12-2018

The Impact of Surfactant on the Transport of *Toxoplasma gondii* Oocysts Through Unsaturated Soil

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THE IMPACT OF SURFACTANT ON THE TRANSPORT OF *TOXOPLASMA GONDII* OOCYSTS THROUGH UNSATURATED SOIL

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Hydrogeology

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by
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December 2018

Accepted by:
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Abstract

Toxoplasma gondii is among the most prevalent parasites affecting warm-blooded animals, including humans. In humans, it poses health risks for immune-compromised populations and for fetuses including damage to the eyes brain and other organs.

Humans may come in contact with *T. gondii* through the consumption of infected animal flesh or accidental contact with cat feces. Its life cycle includes numerous intermediate hosts and felids as the definitive host, which has allowed for its spread through nearly the entire world. *T. gondii* has been detected in open water, soil and animal feeds. Its transport has not been well studied to this point. Understanding of transport of *T. gondii* is necessitated by its presence in soils and human health risks. Surfactants may be introduced in agricultural settings through the application of reused water and sludge to fields, through environmental remediation or the use of pesticides that include surfactants. Surfactants may influence water flow and soil hydrology properties, impacting the flow of water and pathogens conveyed within it. The aim of this study is to assess the way the presence of surfactant influences the transport of *T. gondii* through soil. Continuous rainfall of a KCl solution was simulated on columns of sandy loam and loamy sand soils. Flow within the columns was vertical and gravity driven. Artificial rain on select columns contained an anionic surfactant, Aerosol 22.

After steady state was reached, a pulse containing *T. gondii* oocysts and KBr as a tracer was applied. In those columns where Aerosol 22 was included in the rain,

it was also present in the pulses. *T. gondii* and KBr breakthrough curves were created to characterize transport. Following cessation of the simulated rainfall, soil columns were cut to assess retention of the oocysts within the soil and concentrations of oocysts are given by depth. For both soil and leachate samples, *T. gondii* was quantified using qPCR. *Toxoplasma gondii* oocysts were detected in all columns retained in porous media and in leachate. Surfactant was shown to enhance transport in all soil series studied.

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Chapter 1. Introduction

Toxoplasma gondii is among the most prevalent parasites of warm-blooded animals, including man (Jones and Dubey, 2010; Dubey and Beattie, 1988; Tenter et al., 2000; Hill et al., 2002). It has been estimated that about 8% of hospitalizations and 24% of deaths from foodborne illness each year (Scallan et al., 2011) and 2,839 people develop ocular disease per year because of infection by *Toxoplasma*, or Toxoplasmosis (Jones and Holland, 2010). In the United States and the United Kingdom, between 8 and 22% of people have exhibited seroprevalence (Dubey and Beattie, 1988; Dubey and Jones 2008; Jones et al., 2001; Jones et al., 2003; Jones et al., 2007; Jones et al., 2014) while in Central America, South America and continental Europe, seroprevalence rates range from 30 to 90% (Dubey and Jones, 2008; Dubey, 2010; Mimbaeva et al., 2013). Although infection in healthy adults is often asymptomatic, severe effects may occur. In immunocompromised populations, Toxoplasmosis is a significant cause of death. Approximately 10% of AIDS patients in the United States and 30% of AIDS patients in Europe have died from this disease (Luft and Remington, 1992; Hill and Dubey, 2016). It can also be harmful when acquired transplacentally. In fetuses, infection by *Toxoplasma* may cause abortion, cognitive impairment or other congenital defect in humans as well as other livestock, including sheep (Dubey, 2009; Dubey and Beattie, 1988). Toxoplasmosis has also been linked in several recent studies to suicide and schizophrenia (Pedersen et al., 2012; Torrey et al., 2012).

Toxoplasma has a complex life cycle with numerous intermediate hosts and felids as the definitive hosts. Within the intermediate hosts, *Toxoplasma* exists as tissues cysts that are infectious when consumed without proper cooking. Felids excrete oocysts into the environment in feces. Oocysts can remain viable in moist soil for months or even years (Dubey and Beattie, 1988; Hill and Dubey, 2016). It has been shown that they can remain infective in water at 4°C for 54 months (Dubey, 1998; Dumètre, 2003) and in experimentally infected soils for up to 18 months (Frenkel et al., 1975). Sporulated oocysts may also be able to withstand freezing as it has been shown that they can withstand constant freezing at -21°C for 28 days (Frenkel and Dubey, 1973). The oocysts are subspherical and roughly 10 by 12 μm. They are unsporulated when they are first expelled and after 1 to 5 days, depending on the environment, they become sporulated and therefore infectious. Oocysts are not killed by current municipal water treatment techniques such as chlorine, ozone and ultraviolet though it should be removed via filtration in large and fully-functional water treatment (Jones and Dubey, 2010; Dumètre, 2003). Environmental contamination by oocysts is widespread due to the fecal contamination of soil, surface water and groundwater from the estimated 140 million domestic and feral cats in the United States in addition to wild *Felidae* (Hill and Dubey, 2016). Due to the global presence of felids, *Toxoplasma* has been detected everywhere besides the frozen arctic (Dubey, 2009) including in seawater that had been contaminated by surface water runoff (Lindsay and Dubey, 2009; Miller et al., 2002). Chickens are another key player in infection of humans because

they readily uptake oocysts that are excreted by cats and can cause infection in humans or other animals when their undercooked meat containing tissue cysts is consumed. It has been shown that there is a very high prevalence of *Toxoplasma* in backyard chickens (up to 100%) and in free ranger organic chickens (30 to 50%) (Hill and Dubey, 2016; Dubey, 2010).

Dumetre et al. (2011) summarizes the impact of interaction forces on environmental transmission of protozoa including *T. gondii* and *C. parvuum*. *T. gondii* has a wall thickness of approximately 100 nm and the surface biochemistry consists of possible polymeric cross-links of Cys- and/or Tyr-rich proteins. The wall of *Toxoplasma* encloses two sporocysts. The shell is highly resistant and impermeable. Dumetre et al. also enforces that models would be valuable for risk assessment, but appropriate models do not exist because of the lack of information on the transport properties, survival and prevalence of *Toxoplasma* in the environment (Dumetre and Darde, 2003; Jones and Dubey, 2010; Shapiro et al., 2009). The presence and persistence of *Toxoplasma* in the environment worldwide has been clearly demonstrated (Jones and Dubey, 2010), though to our knowledge there has been no published study of the transport of *T. gondii* in porous media. It has been initially assumed that rain infiltration was not a significant source of transport because of the ability of soil to filter pathogens (Tim et al., 1988). More recent studies have shown the ability of pathogens, specifically *Cryptosporidium parvum*, to be transported through undisturbed, unsaturated soil (Mawdsley et al., 1996) and through packed, saturated soil columns (Brush et al., 1999; Harter et al.,

2000). It may be useful to reference the transport of *C. parvum* because of the large body of work that has been completed related to its transport.

Surfactants introduced to the environment can cause changes to the physical properties of soil including hydraulic conductivity (K). Surfactants are present in many household cleaning products and, as a result, may enter soil water by the use of greywater for irrigation (Al-Jayyousi, O.R., 2003; Hamilton, A.J. et al., 2007).

Approximately 20 million hectares of farmland worldwide are irrigated with greywater currently (Abu-Zreig et al., 2003). Surfactants are widely used because of their amphiphilic property (Laha, et al., 2008), which allow for the reduction of interfacial tension at the liquid/solid interface. Mingorance et al. (2007) demonstrated a shift in infiltration rates for each surfactant studied in each soil type, with some surfactants either increasing or decreasing the infiltration rate for each soil. Peng et al. (2017) specifically studied Aerosol 22, a widely used anionic surfactant, in loamy sands and sandy loams. In this study, saturated hydraulic conductivity (k) was decreased by various amounts in both soil types, though k values fluctuated throughout the experiments and more study is required to fully characterize the impact of Aerosol 22 on sandy loams and loamy sands.

Preferential pathways allow for fast transport of *C. parvum* and other pathogens through soil. Darnault (2003, 2004) and Bradford and Bettahar (2003) showed that *C. parvum* could be transported quickly by fingering flow and macropores in unsaturated soils. In situations where rainfall is not intense enough to produce fingering flow or macropores, pathogens are transported through the

soil matrix where they interact with soil in a variety of physiochemical processes (Darnault et al., 2017). Tufenkji et al. showed that straining and physiochemical processes control the removal of *C. parvum* in natural soil settings (2004). According to Bradford and Bettahar (2003), it has been determined that in saturated column environments, *C. parvum* oocyst transport was controlled by straining and best modeled with a combination of attachment, detachment and straining. It is expected that these retention mechanisms will also play a key role in the transport of *T. gondii* in the subsurface environment.

This work seeks to improve the understanding of transport of *T. gondii* oocysts in natural settings. The understanding of oocyst transport is critical for human health because *Toxoplasma* oocysts have been identified as a key source of large outbreaks of acute toxoplasmosis from soil and water (Dumètre, 2003; Teutsch, 1979; Stagno et al., 1980, Benenson et al., 1982; Coutinho et al., 1982; Tvaerne, 2002) and likely responsible for a significant amount of the infection in animals that may later be consumed by humans (Dumètre, 2003; Tenter, 2000).

This experiment seeks to characterize the transport and retention of *T. gondii* oocysts in soils with varying physiochemical properties that were subjected to a simulated rainfall.

The objective of this study is to investigate the transport of *T. gondii* oocysts in soil and the effect of surfactant on the mobility of the oocysts. Comparisons of the transport behavior in the various soils types were made with breakthrough curves

(BTCs) of *T. gondii* oocysts and distribution profiles of *T. gondii* oocysts in the soil profile created from the soil columns after the cessation of the artificial rain.

Detection and enumeration of *T. gondii* oocysts was done through qPCR for both the water and soil samples. As a secondary outcome, this research seeks to determine future work is necessary to improve our ability to predict *T. gondii* in natural soils.

Chapter 2. Materials and Methods

2.1 Toxoplasma gondii oocysts

Stock suspensions containing a total of about 87.5 million *Toxoplasma gondii* oocysts (Me49 strain) were obtained from the United States Department of Agriculture Agricultural Research Service (USDA-ARS, Beltsville, MD). The concentration of the stock solution was 2.5 million oocysts per milliliter, as determined by USDA-ARS. The oocysts were suspended in a 2% sulfuric acid deionized (DI) water solution. The solution as kept at 4°C prior to use. Prior to addition of the *T. gondii* to the pulses applied to the columns, they were vortexed at 3000 rpm for 5 minutes to ensure that there was a uniform distribution of oocysts throughout the stock solution.

2.2 Soils

The soil physiochemical properties of the soils used in this experiment are presented in Table 1. Soils were previously described in Darnault et al., 2017. These properties were determined by the Utah State University Analytical Laboratories (USUAL). Soil texture and particle size were determined using the hydrometer method (Gee and Bauder, 1986). Analyses for total carbon and inorganic carbon were completed using a Primac^{SLC} Analyzer (Skalar Inc., Buford, GA, USA). Organic carbon was computed by the difference between total and inorganic carbon values. Saturated soil paste extracts were prepared (Rhoades, 1996) to determine electrical conductivity and pH. Cation exchange capacity was measured using the sodium

acetate/ammonium acetate replacement method. Soils were air dried at 37°C, sieved in a 2 mm sieve and stored at room temperature in lidded buckets until used.

The soils used in this study were collected as bulk samples from fallow and cultivated fields in Illinois and Utah, U.S. All four soils have been classified as loamy sand or sandy loam based on textural analysis. Soil from the Greenson series, a sandy loam, was collected in Cache County, Utah. It is classified as a fine-silty, mixed, superactive, mesic Oxyaquic Calcixerolls. Greenson soils are very deep, somewhat poorly drained or moderately well drained soils that formed in lacustrine deposits derived from limestone, sandstone, and quartzite. Greenson soils are on low lake terraces and formed on very deep, somewhat poorly drained ormoderately well drained soils that formed in lacustrine deposits derived from limestone, sandstone, and quartzite. Greenson soils are on low lake terraces. It is somewhat poorly drained or moderately well drained; low to medium surface runoff; slow to moderate permeability and has an organic matter content from 3 to 9% in the A horizon. The second sandy loam, the Lewiston series, was also collected in Cache County, Utah. Lewiston soils are classified as Coarse-loamy, mixed, superactive, mesic Aquic Calcixerolls and is very deep, somewhat poorly drained soils that formed in lacustrine deposits. Lewiston soils are on lake terraces. Lewiston is somewhat poorly drained has slow or very slow runoff and moderate permeability. The soil organic matter content of the A horizon ranges from 1 to 3%. Soil from the Gilford series, a loamy sand, is classified as Coarse-loamy, mixed, superactive, mesic Typic Endoaquolls. It is very deep, poorly drained or very poorly drained soils

formed in loamy over sandy sediments on outwash plains, glacial drainage channels, near-shore zones (relict), and flood-plain steps and has a negligible potential for surface runoff and a moderately high to high saturated hydraulic conductivity in the upper part and high to very high in the lower part. Permeability is moderate or moderately rapid in the upper part and rapid in the lower part. The Gilford series soil in this experiment was collected in Kankakee County, Illinois. The second loamy sand, Sparta, is classified as sandy, mixed, mesic Entic Hapludolls. Sparta soils are very deep, excessively drained soils formed in sandy outwash that has been reworked by wind. These soils are on nearly level to very steep treads and risers on stream terraces in river valleys, outwash terraces, outwash plains, and dune fields. It is an excessively drained soil with a saturated hydraulic conductivity of 10.00 to 100.00 micrometers per second. The Sparta soil is also from Kankakee county, Illinois.

2.3 Artificial rainfall and *T. gondii* inoculum solutions

All solutions were prepared using DI water. The rainfall solutions contained 1mM potassium chloride (KCl). This was continuously applied to the columns at a velocity of about 1 $cm \cdot hr^{-1}$.

Potassium bromide was used as a conservative tracer and applied with the *T. gondii* inoculum at a concentration of 10 mM. This was measured in the inoculum and leachate samples using an Orion™ Bromide Electrode (ThermoFisher Scientific, Waltham, Massachusetts, USA). The inoculum were prepared by adding roughly 25

million *T. gondii* in 1 mL of the stock solution to 49 mL of the 1 mM KCl artificial rainfall solution that was spiked with 10 mM KBr tracer. 45 mL of the inoculum were applied to the columns, while 5 ml was retained in order to quantify the initial concentration (C_0) of *T. gondii.*

In two columns of each soil type, the artificial rainfall also contained 2 critical micelle concentration (CMC) of the surfactant Aerosol 22 (Sigma-Aldrich co., St. Louis, MO, USA).

2.4 Column and rainfall simulator

The columns used in this study were made of plastic rings and were 20 cm in length with a 9.5 cm internal diameter. A 5 cm tall ring was placed at the base of the column atop a mesh fabric and a screen to prevent soil loss. One and 2 cm rings were then placed on top of the base ring to achieve a total height of 30 cm, 20 of which were later filled with soil. To enhance stability, the rings were compressed between top and bottom plastic column holders with four rods positioned parallel to the length of the column, which were bolted to the top and bottom sections. A funnel was clamped separately to the bottom column holder. An additional rod was used behind the column to attach a misting nozzle about 10 cm above the soil surface.

Soil was packed into each column at a uniform bulk density of 1.53 $g \cdot cm^{-3}$. 2,125 g of soil were packed into the columns in three equal amounts and after each addition the soil was compacted with a rod.

Four experimental columns were run simultaneously. A peristaltic pump (Cole-Parmer, Vernon Hills, IL) was fitted with a cartridge that held four tubes to pump the artificial rainfall solution into the misting nozzles that were attached above each column. The misting nozzles (XA nozzle system 1/4, 303 from BETE Fog 218 Nozzle Inc., Greenfield, MA) were connected to the pump on one side with brass couplings and to air on the other. The flow rate through the nozzles was adjusted using Parker Watts miniature precision regulator gauge (1/4 in; 60 psi; Parker Hannifin Corp., 222 Cleveland, OH) to create a mist that covered the entire soil surface.

2.5 Experimental procedure: Rainfall treatments and leachate collection

Once columns were filled with soil and placed in the stands, the artificial rainfall was started. Outflow from each column was observed until it reached steady state, at which point the rainfall was stopped and the *T. gondii* inoculum were added to the surface. The pulses were allowed to infiltrate fully before the rainfall was resumed. Leachate samples were collected in varying volumes throughout the experiment. Samples one through ten were collected as 5 mL samples. Samples eleven through fifteen were 10 mL and sixteen through twenty-seven were 25 mL. After sample twenty-seven, samples of 50 mL were collected. After roughly two pore volumes, samples exceeded 50 mL. Their volumes were recorded and 50 mL subsamples were taken from each sample that exceeded 50 mL. After at least six pore volumes had been collected from each column, rainfall was shut off.

2.6 Soil water content

Each column was sliced into 2 cm slices for analysis of soil water content and *T. gondii* oocyst concentration. Soil water content was determined by taking two 5 g samples from each soil layer. The weight of these samples was recorded; they were placed in aluminum foil cups and dried at 105°C in an oven for 24 hours. The dry weight was recorded and water content was determined gravimetrically.

2.7 Isolation of *T. gondii* oocysts from soil

Following the removal of water content samples, each soil layer was mixed thoroughly to distribute *T. gondii* oocysts evenly throughout the soil. Two soil samples were taken from the aggregated soil layer. Each replicate weighed approximately 25 g and the mass of the sample was recorded. Each sample was placed in a 50 mL centrifuge tube. The method developed by Koken et al. (2013) was used to isolate *T. gondii* oocysts from soil. To cause oocysts that were attached to soil particles to release from soil, 20 mL of a solution containing Tween 80 at 2 critical micelle concentrations (CMC) and 50 mM TRIS buffer was added the each tube. The centrifuge tubes were then attached to a rotational shaker, perpendicular to the axis of rotation, for 24 hours. Following shaking, the tubes were centrifuged at 2,500 x g for 15 min. The resulting supernatant was transferred to a new 50 mL conical-bottomed centrifuge tube and centrifuged again at 2,500 x g for 15 min. The supernatant was removed to leave 4 mL in the tube. For the first wash, the remaining 4 mL was vortexed and 35 mL of deionized water was added to the tube

to resuspend the pellet. A second wash was completed following the procedure of the first wash. For the third wash, the remaining 4 mL were vortexed and moved to 15 mL centrifuge tubes.

2.8 Concentration of *T. gondii* oocysts in effluent

Effluent samples were collected in 50 mL centrifuge tubes for the first 24 hours of the experiment. After this period, greater volumes were collected in beakers. The volume of samples greater than 50 mL was recorded, the samples were mixed with a magnetic mixer, and a 50 mL sample was retained in a centrifuge tube. The method developed by Koken et al. (2013) was again used to isolate oocysts from leachate. Every second sample was evaluated for *Toxoplasma* oocyst concentration. The selected centrifuge tubes were centrifuged at 2,500 x g for 15 minutes to concentrate *T. gondii* in the pellet. Supernatant was removed and discarded, leaving 5 mL in the centrifuge tube. The remaining pellet was vortexed for 20 seconds and 1 mL of the mixed sample was transferred into a microcentrifuge tube for *Toxoplasma* enumeration.

2.9 DNA extraction and qPCR analysis of *T. gondii* oocysts

To enumerate *T. gondii* oocysts, samples were centrifuged at 10,000 x g for 5 minute to concentrate oocysts in a 50 µL sample. Samples were then subjected to six cycles of freezing at -80°C for 5 minutes and thawing at 90°C for 5 minutes. Samples were sonicated in order to weaken the oocyst and sporocyst wall for 10 minutes.

DNA was extracted with the E.Z.N.A.® Tissue DNA kit (OMEGA Bio-Tek, #D3396-02, VWR International S.A.S., Strasbourg, France) in accordance with the manufacturer's instructions, except DNA was eluted in 70 µL TE buffer. The real-time PCR assay targeted the 529 bp repeat region (*REP529*, GenBank accession no. AF487550) of *T. gondii* (Reishl et al., 2003). Real-time PCR reactions were performed on a Roche's LightCycler 480 in a final volume of 25 µL containing 1X LightCycler™ 480 Probes Master (ROCHE Diagnostics, France), 0.5 µmol/L of each primer, 0.25 µmol/L of the Taqman probe, 0.5µl of 1% bovine serum albumin (BSA) and 2 µL of template DNA. The nucleotide sequences of the primers were 5′-AGG AGA GAT ATC AGG ACT GTA G-3′ and 5′-GCG TCG TCT CGT CTA GAT CG-3′. The nucleotide sequence of the Taqman probe was 5′-6- FAM- CCG GCT TGG CTG CTT TTC CTG- TAMRA-3′. The Taqman prove and nucleotide sequence were purchased and designed at EURGENTEC (France). Thermal cycling conditions were as follows: 95°C for 10 minutes, 45 cycles of ten seconds at 95°C, 30 seconds at 58°C, and 10 seconds at 72°C. Results were expressed in cycle threshold values (Ct) and the oocyst number was derived using a samples curbed created by five, 10 times serial dilutions of a sample with a known oocyst concentration (178,571 oocysts/µL). All DNA samples were tested in duplicate and each assay was considered positive if at least one of the duplicated tests was positive. Each PCR run included a positive control and a negative control with no DNA.

Chapter 3. Results

3.1 Soil Physiochemical Characteristics

Table 1 gives the physicohemical properties of each soil series used in this experiment. The Greenson and Lewiston series were characterized as sandy loam soils, while the Gilford and Sparta series were characterized as loamy sand soils. The clay contents in the sandy loam soils were the highest with Greenson containing 19.5% clay and Lewiston containing 12.5% clay. Lower clay contents were found in the loamy sands, Sparta (9.5%) and Gilford (7.9%). The organic matter contents based on lost on ignition in the soils were greatest in Gilford (4.2%) and Greenson (3.8%) soil series. The highest pH values were found in Lewiston (7.50) and Greenson (7.4) while lower pH values were found in Sparta (6.90) and Gilford (5.20) soil series. Total calcium concentrations were lowest in Sparta at 9.36 $mg \cdot kg^{-1}$ and greatest in Greenson at 42.09 $mg \cdot kg^{-1}$.

Flow rate and flow velocity for each experimental column are provided in **Table 2**. The highest average flow velocity was observed in Sparta soils that did not contain surfactant (0.52 $cm \cdot hr^{-1}$). The lowest average flow velocity was observed in Greenson soil containing surfactant (0.29 $cm \cdot hr^{-1}$).

3.2 Prevalence and concentration of *T. gondii* oocysts in soil leachates

Leachate collected from all four soil types was analyzed to determine the concentration of *T. gondii* oocysts. Leachate samples were collected from at least 6 pore volumes of each soil column. The number of *T. gondii* oocysts in the soil

leachates from two replicates of the four soils with and without surfactant are represented in breakthrough curves (BTCs) as C/C0, where C is the concentration of *T. gondii* oocysts detected in leachates and C0 is the concentration of *T. gondii* oocysts on average in the input solutions. The calculation of C0 excluded the pulses of the column where no *T. gondii* was detected (Column 7). For detections of *T. gondii* where C/C0 exceeded 1, values were reduced and instead plotted as 1.

In the Sparta series soil without surfactant, *T. gondii* was only detected within the first pore volume of leachate in minimal concentrations (**Figure 1**). The BTCs of tracer display a fast increase and occur at 0.55 and 0.61 PVs. The heights of the tracer BTCs measured as C/C0 ranged from 0.018 to 0.031.

In the Sparta series soil containing surfactant, *T. gondii* was detected in much greater concentrations and throughout the experiment in replicate 1 (Column 3).

Replicate 2 (Column 4) showed only one detection of *T. gondii*. Peaks of tracer BTCs occurred at 0.02 PV in column 3 and at 0.57 PV in column 4. *T. gondii* peaked in Column 3 at early PVs of leachate. Peaks of tracer in the surfactant-containing columns were lower than columns that did not contain surfactant (**Figure 1**).

In the Lewiston soil series without surfactant, *T. gondii* was detected at low concentrations in both replicates. Detections occurred at Peak tracer concentration occurred at 0.60 and 0.99 PVs. The maximum height of tracer BTCs was 0.15 in both columns (**Figure 1**).

In the Lewiston series soil containing surfactant, *T. gondii* was detected throughout the experiment in both columns. C/C0 of *T. gondii* in column 7 ranged from 0.002 to 1, with the greatest concentrations appearing at 0.32 to 0.48 PV. In Column 8, C/C0 values of *T. gondii* ranged from 0.0003 to 0.011. Column 7 displays an early tracer peak at 0.008 PVs with a height of 0.026 and another peak at 1.43 PVs with a height of 0.021. The tracer peak in Column 8 occurs at 0.83 PV and has a height of 0.016.

In the Gilford soil series without surfactant, *T. gondii* was detected at in several samples at early times in concentrations up to a height of C/C0 of 1. At later times, there are lower detections *T. gondii*. The tracer peak also occurs in Column 9 near 0 PV and a second peak occurs at 0.61 PV with a height of 0.02. Column 10 has a tracer peak at 0.85 PV that has a height of 0.04.

In Gilford soils containing surfactant, *T. gondii* was detected in numerous samples throughout the experiment in both columns in concentrations higher than what were observed in columns without surfactant. The highest concentrations were at or near C/C0 or 1 in Column 12 with lower concentrations in Column 11.

The tracer peak in Column 11 occurs at 1.05 PV and has a height of 0.033. In Column 12, the tracer peak occurs at 1.56 PV and has a height of 0.02.

In the Greenson soil series without surfactant, *T. gondii* was detected mostly early in the experiment. In Column 13, detections of *T. gondii* are low concentrations and occur mostly within the first 2.7 pore volumes of leachate. Detections of *T. gondii* in Column 14 occur mostly within the first pore volume of leachate and are

lower than that of Column 13. The tracer peak in Column 13 occurs near 0 PVs and has a height of 0.07. The tracer peak in Column 14 occurs 0.18 PV and has a height of 0.03.

In Greenson soil series containing surfactant, *T. gondii* detections vary between the two replicate columns. In Column 14, detections are of low concentrations and all occur within the first pore volume of leachate. In Column 16, detections of *T. gondii* are of higher concentrations than that of Column 15 and occur throughout the entire experiment. n Column 15, the tracer peak occurs at2.11 PV and has a height of 0.01. The tracer peak in Column 16 is at 2.47 PV and has a height of 0.01. There are several local peaks that occur before the tracer peaks in Columns 15 and 16.

3.3 Spatial distribution and concentration of *T. gondii* oocysts in soils

Following the flow and transport experiments, soil columns were sliced into layers. Soil layers were subsampled in duplicated and analyzed to determine the spatial distribution of *T. gondii* and water content throughout each column. The number of *T. gondii* oocysts in the soil matrices is represented as number of oocysts per gram of dry soil. Concentrations of *T. gondii* and water content by depth are represented in **Figure 2**.

In the Sparta soil series, oocysts remained concentrated near the top of the columns in columns containing surfactant and those that did not. In both replicates, the concentration of *T. gondii* was highest in the first soil layer. The maximum

concentration was in the uppermost layer of Column 1 and was about 3000 oocysts/g dry soil.

The distribution of oocysts in surfactant-containing Sparta soil columns differed from that of columns without surfactant. In the first replicate containing surfactant (Column 3), detections of a greater number of oocysts throughout the soil column. The greatest concentration was in at about 12 cm and was about 690 oocysts/g dry soil, though there were detections of oocysts within each soil layer.

The second replicate of surfactant-containing Sparta soil (Column 4) was more similar to those that did not contain surfactant in that concentrations were greatest at lower depths of the soil column and comparatively low at greater depths. The concentration of oocysts in the first layer of soil in Column 4 was about 32364 oocysts/g dry soil.

In the Lewiston soil series, *T. gondii* oocysts were concentrated in the uppermost soil layers in those columns that contained and did not contain surfactant. The maximum concentration of *T. gondii* in the first replicate of Lewiston without surfactant is about 188 oocysts/g dry soil and in the second replicate, the maximum is about 119 oocysts/g dry soil.

In the first replicate of surfactant-containing Lewiston soil column has greater detections than any other Lewiston column below the first soil layer but the greatest concentration is about 157 oocysts/g soil and is within the first soil layer. The second surfactant-containing Lewiston soil column has the greatest

concentration of all Lewiston columns, about 23439 oocysts/g dry soil, which is within the first soil layer.

In the Gilford soil series, concentrations of *T. gondii* are highest at shallow depths of the columns in columns that contain surfactant and those that do not. In both replicates of that do not contain surfactant, concentrations of *T. gondii* were greatest in the uppermost layers of soil. In the first replicate (Column 9), the maximum concentration was about 1194 oocysts/g dry soil and in the second replicate (Column 10), the maximum concentration was about 616 oocysts/g dry soil.

In the first replicate of Lewiston columns containing surfactant (Column 11), the maximum concentration was about 192 oocysts/ g dry soil and occurred in the uppermost layer of soil. In the second replicate of Lewiston columns containing surfactant (Column 12), the maximum concentration was 1942 oocysts/g dry soil and occurred in the second layer of soil.

In the Greenson soil series columns that do not contain surfactant, concentrations of *T. gondii* were greatest in the uppermost layer of soil. In the first replicate (Column 13), the greatest concentration was about 175 oocysts/g soil. In the second replicate (Column 14), the greatest concentration was about 1418 oocysts/g dry soil. In Column 13, there were greater concentrations at lower depths than occurred in Column 14.

In the Greenson soil series that contained surfactant, *T. gondii* is better-distributed than in those that did not contain surfactant. The first surfactant

replicate (Column 15) has the greatest concentration within the first layer of soil of about 58 oocysts/ g dry soil, but there are similar concentrations detected at lower depths. In the second replicate, the highest concentration occurs in the lowest depth of the column and is about 33 oocysts/g dry soil.

3.4 Cumulative recovery of *T. gondii* oocysts in leachates and soils

Recovery results are presented in **Table 3**. Calculations are based on the concentration of *T. gondii* detected in the pulses, excluding column 7 where *T. gondii* was not detected. **Table 3** gives the predominant fate of oocysts in each column.

The predominant fate of Sparta and Lewiston soils was retained in the soil. In Sparta and Lewiston soils without surfactant, the predominant fate was in the soil in both replicates. In those that did contain surfactant, the predominant fates varied in the replicates. In Greenson, the majority of columns exhibited breakthrough of *T. gondii* into leachate as the predominant fate. In one column without surfactant, the predominant fate was retention within the soil. In Gilford soil, most oocysts were retained within the soil in the columns without surfactant. In columns containing surfactant, breakthrough into leachate was the predominant fate of *T. gondii*.

Chapter 4. Discussion

The fate and transport of *T. gondii* oocysts in unsaturated soil were investigated in a series of laboratory soil column experiments subject to simulated artificial rainfall events in order to induce unsaturated flow conditions. The transport of *T. gondii* oocysts with infiltrating water through the vadose zone was demonstrated in all soils. Differences in degree of the transport of *T. gondii* oocysts between soil series and whether or not surfactant was present in columns were observed.

T. gondii oocysts were detected in leachate in each soil column in this experiment. Generally, detections of greater concentrations occurred in surfactant-containing soil columns for all soil series.

T. gondii oocysts were detected in the soil matrices of all soil types, in all columns. In most cases, *T. gondii* was detected in the greatest concentration in upper layers of soil. The retention of microorganisms within upper soil depths has been shown in studies of other microorganisms (Mawdsley et al., 1996, Gerba et al., 1975, Darnault et al., 2017). In several columns, *T. gondii* was detected at lower depths indicating that considerable transport occurred. This was particularly the case in Greenson soils containing surfactant. In both replicates, a considerable number of *T. gondii* oocysts were detected in the lowest (Column 16) and second to lowest layer (Column 15). This indicates significantly enhanced transport in the Greenson soil series by surfactant.

The use of repacked, homogenous natural soil columns allows for the investigation of the impact on soil physiochemical properties on transport of *T. gondii*. Similar approaches have been taken in previous studies to assess fate and transport of other microorganisms, including Darnault et al., 2017; Trevors et al., 1990; Gannon et al., 1991; and Elsas et al., 1991. Soil water chemistry, water content and flow rate of water through soil have been determined to have major impacts on the mobility of microorganisms (Kuikman et al., 1990; Trevor et al., 1990; van Elsas et al., 1991; Balthazard-Accou et al., 2014; Shapiro et al., 2008). The oocysts are also hydrophilic, which suggests that *T. gondii* is capable of widespread contamination through water (Shapiro et al., 2008).

Our discussion attempts to provide an understanding of the environmental processes that govern the transport of *T. gondii* oocysts in soil. Transport and retention of oocysts has been clearly demonstrated by this study in all four of the soil series examined. This transport and retention varies by soil series and with the inclusion of an anionic surfactant in the system. *T. gondii* oocysts have a negative charge under typical environmental conditions due to polymeric cross-links of Cys- and/or Tyr-rich proteins on the surface (Dumetre et al., 2011). In our experimental system, the presence of clay particles, organic materials and dissolved ions in the soil-water solutions and soil matrices are likely to have impacted the transport of *T. gondii* oocysts. The inclusion of surfactant in the experimental system has shown an impact on the interactions of soil particles and oocysts but inducing steric forces,

reducing the frequency of oocyst adsorption onto soil grains by coating oocysts and soil grains and reducing air-water surface tension.

4.1 Effects of soil type on *T. gondii* transport

Research on the fate and transport of protozoan cysts has been minimal (Darnault et al, 2017; Gerba et al., 1975; Bitton and Harvey, 1992). Very little is known about the transport of *Toxoplasma* in the environment specifically (Dumetre et al., 2011). It is expected that adsorption, straining and sedimentation will affect the transport of *T. gondii* oocysts as has been observed for other protozoan cysts such as *C. parvuum* (Mawdsley et al., 1996; Bradford and Bettahar, 2005; Darnault et al., 2017). The larger size of *T. gondii* oocysts (10-12 μm) compared to *C. parvuum* oocysts (4-6 μm) may contribute to differences in transport between the two protozoa, including increased straining.

Soil physiochemical properties, including mineralogy, organic matter content, clay content, and pH, have an impact on microbial transport (Dumetre et al., 2011, Bitton et al., 1974; Bashan and Levanony, 1988; Tan et al., 1991; Huysman and Verstaete, 1993; Mawdsley et al., 1996). Organic matter and clay content of soil are particularly influential on the adsorption of microorganisms because of their large surface area and negative charge (Reddy et al., 1981). In addition to physiochemical properties, soil grain size and grain shape play an important role in the extent of straining that occurs (Tufenkji et al., 2004). Increased grain size has been shown to increase straining. Generally, when the ratio of particle diameter to median soil

grain size (d_p/d_{50}) is greater than 0.05, physical straining is thought to play a significant role in particle retention, though several studies have indicated that straining is key when d_p/d_{50} is as low as 0.002 (Tufenkji, 2007). Straining has been identified as an important mechanism for retention of *C. parvuum* in soil by Tufenkji et al., 2004 and given that *T. gondii* oocysts are larger in size than *C. parvuum*, straining is likely a controlling factor in the removal of *T. gondii* from the leachate.

Enhanced transport was observed in Gilford and Greenson soils compared to other soil series studied without surfactant. Gilford is a loamy sand and Greenson is a sandy loam, indicating that the soil type may not be the primary controlling factor in transport of *T. gondii* in this experiment.

4.2 Effects of surfactant on *T. gondii* transport

The surfactant employed in this experiment was Aerosol 22 (A22), which is anionic. Mingorance et al. (2007) studied the effect of A22 on soil water transport properties in a gravity driven flow column with various soils. They showed that in A22 increased saturated hydraulic conductivity (K_S) in the clay loam and decreased K_S in the clay and loam studied. Transport of A22 solutions through soil was affected by Ca^{2+} and Mg^{2+} ions in the soil. A22 precipitates as salts and reduces the porosity of the soil system. Peng et al. (2017) also found that hydraulic conductivity was decreased overall in sandy loam and loamy sand soils studied in saturated soil column experiments, though there was significant variation in K_s values over time and throughout the various layers of soil. The variations in K_s were hypothetically

attributed to the swelling of clay, the collapse of soil aggregates and subsequent particle displacements from surfactant adsorption. Particle displacement caused pore clogging in low layers and higher porosity in layers above. Particle displacement was also suggested in Mingorance, et al. (2007) and was evidenced by coloration of effluent. Coloration of leachate was observed in this experiment as well.

In this experiment, surfactant promoted transport of *T. gondii* in all soil types. Many surfactant-containing columns exhibit tailing concentrations throughout the duration of the experiment. Greater peak concentrations were generally detected in columns with surfactant. Surfactant produces a slight decrease in flow velocity in Sparta, Lewiston, and Greenson soil series when averages of replicates are compared. In Gilford soil, the flow velocity is increased with the inclusion of surfactant. Velocity is not consistent between columns of the same soil type and surfactant status. Decreases in flow velocity with the addition of surfactant may be attributed to the clogging.

Steric forces may be produced when surfactants adsorb onto porous media and prevent microbes from approaching the porous media surface, reducing the interaction of particles (Zhong et al., 2017; Brown and Jaffe, 2001). The presence of steric forces due to the addition of surfactant may contribute to enhanced transport in soil columns containing surfactant.

In unsaturated systems, microbial transport is impacted by the presence of the air-water interface (Wan et al., 1994; Zhong et al., 2017). Surfactants have been

observed to reduce air-water surface tension and allow for the wider spread of microbes through the system (Liu et al., 2011).

4.3 Interaction of *T. gondii* oocysts with soil

Increased transport is exhibited in Gilford (a loamy sand) and Greenson (a sandy loam) soil series as compared to Sparta and Lewiston. These soils contain the greatest amount of organic material (OM) as compared to other soils in the series. The presence of organic matter has been shown to increase cation exchange capacity, surface area, and number of sites for bacterial adsorption (Zhong et al., 2017). An increase in OM has been found to cause a decrease in collision efficiency of sand and *C. parvuum* oocysts, which produces a higher degree of breakthrough (Abudalo et al., 2010). This phenomenon may also be occurring for *Toxplasma* oocysts in this experiment.

Proteins on the surface of *C. parvuum* have been shown to produce steric forces between cell surfaces and soil particles. This produces decreased attachment to soil grains. Considine et al. (2000, 2001, 2002) proposed that these steric forces may be attributed to "hairy" proteins on the surface of the oocyst that cause repulsion between silica grains and oocysts (Tufenkji, 2007). This phenomenon may be occurring between the surface of *T. gondii* and soil grains in this experiment.

More experimentation is necessary to better characterize the surface of *T. gondii* oocysts and their interaction with soil grains.

Surfactants have been shown to enhance the transport of microbes through a variety of mechanisms. Brown and Jaffe (2001) found that a nonionic surfactant enhanced transport of a bacterial culture through porous media by expanding the electric double layer around bacteria and porous media, which increased the electrostatic repulsion of materials and reduced collision frequency.

Surfactants may adsorb onto cell surfaces by van der Waals and hydrophobic forces, which causes a reduction in cell surface hydrophobicity and weakens adsorption by bacteria (Zhong et al., 2017; Gorna et al., 2011; Zhang and Miller et al., 1994). Ishigami et al. (1987) also showed that the adsorption of an anionic surfactant added additional negative charge to cell surfaces, which decreases its zeta potential and weakens the interaction between bacteria and sand surface.

Surfactant may also change the surface charge of soil particles. Zeta potential of sand has been shown to become more negative with increasing concentrations of anionic surfactant, which enhanced bacterial transport (Zhong et al., 2017; Chen et al., 2004; Bai et al., 1997).

4.4 Recovery of *T. gondii* oocysts in soil and water samples

Recovery results are given in **Table 3**. The pulse concentration to which total concentrations detected in leachate and soil were compared was an average of all concentrations detected in the pulses applied to each column. There was a great deal of variability in the pulse concentrations, which likely biased the pulse concentration low. A number of the percentages of recovery calculated in the soil or

leachate exceeded the average number of oocysts in the pulses. The predominant fate of oocysts is still an accurate assessment, though the percentage recovery represents errors in *T. gondii* detection. In order to more accurately calculate recovery of oocysts in the system, it is necessary to more accurately determine the number of oocysts in the pulse of *T. gondii* applied to the system. Recovery of *T. gondii* is greater than values shown in previous experiments with *C. parvuum* (Darnault et al., 2017; Petersen et al., 2012; Mawdsley et al., 1996). Variation in detection of *T. gondii* in both soil and leachate is likely due to the presence of PCR inhibitors such as surfactant and soil organic matter and to the challenges in enumerating *T. gondii* by qPCR which are discussed in Dumètre and Dardé, 2003.

The predominant fate of *T. gondii* in leachate in Gilford and Greenson soils where surfactant was present is consistent with enhanced transport in these soils with the addition of surfactant. In Sparta and Lewiston soils, transport was enhanced by surfactant, but the predominant fates of oocysts varied in the surfactant-containing systems.

Chapter 5. Conclusions

- *Toxoplasma* is a parasite that can be transmitted through a variety of routes that poses threats to human health.
- It is present in the environment and can last there for long periods of time and transport of *T. gondii* through the environment is currently not well understood.
- Based on the results of this experiment, *T. gondii* oocysts are capable of considerable transport.

Surfactant was shown to enhance transport in all soil series studied. Surfactants may cause enhanced transport through steric forces, by increasing the hydrophobicity of either the oocysts or the porous media, or by reducing the air-water surface tension in this unsaturated system.

This study highlights the need for additional study of the transport of *Toxoplasma gondii* through porous media. Saturated flow experiments that allow for modeling would allow for better prediction of the fate and transport of *T. gondii* oocysts in the environment and improve the assessment of risk of exposure through environmental means.

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Appendix: Tables and Figures

		Soil Series			
		Sparta	**Lewiston**	**Gilford**	**Greenson**
Texture	Sand	82.00	79.70	84.40	66.90
	Silt	8.40	7.70	7.70	13.60
	Clay	9.50	12.50	7.90	19.50
	USDA Texture	Loamy sand	Sandy loam	Loamy sand	Sandy loam
pH		6.90	7.50	5.20	7.40
Soil Carbon	TC (%)	1.93	1.28	2.91	2.00
Organic Material	Walkey-Black	2.80	0.60	3.30	2.80
(%)	Loss on Ignition	3.40	0.80	4.20	3.80
Total Nitrogen	(%)	0.15	0.13	0.19	0.08
EC	(dS/m)	0.36	1.01	0.68	0.75
CEC	(mmolc · kg-1)	86	115	84	175
Elements and Ions	Sodium (mg · kg-1)	3.27	11.29	1.87	13.55
	Potassium (mg · kg-1)	13.80	22.00	26.70	10.80
	Magnesium (mg · kg-1)	4.84	14.91	5.71	12.00
	Calcium (mg · kg-1)	9.36	37.10	14.64	42.09
	Chloride (mg · kg-1)	10.40	53.20	44.60	49.90
	Sulfur (mg · kg-1)	1.79	7.50	3.73	8.40
	Nitrate (mg·L-1)	12.60	0.40	44.10	0.90
	Boron (mg · kg-1)	0.02	0.04	0.02	0.02
	CO32-+ HCO32- (mmolc · L-1)	1.80	8.41	0.50	6.04

Table 1: Physiochemical properties of the four series of soils examined—Sparta, Lewiston, Gilford and Greenson.

Soil Type	Surfactant Status	Column	Flow Rate	Ave. Flow Rate	Flow Velocity	Ave. Flow Velocity
			mL/hr		cm/hr	
Sparta	No Surfactant	1	35.25	37.04	0.50	0.52
		2	38.83		0.55	
	Surfactant	3	36.00	35.89	0.51	0.51
		4	35.78		0.50	
Lewiston	No Surfactant	5	35.62	33.92	0.50	0.48
		6	32.21		0.45	
	Surfactant	7	29.58	27.71	0.42	0.39
		8	25.83		0.36	
Gilford	No Surfactant	9	29.95	30.25	0.42	0.43
		10	30.55		0.43	
	Surfactant	11	36.40	30.94	0.51	0.44
		12	25.48		0.36	
Greenson	No Surfactant	13	32.84	32.87	0.46	0.46
		14	32.89		0.46	
	Surfactant	15	17.43	20.58	0.25	0.29
		16	23.73		0.33	

Table 2: Flow rate and flow velocity for each of the four series of soils examined – Sparta, Lewiston, Gilford and Greenson. Averages of replicates are provided.

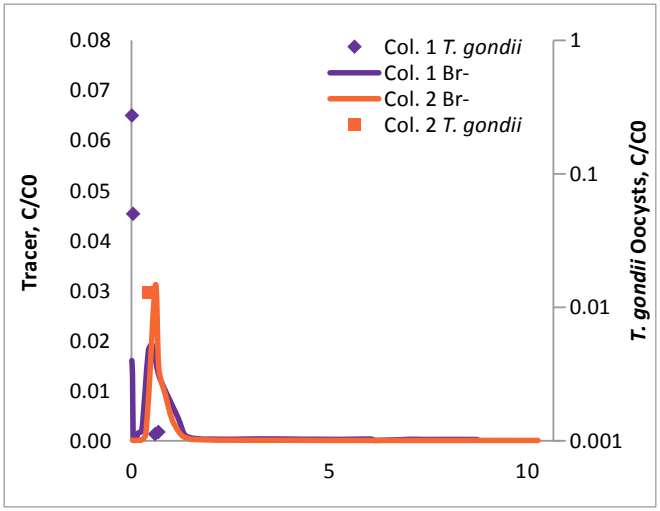
	Column	Total Oocysts in Leachate	Percent in Leachate	Total Oocysts in Soil	Percent in Soil	Total Recovery	Predominant Fate
Sparta	1	39866	0.04	472587	0.52	0.57	SOIL
	2	12952	0.01	187168	0.21	0.22	SOIL
	3	17572242	19.40	315198	0.35	19.75	LEACHATE
	4	7307	0.01	7774378	8.59	8.59	SOIL
Lewiston	5	3342	0.00	25768	0.03	0.03	SOIL
	6	6246	0.01	23557	0.03	0.03	SOIL
	7	6572196	7.26	70948	0.08	7.34	LEACHATE
	8	29873	0.03	5716660	6.31	6.35	SOIL
Gilford	9	165992	0.18	271882	0.30	0.48	SOIL
	10	22172	0.02	132828	0.15	0.17	SOIL
	11	194544	0.21	53435	0.06	0.27	LEACHATE
	12	5942361	6.56	718490	0.79	7.36	LEACHATE
Greenson	13	68084	0.08	55795	0.06	0.14	LEACHATE
	14	40640	0.04	338275	0.37	0.42	SOIL
	15	54929	0.06	27285	0.03	0.09	LEACHATE
	16	186495967	205.95	9742	0.01	205.96	LEACHATE

Table 3: Recovery data for *T. gondii* oocysts in leachate and soil for each column. Purple shading indicates that a column contains surfactant.

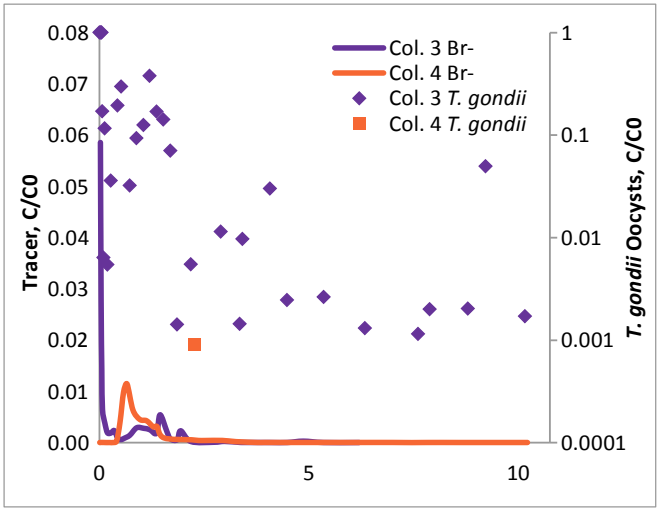
No Surfactant

Surfactant

Sparta (LS)

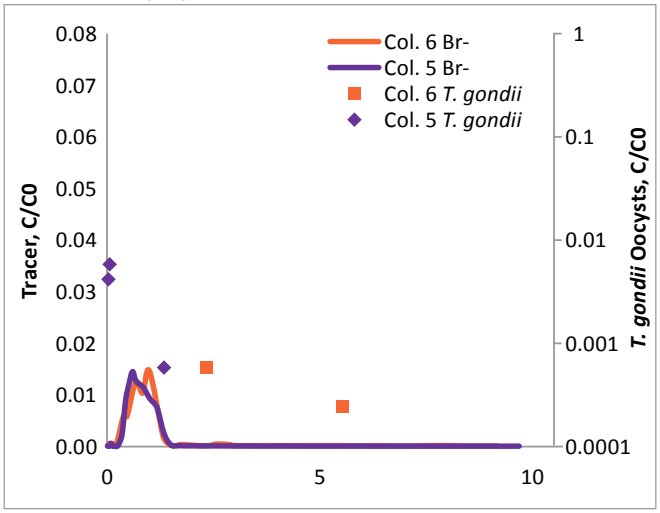


(a)

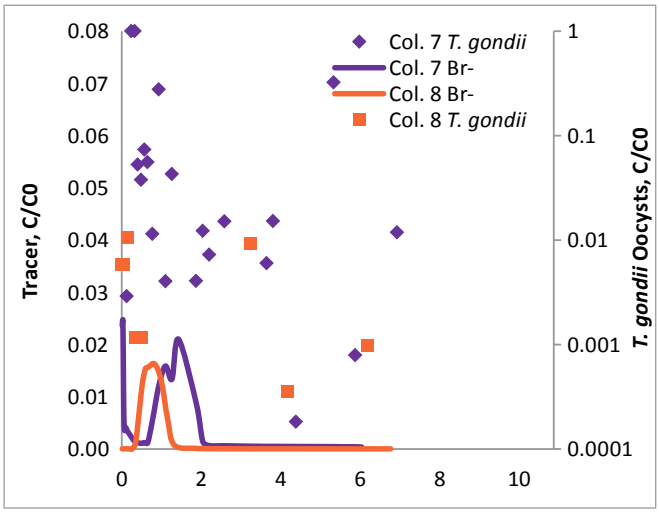


(b)

Lewiston (SL)

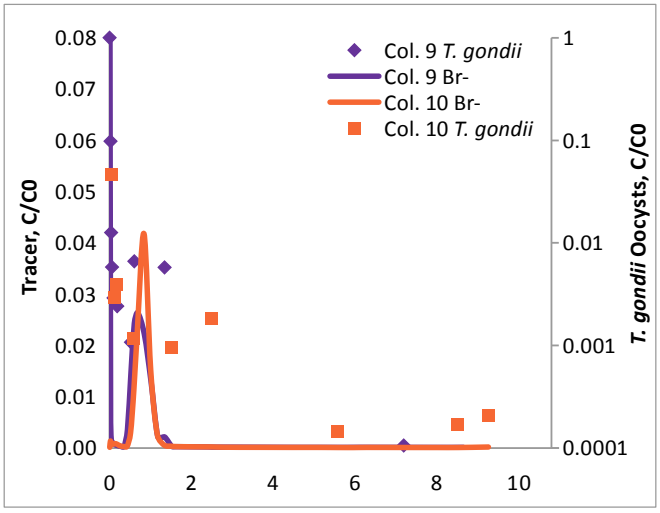


(c)

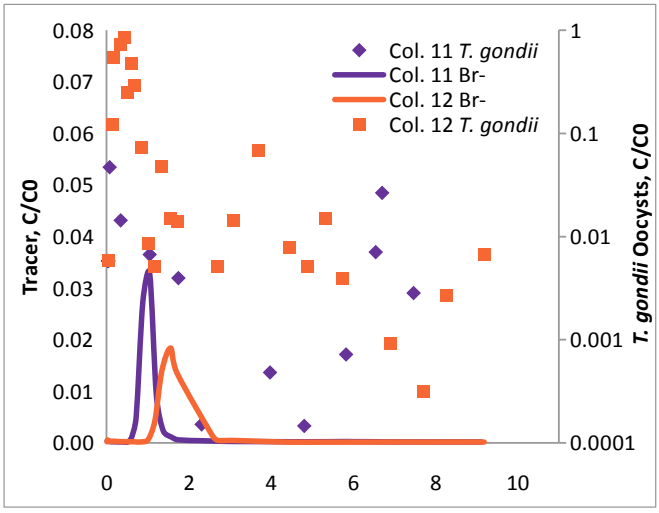


(d)

Gilford (LS)



(e)

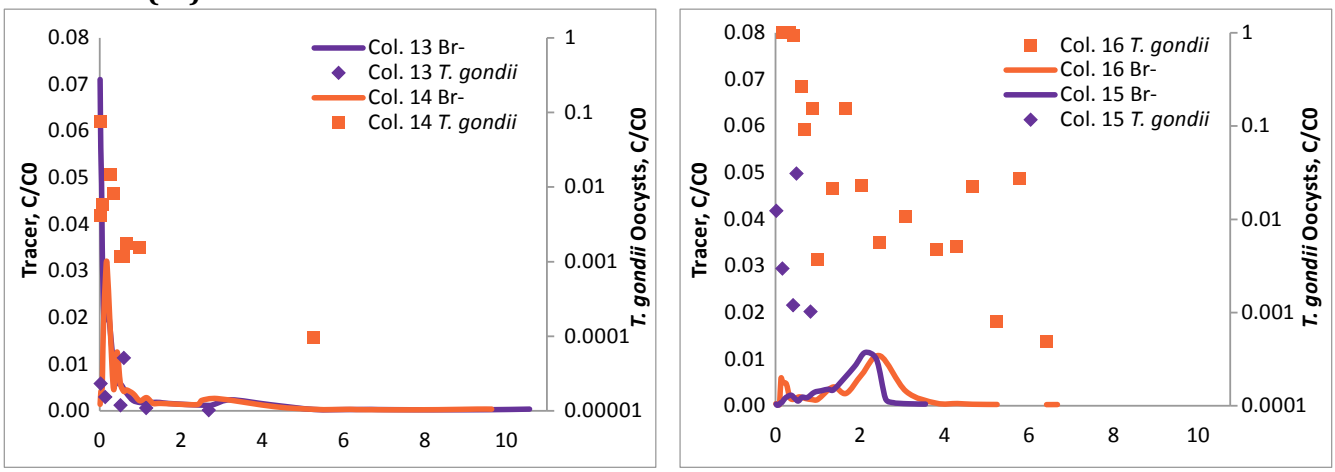


(f)

No Surfactant

Surfactant

Greenson (SL)

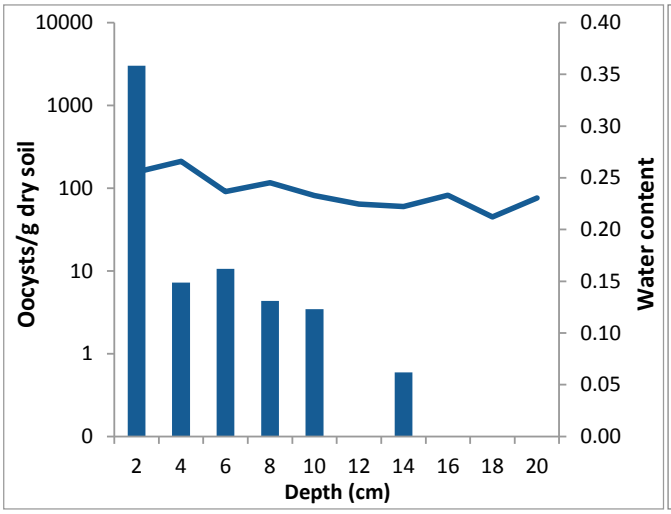


(g) (h)

Figure 1: Breakthrough curves of *Toxoplasma gondii* oocysts and bromide tracer from each of the four soil series studied, with and without surfactant: Sparta (a, no surfactant and b, with surfactant), Lewiston (c, no surfactant and d, with surfactant), Gilford (e, no surfactant and f, with surfactant) and Greenson (g, no surfactant and h, with surfactant).

Sparta Soil Series

No surfactant—Column 1



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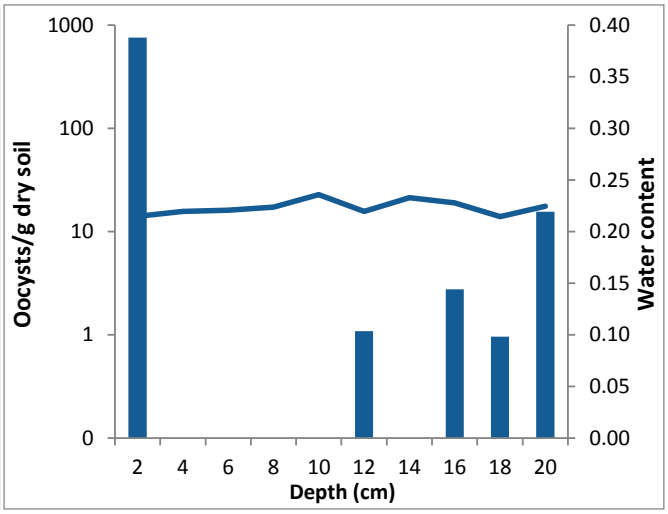
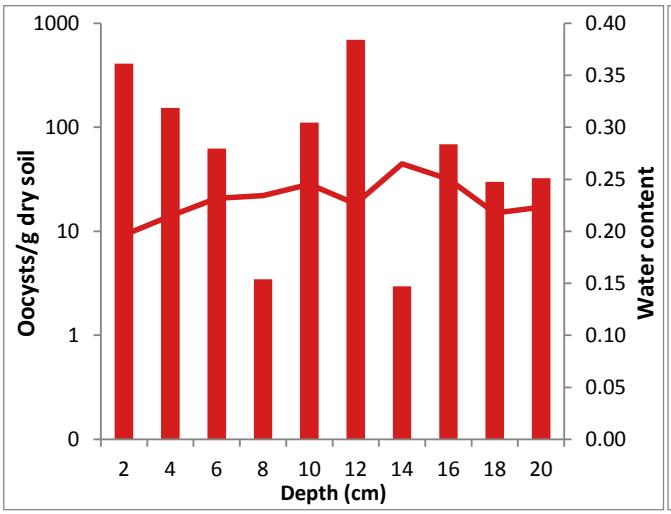
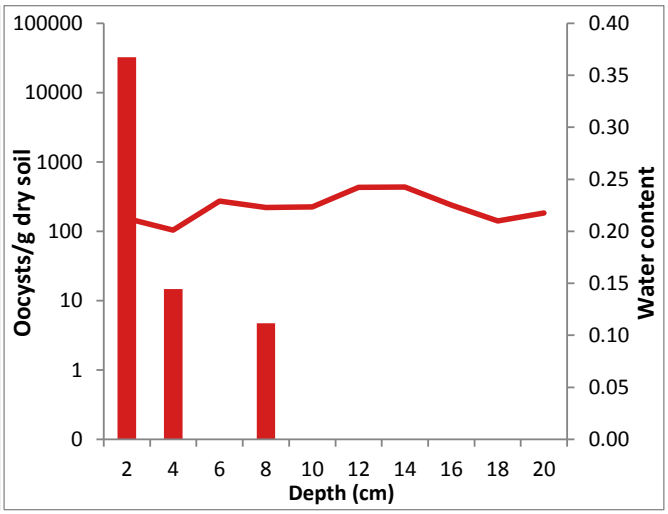


Surfactant—Column 3

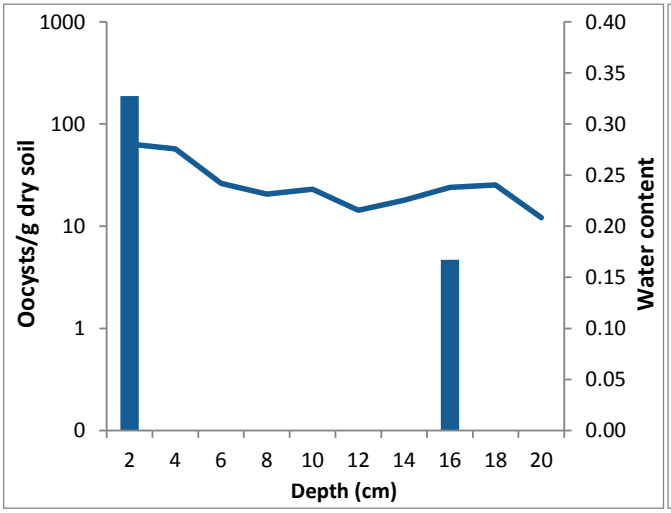


Surfactant—Column 4



Lewiston Soil Series

No Surfactant—Column 5



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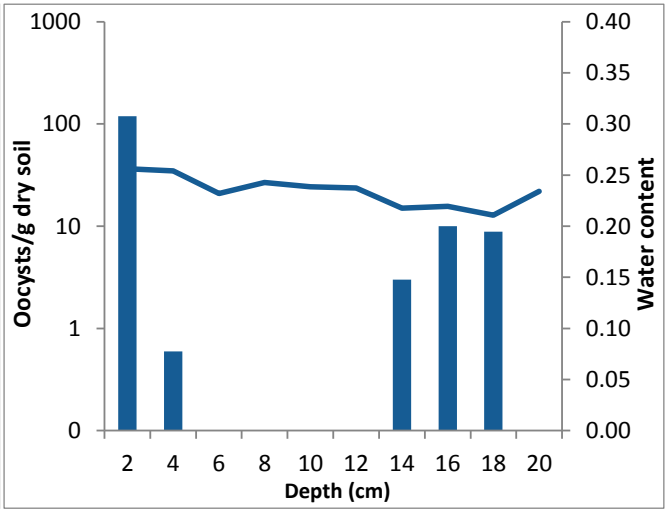
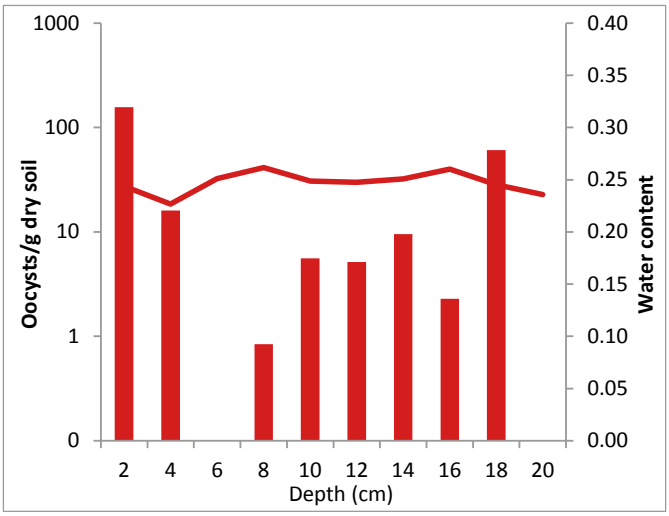
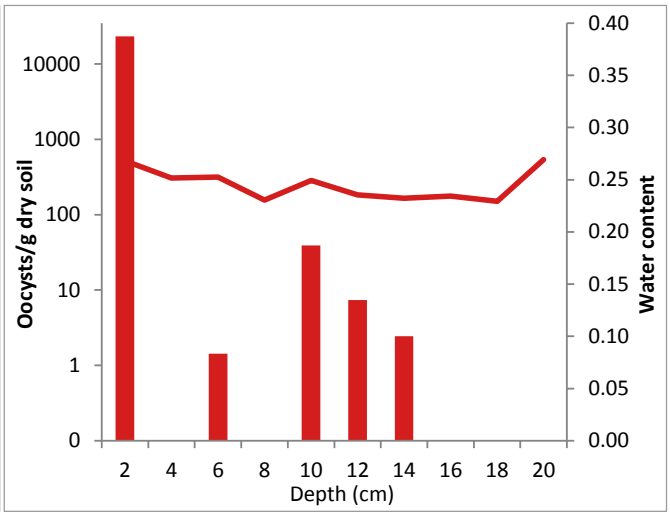


Surfactant—Column 7

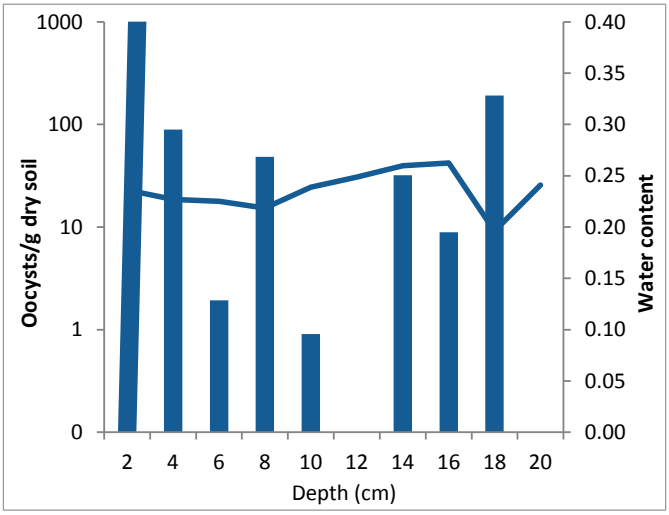


Surfactant—Column 8

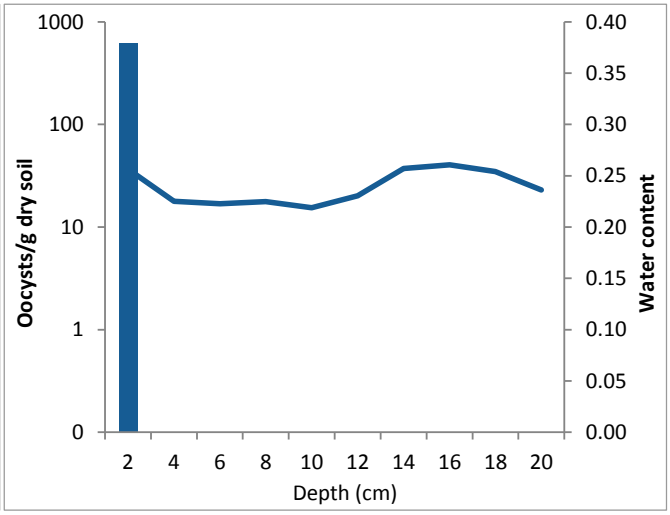


Gilford Soil Series

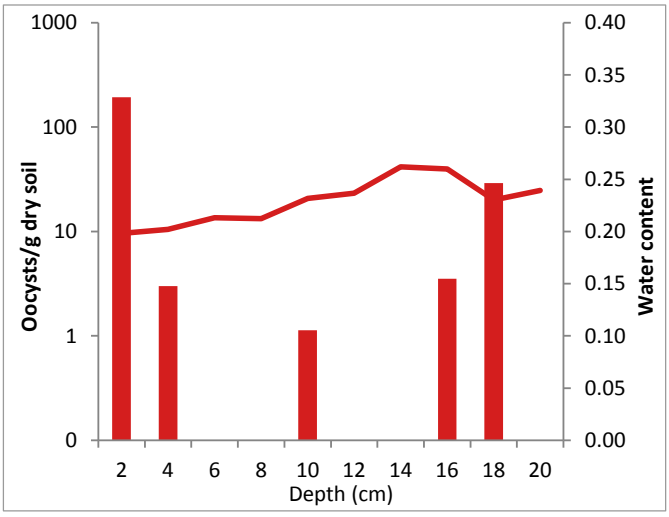
No Surfactant—Column 9



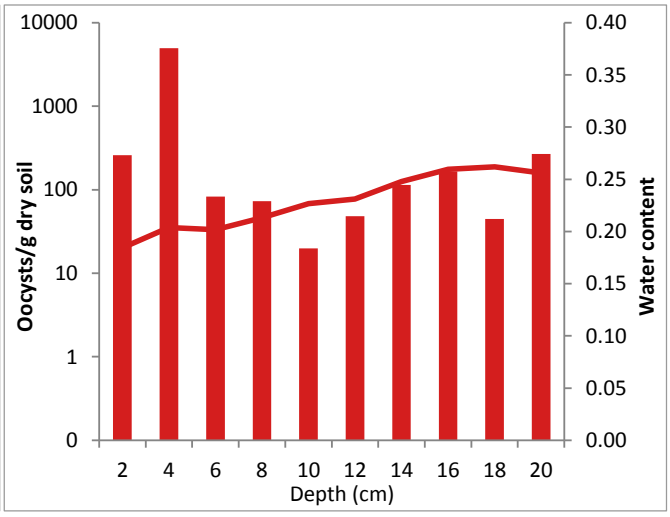
No Surfactant—Column 10



Surfactant—Column 11



Surfactant—Column 12



Greenson Soil Series

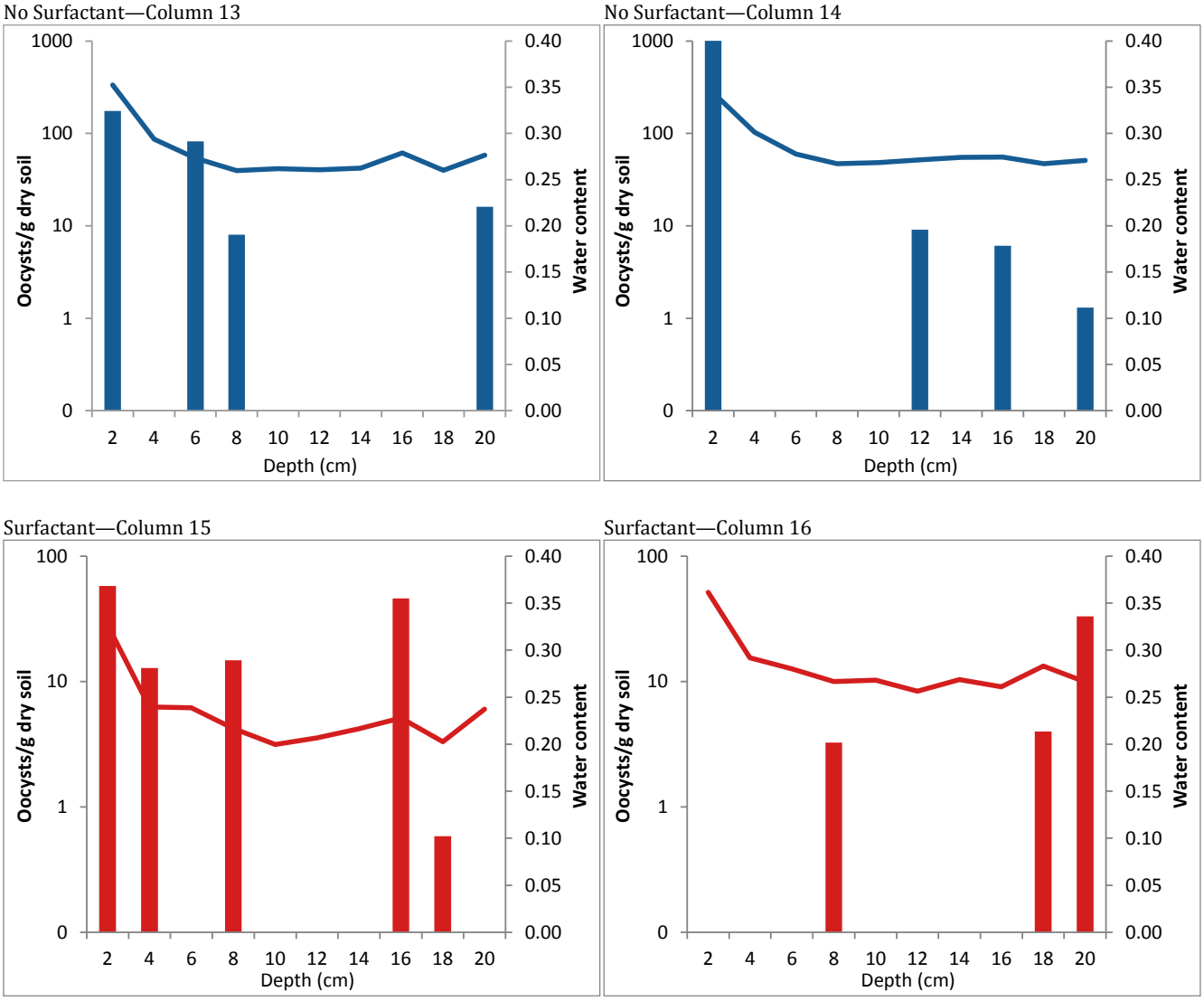


Figure 2: Spatial distribution of *T. gondii* oocysts recovered from soil columns and the water content in soil profiles in each of the soil series examined (Sparta, Lewiston, Gilford and Greenson) following the application of artificial rainfall treatments.