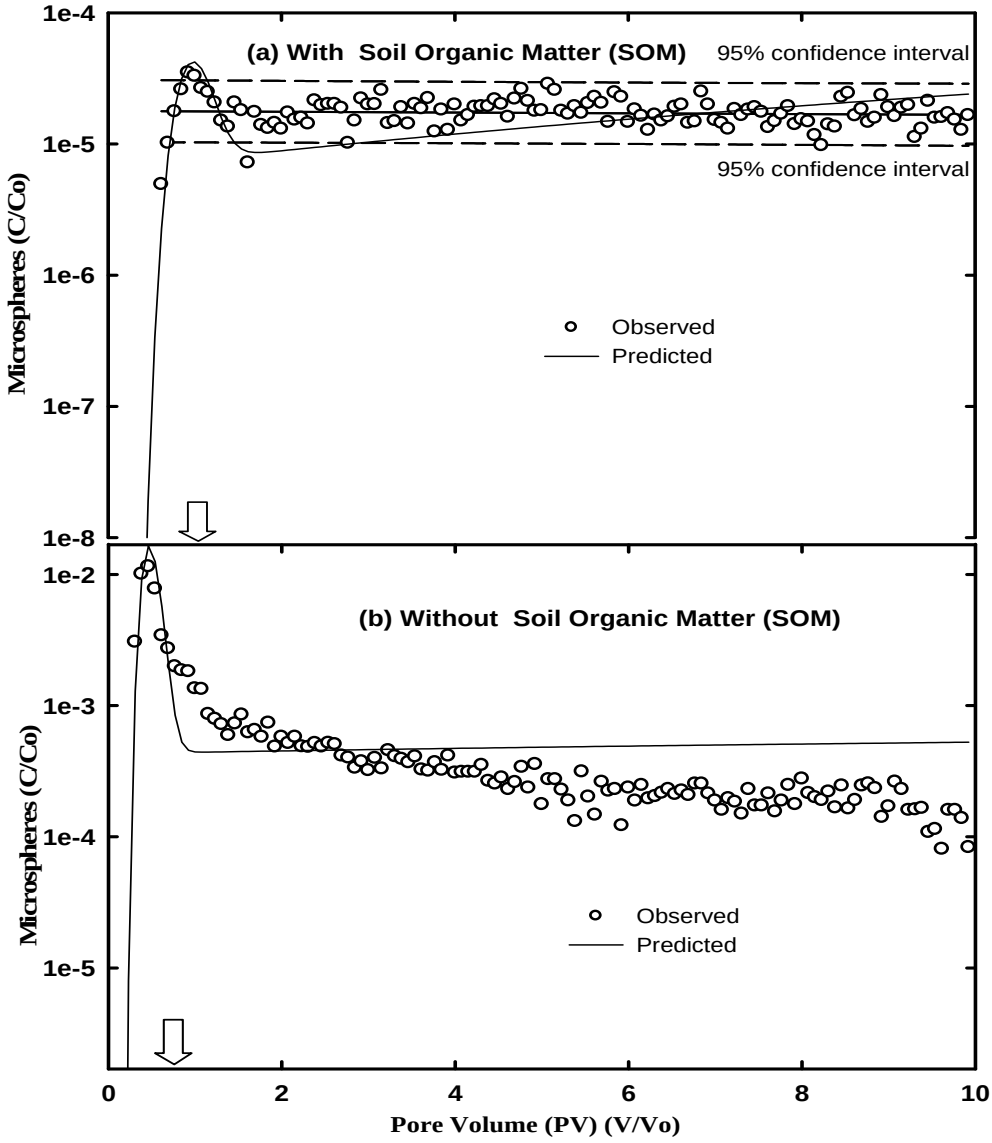

Figure 2.12. Effect of soil organic matter on microspheres transport in Lalamilo soils.

It has been shown that NOM sorption to metal oxide surfaces by electrostatic interactions, and NOM coating on clay minerals blocks the micro pores and naturally occurring preferential flow paths on the soil surface (Munn et al., 1990, Tombacz et al., 2001, Gao et al., 2004). Some studies have shown that NOM coatings on clay minerals physically block access to the clay pores by cation binding (Fitch et al., 1996). So, NOM coating on soils can retard pathogen transport by decreasing soil porosity and hence increase the straining potential of larger particles such as *C. parvum* oocysts.

Indeed, in a recent study (Mohanram et al 2011, under review) the transport of oocysts sized microspheres through clayey soils coated with 100 mgL^{-1} NOM was studied. The recovery of larger (4.9-µm) microspheres was reduced from 4 % (in the absence of NOM) to 0.2 % (presence of NOM) while that of smaller (1.9-µm) microspheres was reduced from 48% to 28%. Oocysts transport was not significantly changed by the presence of NOM. In addition, zeta potential changes in the presence of NOM suggested an increased stability of the fines. So, the dispersion of soil fines in the soil pore space may cause pore blockage and may increase the ability of soils to remove pathogens. When NOM in the clayey soil was replaced by 100 mgL^{-1} anionic surfactant sodium dodecyl benzene sulfonate, the transport of 1.9-µm microspheres increased from 48% to 87% and 4.9-µm increased from 4% to 27%. Surfactants have been found to promote transport of oocysts (Metge et al., 2010), viruses (Foppen et al., 2006, Cao et al., 2010) and bacteria (Brown et al., 2001, Harvey et al., 2010) by masking the heterogeneity occurring in the grains. Thus, it is hypothesized that a negative charge on

SDBS could have neutralized the positive charge on the metal oxide surfaces in the grain surface and lead to the greater transport of microspheres in the presence of SDBS.

The presence of SOM can enhance virus sorption in some soils and inhibits sorption in others. The increased attachment of virus in the presence of SOM was due to hydrophobic interactions (Kinoshita et al., 1993), whereas decreased attachment has been attributed to blocking of viruses attachment sites by organic matter (Moore et al., 1981, Zhuang et al., 2003). The effect of sewage- derived organic matter from secondary sewage effluent on PRD1 (model virus) attachment to iron oxide coated sandy aquifer material was investigated using natural-gradient injections of PRD1 by (Pieper et al., 1997). In the uncontaminated zone, 83% of the PRD1 were attenuated over the first meter of transport, whereas 42% of the PRD1 were attenuated over the first meter of transport in the organically contaminated zone. The difference in immobilization of PRD1 was due to the blocking of favorable sites for attachment by sewage organic matter. Injection of $25mgL^{-1}$ of anionic surfactant, linear alkylbenzene sulfonate (LAS) resulted in the remobilized 87% of attached PRD1 in the contaminated zone, but only 2.2% in the uncontaminated zone. The authors suggested that the number of adsorbed virus were not sufficient to promote release of the attached PRD1 in the uncontaminated zone. Although the process responsible for the apparent contradictory observations is not clear, it is apparently that the effect of SOM depends on the combination of soil type, virus type, and nature of organic matter.

In another study, the pH dependency of bacterial attachment was studied to assess relative effects of the hydrophobic and total fractions of the plume DOC, anionic (LAS)

and nonionic (Imbentin) surfactants, humic and fulvic acids by (Harvey et al., 2010). Anionic surfactant such as LAS caused an 11-33% decrease in bacterial attachment, while the non ionic surfactant, Imbentin caused an increase in attachment even under alkaline conditions. The authors hypothesized that this was due to hydrophobic interactions leading to increased attachment of bacteria to the grain surfaces. These data reveal how different forms of DOC can have profound effects on the attachment characteristics of bacteria and it shows that it may be difficult to predict bacterial transport properties in the natural environment when DOC is present.

Recent evidence indicates that in addition to modifying grain properties, NOM can also affect the surface characteristics of pathogens such as *C. parvum* oocysts by changing their hydrophobicity. Dai and Hozalski (Dai et al., 2003b) reported that low concentration of NOM can lower the hydrophobicity of oocysts. In our own studies (Mohanram et al., 2011, under review) conducted by dosing the oocysts surface with increasing concentration of NOM from 4 mg/L to 120mg/L. It was found that although NOM decreased the hydrophobicity at low concentrations, oocysts hydrophobicity was independent of higher NOM concentration. Abuldalo et al. (Abudalo et al., 2010) reported that NOM has no significant effect on the zeta potential of the oocysts. The reason for the lack of reactivity of oocysts to high NOM concentration is unclear but we speculate that the polyelectrolytic brush like appendages on oocysts offers limited sites for the interaction of NOM moieties which become saturated at low concentration of NOM.

In summary, a large body of literature published studying pathogen transport in sandy soils and aquifer material, has lead to a better understanding of the mechanisms underlying the effect of grain properties such as angularity, surface roughness and grain size distribution as well as heterogeneity in geochemical media that affect pathogen transport. However, soils are rich in clay minerals, organic matter and metal oxides that affect pathogen transport in a complex manner. Sorption of pathogens is enhanced in soils rich in Fe and Al oxides; however their role is suppressed if SOM is present in the soils. Clay minerals stabilize the pathogens and even change their surface characteristics. NOM's role in pathogen transport appears to depend both on the nature of the particle as well as the mineralogy of the grain surface. More study needs to be undertaken to understand the combined effect of these reactive soil components for the protecting of our groundwater sources from waterborne pathogens.

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CHAPTER III

EFFECT OF MINERALOGY ON THE TRANSPORT OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS AND MICROSPHERES IN AGRICULTURAL SOILS

ABSTRACT

In order to gain more information about the fate of *Cryptosporidium parvum* oocysts in tropical volcanic soils, the transport and attachment behaviors of oocysts and oocyst-sized polystyrene microspheres were studied in the presence of two soils. These soils were chosen because of their differing chemical and physical properties, i.e., an organic-rich (43%-46% by mass) volcanic ash-derived soil from the island of Hawaii, and a red, iron (22%-29% by mass), aluminum (29%-45% by mass), and clay-rich (68%-76% by mass) volcanic soil from the island of Oahu. A third agricultural soil, an organic-(13% by mass) and quartz-rich (40% by mass) soil from Illinois, was included for reference. In 100-mm flow-through columns, oocysts and microspheres advecting through the red volcanic soil were almost completely (98% and 99%) immobilized. The minute quantity of breakthrough resulted from preferential flow-path structure inadvertently created by soil-particle aggregation during the re-wetting process. Although a high (99%) removal of oocysts and microsphere within the volcanic ash soil occurred initially, further examination revealed that transport was merely retarded because of highly reversible interactions with grain surfaces. Judging from the slope of the substantive and protracted tail of the breakthrough curve, almost all (>99%) of the colloids predictably would be recovered within ~4000 pore volumes. This suggests that once contaminated with oocysts, the volcanic ash soil could serve as a reservoir for subsequent contamination of surface and groundwater. Surprisingly, oocyst and microsphere attachment to the reference soil from Illinois appeared to be at least as sensitive to changes in pH as was observed for the red, metal-oxide rich soil from Oahu. In contrast, colloidal attachment in the organic-rich, volcanic ash soil was relatively

insensitive to changes in pH in spite of the high iron content. Given the fundamental differences in transport behavior of oocyst-sized colloids within the two volcanic soils of similar origin, agricultural practices modified to lessen *C. parvum* contamination of ground or surface water would necessitate taking the individual soil properties into account.

INTRODUCTION

Groundwater contamination by the pathogenic protozoa *Cryptosporidium parvum* is a cause of serious public health concern. *C. parvum* is an opportunistic protozoan pathogen that infects the small intestine of mammals including humans and cattle, resulting in cryptosporidiosis (LeChevallier et al., 1991). Outside the host, the thick-walled oocysts, the environmental form of this parasite, can remain viable and potentially infective for up to 6 months (Fayer et al., 1997). Oocysts often contaminate drinking water supplies, following their transport from sources such as dairy cattle operations and wastewater effluents (LeChevallier et al., 1991). They are highly resistant to common methods of chemical disinfection employed in water treatment, particularly chlorination (Fayer et al., 1997). Consequently, treatment plants can be faced with a considerable challenge in order to achieve the 4-log removal from source water under the Long Term 2 Enhanced Surface Water Treatment Rule (USEPA, 2006). Cost-effective removal commonly involves immobilization within granular-media, often accomplished by sand filtration or riverbank filtration (Ray et al., 2002, Tufenkji et al., 2002).

Despite the potent health impacts and difficulty of inactivation of *C. parvum*, incomplete data exist regarding the fate and transport of these pathogens in soils and aquifers. A number of controlled laboratory studies on oocysts transport have focused on model granular media such as quartz sand (Tufenkji et al., 2004) or glass beads (Dai and Hozalski, 2002). These studies are helpful for a more mechanistic understanding of oocysts deposition processes. However, more information is needed on how these results might relate to transport and deposition in the multi-component, terrestrial subsurface.

The few studies that have addressed oocyst attachment in natural media (Hijnen et al., 2005; Harter et al., 2000) have involved so-called "permanently charged" soils in which charge derives dominantly from isomorphically-substituted phyllosilicate clay minerals, such as smectite and vermiculite, that impart a net negative structural charge that is relatively independent of pH (Sposito, 2008). However, several cases of oocyst contamination of surface and groundwater have been reported in regions with "variably charged" soils such as in the southeast US (LeChevallier et al., 2000) and Brazil (Bushen et al., 2007), where the clay mineralogy is dominated by kaolinite and (oxy)hydroxides of iron and aluminum, including hematite and gibbsite. These secondary minerals exhibit variable charge whose magnitude and sign depend on the pH, ionic strength (*I*), and ionic composition of the soil water (Uehara and Gillman, 1981). Although it has been reported that metal oxides provide favorable interfacial regions for attachment of oocysts in model sediments (Abudalo et al., 2005), no study to date has reported on the transport behavior of oocysts in tropical soils of volcanic origin, where these mineral phases can predominate. Although these soils may be rich in iron- and aluminum-oxides, high concentrations of organic carbon (e.g., Trumbore, 1993) could mask the electrostatic attraction of the metal oxide surfaces (Pieper et al., 1997), thereby enhancing microbial transport. Consequently, there is a need to assess the efficacy of different types of tropical, volcanic soils to immobilize oocysts under relevant conditions.

The overall objective of this paper is to compare the transport and attachment behaviors of oocysts and oocyst-sized microspheres in two volcanic soils from Hawaii having fundamentally different physicochemical properties. Because considerably more

research has been previously conducted on permanent-charged soils (Bradford and Bettahar, 2005; Tufenkji et al., 2004, Darnault et al., 2004), an agricultural soil from Illinois was also included as a reference. The pH dependency was assessed using static batch experiments, and the magnitude and reversibility of attachment were assessed using flow-through column experiments in order to compare the manner in which oocyst-sized colloids would be transported in the aforementioned soils.

METHODS

CRYPTOSPORIDIUM PARVUM OOCYSTS

Formalin-inactivated oocysts were obtained from Sterling Parasitology Laboratory (SPL) at the University of Arizona and prepared according to the methods outlined by (Abudalo et al., 2005). The final oocyst concentration used for the column experiments was between 2×10^6 - 5×10^6 mL^{-1}. The oocysts were enumerated by epifluorescence microscopy. Samples containing the oocysts were stained with 4,6-diamidino-2-phynylindole (DAPI, 0.1 mg mL^{-1} solutions, 15 min contact time), filtered with vacuum assistance (0.34 bar) onto black polycarbonate membranes (1.0-µm pore diameter, Osmonics), prepared with a cover slide and immersion oil, and counted manually using an epifluorescence microscope (Nikon Optiphot-2, 788X magnification, 350 nm excitation, 470 nm emission). Oocysts were enumerated in at least 100 optical fields for every sample. The average diameter of the oocysts was measured by flow cytometry analysis using DAPI- stained oocysts. The flow cytometer (Biorad, HY Bryte) was calibrated using fluorescent microspheres of 3 different sizes (1.8-µm, 2.9-µm, and 4.9-µm). The electrophoretic mobility of the oocysts was measured at a temperature of

22°C by laser Doppler micro electrophoresis (Zeta Pals-Zeta Potential Analyzer-Brookhaven Instruments) for pH values of 3, 6, and 9 in 10^{-3} M NaCl at a concentration of about 4×10^{5} oocyst mL^{-1}. Electrophoretic mobilities were converted to zeta potential (ζ) using the Smoluchowski equation (Masliyah et al., 2006).

MICROSPHERES

We used a mixture consisting of fluorescent carboxylated, polystyrene microspheres of two diameters: 1.8-µm and 4.9-µm (Type "BB", brilliant blue) and 2.9-µm (Type, fluorescent green) (Polysciences, Warrington, Pennsylvania). These were used to create the polydispersed microsphere suspension that collectively bracketed the 2 to 5-µm variation in oocyst size commonly found in the environment (Harvey et al., 2008). The concentration of the microspheres used in the column studies were $0.5\text{-}1\times10^{7}$ mL^{-1} (1.8-µm) and 4×10^{6} mL^{-1} (2.9-µm and 4.9-µm). The electrophoretic mobility of microspheres was measured by Laser Doppler micro electrophoresis at pH values 3, 6 and 9 in 10^{-3} M NaCl at a concentration of about 4×10^{6} microspheres mL^{-1}.

GRANULAR MEDIA CHARACTERISTICS

Two compositionally different tropical volcanic soils were chosen because they differed substantively in terms of organic and mineral contents and degrees of weathering. The weathered red volcanic clayey (Poamoho) soil was collected on Oahu from the Poamoho Agricultural Experiment Station (College of Tropical Agriculture and Human Resources (CTAHR), University of Hawaii), which is 200 m above sea level and receives ~ 1.0 m of annual rainfall. The red soil (derived from a lava flow of *ca*. 2.5

million years in age) is used for pineapple production and is intensely weathered silty clay Oxisol of the Wahiawa series, and is classified as Rhodic Eutrustox (Wan and El-Swaify, 1997). In contrast, the organic rich-volcanic ash (Lalamilo) soil, collected from a field near the town of Waimea, Hawaii, at an elevation of 350 m, is derived from a lava flow that is *ca.* 150,000 year old, and is subjected to *ca*. 0.3 m of annual rainfall. As a result, this soil is considerably less weathered, and contains a lower mass concentration of iron oxides. The latter soil is used to grow cabbage, lettuce and soybean (Deenik et al., 2006) and is a silty loam Inceptisol of the Waimea series, typic Eutrandepts, medial and amorphic family (Soil survey staff, : http://websoilsurvey.nrcs.usda.gov).

The temperate agricultural soil used for comparative purposes is a 10,000 year old, black loamy Midwest agricultural (Drummer) soil that was collected on the campus of University of Illinois at an elevation of 200 m in a region that receives 0.9 m of annual precipitation. It is used for growing corn and soybeans and is classified as silty clay loam Mollisol of the Drummer series in the Endoaquolls family (David et al., 1997).

The three soils were autoclaved at 121°C for 20 min, oven-dried at 105°C overnight, and sieved to obtain a common grain size corresponding to their d_{50} of the aggregates (red, clayey volcanic soil) and corresponding soil particles. In the rest of the text, the red, clayey volcanic soil; the high-organic volcanic ash; and the black loamy Midwestern agricultural soil will be referred to, respectively, as “Poamoho”, “Lalamilo”, and “Drummer” soils. Physical, chemical and mineralogical data on these three soils are given in Tables 3.1 and 3.2.

X-RAY DIFFRACTION

The mineralogy of the soils were examined by X-ray diffraction (XRD; Siemens model D 500) using Cu Kά radiation from 5°- 65° 2Θ, with 0.02° 2Θ steps and scan time of 2s $step^{-1}$. A graphite monochromator eliminated high background Fe fluorescence. The XRD intensity was converted to mineral weight (%) data using an option in the RockJock computer program and according to the method given in Eberl & Smith (2009).

SOLUTIONS

All solutions were prepared using filter-sterilized deionized water (18 MΩ cm resistivity; Milli-Q, Millipore Corp., Bedford, MA). For the flow-through column studies, influent pH was maintained at 7.2 by adding 10^{-2} M NaOH as necessary. For the experiments in static columns the solution pH was adjusted as necessary with 10^{-2} M NaOH and HCl. Nitrate (10^{-3} M, added as $NaNO_3$) was used as a conservative tracer and measured by ultraviolet absorption (220 nm wavelength) using a spectrophotometer (Spectronic/Unicam, Genesys 10).

STATIC COLUMNS

The attachment studies with oocysts and microspheres were conducted with 20 mL static columns (25 mm diameter, 100 mm height) modified from those described by Scholl and Harvey (1992). Columns were prepared using polypropylene mesh (105-μm pore size) as an underlayment, on top of which was placed about 5 g of soil. Another polypropylene mesh was laid on top of the added soil and 4 g of glass beads (1 mm, diameter) were carefully placed on top of the mesh. This ensured that the soil bed did not

move when washing with background solution and prevented the low bulk density (0.7 gcm^{-3}) Lalamilo soils from floating. The columns were saturated and water level maintained at about 35 mm above the soil bed. The static columns were equilibrated by rinsing with at least 200 pore volumes of background solution. The pore volume of Poamoho and Lalamilo soil columns was approximately 5 mL, whereas that of Drummer soils was approximately 3.5 mL. One mL of Oocysts and microsphere suspensions (2×10^6 - 5×10^6 colloidal particles mL^{-1}) were added to the top of the column. From the bottom of the static columns, 1.0 mL of pore fluid was withdrawn in order to draw the suspension into the granular media. Previous kinetic studies revealed that the > 70 % of microspheres and oocysts attached within the first 2 h and equilibrium was reached in less than 4 h. After 4 h, 12 pore volumes of background solution (10^{-3} M NaCl) were passed through the column to remove unattached particles. The eluent was collected in a 30 mL capacity, acid-washed, baked (400° C) glass vial. Fractional attachment of oocysts and microspheres was calculated based upon the number of oocysts or microspheres recovered compared to the number that were added at pH 3, 6, and 9. All experiments were run in triplicates at 22°C.

FLOW-THROUGH COLUMNS

Transport studies were conducted using flow-through glass chromatography columns (25 mm diameter, 100 mm length). A 5 mL injection loop provided a "pulse input" of colloids to the column. Pore volumes (calculated by the time taken for tracer to break through) for the Poamoho and Lalamilo was 39 mL, while that of Drummer soil

was 30 mL. Polypropylene mesh (105-µm openings) was installed inside the polytetrafluoroethene end caps on both ends of the column.

Two different sets of flow-through column studies were employed. The first set was conducted using the Poamoho and the Drummer soils only. The soil fraction was dry-packed with vibration to minimize any layering. Carbon dioxide (2%) gas was passed for 1 h to remove any residual air in the column. The second set of experiments was conducted with Lalamilo soil using only the 1.8-µm microspheres in the absence of oocysts or 4.9-µm microspheres. Two types of studies were conducted, i.e., one employed unaltered soil and the other employed soil in which the soil organic matter (SOM) was removed by chemical oxidation using sodium hypochlorite (NaOCl) as modified from Siregar et al. (2005). For both experiments, the influent pH was 8.5.

Standard gravimetric methods were used to determine the porosity, bulk density and particle density of the soils. The background solution was fed to the column through polypropylene tubing using a computer-controlled piston pump (stainless steel, 500 mL volume, ISCO model 500D). The tracer solution, oocysts, and microspheres suspensions were added to the background solution using a high performance liquid chromatography injector (stainless steel; Supelco Rheodyne) and injection loop (stainless steel, 5.0 mL volume). For all experiments, the pump was filled with the background electrolyte solution (10^{-3} M NaCl) and the injection loop was filled with the conservative tracer nitrate (added as 10^{-3} M $NaNO_3$; pH 5.6), oocysts (2×10^{6} mL^{-1}), and microspheres (0.5-1×10^{7} mL^{-1} (1.8-µm) and 4×10^{6} mL^{-1} (2.9-µm and 4.9-µm)). The sodium nitrate and oocysts were co-injected as a single pulse. The *I* of the carrier and injection fluids were

the same. A fraction collector and glass test tubes were used to collect the column effluent.

Before each experiment, several hundred pore volumes of background solution were passed though the column until the pH and specific conductance levels of inlet and effluent solutions were the same. Equilibration time for each soil column took approximately one week. The pumping rates were set to produce pore velocities of 1.6 ± 0.1 m d^{-1} for all the experiments. At least three pore volumes of breakthrough were monitored for all experiments except those involving the Lalamilo soils, where 10 pore volumes were collected. Numerical integration of the breakthrough curve was carried out only for the Lalamilo soils. Two replicates were run for the Poamoho, Drummer and the unaltered Lalamilo soil only

TRANSPORT MODELING OF COLLOID TRANSPORT

The one dimensional advective dispersion equation for colloid transport and removal of colloids by physiochemical filtration for homogenous granular porous media under saturated flow conditions is given as:

$$\frac{\partial C}{\partial t} + \frac{\rho_b}{\varepsilon}\frac{\partial S}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x}$$

Where C is the colloid concentration in the aqueous phase at a distance x and time t, D is the hydrodynamic dispersion coefficient, v is the interstitial colloid velocity, S is the concentration of attached colloid, ρ_b is the bulk density of the granular porous media, and ε is the porosity.

The change in microbial concentration due to attachment and detachment processes is given as:

$$\frac{\partial(\rho_b S_{att})}{\partial t} = k_a \varepsilon C - k_r \rho_b S_{att}$$

Where S_{att} is the attached concentration of the colloid, k_a and k_r are the first order attachment and first order detachment rate coefficients respectively.

Colloid filtration theory can be incorporated in this model by using k_a term (Ryan and Elimelech, 1996)

$$k_a = \frac{3}{2}\frac{(1-\varepsilon)}{d_g}\eta\alpha v$$

where d_g is the grain size diameter, η is the single-collector removal efficiency, α is the attachment efficiency and v is the pore water velocity. The value of collector efficiency accounts for the colloid removal due to diffusion, sedimentation and interception and calculated according to Tufenkji and Elimelech (2004). The attachment and detachment rate coefficients, collector efficiency, collision efficiency was calculated previously from "Colloid Filtration Model".

The breakthrough curve data, obtained were used to fit parameters k_a, k_r, dispersivity and collision efficiency using HYDRUS-1D computer code (Simunek et al., 2005). Finally, the extent of colloid removal within the soil columns and the retardation of peaks and center of mass were calculated for all the colloids.

RESULTS

PHYSICAL/MINERALOGICAL COMPOSITION

The data from the XRD analysis are presented in Tables 3.1. The range represents the maximum and minimum values of six samples for each soil type. For the Poamoho soils, the iron oxide composition varied between 22%-29% w/w. Clay minerals comprised between 68%-76% of the total mass, a majority of which were identified as kaolinite. For the Lalamilo soil, the organic matter made up 43%-46% of the total mass, whereas iron oxide (maghemite) made up 14%-16%. In contrast, the Drummer soils were rich in both quartz (40%) and organic matter (13%) with a considerably smaller content (2%) of iron oxide.

Table 3.1. Physical and Chemical characteristics of granular porous media

Property	***Poamoho, Oahu***	***Lalamilo, Hawaii***	***Drummer, Illinois***
Order	Oxisol	Inceptisol	Mollisol
Type	Clay loam	Silt loam	Silty clay loam
Sampled Soil Interval (A-Horizon, mm)	0-300	0-300	0-300
Avg. Grain Density (g/cm^3)	2.66	2.37	2.65
Bulk Density (g/cm^3)	0.92	0.70	1.20
Porosity	0.66	0.70	0.55
pH (1:1, KCl)	5.30	5.80	5.60
d_{50} (mm)	0.65	0.21	1.00
Avg. Grain Fraction (mm)	0.35	0.20	1.00
Pore Volume (mL)	39	39	30

Table 3.2. Mineralogy of granular porous media used for the experiments. The range represents the maximum and minimum values of six samples for each soil type.

Property (w/w %)	*Poamoho soil*	*Lalamilo soil*	*Drummer soil*
Non-clay materials			
Quartz	0	0.9	40
Plagioclase feldspar	0	12-16	0
Potassium feldspar	0	0	8
Magnetite	0	2-3	0
Hematite	5-8	0	0
Maghemite	17-19	15	2
Organic Matter	0	36-43	13
Total non-clay	22-27	66-78	63
Clay Materials			
Smectite	0-5	7-13	14
Amorphous	29-44	0	1
Illite	7-17	7-9	0
Halloysite	14-24	0	2
Muscovite	0	0	8
Total clay	50-90	14-21	27

COLLOID SIZE AND CHARGE

The average diameter of oocysts was measured as 3.6 ± 0.3 μm (± 1 standard deviation) by flow cytometry and confirmed by epifluoresence microscopy. The buoyant density of oocysts was reported previously as 1.075±0.005 g cm^{-3} using Percoll density gradient centrifugation by (Abuldalo et al., 2005). The zeta potential of the microspheres

and oocysts were negative (Figure 3.1) for the pH used in the static and flow-through column experiments.

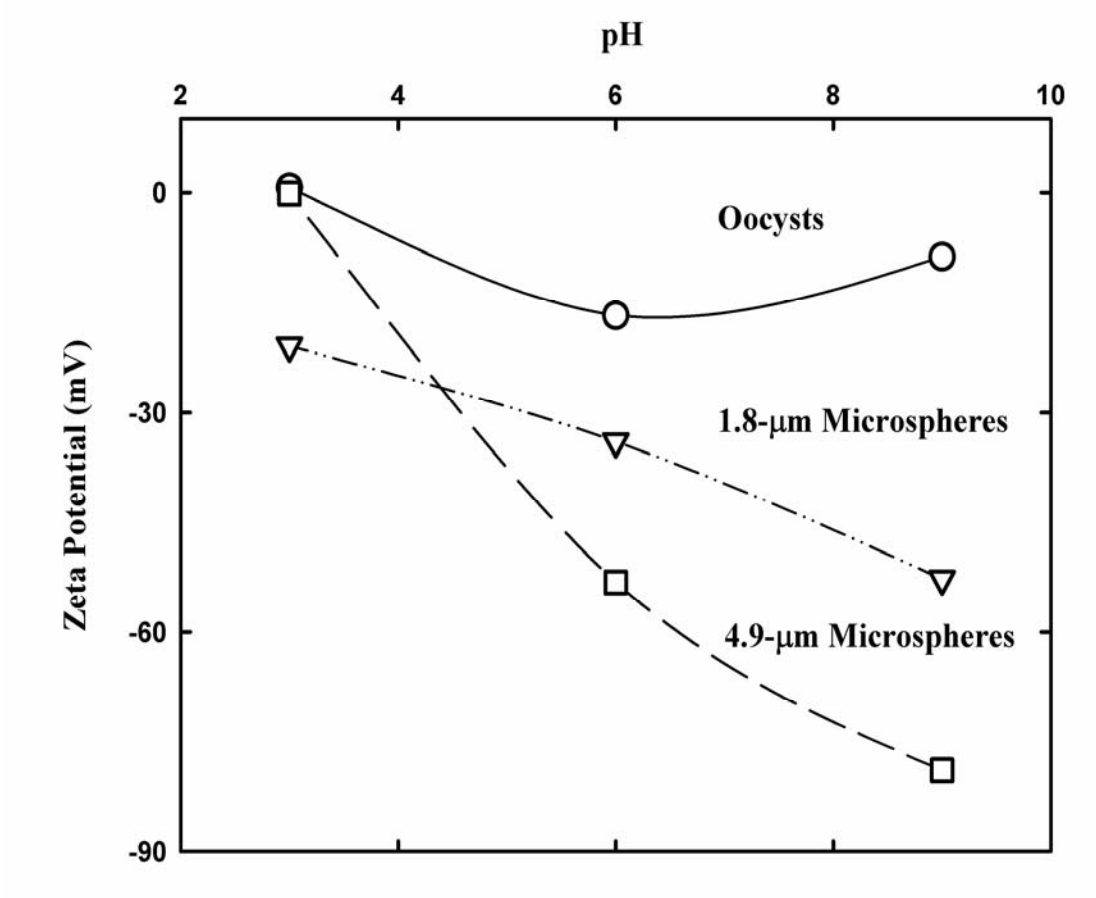

Figure 3.1. ζ- potential of formalin inactivated *C. parvum* oocysts (4×10^5 mL^{-1}) and oocysts sized microspheres (4×10^6 mL^{-1}) suspended in 10^{-3}M NaCl as measured by Doppler micro electrophoresis. 10^{-2} M Sodium hydroxide and Hydrochloric acid were used to adjust the pH.

The microspheres became more negatively charged with increasing pH from 3 to 9. The 4.9-μm microspheres were more negatively charged (-0.12 mV at pH 3 and -79 mV at pH 9) compared with the 1.8-μm microspheres (-21mV at pH 3 and -53mV at pH 9). However, the oocysts exhibited a substantive negative charge (ζ of -17 mV) at pH 6, but

were nearly uncharged at pH 3 and at pH 9 (ζ of only +0.7 mV and -9 mV, respectively). The maximum standard deviation for replicate ζ values determinations was 2.6 mV.

STATIC COLUMNS

A comparison of the pH dependencies of oocyst and microsphere attachment for the three soil types is shown in Figure 3.2. For the microspheres, attachment was strongly pH dependent in the Drummer and Poamoho soils, but relatively independent of pH in the presence of Lalamilo soils. For the 1.8-μm microspheres, fractional attachment in the presence of Drummer soil decreased from 90% at pH 3 to 50% at pH 9 and for Poamoho soil from 99% at pH 3 to 74% at pH 9. However, fractional attachment in the presence of Lalamilo soil remained nearly constant at 67% from pH 3 to 9. Similarly, in response to a pH increase from 3 to 9, fractional attachment of 4.9-μm microspheres dropped from 99% to 79% for Drummer soils and from 99% to 82% for Poamoho soils. For the oocysts, fractional attachment was strongly pH-dependent in the Drummer soils (decreasing from 92% at pH 3 to 29% at pH 9), insensitive to pH (ranging from 98 to 99%) for the Lalamilo soils and slightly pH dependent for Poamoho soil (decreasing from 99% at pH 3 to 86% at pH 9).

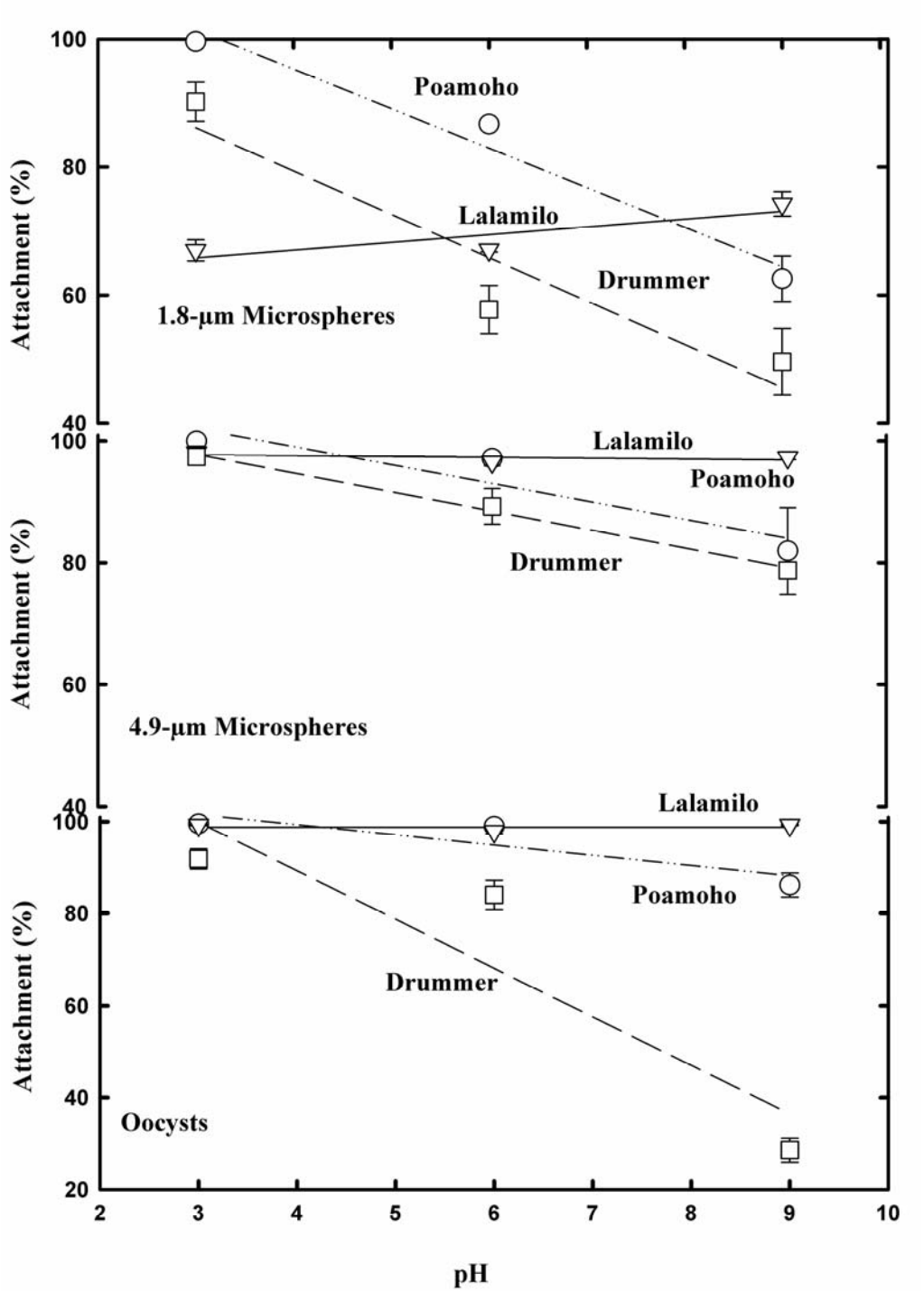

Figure 3.2. Attachment (%) at 22°C±1°C of *C. parvum* oocysts and microspheres (2×10^6 - 5×10^6 mL^{-1}) to 5 g soil packed in 100 mm static columns. Fractional attachment determined by difference between concentration of colloid added and recovered subsequent to flushing. The trend lines indicate best-fit linear regression for each soil. The correlation coefficients varied between 0.78-0.98 at $p \leq 0.001$. The error bars represent standard errors.

FLOW-THROUGH COLUMNS

Poamoho soil. Fractional breakthrough (C/Co) was plotted as a function of the number of pore volumes passing through the 100-mm long column (Figures 3.3, 3.4 and 3.5). For all experiments, peak breakthrough occurred earlier for oocysts and microspheres than for the conservative tracer (nitrate). For the Poamoho soils (Figure 3.3), peak abundance of oocysts arrived at 0.46 pore volume, PV, versus 1.0 PV for nitrate and 0.62 PV and 0.72 PV, respectively, for the 4.9-µm and 1.8-µm microspheres. There were no significant differences between the retardation values of colloids between the replicate values of all the experiments conducted using the three soils. Only about 2% and 1%, respectively, of the 3.6-µm oocysts and 4.9-µm microspheres were transported through the entire length of the column as compared with 18% of the smaller (1.8-µm) microspheres. The retardation factor of the microsphere peak (0.72) through the Poamoho soil was slightly lower than retardation factor estimated for the center of mass (0.79) (Table 3.4). Calculated attachment and detachment rate coefficients for the 1.8-µm microspheres were 39.6 d^{-1} and 1.11 d^{-1}, respectively.

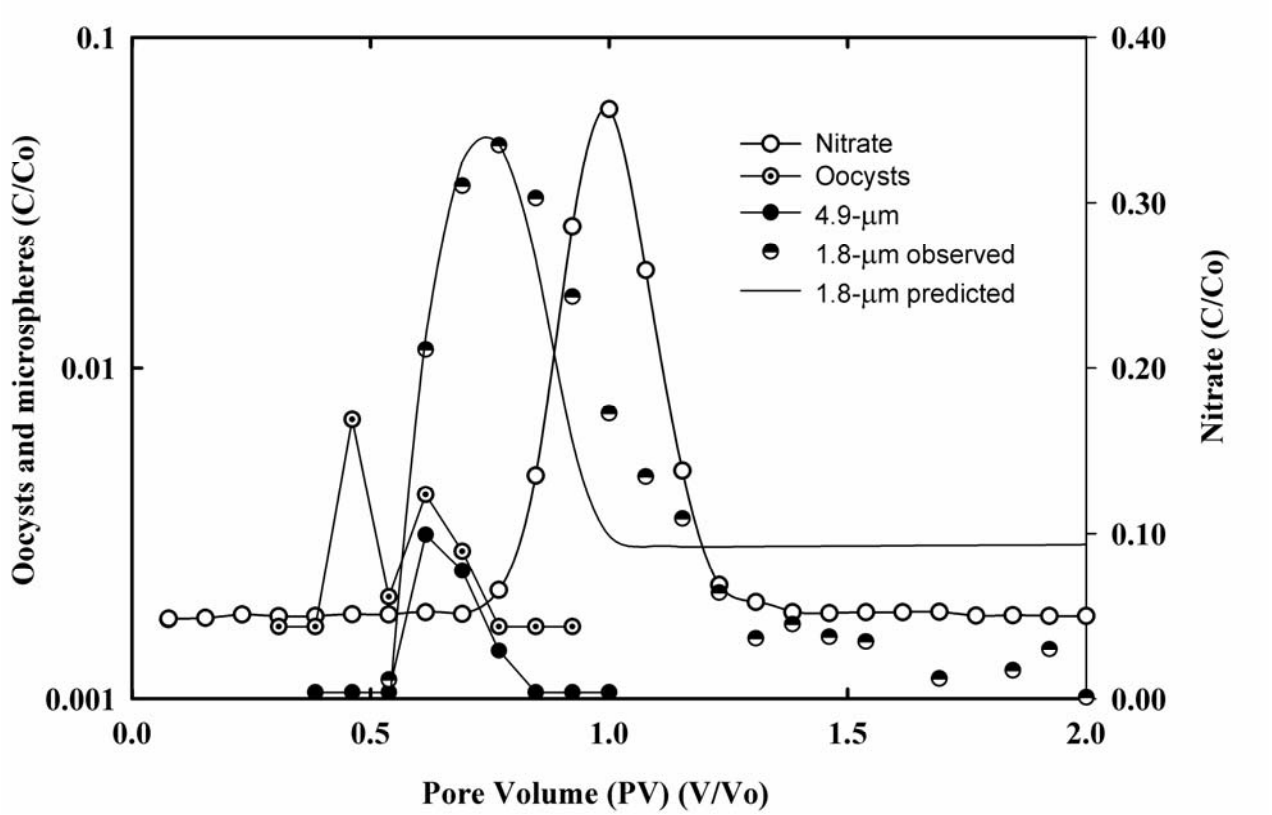

Figure 3.3. Dimensionless concentration histories at 22°C±1°C for conservative tracer, *C. parvum* oocysts, 1.8-µm and 4.9-µm microspheres being advected in 10^{-3}M NaCl through 10 cm Poamoho soil (d_{50} = 0.65 mm) at 1.6 md^{-1} and pH 7.2.

Lalamilo soil. The effect of removal of soil organic matter (SOM) on the transport of 1.8-µm microspheres in the Lalamilo soils is depicted in Figure 3.4. Dimensionless concentrations of microspheres in the effluent of flow-through columns packed with the unaltered organic-rich Lalamilo soil peaked at approximately 3.5×10^{-5} during the first collected pore volume, but exhibited substantive breakthrough (tailing behavior) for the remainder of the experiment. For the subsequent 9 pore volumes following the initial peak, fractional breakthrough of microspheres averaged ~1.8×10^{-5}. Extrapolation based upon the slope of the tailing portion of the breakthrough curve, suggested that substantive breakthrough could have continued for several thousand pore volumes had the test not

been terminated. A subsequent column study employing Lalamilo soil where much of the SOM had been removed by chemical oxidation exhibited a peak in fractional breakthrough of 1.8-µm microspheres (1.2×10^{-2}) that was unexpectedly more than 300 times higher than that observed for the unaltered soil. Also, the onset of detectable microsphere breakthrough (at 0.84 PV) occurred significantly earlier (at 0.54 PV) in the absence of the SOM. Although most (95%) of the 1.8-µm microspheres were also immobilized in the latter column study, attachment was considerably less reversible. The retardation factors for 1.8-µm microspheres also differed substantively between unaltered and chemically oxidized Lalamilo soil. For the unaltered soil, the retardation factor based upon transport of peak breakthrough (0.84) differed from that based upon the center of mass (1800-2100) by more than 2000 fold. On the other hand, retardation factors for the chemically oxidized soil were more similar (0.54 based upon peak breakthrough versus 0.91 based upon the center of mass). Moreover, the attachment rate coefficients (k_a) for the unaltered Lalamilo soil was faster (94.3 d^{-1}) than that observed for the chemically oxidized soil (k_a = 8.6 d^{-1}) while the detachment rates were almost equal (0.36 d^{-1}).

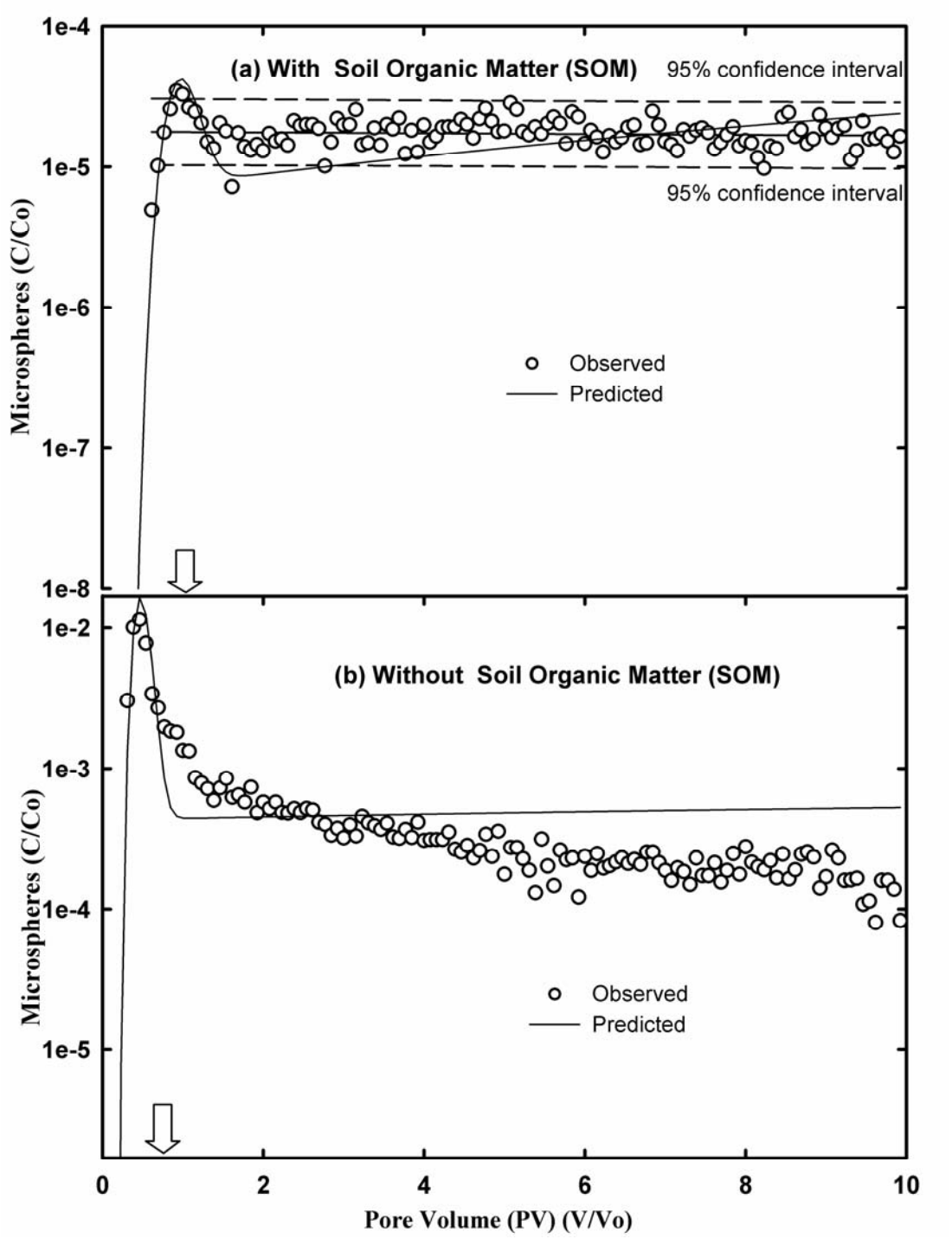

Figure 3.4. Dimensionless concentration histories at 22°C±1°C for 1.8 -µm microspheres being advected in 10^{-3}M NaCl through 100-mm Lalamilo soil column (d_{50} = 0.21 mm) at 1.6 $md^{-1}$and pH 8.5. Arrow indicates the point of nitrate breakthrough.

Drummer soil. Transport of oocysts and microspheres through the quartz-rich Drummer soils is shown in Figure 3.5. Peak abundance of oocysts and microspheres broke through much earlier (at 0.67 PV) relative to that of nitrate (at 1.0 PV). For the 1.8-µm microspheres, retardation based upon peak breakthrough and upon centers of mass were similar (0.67 and 0.64, respectively). As observed for the Poamoho soil, the vast majority of oocysts and 4.9-µm microspheres were immobilized within the column. Only 0.4% of oocysts and 0.7% of 4.9-µm microspheres were recovered in the eluent, although 25% of the smaller (1.8-µm) microspheres were transported through the entire length of the column. The calculated attachment rate coefficient for the latter microspheres (36 d^{-1}) was considerably faster than that for detachment (0.09 d^{-1}).

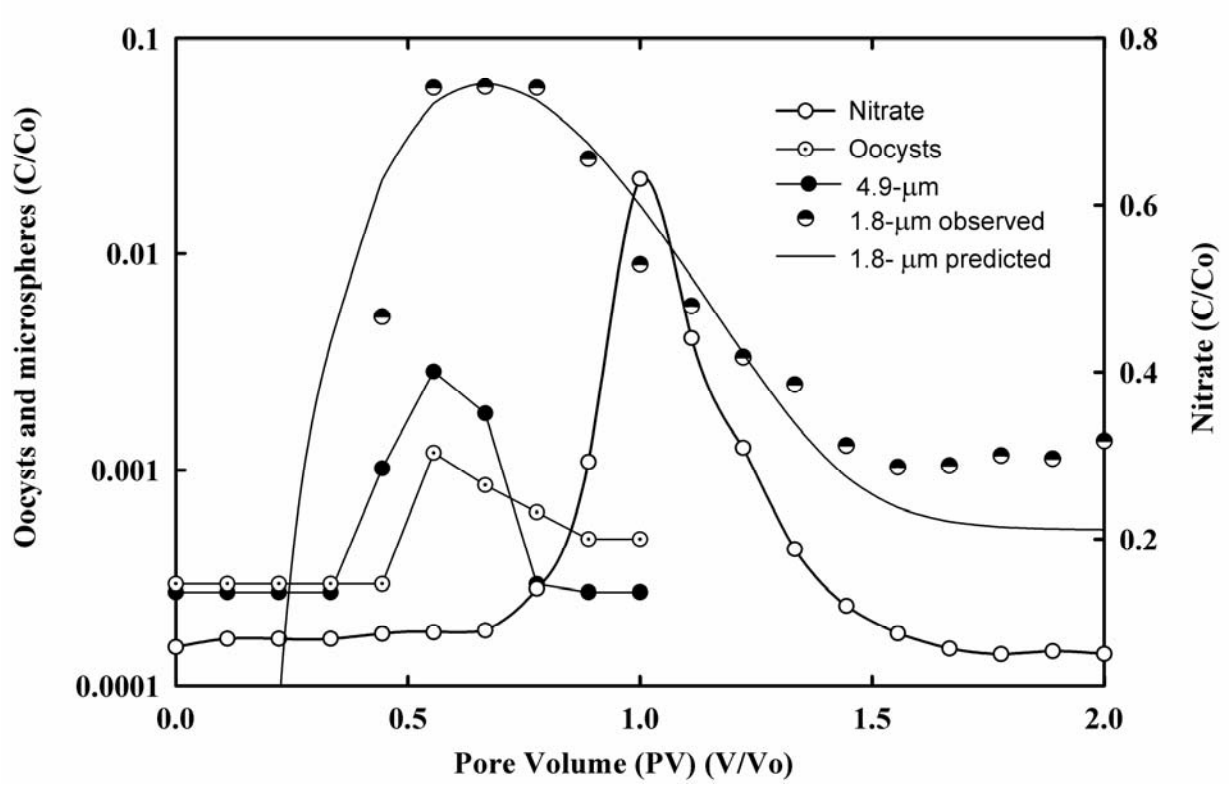

Figure 3.5. Dimensionless concentration histories at 22°C±1°C for conservative tracer, *C. parvum oocysts*, 1.8-µm and 4.9-µm microspheres being advected in 10^{-3}M NaCl through 100- mm Drummer soil (d_{50} = 1 mm) at 1.6 md^{-1} and pH 7.2.

DISCUSSION

pH DEPENDENCY OF COLLOIDAL ATTACHMENT

Although the zeta potentials for oocysts and oocyst-sized microspheres at 1 mM *I* were clearly pH-dependent (Figure 3.2), pH had little effect on the propensity of either colloid for attachment within the Lalamilo soil. The high organic content (43%-46%) in this tropical volcanic ash soil may preclude the microspheres from interacting directly with the otherwise prevalent maghemite surfaces (Table 3.1). The specific manner in which SOM affects the pH-dependency of microbial attachment in soils is difficult to predict, in part, because of its structural complexity. However, findings from other studies suggest that even modest quantities of SOM can greatly diminish the pH-dependency of microbial attachment onto iron-rich soils and sediments (Harvey et al, in press). Consequently, perturbations in pore water pH following precipitation events would be expected to have a lesser effect upon re-entrainment of oocysts in Lalamilo soils than in the other two soils.

In contrast, attachment of oocysts and microspheres in the second weathered volcanic (Poamoho) soil from Hawaii was strongly pH-dependent (Figure 3.2). The pH-dependency of colloidal attachment, particularly for the well-defined, carboxylated microspheres, was surprisingly similar to that observed for our reference Midwestern agricultural (Drummer) soil, despite the disparity in mineral composition (Table 3.1) of the two soils. The steady decrease in attachment (increase in transport potential) with increasing pH was consistent with concomitant increases in the magnitude of negative charge of the colloid, judging from the zeta potential (Figure 3.1). This would occur as

the metal oxide-rich grain surfaces become less electropositive. Consequently, at higher pH, the microspheres and grain surfaces should become more electrostatically repulsive.

It was less clear why oocyst attachment within the Drummer soil was more sensitive to changes in pH than was attachment in the Poamoho soil. Our initial hypothesis was that attachment would be much more pH sensitive in the presence of Poamoho soil than in Drummer soil, because the net charges of the grain surfaces in the former are dominated by abundant iron and aluminum oxides. We suspected that under alkaline conditions (pH 9) less attachment (more transport) of oocysts occurred within the Drummer soil because of the presence of quartz, which constituted up to 40% of the bulk material and presumably promoted stronger negative charges on the grain surfaces relative to the iron/aluminum oxide dominated Poamoho soil. (Harvey et al., 2002) found a 70% decrease in fractional attachment of native bacteria in the presence of iron-coated quartz sand collected from the Cape Cod aquifer when pH was increased from 5.8 to 7.9.

Although the larger (4.9-μm) carboxylated microspheres replicated to a significant extent the pH-dependency of oocyst attachment behavior within the two volcanic soils, the pH dependencies of microsphere- and oocyst attachments in the reference Drummer soil were clearly different. In contrast to the carboxylated latex microspheres, oocyst surfaces are highly complex with multiple layers of glycoprotein and glycolipids that collectively exhibit a different pattern in which zeta potential varies with pH (Figure 3.2). Glycoproteins on the oocyst surface would be expected to exhibit a different pH dependent charge behavior due to the presence of functional groups associated with the cysteine, proline, and histidine amino acids (Tilley and Upton, 1997),

which have different associated points of zero charge (PZC). (Considine et al., 2001) have observed that glycoproteins on the surfaces of oocysts have a tendency to behave like a "polyelectrolytic brush" with charged surfaces extending from the oocysts surface to the solution. Consequently, the more complex surface chemistry of oocysts may make their attachment more pH sensitive in the presence of the quartz-rich Drummer soil than the compositionally simpler microspheres. As was observed in an earlier study involving colloid transport in karst limestone (Harvey et al., 2008), the suitability of microspheres as surrogates for oocysts in soils may depend, at least in part, upon the chemical conditions under which the tests are run.

RELATIVE TRANSPORT VELOCITIES

For the flow-through column studies, faster transport of the peak concentrations of microspheres and oocysts relative to those for nitrate for the Poamoho, Lalamilo, and Drummer soils (Figures, 3.3, 3.4 and 3.5, respectively) suggest that transport of the colloids that have not come into contact with grain surfaces may be more rapid than would be predicted by a conservative tracer. The "velocity enhancement" effect for 1.8-μm colloids was particularly noticeable for the Poamoho and Drummer soils ($RF_p = 0.72$ and 0.67, respectively; Table 3.4) and is likely due to a combination of preferred flow-path structure and size-exclusion effects.

Table 3.3. Removal efficiencies of 1.8-µm microspheres, Retardation coefficients and fitted parameters (ka ,kr) obtained from the data of the breakthrough curve of different granular porous media.
[1]Oocysts- 97.8% (Poamoho soil), 98.6 % (Drummer soil), [2]The corresponding values for oocysts and 4.9-µm colloids were 0.70 and 0.89 respectively. [3]The corresponding values for oocysts and 4.9-µm colloids were 0.99.

Granular media	***% Removed [1]/decimeter travel distance***	k_a $(day)^{-1}$	k_r $(day)^{-1}$	R^2
Poamoho soil (pH-7.2)	82.0	39.60	1.11	0.92[2]
Lalamilo soil (pH-8.5) unaltered soil	99.9	94.30	0.36	0.34
Lalamilo soil (pH-8.5) treated (SOM extracted)	99.6	8.60	0.35	0.89
Drummer soil (pH-7.2)	98.6	36.00	0.09	0.94[3]

The Poamoho soil, which is characterized by a high (>50%) clay content and water-stable aggregates, holds substantial amounts of immobile water (Uehara and Gillman, 1981) and exhibits visible macropore structure when re-wetted. The occurrence of preferential flow paths in granular media has been related to clay content in at least one other study (Kjaergaard et al., 2004) and is commonly observed in aggregated packed soils (Biggar and Nielsen, 1962). Macropores important for colloid transport are generally characterized by higher velocities and less tortuosity, the average velocities of the

colloids appearing in the eluent would be greater than that of the conservative tracer (Grolimund et al., 1998). Presumably because of the size-exclusion effect, the peak concentrations of the larger (4.9-µm) and oocysts (3.6-µm) eluted from the Poamoho soil earlier than the smaller (1.8-µm) microspheres. In a natural-gradient test conducted in a sandy aquifer in Cape Cod, MA, it was found that 1.70-µm sized microspheres broke through earlier than 0.7-µm microspheres (Harvey et al., 1989). Similarly, it has been reported that the 3-µm colloids arrived earlier than 50 nm microspheres and MS2 virus (of diameter 25 nm) (Keller et al., 2004)). Consequently, for Poamoho and Drummer soils, the time required for pathogens to breakthrough down gradient would depend, in part, upon the size of the organism. The "velocity enhancement" for the 1.8-µm microspheres relative to that of the conservative tracer in the eluent of the Lalamilo soil column was clearly related to presence or absence of the SOM. Removal of the organic matter by chemical oxidation had the effect of reducing the (RF_p) from 0.84 to 0.54 (Table 3.4). Although the specific reason(s) for this are not clear, we hypothesize that the SOM, which constitutes up to 46 % of the solid phase, occupies much of what would otherwise be preferred flow-path structure. Consequently, its removal by chemical oxidation re-creates more macroporosity capable of transporting colloids.

Table 3.4. Summary of the Retardation coefficients, and cumulative recovery of all colloids for replicate experiments

Soil	*Colloid*	*Run*	*Retardation [1] factor for peak breakthrough(Rf_p)*	*Retardation factor for center of mass (Rfcm)*	*Σ(C/Co)*	*P value*
Poamoho	1.8-µm	1	0.72	0.79	0.180	0.101
		2	0.85	0.78	0.012	0.144
	4.9-µm	1	0.62	0.65	0.010	0.177
		2	0.77	0.74	0.001	0.183
	Oocysts	1	0.46	0.54	0.018	0.172
		2	0.54	0.51	0.004	0.181
Drummer	1.8-µm	1	0.67	0.64	0.250	0.062
		2	0.62	0.70	0.088	0.134
	4.9-µm	1	0.55	0.54	0.007	0.178
		2	0.55	0.64	0.009	0.180
	Oocysts	1	0.67	0.59	0.004	0.183
		2	0.27	0.24	0.005	0.177
Lalamilo soil (pH-8.5) (with SOM)	1.8-µm	1	0.67	1800-2100[2]	0.003	NA
		2	0.69	1200-1300[3]	0.001	NA
Lalamilo soil (pH-8.5) treated (SOM extracted)	1.8-µm	1	0.54	0.91	0.004	NA

[1] Calculated as ratios of time required to reach peak abundance for microspheres to time to peak concentration for the conservative tracer.

[2,3] Simulated data, extrapolated for 99% recovery of mass.

REVERSIBILITY OF COLLOIDAL ATTACHMENT

The model could capture the tail of the breakthrough curve for the Poamoho and Drummer soils but not for the Lalamilo soil without organic matter. Colloidal attachment appeared to be much weaker in the organic-rich Lalamilo soil than in the other two soils. For the Poamoho soil (Figure 3.3), chemically oxidized Lalamilo soil (Figure 3.4), and Drummer soil (Figure 3.5), only a modest degree of tailing was evident from the breakthrough curves of the 1.8-μm microspheres relative to those of the conservative tracer. Tailing is generally caused by the release of microspheres previously attached to grain surfaces. A subsequent experiment designed to assess reversibility of colloid detachability in Poamoho and Drummer soils indicated that few attached microspheres detached during the passage of 10 pore volumes of deionized water at pH 7, suggesting that deposition in these soils is essentially irreversible (Mohanram et al., unpublished data).

In contrast, for the 1.8-μm microspheres advecting through Lalamilo soil, the model could only capture few of the data points because deposition on this soils was found to be highly reversible. This was evidenced by the substantive and protracted tail that was about one half the height of the peak. We hypothesize that the reversibility of colloidal attachment in this soil type is due to the abundance of SOM- because attachment was less reversible after the extraction of SOM and the breakthrough curve changed to a steadily decreasing tail (Figure 3.4). This suggests that the organic-rich Lalamilo soil may act as a reservoir for the accumulation of pathogens and detachment of colloids into pore water would be likely, following precipitation events. In spite of the

high reversibility of microspheres, it is not clear that this soil would be ineffective in permanently removing oocysts. This is largely because of the 3.6 micron oocysts have difficulty passing through these very fine-grained sediments. However, there is a good possibility that smaller pathogens, particularly those < 2-microns in size that would be subject to straining to a much lesser degree, would slowly be transported through this soil, largely because of the highly reversible nature of attachment in this soil. Because viable oocysts can persist in some environments for up to one year (Current, 1988) and because ingesting even a few oocysts can cause cryptosporidiosis (Rose, 1997; Casemore et al., 1997), more caution may need to be exercised when grazing cattle on Lalamilo soil to preclude contamination of groundwater or nearby surface water. Although the Poamoho soil in Oahu appears to be an efficient filter in spite of the macropore structure that develops upon rewetting, the organic-rich Lalamilo from the island of Hawaii clearly is not.

COMPARISON OF TRANSPORT BEHAVIOR OF OOCYSTS AND MICROSPHERES

All our experiments were conducted to check the efficacy of microspheres in simulating the transport of oocysts. A polydispersed mixture of 1.8-µm, 2.9-µm, and 4.9-µm were used to bracket the size range of oocysts used in our study. It was found that although the 4.9-µm microspheres are slightly larger than the oocysts (3.6-µm), the 4.9-µm microspheres seem to closely match the oocysts behavior in terms of retardation and filtration efficiency. On average, peak arrival times for the oocysts and 4.9-µm microspheres varied by only ±0.1 PV in both Poamoho and Drummer soils. Moreover,

4.9-µm colloid and oocysts exhibited similar attenuation within the soil. Although microsphere applications have been shown to be safe, the structures of oocysts are much more complex. Also, it has also been reported that microspheres transport is quite different from oocysts and over-predict or under-predict oocysts transport in laboratory and field scale studies (Harvey et al, in press). Consequently, experimental findings resulting from using microspheres as transport surrogates should be treated with caution.

CONCLUSIONS

All three soils examined in our study appear to be good filters for removing *C. parvum* oocysts over the short-term (first few pore volumes). In particular, the fast attachment rates combined with very slow detachment and high filtration capacities suggest that the red iron-, aluminum-, and clay- rich (Poamoho) soil from Oahu and the reference agricultural (Drummer) soil from Illinois should be highly effective at filtering out *C. parvum* oocysts. However, we hypothesize that the highly reversible attachment behavior caused by the abundant SOM (43%-46% w/w) in the volcanic ash-derived (Lalamilo) soil from the island of Hawaii could serve as a reservoir of oocysts for their reintroduction into ground or surface water over a protracted period. This was in spite of the high iron oxide content of these soils, which provided favorable sites for the irreversible attachment of microspheres. Clearly, the relative abundance of SOM critically affects the efficacy of tropical volcanic soils for removing pathogens and needs to be taken into account when optimizing agricultural practices to lessen the potential for pathogen contamination of underlying groundwater resources. Future studies will

include the roles of cow manure, surfactants, and clays on oocysts transport behavior in tropical volcanic soils.

Major findings from this study include:

- Attachment was pH dependent in the Poamoho and Drummer soil but pH independent in the organic-rich Lalamilo soil.
- "Velocity enhancement" of oocysts relative to a conservative tracer was observed for Poamoho and Drummer soils, indicating that oocysts and oocyst-sized microspheres are transported primarily through preferential flow paths in these complex soils. In spite of the presence of macropores, these soils were efficient filters for removing oocysts and microspheres.
- 'Tailing' caused by highly reversible attachment dominated the colloidal transport behavior in the volcanic ash (Lalamilo) soil indicating that the fate and transport of colloids across these soils is clearly different from Poamoho and Drummer soils. Although oocysts would have difficulty in passing through these fine grained soils, water borne pathogens of smaller diameter (< 2-μm) can be slowly be transported because of the reversible nature of attachment in this soil.
- Finally, it was found that 4.9-μm carboxylated microspheres appeared to be better surrogates for assessing the abiotic aspects of oocyst transport behavior than the 1.8-μm microspheres, although the suitability of microspheres as surrogates was clearly pH-dependent.

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CHAPTER IV

EFFECT OF DISSOLVED ORGANIC CARBON ON THE TRANSPORT OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS AND MICROSPHERES IN AGRICULTURAL SOILS

ABSTRACT

Transport of *Cryptosporidium parvum* oocysts and microspheres in two disparate agricultural soils (a clay-and Fe-rich, tropical, volcanic soil and a temperate, humic soil) were studied in presence and absence of 100 mgL^{-1} of dissolved organic carbon (DOC)-Sodium dodecyl benzene sulfonate (SDBS), and Suwannee river humic acid (SRHA) at pH 5.0-6.0. Transport of carboxylate-modified, 1.8-μm microspheres was highly sensitive to the nature of the DOC, whereas oocysts transport was more affected by soil mineralogy. The presence of SDBS increased transport of microspheres from 48% to 87% within a column packed with tropical soil, and from 43% to 93% in temperate soil. However, SRHA reduced transport of microspheres from 48% to 28% in tropical soil and from 43% to 16% in temperate soil. Oocysts transport through the temperate soil increased from 0.40% to 2.0% in the presence of the SDBS, whereas no oocyst transport was detected in tropical soil. Oocysts transport with SRHA increased from 0.11% to 0.17% in tropical soil and from 0.40% to 2.4% in temperate soil. Adding 4 mgL^{-1} SRHA and 4 mgL^{-1} SDBS decreased oocyst hydrophobicity from 66% to 20% and from 66% to 5%, respectively. Addition of 4 mgL^{-1} SDBS increased microspheres hydrophobicity from 16% to 33%. The soil fines component and 4 mgL^{-1} SRHA increased the magnitude of oocysts zeta potential (ζ), but decreased microspheres ζ. The disparate behaviors of the two colloids in the presence of an ionic surfactant and natural organic matter suggest that microspheres are probably not suitable particulate surrogates for oocysts in transport studies involving soils. Results from this study indicate that whether or not DOC inhibits or promotes transport of oocysts and microspheres in agricultural

soils and by how much depends not only on the surface characteristics of the colloid, but the nature of the DOC and the mineralogy of granular porous media.

INTRODUCTION

Agricultural runoff and seepage is typically high in DOC because of applications of manure, pesticides, and recycle water. Agriculture involving livestock can also produce high numbers of pathogens, including oocysts of the protozoan parasite, *Cryptosporidium parvum* (Rose 1997). It has been shown that the presence of DOC promotes the transport of bacteria (Johnson et al., 1996), viruses (Jin et al., 2000), and *C. parvum* oocysts (Abudalo et al., 2010). However, the aforementioned studies were conducted using model porous media, not natural soils. The few studies that have examined transport in natural granular media have shown that oocysts do not readily attach to soil particles (Dai et al., 2003a) and they can be transported through preferential flow paths (Darnault et al., 2004b). It has been reported that viability of oocysts depends, at least in part, on the soil type (Jenkins et al., 2002) and that oocysts can remain infective for at least 6 months in the subsurface around dairy farms (Kato et al., 2004). There is a dearth of information available on the transport of oocysts through agricultural soils in the presence of elevated DOC.

Many researchers have provided insight into the complex surface characteristics of the oocyst wall by looking at their macromolecular composition (Jenkins et al., 2010), hydrophobicity (Kuznar et al., 2006), interaction with model porous media using atomic force microscopy (Considine et al., 2002), and molecular spectroscopy studies (Gao et al., 2009). Although the surfaces of carboxylate-modified microspheres are simple by comparison, they have been used as particulate surrogates for oocysts in field-scale

(Harvey et al., 2008), pilot-scale (Dai et al., 2003b, Emelko et al., 2004), and column-scale studies (Bradford et al., 2007, Mohanram et al., 2010).

We undertook a systematic study to understand better the effects of humic acid and anionic surfactants on the transport of oocysts and microspheres in two contrasting agricultural soils from a temperate and a tropical region of the US. Iron-oxide coatings on grain surfaces can promote attachment of oocysts (Abudalo et al., 2005), but recent findings shows that the presence of anionic surfactants such as SDS diminishes oocysts complexation on hematite surfaces, and possibly increases oocysts mobility (Metge et al., 2010) . Given the compositional differences of the two disparate agricultural soils and the two colloids, our major hypothesis was that the differences in the degrees of attachment exhibited by oocysts and oocyst-sized microspheres during advective transport will depend strongly on both the nature of the dissolved organic matter as well as the differences in their respective surface characteristics.

METHODS

CRYPTOSPORIDIUM PARVUM OOCYSTS

Formalin-inactivated oocysts (3.6-µm, average diameter) were obtained from Sterling Parasitology Laboratory (SPL) at the University of Arizona and prepared according to the methods outlined by Abudalo et al. (Abudalo et al., 2005). The final oocyst a used for the column experiments was between $2\text{-}6\times10^{6}$ mL^{-1}. Enumerations were performed using epifluorescence microscopy (Mohanram et al., 2010).

MICROSPHERES

Carboxylate-modified, polystyrene microspheres in the1.8-µm and 4.9-µm diameter size classes (Type BB, brilliant blue) and 2.9-µm diameter size class (Type YG, fluorescent green) (Polysciences, Warrington, Pennsylvania) were used to create a polydispersed microsphere suspension that collectively bracketed the 2-5-µm variation in oocyst size commonly found in the environment (Harvey et al., 2008).

ZETA POTENTIAL

Electrophoretic mobilities of microspheres and oocysts were measured at 22°C in a 10^{-3} M NaCl solution by Laser Doppler micro-electrophoresis (Zeta Pals-Zeta Potential Analyzer- Brookhaven Instruments) using approximately 4×10^{5} oocysts mL^{-1} or 4×10^{6} microspheres mL^{-1}. Electrophoretic mobilities were converted to zeta potential (ζ) using the Smoluchowski equation (Masliyah et al., 2006).

DISSOLVED ORGANIC CARBON (DOC)

We used Suwannee River humic acid (SRHA) to assess the effect of natural organic matter (NOM) on the transport behavior of oocysts and oocyst-sized microspheres in soils. SRHA was obtained from the International Humic Substance Society (IHSS Code: S101H). It has an average molecular weight of 3820 $gmole^{-1}$ and contains 15% aliphatic and 42% aromatic compounds (Chin et al., 1997). SDBS (Sigma Aldrich, St. Louis, MO, molecular weight 348.5 $gmole^{-1}$) was added to a background solution of 10^{-3}M NaCl. Sodium hydroxide at 10^{-2}M was used to adjust the pH of both SRHA and SDBS solutions.

SOIL FINES

The soil fines, which include the clay minerals, from the two soils examined were isolated according to an earlier published procedure (Moore et al., 1989). Briefly, 1g of < 63-µm particle size soil fraction was added to 250 mL of purified water (Milli-Q, 18 MΩ resistivity) , sonicated for 1 min and centrifuged in Sorval RC-5 (Rotor: HS4, bucket type) at 192 *g* for 2 min. in order to separate the < 1-µm fraction of fines from the coarser materials. The size distribution of the fines was confirmed by Dynamic Light Scattering (Brookhaven Instruments) technique. The process was repeated until the supernatant was relatively clear and then siphoned to another container. The collected supernatant was then centrifuged on Beckman J2-21 (rotor JA-14) at 22060 *g* for 2 hrs to precipitate the soil fines, which were then dried at 70°C overnight.

BATCH EXPERIMENTS

Batch experiments were conducted to study the electrophoretic mobility of oocysts and microspheres in the presence of soil fines and SRHA. The soil fines at concentration of 200 mgL^{-1} were dissolved in 10^{-3} M NaCl and sonicated for 1 min to disperse the aggregates. *C. parvum* oocysts (5×10^{5} mL^{-1}) or 1.8-µm microspheres (6×10^{6} mL^{-1}) were added to 10 mL of either (1) soil fines solution (2) soil fines solution containing 4 mgL^{-1} of SRHA, (3) 4 mgL^{-1} SRHA in 15-mL polypropylene centrifuge tubes and mixed for 24 hrs at a speed of 25 rpm. All the experiments were conducted at a pH between 5.0 and 6.0, which is the collective natural pH range of these agricultural soils (Mohanram et al., 2010). After 24 hrs, the zeta potentials of the samples were

analyzed using the aforementioned procedure outlined earlier. The experiments were conducted in duplicates.

MICROBIAL ADHESION TO HYDROCARBONS (MATH) TEST

The hydrophobicities of the oocysts were evaluated using the microbial adhesion to hydrocarbons test (MATH). The effect of DOC on the hydrophobicities of oocysts and 1.8-μm microspheres were evaluated in the presence and absence of SDBS (4 mgL^{-1}) and as a function of SRHA concentration (4 mgL^{-1} to 120 mgL^{-1}), according to the water:hexadecane partitioning procedure outlined by Dai and Hozalski (Dai et al., 2003c). All experiments were repeated 3 to 5 times at 22°C.

SOIL CHARACTERISTICS

Two agriculture soils of contrasting mineralogy were used in the experiment. A weathered red volcanic clayey (Poamoho) soil was collected on Oahu from the Poamoho Agricultural Experiment Station (College of Tropical Agriculture and Human Resources (CTAHR), University of Hawaii). The soil is an intensely weathered silty clay Oxisol of the Wahiawa series. X-ray Diffraction (XRD) data indicate high amorphous iron (22%-29% w/w) and clay mineralogy (68%-76% w/w). The temperate agricultural soil used for comparison was a black loamy Midwest agricultural (Drummer) soil collected on the campus of the University of Illinois. The soil is classified as silty clay loam Mollisol of the Drummer series containing 40% quartz, 27% clay, and 13% organic matter by mass (Mohanram et al., 2010). The two soils were autoclaved at 121°C for 20 min, oven-dried at 105°C overnight, and sieved to obtain a common grain size that corresponds to the d_{50}

of the aggregates (red, clay volcanic soil) and soil grains. Henceforth, the red, clay volcanic soil; and the black loamy Midwestern agricultural soil will be referred to, respectively, as "Poamoho" and "Drummer" soils.

SOLUTIONS

All solutions were prepared using filter-sterilized, deionized water (18 MΩ cm resistivity; Milli-Q, Millipore Corp., Bedford, MA). For the flow-through column studies, influent pH was maintained between 5.0-6.0 by adding 10^{-2} M NaOH to background solution of 10^{-3}M NaCl. Nitrate (10^{-3} M, added as $NaNO_3$, pH 5.3-5.6) was used as a conservative tracer and measured spectrophotmetrically (Spectronic/Unicam, Genesys 10) by ultraviolet (220 nm wavelength).

FLOW-THROUGH COLUMNS

Transport studies were conducted using flow-through glass chromatography columns (2.5-cm diameter, 10-cm length). A 5-mL injection loop provided a "pulse input" of colloids to the column. Pore volumes (calculated on the basis of elapsed time between tracer injection and 50% breakthrough in the eluent) for the Poamoho and Drummer soils were 39 and 33 mL respectively. Polypropylene mesh (105-µm openings) was installed inside the polytetrafluoroethene retention caps on both ends of the columns. A more detailed experimental description is given elsewhere (Mohanram et al., 2010). For studies involving DOC amendments, 100 mgL^{-1} of SDBS (348.5 $gmole^{-1}$) or SRHA (3820 $gmole^{-1}$) were added to the background solutions throughout the course of the experiment (i.e. during initial column equilibration, colloids injection, and subsequent

sample collection). The effluent concentration of SDBS was monitored by ultraviolet absorption (224 nm), whereas effluent SRHA was analyzed using an Oceanographic International (OI) organic carbon analyzer (Geertsemadoornbusch et al., 1993). Colloids were injected only after the concentrations of DOC were the same in the influent and effluent. At least five pore volumes of effluent were collected. Sodium nitrate was injected prior to equilibration with DOC to preclude interference with the spectrophotometric determinations of DOC. Equilibration took approximately 20 days because of the high buffering capacity of the soils. Duplicates were run for each experiment.

RESULTS

FLOW-THROUGH COLUMNS

Poamoho soil. Fractional breakthrough (C/C_o) was plotted as a function of the number of pore volumes passing through the 10-cm long column (Figures 4.5 and 4.7 of Appendix). Summary of breakthrough curve (BTC) information for the microspheres and oocysts is shown in Table 4.1. For all experiments, peak breakthrough occurred earlier for oocysts and microspheres than for the conservative tracer (Nitrate) indicating preferential flow paths likely formed during packing the column. Only 0.11% of the oocysts were transported through the column, whereas 4% of 4.9-µm microspheres and 48% of 1.8-µm colloids were collected in the effluent. In the presence of 100 mgL^{-1} of SDBS, no oocysts could be detected in the column eluent, whereas 27% of 4.9-µm microspheres and 87% of 1.8-µm microspheres were transported all the way through the

column. In the presence of 100 mgL^{-1} of SRHA, 0.17% of oocysts and 0.20% of 4.9-µm microspheres and 28% of 1.8-µm microspheres were recovered.

Drummer soil. Fractional breakthrough (C/Co) was plotted as a function of the number of pore volumes passing through the 10-cm long column (Figures 4.6 and 4.8, Appendix). Transport of oocysts and microspheres through the quartz-rich Drummer soil is shown in Table 5.1. Only 0.4% of oocysts and 2% of 4.9-µm microspheres were recovered in the effluent, whereas 43% of the 1.8-µm microspheres were transported. In the presence of 100 mgL^{-1} of SDBS, 2% of oocysts, 17% of 4.9-µm microspheres, and 93% of 1.8-µm microspheres were transported through the column. In the presence of 100 mgL^{-1} SRHA, 2.4% of oocysts, 1.9% of 4.9-µm microspheres, and 16% and 1.8-µm microspheres were transported.

Table 4.1. Summary of the transport data on the effect of DOC on the transport of colloids through Poamoho and Drummer soil

Soil	*Colloid*	*Absence of DOC (pH 5.0-6.0)*			*Presence of DOC (100 mgL^{-1}, pH 5.0-6.0)*					
			Retardation factor		*Total recovery of colloids (%)*		*Retardation factor in the presence of SDBS*		*Retardation factor in the presence of SRHA*	
		Total recovery of colloids (%)	*Peak breakthrough* RF^{1}_{peak}	*Center of mass* RF_{CM}	*SDBS*	*SRHA*	*Peak breakthrough* RF_{peak}	*Center of mass* RF_{CM}	*Peak breakthrough* RF_{peak}	*Center of mass* RF_{CM}
Poamoho	**1.8-µm**	48.0	0.62	0.62	87.0	28.0	0.62	0.67	0.54	0.80
	4.9-µm	4.00	0.54	0.57	27.0	0.20	0.54	0.67	0.54	1.10
	Oocysts	0.11	0.46	0.60	Not detected	0.17	na	na	0.62	0.55
Drummer	**1.8-µm**	43.0	0.76	0.66	93.0	16.0	0.47	0.54	0.57	0.67
	4.9-µm	2.00	0.47	0.55	17.0	1.86	0.40	0.53	0.57	0.58
	Oocysts	0.40	0.47	0.56	2.00	2.40	0.40	0.33	0.47	0.48

[1] Calculated as ratios of time required to reach peak abundance for microspheres to time to peak concentration for the conservative tracer, Nitrate.

RF- Retardation Factor, na- not applicable

HYDROPHOBICITY OF COLLOIDS IN THE PRESENCE OF DOC

The effects of 4 mgL^{-1} DOC on the colloid hydrophobicity, as estimated by partitioning to the organic phase (P_{org}) in the MATH assay, are shown in Figure 4.1. In the absence of DOC (control), P_{org} was 65.8% for the oocysts, but only 16.2% and 17.2% for the 1.8-µm and 4.9-µm microspheres, respectively. Addition of 4 mgL^{-1} SRHA caused the P_{org} of oocysts to decrease from 65.8% to 20.4%, whereas there was a slight increase in P_{org} for the microspheres (from 16.2% to 16.6% for the 1.8-µm and from 17.2%.to 20.1% for the 4.9-µm size classes). When 4 mgL^{-1} of SDBS was added, the oocysts also became less hydrophobic, as evidenced by a decrease in P_{org} from 65.8% to 4.7%. In contrast, the microspheres became more hydrophobic as evidenced by an increase in P_{org} from 16.2% to 33.2% and from 16% to 54.6% for the 1.8 and 4.9-µm microspheres, respectively.

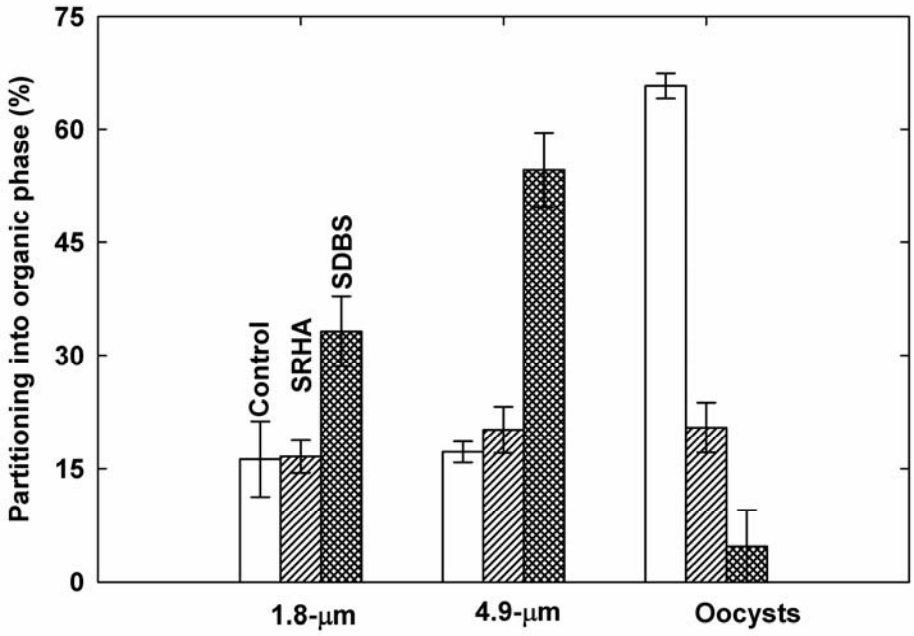

Figure 4.1. Partitioning of 1.8-µm and 4.9-µm microspheres and *C. parvum* oocysts into the organic phase (P_{org}) as determined by MATH test in the presence of 4 mgL^{-1} of Suwannee river humic acid (SRHA) and 4 mgL^{-1} of Sodium dodecyl benzene sulfonate (SDBS). Error bars represent ≤ 5% standard errors.

The changes in P_{org} for the oocysts and 1.8-μm microspheres as a function of SRHA concentration are shown in Figure 4.2. P_{org} increased from 20.4% at 4 mgL^{-1} SRHA to 31.7% at 120 mgL^{-1} SRHA. For microspheres, P_{org} increased from 16.6% at 4 mgL^{-1} to 95.9% at 120 mgL^{-1} SRHA.

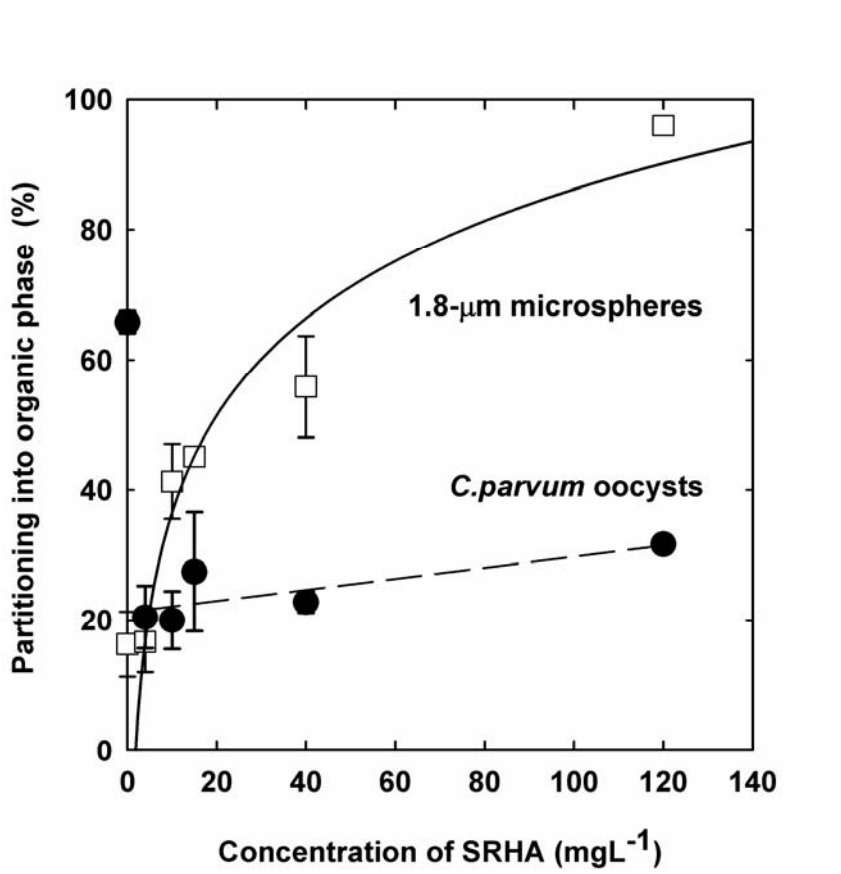

Figure 4.2. Changes in partitioning to organic phase (P_{org}) for 1.8-μm microspheres and *C. parvum* oocysts as function of Suwannee river humic acid (SRHA) concentration. Error bars represent ≤ 5% standard errors. The coefficient of regression for 1.8-μm is: $y = 21.57\ln(x)-13.23$, $R^2= 0.96$, $p < 0.005$, for oocysts: $y = 0.0085x+21.23$, $R2= 0.67$, $p < 0.005$.

ZETA POTENTIAL OF COLLOIDS IN THE PRESENCE OF SOIL FINES AND SRHA

Surface modifications of oocysts and 1.8-μm microspheres in the presences of soil fines and SRHA are shown in Figure 4.3. The oocysts ζ decreased from -17 mV in the control to -20 mV in the presence of the Poamoho soil fines and to -29 mV in the presence of both the Poamoho fines and 4 mgL^{-1} SRHA. For the microspheres, the ζ increased from -61 mV (control) to -47 mV in the presence of the Poamoho fines to -55 mV in the presence of both the fines and SRHA. The net surface charge of the oocysts became more negative (from -17 mV to -31 mV) in the presence of the fines from the Drummer soil and in the presence of both Drummer fines and SRHA. For the microspheres, the ζ decreased from -61 mV to -39 mV in the presence of both the Drummer fines and 4 mgL^{-1} SRHA.

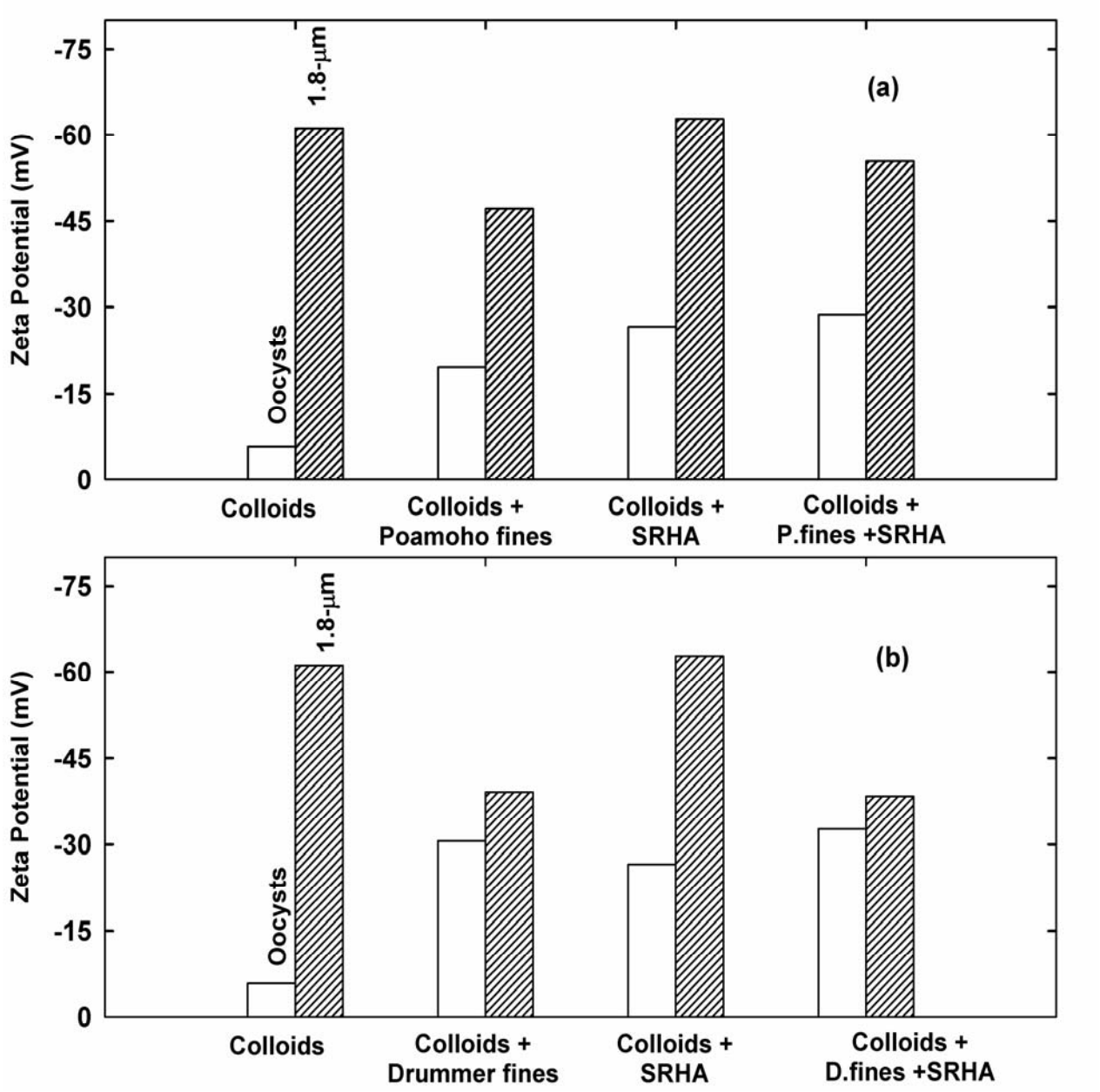

Figure 4.3. Surface modifications of *C. parvum* oocysts and 1.8-µm microspheres in the presence of (a) Poamoho and (b) Drummer soil fines (< 1-µm, 200mgL^{-1}) and 4 mgL^{-1} of Suwannee river humic acid (SRHA) at pH 5.0-6.0. Error bars < 2%.

Variations in ζ of the soil fines as a function of pH in the presence of 4 mgL^{-1} SRHA are depicted in Figure 4.4 for the Poamoho and Drummer soils. Addition of SRHA caused the fines component to become more negatively charged in the Poamoho and less negatively charged in Drummer soils.

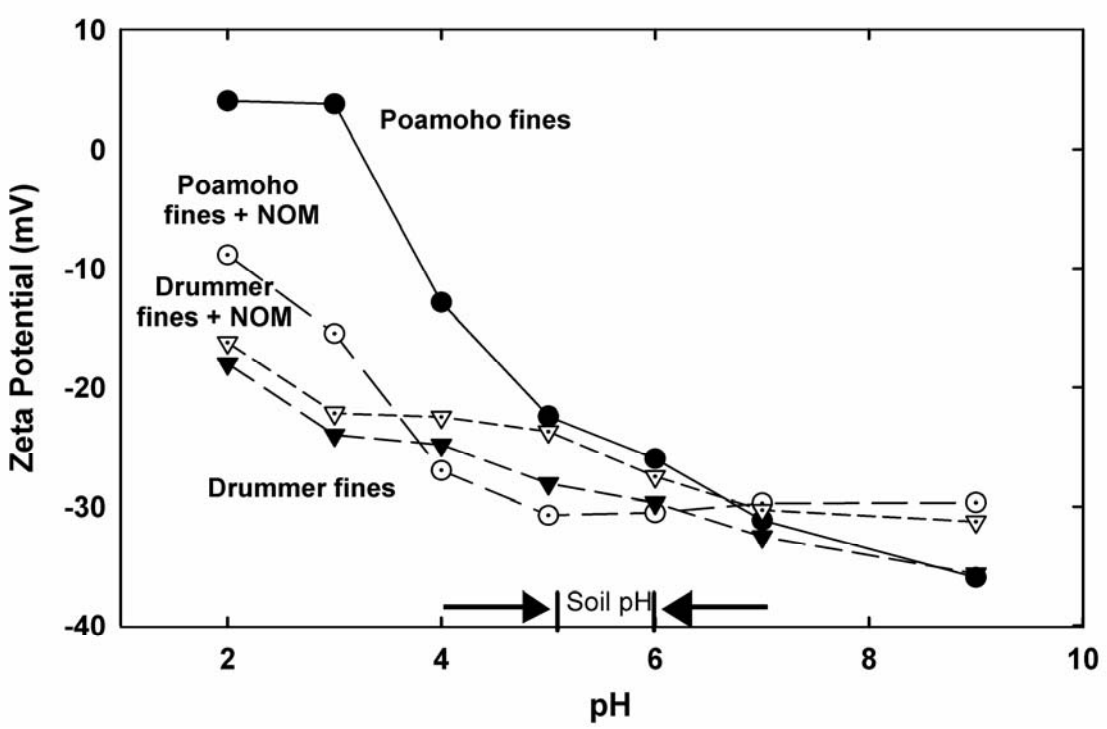

Figure 4.4. Variations in ζ of the soil fines in the presence of 4mgL^{-1} Suwannee river humic acid (SRHA). The size of fines is ≤ 1-μm at concentration of 200mgL^{-1}. The arrow marks indicate the pH of the soils (5.0-6.0).

DISCUSSION

EFFECT OF DOC

That transport behaviors of microspheres, particularly the smaller (1.8-μm) size class, through the two disparate agriculture soils were considerably more sensitive to the nature of the DOC than to soil mineralogy (Table 4.1) underscores the complexity of subsurface microbial transport (Figures 4.5-4.8, Appendix). The observations that SDBS promoted transport of microspheres in both Poamoho and Drummer soils, whereas the addition of SRHA inhibited transport, suggests that the two organic compounds were affecting transport in fundamentally different manners. In the presence of SDBS, retardation factors considerably less than 1.0 for peak breakthrough and center of mass were consistent with the high recoveries observed for the microspheres (Table 4). Anionic surfactants such as SDBS have been reported to promote microbial transport in subsurface sediments; by altering (decreasing) the net positive charges associated with iron oxyhydroxide patches on grain surfaces (Harvey et al., 2010, Pieper et al., 1997). This phenomenon has been shown to be highly pH-sensitive at pH values relevant to many soils and aquifers (Harvey et al., 2010). In the present study, we surmise that enhanced transport in the presence of SDBS is likely due to enhanced electrostatic repulsion between the grain surfaces and the carboxylated microspheres.

Decreased microsphere transport in both soils in the presence of SRHA was accompanied in the Poamoho soil by a later arrival of the center of mass, suggesting longer surface residence times, more frequent attachments, or both. Because SRHA rendered the highly hydrophilic microspheres considerably more hydrophobic (Figure 4.1

and 4.2), but had little effect on their net surface charge (Figure 4.3), it appears that the decreased transport of carboxylated microspheres in the presence of SRHA was probably due, at least in part, to an enhanced hydrophobic effect. Dai and Hozalski (Dai et al., 2003b) reported similar observations where SRHA modified the surfaces of colloids depending on the initial surface chemistry of the particles.

In contrast to the transport behaviors of the microspheres, transport of the structurally more complex oocysts was much less sensitive to both the nature of DOC and to soil mineralogy. No oocysts were found in the effluent in the presence of SDBS in Poamoho soil, but SDBS promoted oocyst transport in Drummer soil. Figure 4.1 shows that oocyst surfaces exhibit ~3-fold decrease in hydrophobicity when in the presence of just $4mgL^{-1}$ of SDBS. A diminished hydrophobic effect would help explain the higher recovery in the high organic (13% w/w) and quartz (40% w/w) Drummer soil relative to the Poamoho soil having very little organic material. Interestingly, amendments with SRHA caused the same effect. When SRHA caused oocysts to become less hydrophobic (Figure 4.1), it caused an increase in negative surface charge (Figure 4.3). The decrease in a microorganism's hydrophobicity is often accompanied by an increase in its net surface charge (ζ) (Vanloosdrecht et al., 1987). This has also been reported to be the case for oocysts (Considine et al., 2002). Thus, the enhanced transport of oocysts in Drummer soils in the presence of SRHA is due to decreases in hydrophobicity as well as electrostatic repulsion between colloid and collector surfaces.

Because SDBS and SRHA amendments did not affect appreciably oocyst transport in the Poamoho soil columns, it appears that soil mineralogy may dominate oocysts transport potential in this soil type. In general, SDBS has been shown to enhance transport and re-entrainment of microorganisms in subsurface sediments, which is thought to involve charge modification of Fe- and Al-oxide minerals on grain surfaces (Ryan et al., 2002). The complete absence of oocyst breakthrough in the Poamoho soil in the presence of SDBS shows that that the presence of this surfactant may be insufficient to overcome the net positive charge of these volcanic soils that consist largely of Fe (22-29% w/w) or straining because of the high fraction of clay (68-76% w/w).

Our observations of colloid transport indicate that oocyst transport through agricultural soil may be governed by a complex mix of factors affecting the nature of the interactions between colloid and collector surface, including Coulombic and hydrophobic forces. Although the water: hexadecane partition assay indicated that the microspheres and oocysts have disparate surface characteristics, their transport also appears to depend strongly on the characteristics of granular media and the nature of the dissolved organic carbon.

EFFECT OF SOIL FINES

In order to better understand the ternary interactions between grain surface, DOC, and oocysts we used soil fines. Soil fines have been reported to be reasonable indicators of processes occurring at grain surfaces (Grolimund et al., 1998). Figure 4.4, which depicts the variations of zeta potential exhibited by Poamoho and Drummer fines in

response to changes in pH, shows a strongly negative net charge for both types of fines within the soils pH range regardless of whether SRHA is present or not. However, $4mgL^{-1}$ SRHA caused the Poamoho fines, which consist mainly of 1:1 clay minerals, largely kaolinite with little organic matter, to become more negatively charged. Kaolinite clays have both a pH-dependent positive charge (on the edges) and a permanent negative charge (on the face) occurring simultaneously on one surface. Under low pH, near the point of zero charge (PZC), electrostatic and van der Waals forces between the face and edge regions cause the clay surface to be positively charged. At higher pH values most of the soil fines are negatively charged and face-to-edge interaction is not possible due to electrostatic repulsion. Under these conditions the addition of low concentration of SRHA enhances stabilization of the fines due to the sorption of humic acid on their surfaces causing an increase in negative charge. This phenomenon has been termed *electrostatic stabilization* (Kretzschmar et al., 1998). It should be noted that under neutral pH conditions the zeta potential of the soil fines was the same as that of soil fines in the presence of SRHA.

For the titration studies conducted in the Drummer fines, a reverse trend was found; i.e. the presence of SRHA caused the soil fines to become less negatively charged. A closer look reveals that these soils have 2:1 expandable clays with smectite as the major mineral component with permanent negative charge. The 2:1 clays are more reactive (than 1:1 clays) and have higher tendency to aggregate (Schulten et al., 2000) and the presence of organic matter acts like a bridge in between the aggregates (Six et al., 2000). We hypothesize that the decrease in zeta potential of the Drummer clay in the

presence of SRHA is due to SRHA-promoted clay aggregation. The decrease in zeta potential is due to destabilization of the fines by SRHA, an initial step in particle coagulation (Elimelech 1998). These studies show that SRHA can stabilize or destabilize fines according to the soil mineralogy and the particle stability can change with pH conditions.

COMBINED EFFECTS OF SOIL FINES AND DOC

To further understand the type of interactions occurring at the surfaces, we measured the zeta potential of oocysts and microspheres in the presence of soil fines and SRHA, separately, and the presence of soil fines and SRHA together (Figure 4.3). It is clear that oocysts had a higher susceptibility to surface modification than that of the microspheres. This was found to be true for both the soils studied. The oocyst surfaces are hydrophobic as well as having a pH-dependent charge due to the presence of surface active protein (Jenkins et al., 2010).

For the oocysts in Poamoho soil and Drummer soil, addition of soil fines or SRHA led to a more negative zeta potential. Abudalo et al.(Abudalo et al., 2005b) found the oocysts surfaces to be insensitive to zeta potential changes with addition of fulvic acid. They hypothesized that the anionic moieties of fulvic acid would preclude its interaction with negatively charged oocysts. Although the concentration of NOM they used was similar to our study, the nature of organic matter is different. We used SRHA which has weakly charged cationic and anionic moieties, unlike fulvic acid. We hypothesize the polyelectrolytic moieties in SRHA to be interacting through Coulombic

forces with the oocysts surfaces. The net effect appears to cause oocysts to become more negatively charged. This is a very significant finding because the data show that oocysts are very reactive to small changes in environmental conditions. Therefore, it appears that the oocysts are vulnerable to surface modification in the presence of soil fines, and upon the soil type, to the presence of humic acid.

The surface characteristics of the microspheres were also more sensitive to the soil fines than SRHA. Our observations that the presence of suspended soil fines caused the zeta potentials of the microspheres to become less negative and those of the oocysts more negative can be explained, in part, by the fact that there is an initial disparity in the surface hydrophobicities and electrical properties between the two colloids. The soil fines from the Drummer soil decreased the zeta potential more so than that from the Poamoho soil. The difference is due to the 2:1 clay minerals in Drummer soil which promotes aggregation of the microspheres to higher extend than 1:1 clay minerals in Poamoho soil. From these experimental data, we have sufficient evidence to say that the behavior of the microspheres is substantially different from that of the oocysts and they may not be an appropriate surrogate for studying oocysts transport.

Fitch et al (Fitch et al., 1996) found that NOM coatings on clay minerals physically blocks access to the clay pores by cation binding. Although SRHA caused an increase in negative charge on the surface of fines, the transport of negatively charged microspheres was reduced in Poamoho soil. We speculate that in the Poamoho soil, SRHA promoted dispersion of soil fines could have migrated into the finer porosity of the

soil column, thereby blocking the access of pore space for movement of microspheres, while SRHA mediated aggregation could have caused clogging of access pores for microspheres transported through Drummer soil. So, the microspheres are strained out by the presence of soil fines as well as SRHA moieties blocking the soil porosity. We suspect that lower transport of microspheres could also be due to the blockage of the finer porosity and preferential flow paths by SRHA and soil fines thereby trapping them and retarding transport

To summarize, oocysts and microspheres transport appears to depend strongly on the characteristics of granular media, the nature of the colloids, and the type of organic surface interacting with the colloids. Thus, it is not possible to generalize the effect of DOC on the transport of oocysts and microspheres in agricultural soils. Our data also show that the oocysts surfaces are extremely reactive and sensitive to small changes in environmental conditions, presence or absence of DOC, and soil colloids. These, in turn can have significant impact on their transport characteristics. Finally our studies have also showed that the oocyst and microsphere surfaces are clearly different because of the observed difference in attachment, zeta potentials and hydrophobicity. Microspheres may not be a reliable surrogate for studying oocyst transport in soils. Finally, soil fines, SRHA, and SDBS all appear to substantively affect the transport process under certain sets of conditions. The manner in which soil fines and dissolved organic matter affect colloid transport in a wider variety of soil types is worthy of further study.

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APPENDIX

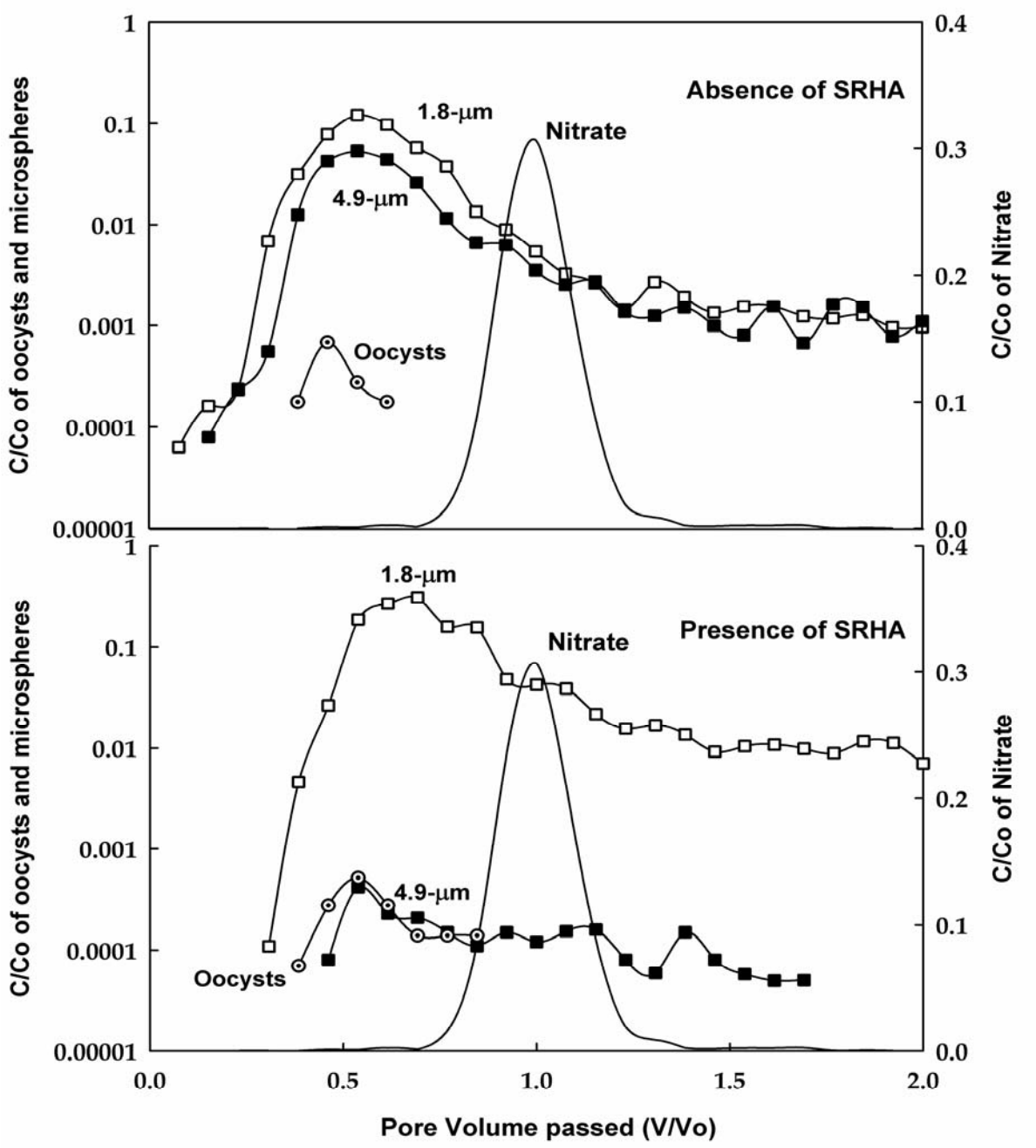

Figure 4.5. Dimensionless concentration histories at 22°C±1°C for conservative tracer, *C. parvum* oocysts, and 1.8-μm and 4.9-μm microspheres being advected in 10^{-3}M NaCl through 10 -cm Poamoho soil (d_{50} = 0.65 mm) at 1.6 md^{-1} and pH 5.5 in the absence and presence of 100 mgL^{-1} Suwannee River Humic Acid (SRHA).

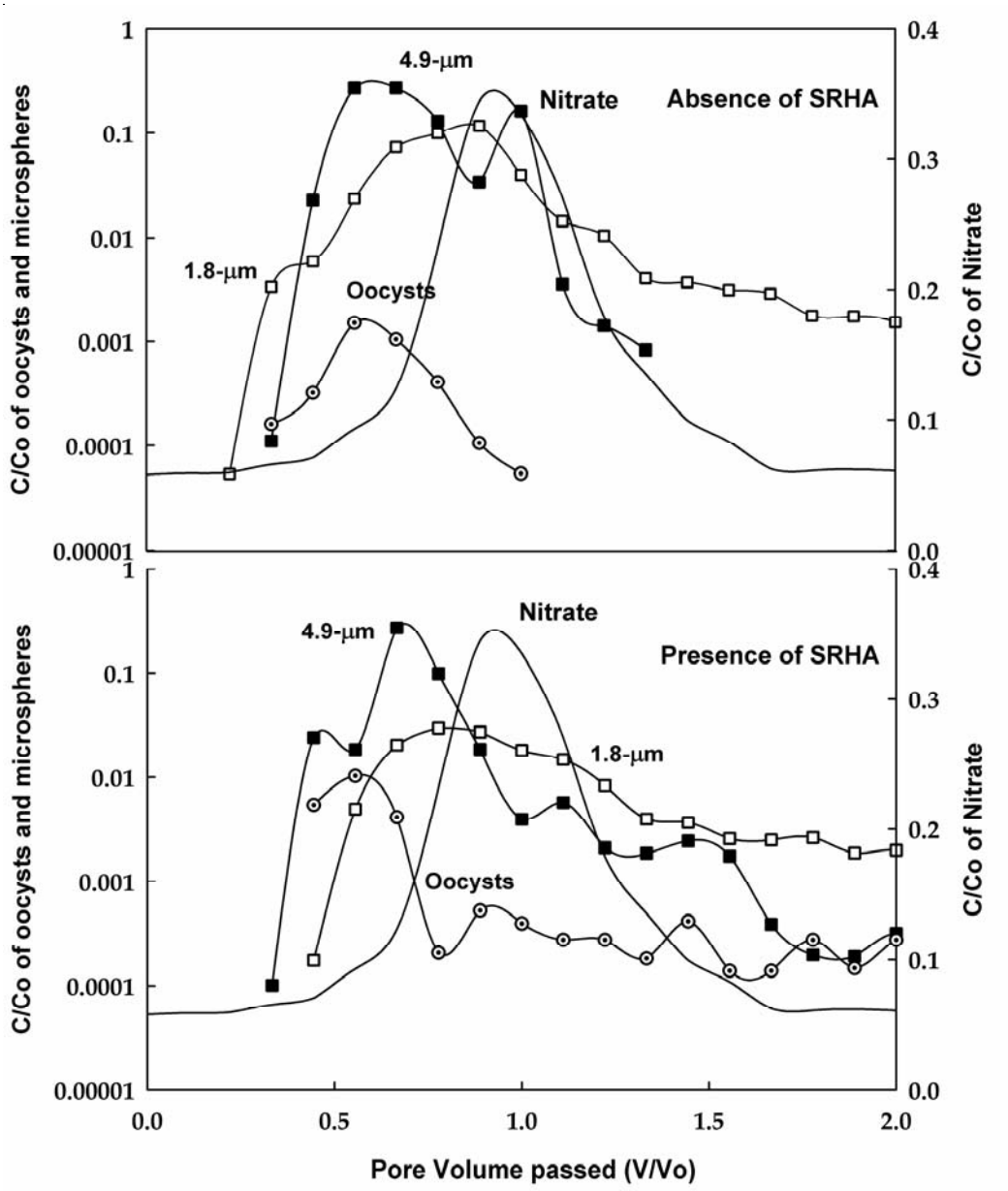

Figure 4.6. Dimensionless concentration histories at 22°C±1°C for conservative tracer, *C. parvum* oocysts, and 1.8-μm and 4.9-μm microspheres being advected in 10^{-3}M NaCl through 10 -cm Drummer soil (d_{50} = 1 mm) at 1.6 md^{-1} and pH 5.5 in the absence and presence of 100 mgL^{-1} SRHA.

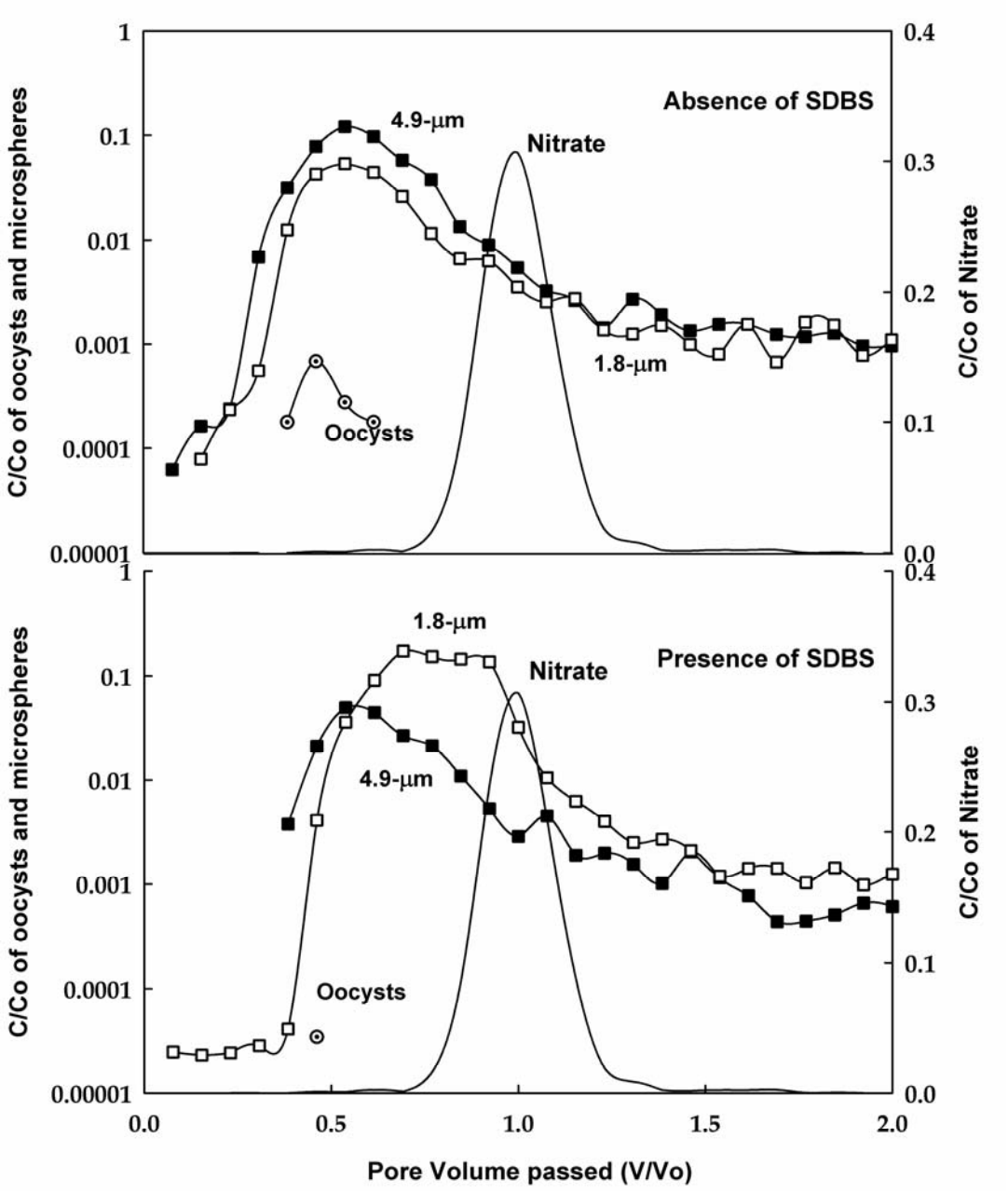

Figure 4.7. Dimensionless concentration histories at 22°C±1°C for conservative tracer, *C. parvum* oocysts, and 1.8-μm and 4.9-μm microspheres being advected in 10^{-3}M NaCl through 10 -cm Poamoho soil (d_{50} = 0.65 mm) at 1.6 md^{-1} and pH 5.5 in the absence and presence of 100 mgL^{-1} Sodium Dodecyl Benzene Sulfonate (SDBS).

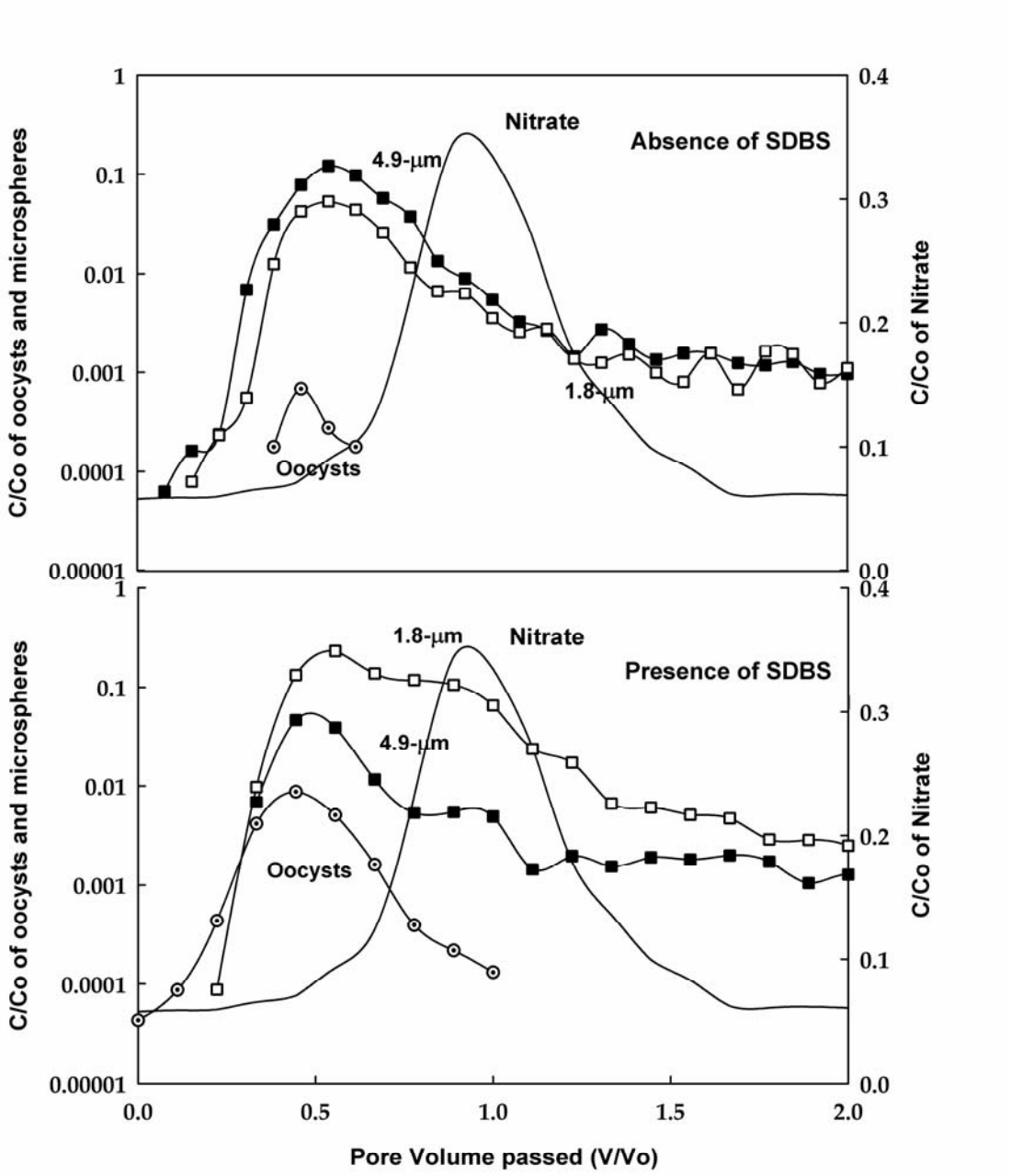

Figure 4.8. Dimensionless concentration histories at 22°C±1°C for conservative tracer, *C. parvum* oocysts, and 1.8-μm and 4.9-μm microspheres being advected in 10^{-3}M NaCl through 10 -cm Drummer soil (d_{50} = 1 mm) at 1.6 md^{-1} and pH 5.5 in the absence and presence of 100 mgL^{-1} SDBS.

CHAPTER V

CONCLUSIONS

Transport of *Cryptosporidium parvum* oocysts and microspheres were studied in agricultural soils of disparate mineralogy and some of the major conclusions are:

1. Agricultural soils in Hawaii are efficient filters of pathogens such as *C. parvum* oocysts. Transport of pathogens strongly depends on the mineralogy of these soils. While the majority of oocysts are filtered out in the high clay, metal oxide rich Poamoho soils, the organic-rich volcanic ash soils from the island of Hawaii were poor filters of microspheres due to the high particulate organic matter content. These soils can serve as reservoirs of pathogens in farming areas.

2. Soil fines, clay minerals, natural and dissolved organic matter content modify the oocysts characteristics in a complex manner. Oocyst transport in the presence of organic carbon depends on the type of organic carbon (i.e. anthropogenic or natural), the mineralogy of the soil grains as well as the surface characteristics of the particles. These findings indicate that it might be difficult to predict pathogen transport in the presence of DOC. Consequently, it is necessary to take into account the individual soil properties to predict pathogen transport in agricultural soils.

3. The presence of surfactants promotes transport of pathogens in agricultural soils. So, irrigation with recycled water rich in surfactants can enhance oocyst transport. Considerable caution needs to be exercised before using recycled water for irrigation.

4. Carboxylated microspheres are not a reliable surrogate for *Cryptosporidium parvum* oocysts. Our experiments indicate that due to the differences in surface characteristics between the microspheres and oocysts, the microspheres may not be used to predict oocysts transport in soils.

Based on our present findings there could be several possible ideas worth pursuing, such as studying the microspheres transport in organic rich volcanic ash soil in the absence of SOM and in the presence of DOC. It is expected that SOM removal can expose the mineral surface which can interact with DOC and affect transport potential of oocysts and microspheres.

Because oocysts occur in farmlands rich in organic matter loadings, oocysts attachment and transport in these agricultural soils in the presence of DOC isolated from manure lagoons would give insight on difference in oocysts retention and transport from other forms of DOC such as humic acid and surfactants.

Our most promising research arena is to use clay minerals and soil fines as probes in understanding the complex surface topography of *Cryptosporidium parvum* oocysts.