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Effects of Sediment-Associated Extractable Metals, Degree of Sediment Grain Sorting, and Dissolved Organic Carbon upon *Cryptosporidium parvum* Removal and Transport within Riverbank Filtration Sediments, Sonoma County, California

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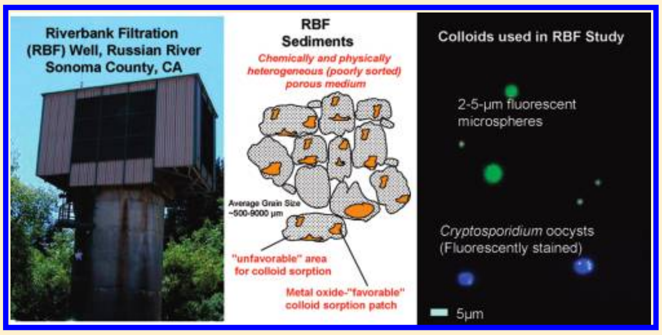
ABSTRACT: Oocysts of the protozoan pathogen *Cryptosporidium parvum* are of particular concern for riverbank filtration (RBF) operations because of their persistence, ubiquity, and resistance to chlorine disinfection. At the Russian River RBF site (Sonoma County, CA), transport of *C. parvum* oocysts and oocyst-sized (3 μm) carboxylate-modified microspheres through poorly sorted (sorting indices, σ_1, up to 3.0) and geochemically heterogeneous sediments collected between 2 and 25 m below land surface (bls) were assessed. Removal was highly sensitive to variations in both the quantity of extractable metals (mainly Fe and Al) and degree of grain sorting. In flow-through columns, there was a log−linear relationship ($r^2 = 0.82$ at $p < 0.002$) between collision efficiency (α, the probability that colloidal collisions with grain surfaces would result in attachment) and extractable metals, and a linear relationship ($r^2 = 0.99$ at $p < 0.002$) between α and σ_1. Collectively, variability in extractable metals and grain sorting accounted for ~83% of the variability in α (at $p < 0.0002$) along the depth profiles. Amendments of 2.2 mg L^{-1} of Russian River dissolved organic carbon (DOC) reduced α for oocysts by 4−5 fold. The highly reactive hydrophobic organic acid (HPOA) fraction was particularly effective in re-entraining sediment-attached microspheres. However, the transport-enhancing effects of the riverine DOC did not appear to penetrate very deeply into the underlying sediments, judging from high α values (~1.0) observed for oocysts being advected through unamended sediments collected at ~2 m bls. This study suggests that in evaluating the efficacy of RBF operations to remove oocysts, it may be necessary to consider not only the geochemical nature and size distribution of the sediment grains, but also the degrees of sediment sorting and the concentration, reactivity, and penetration of the source water DOC.



INTRODUCTION

Riverbank filtration employs bank and adjacent aquifer sediments to reduce concentrations of microbial and chemical contaminants in a cost-effective manner. Of major concern for RBF operations are oocysts of the protozoan parasite, *Cryptosporidium parvum,* because of their ubiquity,[1] resistance to chemical disinfection,[2] very low infective dose rate,[3] and environmental persistence.[4] Because RBF operations use groundwater that is in close hydrologic connection with surface water, they are covered by the USEPA's Long-Term 2 Enhanced Surface Water Treatment (aka LT2) Rule whose purpose is "to reduce disease incidence associated with *Cryptosporidium* and other enteric disease-causing microorganisms in drinking water".[5] Consequently, understanding the efficacy of aquifer sediments between the river bank and collector well to remove oocysts becomes important in determining what additional treatment steps may be needed in order to meet regulatory requirements.

Removal of *C. parvum* oocysts in sand columns is often well-described by colloid filtration theory (CFT), e.g., ref 6. However, many uncertainties remain in the application of CFT-based models for predicting oocyst removal in operating RBF sites. In addition to the well-documented limitation of CFT to predict colloid immobilization under unfavorable ("electrostatically repulsive") conditions,[7] there are a number of poorly understood factors affecting field-scale transport of this pathogen that are not generally considered in CFT-based models. In a recent study involving virus, bacteria, and *C. parvum* oocyst transport through 3- and 9-m columns of RBF sediments, Gupta et al.[8] found qualitative agreement with CFT regarding optimal microbial size

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for transport and increased transport with increased flow rate and decreased ionic strength. However, the spatial pattern of microbial deposition deviated substantively from the log−linear pattern predicted by CFT.[8] Clearly, there are important physical−chemical factors not accounted for in colloid filtration that are important determinants of field-scale transport at RBF sites.

Important physicochemical factors affecting oocysts transport in sandy bank filtration sites can include the nature of dissolved organic carbon (DOC) in the river, and the mineralogy and degree of grain sorting that characterize underlying aquifer sediments. These parameters tend to vary spatially in aquifers and, consequently, removal of *C. parvum* oocysts by sorptive filtration likely varies a great deal along the flow path between the riverbed and collector well. Deposition of oocysts in sandy media is very sensitive to surface coverage of iron oxides on individual grains[9] and to the presence of humic acid.[10] Recent small-scale studies have confirmed that this is also the case in RBF sediments.[11] However, much less is known about the contribution of grain sorting in the transport of *C. parvum* oocysts through aquifer sediments.

The goal of this study was to obtain information on how spatial variability in metal oxide content and grain sorting independently and collectively affect transport behavior of *C. parvum* oocysts through poorly sorted, iron- and aluminum-rich subsurface sediments that characterize the Russian River RBF site. An earlier study at this same site established that near-surface (upper meter) sediments were highly effective in removing oocyst-sized colloids.[12] However, the efficacy of the deeper sediments (1−24 m) to remove oocysts remained unknown. More recently, scanning electron microscopy/energy dispersive X-ray microanalyses confirmed that iron and aluminum oxides on the grain surfaces were serving as preferred binding sites for oocysts and microspheres within aquifer sediments at this site.[11] In the present study, we evaluate the effect of spatial variability along a vertical transect in extractable metal oxides and grain sorting upon removal by sorptive-filtration of oocysts and oocyst-sized microspheres. We also assessed the effects of low concentrations (1−2 mg L^{-1}) of Russian River DOC and the anionic surfactant sodium dodecyl benzene sulfonate (SDBS) on the collision efficiencies (α) of oocysts and oocyst-sized (2−5 μm) microspheres. Our main hypothesis was that although metal oxides within the sediments would have the major role in controlling the transport behavior of oocyst-sized colloids, the degree of grain sorting could also have a substantive effect. Our second hypothesis based, in part, upon earlier, more-qualitative observations was that transport of oocyst-sized colloids could be enhanced in the presence of trace (1−2 mg L^{-1}) concentrations of river DOC and SDBS.

■ EXPERIMENTAL SECTION

Site. The Sonoma County Water Agency (SCWA) operates a RBF facility along the lower reach of the Russian River (160 km length and 3850-km^2 watershed) in northern California, USA that provides water for ∼600,000 people. The facility consists of 6 lateral, Ranney-type collector wells (14,500 m^3 h^{-1} collective capacity) that draw water from the river through the unconsolidated alluvial aquifer.[13] At a U.S. Geological Survey (USGS) gauging station near Guerneville, daily river flows vary from 4.5 (summer) to (spring) 265 m^3 s^{-1}. Measured hydraulic conductivities for the shallow aquifer sediments at the RBF site range from 5.5×10^{-5} to 4.1×10^{-4} m s^{-1} (ref 14) and from 2×10^{-5} m s^{-1} in sediments beneath the adjacent recharge basins to 8×10^{-5} m s^{-1} in coarser sands in riverbed sediments.[12] Specific conductance, pH, and ionic strength (Ic) in the river and adjacent aquifer vary 214−598 μS cm^{-1}, 7.3−8.5, and ∼1−3 mM, respectively).

Aquifer Sediments. Sediment cores, using split-spoon recovery techniques,[15] were collected along vertical transects from 1.7 to 13.7 m below land surface (bls) in 2008 and from 0.7 to 15.2 m bls in 2005, each from the two locations along the river. The two coring locations were ∼220 m apart, one near SCWA collector well 1 and the other near collector well 2.[11] Recovered core materials were shipped in 0.3-m sections to the USGS laboratory in Boulder, CO. For each 0.3-m core section, grain-size distributions were determined using standard sieving techniques. Grain-size distribution variations along the vertical coring transects were determined,[12] and used to calculate vertical profiles of grain sorting (deviations from grain-size uniformity), using the statistical equation of Poppe et al.,[16] i.e.:

$$\sigma_1 = \frac{d_{84} - d_{16}}{4} + \frac{d_{95} - d_5}{6.6}$$

where σ_1 is the unitless sorting coefficient and d_5, d_{16}, d_{84}, and d_{95} are the grain diameters (in mm) for the respective 5, 16, 84, and 95% size fractions of bulk sediments. The vertical transect subcore sorting indices were subsequently combined into categories relating to sorting nature (i.e., evenly sorted, poorly sorted, and very poorly sorted).

The d_{10} values were used for calculating hydraulic conductivities (K) using the Hazen equation as published in Odong:[17]

$$K = \frac{g}{\nu} \cdot C \cdot f(n) \cdot d_{10}^2$$

where K is the hydraulic conductivity (cm s^{-1}), g is the acceleration due to gravity (9.81 m s^{-2}), ν is the viscosity (1.14 mm^2 s^{-1}), C is a unitless coefficient (0.06), and f is a function of porosity ($[1 + 10 \times [\text{porosity} - 0.26]]$), n is porosity, and d_{10} is diameter, mm, 10% size fraction of bulk sediment. These estimations were compared with results obtained with a falling-head permeameter and published values of hydraulic conductivity for the RBF site.[14,18] Permeameter measurements were made according to methods outlined in Fetter[19] and summarized in Peirce.[20] Extractable metals analyses were performed using EPA method 3050 at the Laboratory for Environmental and Geological Studies at the University of Colorado, Boulder.

Microspheres and C. parvum Oocysts. We used 1.71, 2.89, and 4.90 μm nominal diameter carboxylate-modified, fluorescent (type YG) polystyrene microspheres (hereafter referred to as 2-, 3-, and-5 μm microspheres) obtained from Polysciences, Warrington, PA and Invitrogen-Molecular Probes, Carlsbad, CA. Use of the microspheres facilitated comparison to an earlier in situ transport study involving the shallow (0−1 m) riverbank sediments at the Russian River RBF site.[12]

Purified, formalin-inactivated suspensions of *C. parvum* oocysts were obtained from Sterling Parasitology Laboratory (SPL, University of Arizona, Tucson) as described in Abudalo et al.[9] Prior to the column studies, the oocysts were pelleted as previously described,[21] stained with the DNA-specific fluorescent dye 4′,6-diamidino-2-phenyl-indole (DAPI, 0.1 mg L^{-1}, final concentration, for 30−90 min staining time), filtered through a 1.0-μm pore size filter, washed with 1 mM NaCl to remove excess stain and any bacteria in suspension, and resuspended in 1 mM NaCl to final abundances between 10^5 and

10^7 oocysts mL^{-1}. The peak excitation and emission wavelengths for DAPI-stained oocysts are 359 and 461 nm, respectively. Further details regarding the preparation and enumeration of microsphere and oocysts were reported earlier.[11,21]

Organic Carbon. Large-volume river water samples were shipped on ice via overnight courier to the USGS laboratory in Boulder, CO and immediately filtered using Gelman AquaPrep 600 capsule filters (0.45 μm). Dissolved organic carbon (DOC) concentration was determined using an OI Analytical 700 TOC analyzer.[22] UV−visible absorbance measurements were performed on a Hewlett-Packard photodiode array spectrophotometer (model 8453) using a 10-mm width quartz cell. Specific UV absorbance (SUVA254) was determined by dividing the UV absorbance measured at 254 nm by the DOC concentration and reported in units of milligrams carbon per liter according to methods outlined in Weishaar et al.[23] The relatively more-reactive hydrophobic organic acid fraction of the DOC was isolated. Hydrophobic organic acid (HPOA, 5 mg L^{-1}, 2.2 mg C L^{-1}) isolation from Russian River water was performed using Amberlite XAD-4 and XAD-8 resins in a column series as described in Aiken et al.[22]

Static Mini-Column Experiments. A series of static columns packed with aquifer sediments from different depths were employed to assess the manner in which grain size and extractable metals affect oocyst and microsphere attachment. These studies employed 20-mL static mini columns (2.5-cm diameter, 12-cm height) with 105-μm pore size bed support and modified from previous studies described by Scholl and Harvey.[24] Triplicate acid-washed and baked (450 °C) glass columns were packed with aquifer sediments taken from the vertical coring transects. Each column was flushed with ~20 pore volumes (PV) artificial groundwater (AGW) consisting of 2.4 mM $NaHCO_3$, 0.3 mM $CaCl_2$, and 0.3 mM $MgCl_2$ at pH 7.5−7.6 to geochemically steady state conditions (constant pH and conductivity). One-mL colloidal suspensions, consisting of AGW, oocysts, 3-μm microsphere (1.4×10^7 colloidal particles), were then drawn into the aquifer sediments. After a 4-h incubation at 22−25 °C, the oocysts and microspheres that remained unattached were recovered using ~20 PV of AGW gently flushed through columns. Recovered oocysts and microspheres (eluent colloids) were enumerated by epifluorescent microscopy and flow cytometry as previously described.[11] Fractional attachment within the column was calculated by comparing the number of oocysts and microspheres amended to the column versus the number recovered. To assess the effects of DOC upon re-entrainment, total numbers of oocysts and microspheres appearing in the minicolumn eluents during a ~20 PV flush with 5 mg L^{-1} Russian River were determined.

Flow-Through Column Experiments. Flow-through columns were used to determine the relationships between α (probability that colloid collisions with grains surfaces result in attachment for microspheres and oocysts) and the amounts of extractable metals, the degrees of grain-size sorting, and the effect of DOC amendments. Nitrate (added as 1−5 mM $NaNO_3$ as a conservative tracer), polydispersed suspensions of 2- to 5-μm microspheres (1.25×10^7 mL^{-1}), and fluorescently labeled oocysts (0.05×10^7 mL^{-1}) were added as a pulse injection using a 5-mL injection loop, and subsequently collected on a fraction collector. Eluted fractions were collected and measured as described previously.[11] Approximately 10−50 PV of degassed 1 mM NaCl solution (ionic strength, 1 mM, pH 5.6−5.8) was passed through the column. Effluent pH and specific conductance of inlet and outlet solutions reached geochemically steady state conditions before introducing conservative tracer or oocyst and microspheres. Porosities in the packed columns ranged from 29.1 to 41.6%. Attachment of oocysts and microspheres was estimated from the number of colloids recovered versus the number added and plotted as a function of grain size and extractable metals to assess relative effects of physical or chemical sorption upon transport through these sediments.

Table 1. Variability in Extractable Metal Content along a Vertical Transect (0.7−15.2 m bls) through Aquifer Sediments at the Russian River Bank Filtration Site (Santa Rosa, CA)

extractable metal	concentration (mg g^{-1})	percentage (%) of total composite mass of metals
iron (Fe)	20.2−39.2	58.3%
aluminum (Al)	13.2−30.5	40.3
manganese (Mn)	0.4−0.8	1.2
Pb, Cu, Zn	0.05−0.15	<0.2
total metals	35.9−70.6	100%

Estimation of collision efficiency was performed using equations outlined in Yao et al.[25] and modified by Tufenkji and Elimelech.[26] For our purposes, we have neglected dispersivity and detachment terms. The oocysts and microspheres were monitored until no further colloid breakthrough tailing was detected and colloid concentrations returned to undetectable levels. The mean α values were normalized to the maximum determined α value to account for the observation that some averaged collision efficiencies were greater than 1. This may reflect the complexities in using natural sediments with colloid filtration relationships developed for idealized colloids and collectors.

$$\text{Collision Efficiency}: \alpha = -\frac{2}{3}\cdot\frac{d_c \ln(\mathrm{RB})}{(1-f)\cdot\eta_0\cdot x_1}$$

where α is the collision efficiency (unitless), RB is the relative breakthrough of colloid normalized to that of the conservative tracer, f is the fractional porosity, η_0 is the single collector contact efficiency (unitless), x_1 is the column length (m), and d_c is the average grain diameter (m).

As a statistical evaluation, we calculated Akaike information criterion (AIC,[27]), as later corrected (AIC_c) and Bayesian information criteria (BIC) values (equation sources: Hill and Tiedeman[28]) for finite samples. These helped assess the goodness of fit for oocyst and microsphere results in which calculated α (collision efficiency equations developed and modified for colloid removal in nonideal sediments) was plotted as a function of sediment extractable metal content. For comparative purposes, we calculated AIC_c and BIC statistics for collision efficiency as a function of sorting index and those for colloid recovery as a function of sediment extractable metal content.

$$\mathrm{AIC_c} = n\ln(\textstyle\sum \mathrm{SS\ WR}/\mathrm{n}) + 2\mathrm{k} + (2\mathrm{k}[\mathrm{k}+1]/(\mathrm{n}-\mathrm{k}\text{-}1))$$

$$BIC = \mathrm{n}\ln(\textstyle\sum \mathrm{SS\ WR}/n) + k\ln(n)$$

where n is the number of observations (n = 3−16), k is the number of parameters, and SS WR is the sum of squares weighted residuals. This allowed comparison of results from flow-through and static column experiments.

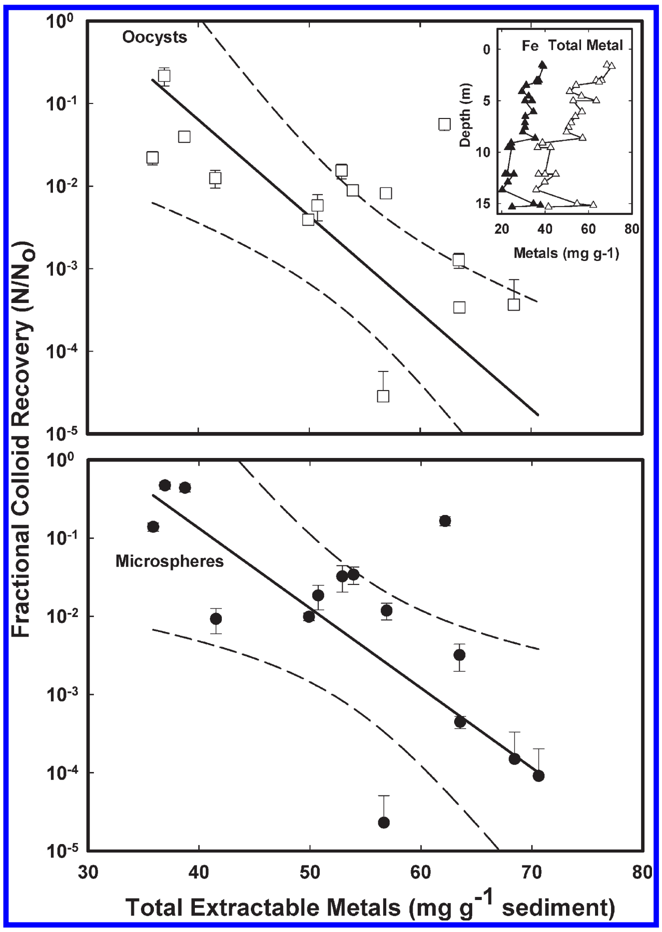

Figure 1. *Cryptosporidium parvum* oocyst (top graph, ☐) and 3-μm microsphere (bottom graph, ●) recoveries plotted as a function of total extractable metals on RBF sediments (static column experiments). Dashed areas represent 95% confidence intervals (top graph is *C. parvum* oocysts and bottom graph is 3-μm CCM). Removal was significantly related to metal content for each colloid (3 μm microspheres $y = 1600\ e^{-0.24x}$, $R^2 = 0.50$, $p < 0.008$; *C. parvum* oocysts $y = 2987\ e^{-0.27x}$, $R^2 = 0.48$, $p < 0.03$). Inset: depth profile of total extractable metals (Δ) and iron (Fe, ▲). Metals were assayed by EPA 3050 protocol at the CU, Boulder Laboratory for Environmental and Geological Sciences (LEGS).

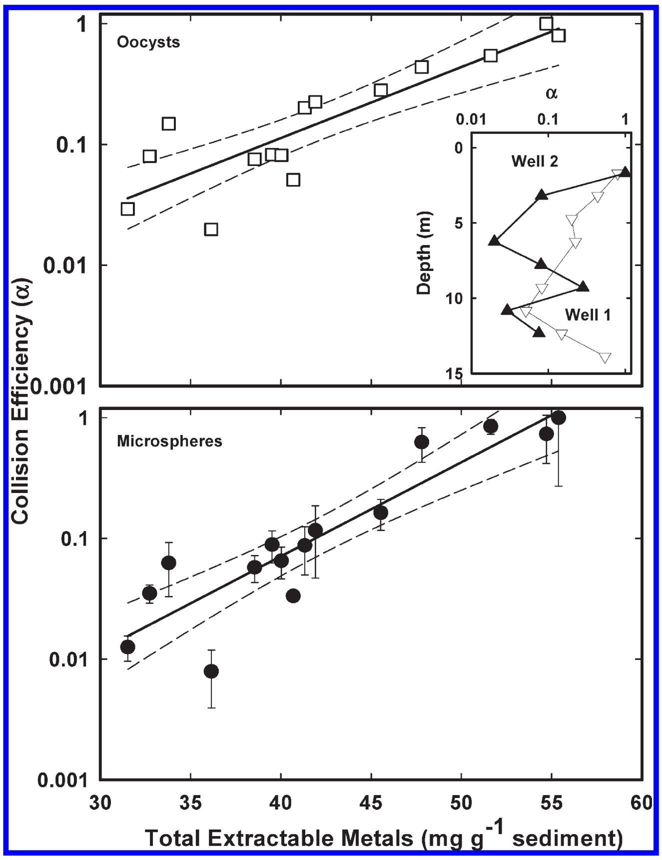

Figure 2. Relationship between normalized average collision efficiencies (*Cryptosporidium parvum* oocysts, ☐; and microspheres, averages ± standard error for 2-, 3-, and 5-μm microspheres, ●) and sediment extractable metal content. Collision efficiencies for *C. parvum* oocysts ($y = 5 \times 10^{-4}\ e^{0.14x}$, $R^2 = 0.76$, $p < 0.001$) were significantly related to metal content as were microspheres ($y = 5 \times 10^{-5}\ e^{0.18x}$, $R^2 = 0.83$, $p < 0.0001$). Dashed lines are 95% confidence intervals for microspheres and *C. parvum* oocysts . Inset: Depth profile of oocyst collision efficiency for two coring intervals (Well 1, ▽ and Well 2, ▲).

RESULTS

Sediment Characterization. Table 1 summarizes the variations in concentrations and relative abundances of extractable metals within continuous vertical (0.7−15.2 m bls) cores of aquifer sediment. Although Fe comprised, on average, more than half (~58%) of the extractable mass, a substantial fraction (~40%) consisted of Al. In contrast, Mn constituted only ~1% of the extractable mass and Pb, Cu, and Zn collectively made up less than 0.2%. Despite differences in average grain size (d_{50} = 0.21−9.13 mm) with depth, there was a high degree of consistency with respect to the fractional contribution of the individual extractable metals to the total extractable mass. In general, the higher extractable metal contents were associated with sediments characterized by lower *K* and smaller average grain sizes, although the correlation between extractable metals and *K* was weak ($r^2 = 0.08$ at $p < 0.31$). Whereas the sediments had substantive amounts (16.7−27.1% by XRD chemistry) of clay minerals (e.g., chlorite and smectite), they constituted <10% of the sieved bulk fractions. Based upon grain-size distribution, *K* varied from 0.002 to 0.33 cm s^{-1} along the vertical transect. Indices of sorting, σ_1, varied between 0.06 and 3.13 along the same vertical transect. Although four subcores had σ_1 values <1, indicating well-sorted sediments at the corresponding depths, six other subcores had σ_1 values of between 1 and 2, indicative of poorly sorted sediments, and five subcores had σ_1 values between 2 and 4, indicative of very poor sorting.

Extractable Metals Effects. For both oocysts and 3-μm microspheres, there was a log−linear relationship between recovery and extractable metal content (Figure 1). Correlation coefficients (r^2) for the latter regressions were 0.48 (at $p < 0.03$) and 0.50 (at $p < 0.008$) for the oocysts (top graph) and microspheres (bottom graph), respectively. There was a general trend of decreasing extractable iron and total metals with increasing depth along the 14.5-m transect (Figure 1, inset graph) and, consequently, an increasing potential for colloidal transport. In contrast, the relationships between oocyst and microsphere attachment as a function of average grain size were poor, judging from the respective linear correlation coefficients of only 0.05 (at $p < 0.61$) and 0.004 (at $p < 0.83$). AIC$_c$ and BIC values helped evaluate goodness of fit for colloid recoveries plotted as a function of metal content. These are summarized in Table 3.

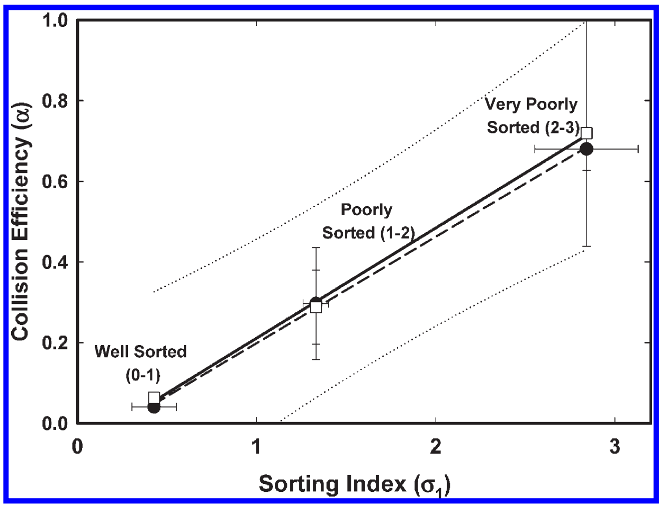

Figure 3. Relationship between average collision efficiencies (●, averages ± standard error for 2-, 3-, and 5-μm microspheres; and □, *Cryptosporidium parvum* oocysts) and composited sorting indices (averages ± standard error). Linear regressions were statistically significant (microspheres: $y = 0.26x - 0.065$, $R^2 = 0.99$, $p < 0.018$; and *C parvum* oocysts: $y = 0.27x - 0.062$, $R^2 = 0.99$, $p < 0.023$). Dotted lines represent 95% predictive intervals of regression lines.

The BIC and AIC_c values for recovery as a function of metals were lower for the oocysts relative to the microspheres. Strong first-order relationships ($r^2 = 0.76$ at $p < 0.001$ and $r^2 = 0.83$ at $p < 0.0001$, respectively) were also observed between the log α and extractable metals in flow-through columns (Figure 2). For both oocysts and polydispersed (2−5 μm) microspheres, 2-fold increases in sediment-extractable metals corresponded to 2-log increases in α. The AIC_c values for collision efficiency as a function of metals (from Figure 2, bottom) were again smaller for the oocysts (−21) compared to the microspheres (−16) and are summarized in Table 3. Values of α for microspheres and oocysts contacting grains within the Fe- and Al-rich aquifer sediments varied along the transect from 0.008 to 1.0 and from 0.019 to 1.0, respectively (Figure 2, inset graph).

Grain-Sorting Effects. Correlation coefficients (r^2) and corresponding significance levels (p values) were determined for the log−linear relationships between α and sediment extractable metals, and α and degree of grain sorting for composite colloidal suspensions (oocysts and microspheres) being advected through sediments collected along the two vertical transects. Additionally we determined the r^2 between α and extractable metals and sorting (multivariate regression). The variability in extractable metals accounted for a majority of the variability in α, as evidenced by a correlation coefficient of 0.82 (at $p < 0.002$). Collision efficiency correlated less strongly with the degree of grain sorting ($r^2 = 0.45$, $p < 0.05$). Subcores were composited into categories of well to very poorly sorted and a significant relationship between collision efficiency and sorting index resulted (Figure 3) for both microspheres and *C. parvum* oocysts. Smaller BIC and AIC_c values for microspheres (8.0 and −5.8) than those of oocysts (8.2 and −5.6) were obtained and indicated a slightly better description of fit for microspheres than the C. *parvum* oocysts. Although a multivariate regression involving both extractable metals and sorting yielded an r^2 value that was only slightly higher (0.83) than that calculated for extractable metals alone (0.82), the inclusion of both parameters increased the level of significance by a factor of 10 to $p < 0.0002$.

Table 2. Collision Efficiency (α) for Oocysts and Oocyst-Sized Microspheres in Columns of Infiltration Basin Sediments in the Presence and Absence of DOC Amendments

		collision efficiency		
colloid	diameter (μm)	(NO NOM)	SDBS (1 mg L^{-1})	NOM (2.2 mg L^{-1})
microspheres	2	0.51	0.20	0.06
	3	0.82	0.41	0.09
	5	0.96	0.95	0.14
C. parvum oocysts	3−5	0.96	0.53	0.22

DOC Effects. Results of DOC- (NOM- or SDBS-) amended and unamended flow-through column studies involving transport of microspheres and oocysts through packed sediments collected from beneath the infiltration ponds are summarized in Table 2. In the absence of amended DOC, α values for the aforementioned four, oocyst-sized colloids ranged from 0.51 (2-μm microspheres) to 0.96 (*C. parvum* oocysts). An amendment of 1 mg L^{-1} (final concentration) SDBS led to decreases in α by a factor of 1.0−2.5 relative to unamended values and, in the presence of 2.2 mg C L^{-1} Russian River DOC, decreases by factors of 4.4−9.1. For the columns involving DOC amendments, peak breakthrough of oocysts and microspheres occurred up to four times earlier than those of the conservative tracer. The NOM-treatment lengthened (slightly to 8-fold) the number of pore volumes required to reduce the colloid concentration by 4 log units. Addition of Russian River HPOA at 5 mg L^{-1} enhanced re-entrainment of previously entrained microspheres (Figure 4). For the sediments collected between 5 and 8 m bls, re-entrainment in the presence of HPOA was several-fold higher than that caused by flushes with AGW alone.

DISCUSSION

Effect of Physiochemical Sediment Variability. It has been shown that physicochemical variability in sediments can lead to substantial depth-specific differences in velocity, retardation, attenuation, and apparent dispersion of microorganisms and microspheres being advected through granular aquifers.[29] Recent studies suggest that a variety of factors affect oocyst removal in RBF and sandy aquifer sediments, including grain size, ionic strength, flow rate, and the presence of grain-surface metal oxides.[8] In the present study, the log−linear relationships between fractional recovery of oocysts in static columns and extractable metals in the sediments (Figure 1, top graph) suggests that transport of *C. parvum* from the river to the underlying collector wells will be extremely sensitive to relatively small changes in Fe and Al oxides associated with grain surfaces along the flow path. Our observations that less than a 2-fold increase in extractable metals results in an ∼2-log increase in α suggests that the quantity of grain-surface metal oxides will be a (the) major determinant of oocyst removal within the sediments. For columns packed with model sediments in which quartz grains were coated to varying degrees with iron oxides, α for *C. parvum* oocysts was found to be related directly to the surface coverage of iron oxides.[9] In the present study, the general trend of decreasing amounts of extractable metals with increasing depth along the vertical coring transect (Figure 1, inset) further suggests that the best removal of this pathogen should occur in the shallower (2−3 m bls) sampled sediments. In flow-through columns, α values of ∼1.0 were observed for oocysts being advected through the shallower sediments characterized by extractable metal contents of up to ∼55 mg g^{-1}, suggesting that almost all collisions with grain surfaces would result in immobilization. The strong log−linear relationships between α and the amount of extractable metals in the sediments indicates that the latter parameter could be useful in obtaining estimates of oocyst transport potential at different depths within the RBF sediments, at least for the depth interval represented by the coring transects (1−15 m bls).

The ability of the upper meter of sediments immediately beneath the river to remove *C. parvum* was not assessed in the present study. However, earlier field studies[12] employing oocyst-sized (2−5 μm) microspheres in an in situ column indicated that oocyst removal in the fine-grained surficial sediments was strongly affected by "patchiness" in near-surface vertical transmissivities. Removal in the surficial sediments ranged from 50 to >99%. Because the fine-grained, surficial sediments at the river bottom get scoured out seasonally due to winter and spring floods and because of the aforementioned patchiness in vertical *K*, the efficacy of the deeper sediment for removing *Cryptosporidium* is an important consideration for the continued safe operation of the RBF facility. Judging from the static and flow-through column results in the present study, >99.99% removal of the oocysts should occur within the deeper (2−25 m) aquifer sediments overlying the collector wells. This >4-log predicted removal is based upon laboratory-derived α values between 0.01 and 1.0 (Figure 2).

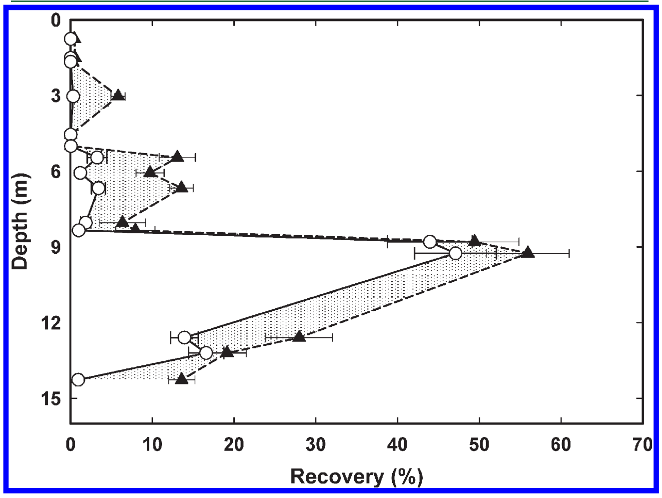

Figure 4. Results of static column experiments to assess effect of NOM upon remobilization of 3-μm microspheres. Two treatments are illustrated (NOM-free flush [○] and additional recovery (shaded area) following an amendment with 5 mg L^{-1} HPOA [▲]). Error bars reflect standard errors for results from three replicate columns.

Little information is available on the efficacy of *Cryptosporidium* removal as a function of grain sorting. The poorly sorted nature of the sediments at the Russian River site suggests that nonuniformity in grain size may also affect the efficacy of the sediments to remove oocyst-sized colloids. Our observations that the degree of grain sorting varies substantially with depth suggests that this parameter may also be important in determining variability in transport potential as oocysts are advected downward toward the collection well. The strong linear relationships between α (for both oocysts and 2- to 5-μm microspheres) and the grain-sorting index for the series of flow-through columns packed with aquifer sediments collected at different depth intervals (Figure 3) suggest that this is indeed the case. The strong linear correlation between α and σ_1 indicates that, not surprisingly, removal of oocysts by sorptive filtration occurs to a greater degree in poorly sorted (vs well-sorted) sediments. However, colloid removal appears unrelated to grain size (R^2 = 0.19−0.27, $p < 0.08$−0.12) and the colloid/grain-size ratios reported necessary for straining to occur[30] were not met as discussed earlier.[11] In a study investigating *E. coli* transport in flow-through columns containing model quartz sands, AIC data helped evaluate models employing attachment−detachment and straining.[31] The grain sizes employed in the latter study ranged from 150 to 710 μm and were generally smaller than those used in our study. With respect to sediment sorting in this study, the AIC_c and BIC values for collision efficiency as a function of sorting were greater (−5.8 to 8.2) than values derived for collision efficiency as a function of metals (Table 3, −16 to −32) with negligible differences between the statistics. Smaller AIC_c and BIC values obtained when evaluating different models offer better data descriptions than models with larger AIC and BIC values.[27,28,32] Thus, our results, which incorporated α, described colloid removal better than relationships which only included colloid recovery as smaller differences between AIC_c and BIC values were obtained. In addition, AIC_c information suggests predictive models for colloid removal within natural systems could be improved by incorporation of sediment sorting information.

In earlier experiments, immobilization per meter of transport for 2-μm microspheres being advected through sandy aquifer

Table 3. Akaike Information Criterion (AIC_c) and BIC Values for Experimental Results Using Colloid Recovery (Data from Figure 1), Collision Efficiency Model Calculations (Data from Figure 2) as a Function of RBF Sediment Extractable Metals, and Collision Efficiency as a Function of RBF Sediment Sorting (Data from Figure 3)[a]

colloid	variables (dependent, control)	model statistics				
		R^2	p-value	AIC_c	BIC	SS weighted
microspheres	recovery, metals	0.50	0.008	−67 (20)	−67 (20)	0.172
C. parvum oocysts	recovery, metals	0.48	0.03	−87	−87	0.049
microspheres	collision efficiency (α), metals	0.83	0.0001	−16 (5)	−27 (5)	1.693
C. parvum oocysts	collision efficiency (α), metals	0.76	0.001	−21	−32	1.251
microspheres	collision efficiency (α), sorting	0.99	0.018	8.0 (0.2)	−5.8 (0.2)	0.207
C. parvum oocysts	collision efficiency (α), sorting	0.99	0.023	8.2	−5.6	0.222

[a] The number of parameters (k) in all cases was 2, and numbers in parentheses reflect differences between criteria values.

sediments in in situ transport tests were 5−6 log units greater in the poorly sorted, Fe- and Al-rich sediments underlying the Russian River[12] relative to the well-sorted, less Fe and Al-enriched sediments[24,33] of the Cape Cod aquifer.[34] For a polydispersed mixture of oocysts and 2- to 5-μm microspheres, the multivariate (collective) correlation of α with both extractable metals and grain sorting has a 10-fold higher significance level (p value) than the correlation that only includes extractable metals as the independent variable. Consequently, both sediment characteristics (extractable metals and grain-sorting) need to be considered when assessing the efficacy of the Russian River RBF sediments to remove oocysts along the flow path from the river to underlying lateral collector wells. In contrast, the very weak correlations between α and median grain size and between α and ln K lead to the counterintuitive conclusion that permeability may not be a good predictor of oocyst transport, at least in this particular system.

Carboxylate-Modified Microspheres As Surrogates. Although carboxylated, polystyrene microspheres (CMM) often have proven to be less-than-ideal analogs for capturing the abiotic transport behavior of viruses and bacteria, there is evidence to suggest their utility as surrogates of *C. parvum* oocysts at RBF sites.[35] Similarly, field-scale studies by Passmore et al.[36] found that microspheres with surface properties and size similar to microorganisms of interest can be useful surrogates to trace transport pathways of pathogens in the subsurface. In particular, polydispersed suspensions of microspheres that bracket the size range of oocysts typically found in natural waters have proven useful as surrogates in field injection-and-recovery tests at the Russian River RBF site[12] and at a water-supply well drawing water, at least in part, from nearby surface water.[21] Because removal of microspheres and oocysts are influenced similarly by variability in extractable metals (Figure 2) and grain sorting (Figure 3), microspheres may be useful in assessing relative differences in oocyst transport behavior that results from variations in sediment properties along the flow path from the near-surface sediments to the underlying collector wells. However, judging from comparisons of attachment data (Figure 1), more caution needs to be exercised in using microspheres to predict the magnitude of oocyst transport at RBF sites in field studies. From our observations, transport of microspheres through the aquifer sediments beneath and adjacent to the Russian River would be expected to be more extensive than that of similar-sized oocysts.

The observation in the present study that microspheres would overpredict oocyst transport does not appear to be the case in all geologic media. For example, within the matrix porosity of karst limestone cores, the same microspheres underpredicted transport of similar-sized oocysts by 4−6 fold.[21] The effectiveness of microspheres as surrogates for *C. parvum* oocysts in subsurface transport studies depends upon how well they represent the oocyst surface properties for a particular set of physical and chemical conditions. In several studies, microsphere zeta potentials (ζ) were more negative than those of oocysts under neutral to slightly alkaline conditions[21,37,38] germane to the Russian River. Reported differences in oocyst ζ where similar physicochemical conditions were used are likely due to a variety of factors, including source,[39] age, exposure to antibiotics, and method of purification.[40] Consequently, given the variability of surface characteristics of oocysts and differing geologic media from site to site, a side-by-side comparison of attachment behaviors of microspheres and oocysts in the presence of aquifer sediment collected from different depths proved helpful in assessing the utility and limitations of using microspheres as surrogates. The differences in comparative attachment behaviors between microspheres and oocysts in the presence of different types of aquifer materials suggest that whether or not microspheres under-predict or over-predict transport of oocysts in a field-scale test would depend, at least in part, upon the physicochemical nature of the aquifer.

In Table 3, the lower $\mathrm{AIC_c}$ values for *C. parvum* oocysts compared to those of microspheres for Figures 1 and 2 may not be surprising given the nature of metal oxide−colloid sorption interactions, colloid surface charge uniformity, and discrete size classes of the microspheres used. For Figure 3, we obtained smaller $\mathrm{AIC_c}$ critierion differences with α values and sorting indices (σ_1) relative to colloid recovery alone. These likely occur because the collision efficiency and sorting index relationships take into account additional factors (e.g., colloid and grain size, collector efficiency, flow rate, sorting) that are absent in recovery determinations. The inclusion of collision efficiency and sorting information may provide a better predictive tool in assessing colloid removal efficiencies within RBF systems.

Effect of Organic Carbon. Table 2 indicates that modest amendments of anthropogenic or natural organic carbon (1 mg $\mathrm{L^{-1}}$ of SDBS surfactant or 2.2 mg $\mathrm{L^{-1}}$ Russian River HPOA) decreased by up to 30-fold the sorptive capabilities of aquifer sediments for oocysts and microspheres. Anionic surfactants have been employed in field[41] and laboratory studies[11] to examine their effect upon microbial transport in granular aquifers. Pieper et al.[41] reported ∼90% remobilization for previously sorbed bacteriophage (PRD-1) in the presence of 25 mg $\mathrm{L^{-1}}$ linear alkylbenzene sulfonate (LAS) and a lessening of the net positive charge associated with patches of amorphous iron oxyhydroxides on the sediments.[42] Additional work by Abudalo[10] found that fulvic acid isolated from south Florida had no effect on the surface charge of the oocysts, but even small amounts (e.g., 2 mg $\mathrm{L^{-1}}$) could substantively lessen the surface charge of the ferric oxyhydroxide-coated sediments. In the present study, no measurable changes in oocyst surface charge were observed in response to amendments of HPOA ranging up to 20 mg $\mathrm{L^{-1}}$. Consequently, the role of the river DOC in affecting the extent of colloid transport within the underlying aquifer sediments appears to involve DOC-induced modifications of grain-surface chemistry, rather than DOC-induced alterations of the oocysts or microsphere surfaces.

In our experiments examining oocyst remobilization, we found that modest HPOA addition would re-entrain previously sorbed oocysts and microspheres, particularly for sediments having the lowest amounts of extractable Fe and Al (Figure 4). Consequently, vulnerability to re-entrainment due to NOM was greatest for the sediments collected between 9 and 12 m bls that are characterized by the lower amounts of extractable metals (Figure 1, inset). Other research involving the effects of DOC upon remobilization of previously sorbed microbes and microspheres in aquifer sediments has demonstrated the high effectiveness of linear alkyl benzene sulfonates (LAS) surfactants in colloid re-entrainment.[41,43]

The degree of remobilization was somewhat dependent upon sediment metal oxide content, suggesting the sediments contain/retain a considerable colloid-removal capacity. For the deeper interval sediments, the amount of re-entrained colloids was about an order of magnitude larger, indicating a sorption capacity that could be overcome by small amounts of HPOA. These deeper interval sediments contain lower amounts of sediment surface associated extractable metals which show these sediments have lower capacities for colloid removal than sediments collected from shallower intervals. Other research has examined the effect of geochemistry, including organic carbon, upon remobilization of previously sorbed colloids,[41,43] and found that anionic surfactants will remobilize previously sorbed microbes and CMM. Current flow-through column studies employing untreated and NOM-amended sediments suggest that the dissolved organic matter in the Russian River has the potential to enhance transport of microspheres and colloids by reducing sorption to grain surfaces (Table 2). Indeed, the presence of only 2.2 mg C L^{-1} natural organic matter led to decreases in α for microspheres up to 9 fold, which would correspond to a 53-fold increase in their penetration distance within sediments. Although the Russian River DOC clearly has the ability to facilitate transport of oocysts-sized colloids, it does not appear that its transport-enhancing effects penetrate very deeply into the aquifer sediments. For example, the collision efficiencies for oocysts and oocysts-sized were ∼1.0 for sediments collected only 2 m bls. The reasons for a lack of a DOC effect in the deeper sediments at the RBF site are not entirely clear, although its low concentration ($1-2$ mg L^{-1}) in the river suggests that that the most-reactive components of the DOC may not penetrate beyond the near-surface (upper meter) of sediments.

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