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Effect of Dissolved Organic Carbon on the Transport and Attachment Behaviors of *Cryptosporidium parvum* oocysts and Carboxylate-Modified Microspheres Advected through Temperate Humic and Tropical Volcanic Agricultural soil

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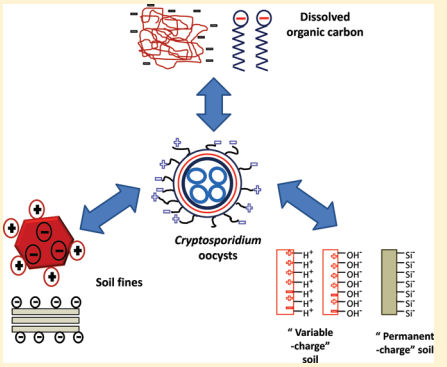
Supporting Information

ABSTRACT: Transport of *Cryptosporidium parvum* oocysts and microspheres in two disparate (a clay- and Fe-rich, volcanic and a temperate, humic) agricultural soils were studied in the presence and absence of 100 mg L^{-1} of 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 in soil columns was highly sensitive to the nature of the dissolved organic carbon (DOC), whereas oocysts transport was more affected by soil mineralogy. SDBS increased transport of microspheres from 48% to 87% through the tropical soil and from 43% to 93% in temperate soil. In contrast, SRHA reduced transport of microspheres from 48% to 28% in tropical soil and from 43% to 16% in temperate soil. SDBS also increased oocysts transport through the temperate soil 5-fold, whereas no oocyst transport was detected in tropical soil. SRHA had only a nominal effect in increasing oocysts transport in tropical soil, but caused a 6-fold increase in transport through the temperate soil. Amendments of only 4 mg L^{-1} SRHA and SDBS decreased oocyst hydrophobicity from 66% to 20% and from 66% to 5%, respectively. However, SDBS increased microsphere hydrophobicity from 16% to 33%. Soil fines, which includes clays, and SRHA, both caused the oocysts zeta potential (ζ) to become more negative, but caused the highly hydrophilic microspheres to become less negatively charged. The disparate behaviors of the two colloids in the presence of an ionic surfactant and natural organic matter suggest that microspheres may not be suitable surrogates for oocysts in certain types of soils. These results 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 soil mineralogy.



INTRODUCTION

Agricultural runoff and seepage is typically high in DOC because of applications of manure, pesticides, and recycled water. Agriculture involving livestock can also produce high numbers of pathogens, including oocysts of the protozoan parasite, *Cryptosporidium parvum*,[1] which can remain infective in the subsurface near dairy farms for at least 6 months.[2] Much has been learned about the fate and transport of oocysts in soils from column-scale studies. For example, such studies have shown that oocysts do not readily attach to soil particles under certain conditions,[3] that oocysts can be transported through preferential flow paths in the vadose zone,[4] that the viability of oocysts depends, at least in part, on soil type,[5] and that their transport can be greatly facilitated by the presence of natural organic matter (NOM) and anionic surfactants.[6,7] However, it has been shown that it is difficult to extrapolate column test results to the field [8,9] and finding suitable surrogates for oocysts can be problematic.[10]

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Recent insight into the complexity of the oocyst wall has been gained by examining its macromolecular composition,[11] hydrophobicity,[12] interaction with model porous media using atomic force microscopy,[13] and molecular spectroscopy studies.[14] Although the surfaces of carboxylate-modified microspheres are simple by comparison, in that they are dominated by only one type of ionizable functional group and lack the three-dimensional structures that characterize the surfaces of oocysts, they have been used as particulate surrogates for oocysts in field-scale,[15] pilot-scale,[16,17] and column-scale [18,19] studies.

We undertook a systematic study to understand better the effects of anionic surfactants, a common surface water contaminant, and NOM (humic acid) on the transport of both oocysts and carboxylate-modified microspheres in two compositionally disparate agricultural soils. Because some of the soil clays can become mobilized under transient saturated conditions, we also examined the role of suspended soil fines. The test soils consisted of a humic-rich, permanent-charge soil from Illinois and a high-iron, variable-charge, volcanic soil from Hawai'i. Little is known about the fate of *Cryptosporidium* in variable charge soils (VCS), although oocyst-contamination of groundwater in regions characterized by VCS have been reported.[20] Given the compositional differences between the two test soils and between the two colloids, our major hypothesis was that the dissimilarities in attachment behaviors between oocysts and microspheres will depend strongly on the nature of the soil. Our second hypothesis was that the presence of humic acid and anionic surfactants would affect the extent of attachment of the highly hydrophilic microsphere and the more hydrophobic oocysts differentially.

MATERIALS AND METHODS

***Cryptosporidium parvum* Oocysts.** We used formalin-inactivated oocysts in our laboratory studies for reasons of laboratory safety and in order to more directly compare our results to the many published studies where formalin-inactived oocysts were employed. Although a few studies have shown formalin- inactivation enhances deposition,[12,22] other studies have shown that the surface characteristics of infective and formalin-inactivated oocysts at circumneutral pH are similar.[21,23] 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.[21] The final oocyst concentrations used for the column experiments were between 2 and 6 $\times$ 10^6 mL^{-1}. Enumerations of oocysts were performed using epifluorescence microscopy.[21] At least 100 microscopic fields were examined for each sample.

Microspheres. Carboxylate-modified, polystyrene microspheres in the 1.8 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 size variation in *C. parvum* oocysts that are commonly reported for aquatic environments.[15] The microspheres were enumerated by epifluorescence microscopy as outlined by methods previously described in Mohanram et al.[19] At least 100 fields were counted for every effluent sample.

ζ Potential. Electrophoretic mobilities of microspheres and oocysts were measured at 22 °C in a 10^{-3} M NaCl solution by Laser Doppler microelectrophoresis (Zeta Pals-Zeta Potential Analyzer, Brookhaven Instruments) using ~4 $\times$ 10^5 oocysts mL^{-1} or 4 $\times$ 10^6 microspheres mL^{-1}. Electrophoretic mobilities were converted to ζ potentials using the Smoluchowski equation.[24]

Dissolved Organic Carbon (DOC). SRHA was obtained from the International Humic Substance Society (IHSS Code: S101H) was employed to assess the effect of natural organic matter (NOM) on the transport behavior of oocysts and oocyst-sized microspheres in soils. It has an average molecular weight of 3820 g mol^{-1} and contains 15% aliphatic and 42% aromatic compounds.[25] SDBS (Sigma Aldrich, St. Louis, MO, molecular weight 348.5 g mol^{-1}) was employed to assess the role of anionic surfactants on the transport behavior of the two colloids. Sodium hydroxide (0.01 N) was used to adjust the pH of both SRHA and SDBS solutions. The ionic strength of the background, SRHA, and SDBS solutions was adjusted to ~10^{-3} M using NaCl.

Soil Fines. The soil fines, which include the clay minerals, from the two soils examined were isolated according to an earlier published procedure.[26] Briefly, 1 g of <63-μm soil-particle size fraction was added to 250 mL of purified water (Milli-Q, 18 MΩ cm resistivity), sonicated for 1 min and centrifuged using a 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 the 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 h to precipitate the soil fines, which were then dried at 70 °C overnight.

Batch Experiments to Assess Changes in ζ Potential. 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 mg L^{-1} were dissolved in 10^{-3} M NaCl and sonicated for 1 min to disperse the aggregates. *C. parvum* oocysts (5 $\times$ 10^5 mL^{-1}) or 1.8 μm microspheres (6 $\times$ 10^6 mL^{-1}) were added to 10 mL of either (1) soil fines solution, (2) soil fines solution containing 4 mg L^{-1} of SRHA, or (3) 4 mg L^{-1} SRHA in 15 mL polypropylene centrifuge tubes and mixed for 24 h at a speed of 25 rpm. All experiments were conducted in duplicate at pH 5.0−6.0, which is the collective natural pH range of these agricultural soils.[19] After 24 h, the ζ potentials of the samples were analyzed using the procedure outlined earlier.

Microbial Adhesion to Hydrocarbons (MATH) Assay. Changes in hydrophobicities of oocysts and microspheres were evaluated using the microbial adhesion to hydrocarbons (MATH) test. Although there are limitations with this procedure,[27] it was chosen because it is simple to perform, was repeatable for our particular set of experimental conditions, and correlated inversely with ζ potential in a previous study.[28] The effects of SDBS (4 mg L^{-1}) and SRHA (4 mg L^{-1} to 120 mg L^{-1}) on the microsphere and oocyst hydrophobicity were assessed using the water: hexadecane partitioning procedure outlined by Dai and Hozalski.[17] All determinations were repeated 3−5 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 operated by the University of Hawai'i. 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

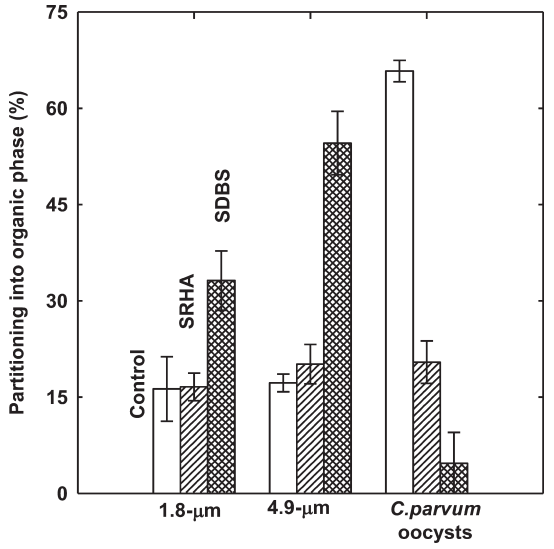

Figure 1. Partitioning of 1.8 μm and 4.9 μm microspheres and *C. parvum* oocysts into the organic phase (P_{org}) as determined by the MATH test in the presence of 4 mg L^{-1} of Suwannee River Humic Acid (SRHA) and 4 mg L^{-1} of sodium dodecyl benzene sulfonate (SDBS). Error bars represent the 95% confidence interval based on replicate measurements.

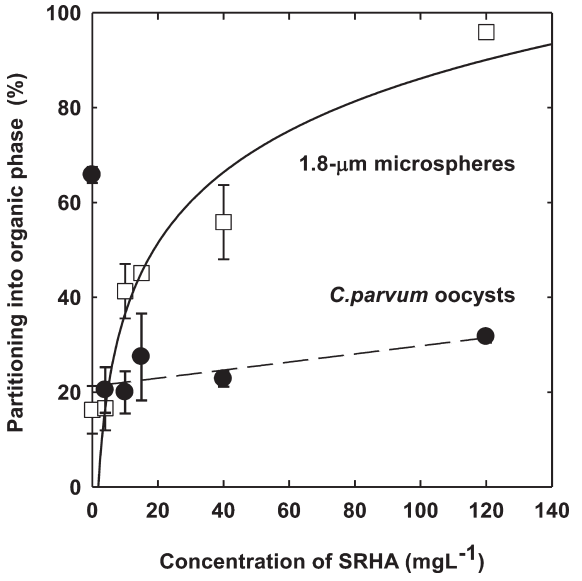

Figure 2. Changes in partitioning to organic phase (P_{org}) for 1.8 μm microspheres and *C. parvum* oocysts as a function of Suwannee of River Humic Acid (SRHA) concentration. The best-fit regression for the microspheres is $y = 21.57\ln(x) - 13.23$, $R^2 = 0.96$, $p < 0.005$. For the oocysts, it is $y = 0.0085x + 21.23$, $R^2 = 0.67$, $p < 0.005$. Error bars represent the 95% confidence interval based on replicate measurements.

(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. 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. Detailed mineralogical data for the two disparate soils were reported by Mohanram et al.[19]

Solutions. All solutions were prepared using filter-sterilized, deionized water (18 M′Ω cm resistivity; Milli-Q, Millipore Corp., Bedford, MA). For the flow-through column studies, influent pH was maintained between 5.0 and 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) at 220 nm wavelength.

Transport Studies. Transport studies were conducted using flow-through glass chromatography columns (2.5-cm diameter, 10-cm length) packed with Poamoho or with Drummer soils having final pore volumes of 39 and 33 mL, respectively. A 5 mL injection loop provided a "pulse input" of colloids to the column. 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.[19] For studies involving DOC amendments, 100 mg L^{-1} of SDBS (348.5 g mol^{-1}) or SRHA (3820 g mol^{-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.[29] Colloids were injected only after DOC and pH values in the eluent matched those in the influent. Equilibration took approximately 20 days because of the high pH-buffering capacity of the soils. At least 5 pore volumes of effluent were collected. Duplicates were run for each experiment.

RESULTS

Hydrophobicity of Colloids in the Presence of DOC. The effects of a 4 mg L^{-1} amendment of SRHA and SDBS on the colloid hydrophobicity, as estimated by partitioning to the organic phase (P_{org}) in the MATH assay, are shown in Figure 1. In the absence of DOC (control), two-thirds of the oocysts partitioned into the organic phase, indicating a high hydrophobicity. In contrast, >82% of the microspheres partitioned into the aqueous phase, indicating that these colloids are highly hydrophilic. Addition of a modest concentration (4 mg L^{-1}) of SRHA caused the P_{org} of oocysts to decrease more than 3-fold, but had little to effect on the P_{org} of the microspheres. Addition of 4 mg L^{-1} of SDBS also caused the oocysts to become much less hydrophobic. However, unlike the SRHA amendment, SDBS made the microspheres more hydrophobic. The hydrophobicity of the 1.8 μm microspheres (as estimated by P_{org}) was extremely sensitive to the concentration of SRHA, whereas the hydrophobicity of the oocyts changed little over the SRHA concentrations of 1−120 mg L^{-1}. The maximum adsorption of SRHA was reached at much lower concentrations for oocysts than for microspheres (Figure 2).

ζ Potential of Colloids in the Presence of Soil Fines and SRHA. Surface charge modifications of oocysts and 1.8 μm microspheres in the presences of soil fines and SRHA are shown in Figure 3. The microspheres' ζ were always more negative than those of the oocysts, but became less negative in the presence of Drummer or Poamoho fines. The presence of SRHA had little effect. The oocysts' ζ became more negative in the presence of SRHA, Poamoho and Drummer fines.

Transport Studies. *Poamoho Soil.* Fractional breakthroughs (C/C_o) of the three colloids in the 10-cm long columns packed with the iron-rich volcanic soil are depicted in Figures 1S and 3S

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(Supporting Information). For all experiments, peak breakthrough occurred earlier for oocysts and microspheres than for the conservative tracer (nitrate) indicating that the pore volumes that were readily accessible by the colloids were smaller than those accessible by the solutes (conservative tracers). It has been suggested that visual comparison of breakthrough where colloid and conservative tracer are plotted on disparate scales can lead to interpretational errors.[30] In this study, direct comparison of colloid and tracer breakthrough is valid despite the use of separate scales, because peak colloids concentrations are generally significant on the tracer scale. Summary recovery and retardation data for the microspheres and oocysts are shown in Table 1. Compared with the microspheres, few oocysts were transported. Although the presence of SDBS enhanced, by respective factors of ~2 and ~7, transport of the 1.8 and 4.9 μm microspheres, it did not measurably enhance transport of oocysts. In contrast, SRHA decreased by factors of ~2 and 5 the transport of the smaller and larger microspheres, respectively, but caused a slight increase in transport of oocysts.

Drummer 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 2S and 4S). Transport of oocysts and microspheres through the quartz- and humic-rich Drummer soil is shown in Table 1. As was the case for the Poamoho-soil columns

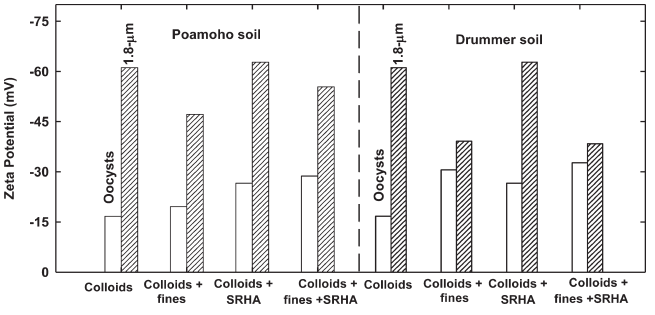

Figure 3. Surface modifications of *C. parvum* oocysts and 1.8-μm microspheres in the presence of soil fines (<1 μm, 200 mg L^{-1}) and 4 mg L^{-1} of Suwannee River Humic Acid (SRHA)) at pH 5.0−6.0. Error bars represent the 95% confidence interval based on replicate measurements.

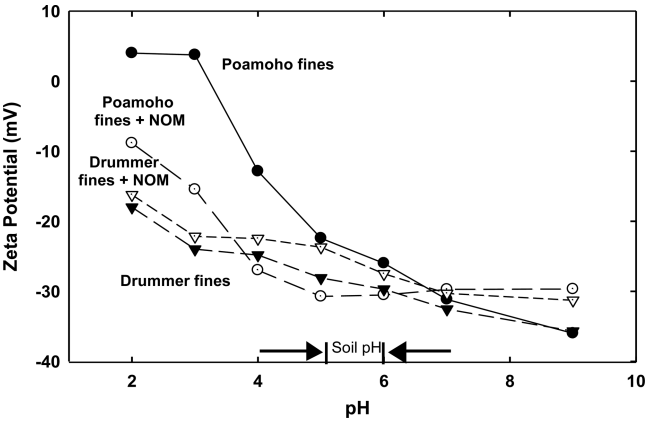

Figure 4. Variations in ζ of the soil fines in the presence of 4 mg L^{-1} Suwannee River Humic Acid (SRHA). The size of fines is ≤1 μm at concentration of 200 mg L^{-1}. The standard error for replicate determinations of ζ was ± 2%.

described in the preceding section, a substantive fraction of the 1.8 μm microspheres, a much smaller fraction of the 4.9 μm microspheres, and very few oocysts were transported through the unamended soil column. However, the presence of SDBS substantively enhanced transport of all colloids. The presence of SRHA again caused a decrease in transport of the smallest microspheres, this time by a factor of 2−3. However, in contrast to the Poamoho soil studies, it had little effect on the transport of the larger microspheres, and caused a 6-fold increase in transport of oocysts. Surprisingly, addition of SRHA caused the Poamoho soil fines to become more negatively charged and the Drummer soil fines to become less negatively charged (Figure 4).

DISCUSSION

Effect of DOC. The substantive changes in retardation and attachment behaviors for microspheres, particularly the smaller (1.8 μm) size class, caused by the presence of DOC are consistent with the well-publicized complexities of subsurface microbial transport (SI Figures 1S−4S). The observations that

Table 1. Summary of the Transport Data on the Effect of DOC on the Transport of Colloids through Poamoho and Drummer Soils

		no amendment			SDBS amendment (100 mg L^{-1})			SRHA amendment (100 mg L^{-1})		
		recovery	retardation		recovery	retardation		recovery	retardation	
soil	colloid	(%)	$(RF_{peak})^a$	$(RF_{CM})^b$	(%)	(RF_{peak})	(RF_{CM})	(%)	(RF_{peak})	(RF_{CM})
Poamoho										
	$MS_{1.8\ \mu m}$[c]	48.0	0.62	0.62	87.0	0.62	0.67	28.0	0.54	0.80
	$MS_{4.9\ \mu m}$[d]	4.00	0.54	0.57	27.0	0.54	0.67	0.20	0.54	1.10
	oocysts[e]	0.11	0.46	0.60	ND[f]	NA[g]	NA[g]	0.17	0.62	0.55
Drummer										
	$MS_{1.8\ \mu m}$	43.0	0.76	0.66	93.0	0.47	0.54	16.0	0.57	0.67
	$MS_{4.9\ \mu m}$	2.0	0.47	0.55	17.0	0.40	0.53	1.86	0.57	0.58
	oocysts	0.40	0.47	0.56	2.00	0.40	0.33	2.40	0.47	0.48

[a] Retardation factor (tracer velocity/colloid velocity) based on peak concentrations in the breakthrough curve. [b] Retardation factor (tracer velocity/colloid velocity) based on movement of centers of mass in the breakthrough curve. [c] Carboxylate-modified polystyrene microspheres with a diameter of 1.8 μm. [d] Carboxylate-modified polystyrene microspheres with a diameter of 4.9 μm. [e] *Cryptosporidium parvum* oocysts, 3−5 μm diameters. [f] ND: none detected. [g] NA: not applicable because of no recovery.

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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 concentrations and center of mass were consistent with the high recoveries observed for the microspheres (Table 1). 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.[31] Metge et al.[7] showed that the extent of oocyst and oocyst-sized microsphere attachment in sandy aquifer sediments, which correlated strongly with extractable Fe and Al, could be decreased up to 30-fold in the presence of only 1 mg L^{-1} of SDBS. In the present study, we surmised 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 by a later arrival of the center of mass, suggesting longer contact times, more extensive attachment, or both. Because SRHA modified the surfaces of the highly hydrophilic microspheres in a manner that made them more hydrophobic (Figures 1 and 2), but had little effect on their net surface charge (Figure 4), it appears that the decreased transport of carboxylated microspheres in the presence of SRHA was due, at least in part, to an enhanced hydrophobic effect. Dai and Hozalski[17] reported similar observations where SRHA modified the surfaces of colloids differentially depending on the initial surface chemistry of the particles.

In contrast to what was observed for the microspheres, amendments of SRHA caused oocysts to become less hydrophobic (Figure 1) with a concomitant increase in negative surface charge (Figure 3). This is consistent with observations that decreased microbial hydrophobicity is often accompanied by increased negative surface charge.[32] Thus, the enhanced transport of oocysts in Drummer soils in the presence of SRHA is likely due to decreased hydrophobicity as well as increased 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 transport in this soil type. In general, SDBS has been shown to enhance transport and reentrainment of microorganisms in subsurface sediments, which is thought to involve charge modification of Fe- and Al-oxide minerals on grain surfaces.[7,31] The relative 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 red volcanic soils that have a very high (22−29%) w/w Fe content and (or) because of straining within the 68−76% (w/w) clay fraction.

Effect of Suspended Soil Clays and Fines. In order to better understand the ternary interactions between grain surface, DOC, and oocysts, we used soil fines. Soil fines, which include the clay fraction, have been reported to be reasonable indicators of processes occurring at grain surfaces[33] and can occur in suspension when water moves through the soils under saturated conditions. Figure 4, which depicts ζ variations exhibited by Poamoho and Drummer fines in response to changes in pH, shows a strongly negative net charge for both types of fines regardless of whether SRHA is present or not. However, 4 mg L^{-1} of 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 faces) occurring simultaneously on one surface.[34] Under low pH conditions, near the point of zero charge (PZC), electrostatic and van der Waals forces between the face and edge regions cause the clay surfaces to be positively charged. At higher pH values, most of the soil fines are negatively charged and face-to-edge interaction are not possible due to electrostatic repulsion. Under these conditions the addition of low concentrations 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*.[35]

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. These soil fines have 2:1 expandable Smectite clays (a group that includes montmorillonite, nontronite and saponite where the silicate layers possess a slight negative charge because of ionic substitutions in the octahedral and tetrahedral sheets) 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.[36] The presence of organic matter can act like a bridge between the aggregates.[37] Consequently, the decreased ζ is due to destabilization of the fines by SRHA, an initial step in particle coagulation.[38] These studies show that SRHA can stabilize or destabilize fines according to the soil mineralogy and that particle stability can change with pH conditions.

Fitch et al.[39] found that NOM coatings on clay minerals physically blocks access to the clay pores by cationic binding. Although SRHA caused an increase in negative charge on the surface of fines, transport of negatively charged microspheres was surprisingly reduced in Poamoho soil. It is possible that in the Poamoho soil, SRHA promoted dispersion of soil fines, which could have migrated into the finer porosity of the soil columns, thereby blocking access by microspheres and contributing to straining. In the Drummer soil, SRHA-mediated aggregation of fines could have caused clogging of pores. Our transport results suggest that NOM may have two roles in affecting oocyst transport through soils. In addition to affecting the surfaces characteristics of oocysts and test surrogates described in the preceding section, NOM can also affect the extent to which the soil fines affect the transport process by promoting their dispersion or aggregation. Consequently, whether humic acid promotes or inhibits oocyst transport in soils may depend, in part, on the nature and quantity of the soil fines, particularly the highly reactive clay fraction.

Microspheres as Surrogates in Agricultural Soils. Although the differences in recovery for the two sizes of microspheres being advected through the soil columns in the absence of DOC can be predicted, in large part, by colloid filtration theory, the differences between the transport behaviors of microspheres and oocysts cannot. The apparently opposite trends in attachment behaviors of oocysts and microspheres in response to amendments with SRHA are a reflection of the differences in their respective surface characteristics. Relative to the surfaces of carboxylate-modified microspheres, the surfaces of oocysts are considerably more hydrophobic. They are also more complex because of multiple layers of proteins and lipids that extend out from the surface and have been shown to hinder attachment to grain surfaces.[12] In contrast to the transport behavior of the microspheres, transport of oocysts was less sensitive to both the nature of the DOC and to soil mineralogy. Figure 1 shows that

oocysts, in contrast to microspheres, exhibited ~3-fold decrease in hydrophobicity when in the presence of just 4 mg L^{-1} of SDBS. A diminished hydrophobic effect would help explain the higher recovery of oocysts in the high organic (13% w/w) Drummer soil relative to the organic-poor Poamoho soil.

That suspended fines caused ζ of the microspheres to become less negative and those of the oocysts more negative can be explained, in part, by an initial disparity in the respective electrical properties of these two colloids. In contrast to oocysts, carboxylate-modified microspheres that are suspended in simple electrolytic solutions exhibit a much more negative charge. However, the polyfunctional nature and the more hydrophobic moieties associated with the surface macromolecules on the oocysts may make them more susceptible to alteration by DOC and by suspended clays. To further understand the manner in which the surface properties of oocysts and microspheres might be affected in the agricultural soils, we measured ζ of oocysts and microspheres in the presence of soil fines and SRHA, separately, and the presence of soil fines and SRHA together (Figure 3). From those measurements, it was clear that oocysts had a higher susceptibility to surface modification than that of the microspheres, regardless of soil type. For the oocysts, addition of either Poamoho or Drummer fines led to a more negative ζ. Further addition of SRHA just magnified this effect. This pattern was not observed for the microspheres, suggesting that that the oocysts are more vulnerable to surface modification in the presence of soil fines and humic acid.

In summary, oocysts and microspheres transport appears to depend strongly on the characteristics of granular media, the nature of the colloid surface, and the type of organic matter interacting with the colloids. Thus, it is difficult to generalize the effect of DOC on the transport of oocysts and microspheres in agricultural soils. Although NOM can affect the surface characteristics (charge, hydrophobicity) of oocysts, it can also interact with the soil fines/clays, thereby influencing the magnitude of their contribution to oocyst retention within the soils. Our studies suggest that interactions of the DOC with the oocysts and with the soil fines/clays, in addition to the overall soil composition, are important for better predictions of the subsurface fate and transport of oocysts in agricultural environments. Our studies have also shown that the oocyst and carboxylate-modified microsphere surfaces can have very different sensitivities to changes in their electrical and hydrophobic properties in the presence of soil fines/clay, humic acid, and anionic surfactants. Consequently, microspheres may not be a reliable surrogate for studying oocyst transport, at least in the types of soils we employed in our study. 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.

■ ASSOCIATED CONTENT

Supporting Information. Figures 1S−4S. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Rose, J. B. Environmental ecology of *Cryptosporidium* and public health implications. *Annu. Rev. Public Health.* **1997**, *18*, 135–61.

(2) Kato, S.; Jenkins, M.; Fogarty, E.; Bowman, D. *Cryptosporidium parvum* oocyst inactivation in field soil and its relation to soil characteristics: analyses using the geographic information systems. *Sci. Total Environ.* **2004**, *321*, 47–58.

(3) Dai, X.; Boll, J. Evaluation of attachment of *Cryptosporidium parvum* and *Giardia lamblia* to soil particles. *J. Environ. Qual.* **2003**, *32*, 296–304.

(4) Darnault, C. J. G.; Steenhuis, T. S.; Garnier, P.; Kim, Y. J.; Jenkins, M. B.; Ghiorse, W. C.; Baveye, P. C.; Parlange, J. Y. Preferential flow and transport of *Cryptosporidium parvum* oocysts through the vadose zone: Experiments and modeling. *Vadose Zone J.* **2004**, *3*, 262–270.

(5) Jenkins, M. B.; Bowman, D. D.; Fogarty, E. A.; Ghiorse, W. C. *Cryptosporidium parvum* oocyst inactivation in three soil types at various temperatures and water potentials. *Soil Biol. Biochem.* **2002**, *34*, 1101–1109.

(6) Abudalo, R. A.; Ryan, J. N.; Harvey, R. W.; Metge, D. W.; Landkamer, L. Influence of organic matter on the transport of *Cryptosporidium parvum* oocysts in a ferric oxyhydroxide-coated quartz sand saturated porous medium. *Water Res.* **2010**, *44*, 1104–1113.

(7) Metge, D. W.; Harvey, R. W.; Aiken, G. R.; Anders, R.; Lincoln, G.; Jasperse, J. Influence of organic carbon loading, sediment associated metal oxide content and sediment grain size distributions upon *Cryptosporidium parvum* removal during riverbank filtration operations, Sonoma County, CA. *Water Res.* **2010**, *44*, 1126–1137.

(8) Hijnen, W. A. M.; Brouwer-Hanzens, A. J.; Charles, K. J.; Medema, G. J. Transport of *MS2 phage, Escherichia coli, Clostridium perfringens, Cryptosporidium parvum* and *Giardia intestinalis* in a gravel and a sandy soil. *Environ. Sci. Technol.* **2005**, *39*, 7860–7868.

(9) Davies, C.; Kaucner, C.; Altavilla, N.; Ashbolt, N.; Fuerguson, C.; Krogh, M.; Hijnen, W.; Medema, G.; Deere, D. *Fate and Transport of Surface Water Pathogens in Watersheds*; AWWA Research Foundation, Denver, CO. 2005.

(10) Hijnen, W. A. M.; Dullemont, Y. J.; Schijven, J. F.; Hanzens-Brouwer, A. J.; Rosielle, M.; Medema, G. Removal and fate of *Cryptosporidium parvum, Clostridium perfringens* and small-sized centric diatoms (*Stephanodiscus hantzschii*) in slow sand filters. *Water Res.* **2007**, *41*, 2151–2162.

(11) Jenkins, M. B.; Eaglesham, B. S.; Anthony, L. C.; Kachlany, S. C.; Bowman, D. D.; Ghiorse, W. C. Significance of wall structure, macromolecular composition, and surface polymers to the survival and transport of *Cryptosporidium parvum* oocysts. *Appl. Environ. Microbiol.* **2010**, *76*, 1926–1934.

(12) Kuznar, Z. A.; Elimelech, M. *Cryptosporidium* oocyst surface macromolecules significantly hinder oocyst attachment. *Environ. Sci. Technol.* **2006**, *40*, 1837–1842.

(13) Considine, R. F.; Dixon, D. R.; Drummond, C. J. Oocysts of *Cryptosporidium parvum* and model sand surfaces in aqueous solutions: an atomic force microscope (AFM) study. *Water Res.* **2002**, *36*, 3421–3428.

(14) Gao, X. D.; Metge, D. W.; Ray, C.; Harvey, R. W.; Chorover, J. Surface complexation of carboxylate adheres *Cryptosporidium parvum* oocysts to the hematite−water interface. *Environ. Sci. Technol.* **2009**, *43*, 7423–7429.

(15) Harvey, R. W.; Metge, D. W.; Shapiro A. M.;Renken R. A.; Osborn, C. L.; Ryan, J. N.; Cunningham K. J.; Landkamer L. Pathogen and chemical transport in the karst limestone of the Biscayne aquifer: 3. Use of microspheres to estimate the transport potential of *Cryptosporidium parvum* oocysts. *Water Resour. Res.* **2008**, *44*, W08431. DOI: 10.1029/2007WR006060.

(16) Emelko, M. B.; Huck, P. M. Microspheres as surrogates for *Cryptosporidium* filtration. *J. Am. Water Works Assoc.* **2004**, *96*, 94–105.

(17) Dai, X. J.; Hozalski, R. M. Evaluation of microspheres as surrogates for *Cryptosporidium parvum* oocysts in filtration experiments. *Environ. Sci. Technol.* **2003**, *37*, 1037–1042.

(18) Bradford, S. A.; Torkzaban, S.; Walker, S. L. Coupling of physical and chemical mechanisms of colloid straining in saturated porous media. *Water Res.* **2007**, *41*, 3012–3024.

(19) Mohanram, A.; Ray, C.; Harvey, R. W.; Metge, D. W.; Ryan, J. N.; Chorover, J.; Eberl, D. D. Comparison of transport and attachment behaviors of *Cryptosporidium parvum* oocysts and oocyst-sized microspheres being advected through three minerologically different granular porous media. *Water Res.* **2010**, *44*, 5334–5344.

(20) Gamba, R. C.; Ciapina, E. M.P.; Espindola, R. S.; Pacheco, A.; Pellizari, V. H. Detection of *Cryptosporidium* sp. oocysts in groundwater for human consumption in Itaquaquecetuba city, Sao Paulo, Brazil. *Braz. J. Microbial.* **2000**, *31*, 151–153.

(21) Abudalo, R. A.; Bogatsu, Y. G.; Ryan, J. N.; Harvey, R. W.; Metge, D. W.; Elimelech, M. Effect of ferric oxyhydroxide grain coatings on the transport of bacteriophage PRD1 and *Cryptosporidium parvum* oocysts in saturated porous media. *Environ. Sci. Technol.* **2005**, *39*, 6412–6419.

(22) Byrd, T. L.; Walz, J. Y. Investigation of the interaction force between *Cryptosporidium parvum* oocysts and solid surfaces. *Langmuir.* **2007**, *23*, 7475–7483.

(23) Butkus, M. A.; Bays, J. T.; Labare, M. P. Influence of surface characteristics on the stability of *Cryptosporidium parvum* oocysts. *Appl. Environ. Microbiol.* **2003**, *69*, 3819–3825.

(24) Masliyah, J. H.; Bhattacharjee, S.; Wiley, J.; InterScience, W. *Electrokinetic and Colloid Transport Phenomena*; Wiley Interscience: New York, 2006.

(25) Chin, Y. P.; Aiken, G. R.; Danielsen, K. M. Binding of pyrene to aquatic and commercial humic substances: The role of molecular weight and aromaticity. *Environ. Sci. Technol.* **1997**, *31*, 1630–1635.

(26) Moore, D. M.; Reynolds, R. C. *X-ray Diffraction and the Identification and Analysis of Clay Minerals*; Oxford University Press: Oxford, 1989.

(27) Geertsemadoornbusch, G. I.; Vandermei, H. C.; Busscher, H. J. Microbial cell-surface hydrophobicity - the involvement of electrostatic interactions in microbial adhesion to hydrocarbons (MATH). *J. Microbiol. Meth.* **1993**, *18*, 61–68.

(28) Harvey, R. W.; Metge, D. W.; Mohanram, A.; Gao, X.; Chorover, J. Differential effects of dissolved organic carbon upon reentrainment and surface properties of groundwater bacteria and bacteria-sized microspheres during transport through a contaminated, sandy aquifer. *Environ. Sci. Technol.* **2011**, *45*, 3252–3259.

(29) Aiken, G. R.; McKnight, D. M.; Thorn, K. A.; Thurman, E. M. Isolation of hydrophilic organic-acids from water using nonionic macroporous resins. *Org. Geochem.* **1992**, *18*, 567–573.

(30) Zhang, P.; Johnson, W. P.; Piana, M. J.; Fuller, C. C.; Naftz, D. L. Potential artifacts in interpretation of differential breakthrough of colloids and dissolved tracers in the context of transport in a zero-valent iron permeable reactive barrier. *Ground Water.* **2001**, *39*, 831–840.

(31) Pieper, A. P.; Ryan, J. N.; Harvey, R. W.; Amy, G. L.; Illangasekare, T. H.; Metge, D. W. Transport and recovery of bacteriophage PRD 1 in a sand and gravel aquifer: effect of sewage-derived organic matter. *Environ. Sci. Technol.* **1997**, *31*, 1163–1170.

(32) Vanloosdrecht, M. C. M.; Lyklema, J.; Norde, W.; Schraa, G.; Zehnder, A. J. B. Electrophoretic mobility and hydrophobicity as a measure to predict the initial steps of bacterial adhesion. *Appl. Environ. Microbiol.* **1987**, *53*, 1898–1901.

(33) Grolimund, D.; Elimelech, M.; Borkovec, M.; Barmettler, K.; Kretzschmar, R.; Sticher, H. Transport of in situ mobilized colloidal particles in packed soil columns. *Environ. Sci. Technol.* **1998**, *32*, 3562–3569.

(34) Bergaya, F.; Theng, B.; Lagaly, G. *Handbook of Clay Science*, Elsevier Science Ltd. 2006; pp 141−245.

(35) Kretzschmar, R.; Holthoff, H.; Sticher, H. Influence of pH and humic acid on coagulation kinetics of kaolinite: A dynamic light scattering study. *J. Colloid Interface Sci.* **1998**, *202*, 95–103.

(36) Schulten, H. R.; Leinweber, P. New insights into organic-mineral particles: Composition, properties and models of molecular structure. *Biol. Fertil. Soils.* **2000**, *30*, 399–432.

(37) Six, J.; Elliott, E. T.; Paustian, K. Soil structure and soil organic matter: II. A normalized stability index and the effect of mineralogy. *Soil Sci. Soc. Am J.* **2000**, *64*, 1042–1049.

(38) Elimelech, M.; Gregory, J.; Jia, X.; Williams, R. A. *Particle Deposition and Aggregation: Measurement, Modelling, and Simulation*: Butterworth-Heinemann: Oxford, U.K., 1995.

(39) Fitch, A.; Du, J. Solute transport in clay media: Effect of humic acid. *Environ. Sci. Technol.* **1996**, *30*, 12–15.