

An evaluation of the mobility of pathogen indicators, *Escherichia coli* and bacteriophage MS-2, in a highly weathered tropical soil under unsaturated conditions

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ABSTRACT

Laboratory column experiments were conducted to study the effects of anionic polyacrylamide (PAM) polymer and surfactant linear alkylbenzene sulfonate (LAS) on the movement of *Escherichia coli* and the FRNA phage MS-2. The study was designed to evaluate if PAM or PAM + LAS would enhance the mobility of human pathogens in tropical soils under unsaturated conditions. No breakthrough of phage was observed in a 10 cm column after passing 100 pore volumes of solution containing 1×10^8 plaque-forming units (PFU)/ml. In later experiments, after passing 10–20 pore volumes of influent containing 1×10^8 /ml MS-2 or *E. coli* through 15 cm columns, the soil was sliced and the organisms eluted. Phage moved slightly deeper in the polymer-treated column than in the control column. There was no measurable difference in the movement of *E. coli* in either polymer-treated or control columns. The properties of the soil (high amounts of metal oxides, kaolinitic clay), unsaturated flow conditions, and relatively high ionic strengths of the leaching solution attributed to significant retention of these indicators. The impacts of PAM and LAS on the mobility of *E. coli* or MS-2 phage in the chosen soils were not significant.

Key words | *E. coli*, linear alkylbenzene sulfonate, MS-2 phage, polyacrylamide, soil column, tropical soils

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INTRODUCTION

The islands of Hawaii have many similarities to other tropical regions of the world. They have highly weathered soils, seasonal rainfall that is high in intensity, and a high population density that relies on ground water as the primary source of drinking water. Prevention of pathogen contamination of ground water and coastal waters is among the top priorities in Hawaii. Some areas of the Hawaiian islands are not serviced by sewers. Instead, they are serviced by septic tanks and cesspools. Many communities along coastline areas have high-capacity (serving multiple dwellings and businesses) cesspools that are very close to the

ocean, and they are potential contamination sources for both underground waters as well as coastal recreational waters. Currently, the US Environmental Protection Agency (EPA) has issued a ban on high-capacity cesspools in Hawaii. However, users have yet to fully comply with the ruling. Further, the rule does not apply to the cesspools of individual homeowners. In some regions of the state, land is also being used for the disposal of treated wastewater and sludge as ocean disposal is becoming increasingly regulated.

No systematic studies have been conducted in Hawaii on the transport behavior of pathogens in unsaturated soils.

Although a limited number of field and laboratory studies have been conducted to evaluate the transport of bacteria (total and fecal coliforms) and viruses (phage T4, coxsackie, and polio type 2) in selected soils (Tanimoto *et al.* 1968; Hori *et al.* 1970; Lau *et al.* 1975; Chang & Young 1977), no concerted efforts were made to control the moisture regime, pH, or boundary conditions in the laboratory studies, even though these are some of the important processes that affect the transport of microorganisms in soil. As reported in the above studies, virus concentrations in the effluent from the soil columns or field lysimeters varied from no detection to a small fraction (3%) of the applied concentrations depending on soil type. In the above literature, field studies were conducted at agronomic rates on soils rich in oxides of iron and aluminum. Water samples collected from lysimeters at 1.5 m depths contained no viruses, suggesting their limited movement in the soils tested.

In the western United States, anionic polymers such as polyacrylamides (PAMs) are being used in agricultural lands to reduce erosion and enhance water infiltration (Trout *et al.* 1995; Zhang & Miller 1996; Ben-Hur & Keren 1997; Aase *et al.* 1998; Sojka *et al.* 1998). Enhanced infiltration may increase the movement of pathogens through the soil and, consequently, increase the risk of their migration to the aquifer. PAM is also used as a flocculant in water and wastewater treatment (Gardiner 1996; Vanotti *et al.* 1996). Residual PAM can be found in treated wastewater or sludge. Thus, the use of PAMs on agricultural land for erosion control or the presence of residual PAMs in effluent or biosolids from treatment plants may increase the chance of ground-water contamination due to enhanced infiltration. Similarly, linear alkylbenzene sulfonate (LAS), the most widely used surfactant in cleaning products and laundry detergents, is typically found in wastewater and sludge (Rapaport & Eckhoff 1990).

Ongoing field studies of effluent application at agronomic rates require a long time to observe any effects in deeper soils or in the underlying ground water. Thus, column experiments at high loading rates of water and pathogens needed to be conducted to examine their transport behavior in relatively short periods of time.

The overall goal of this research was to evaluate if the ground water beneath the highly weathered tropical soils would be susceptible to contamination from pathogens

present in wastewater and sludge. The specific objective was to study the transport behavior of *E. coli* and the bacteriophage MS-2 (often used as a surrogate for certain human enteric viruses) in packed unsaturated soil columns of Wahiawa Oxisol under conditions where the soil surface was treated with PAM and the leaching solution was amended with LAS typically found in effluent. The relatively high ionic strength of the leaching solution was used to represent the coastal water quality.

MATERIALS AND METHODS

Materials

Soil

The Wahiawa Oxisol, an extremely weathered tropical soil of basaltic origin, was collected from Central Oahu at the depth ranges of 25 to 40 cm to exclude much of the surficial organic matter. Kaolin is the primary mineral in the soil. The soil is classified as silty clay loam, with large fractions of water-stable aggregates. The soil contains a few weatherable minerals and is rich in oxides of iron and aluminum. Iron oxides (hematite) give the soil a distinct red color. The particle-size analysis of dispersed aggregates revealed that the soil contains 83% clay, 12% silt, and 5% sand. The average pH (1 part soil with 1 part deionized water) of the soil is 5.7. The particle densities of the Wahiawa soil samples range from 2.78 to 3.23 g/cm³ (Miller 1987). It is believed that such high particle densities are due to the presence of heavy metals such as iron and titanium (15 to 18% and 2.5 to 3.5%, respectively, determined by X-ray fluorescence analysis). Among the base cations, approximately 70% is Ca²⁺, 17% is Mg²⁺, 7% is Na⁺, and 6% is K⁺ (Teo *et al.* 2006). In the current study, the soil was sterilized by irradiation with a Co⁶⁰ source for 60 hours and was refrigerated until use.

Triplicate soil samples were analyzed for their mineral composition using X-ray fluorescence and they showed high fractions of iron and aluminum oxides. The average respective percentages of Fe₂O₃, Al₂O₃, and MnO were 18.7%, 32.5%, and 1.1%. In addition, TiO₂ in the soil averaged around 3%.

PAM + LAS

The anionic PAM (Superfloc A-836) was received from Cytec Industries, Inc. (Stamford, Conn.). The approximate molecular weight was 15×10^6 g/mole, and it had a medium charge density (18% charge substitution). Its pH ranged from 5 to 7 in aqueous solution. A stock solution having a concentration of 1,000 mg/L was prepared for soil application at the rate of 10 kg/ha. The sodium salt of dodecylbenzenesulfonic acid, an LAS or surfactant, was obtained from Sigma Chemical Co. (St. Louis, Mo.) and was used at 25 mg/L in the buffer.

Microorganisms

The *E. coli* strain used for all experiments was American Type Culture Collection 25922. The male-specific RNA coliphage, MS-2, which infects *E. coli* HS(pFamp)R, was also utilized in all experiments. It has a diameter of 26.0 to 26.6 nm (van Duin 1988) and an isoelectric point of pH 3.9 (Zerda 1982; Zerda *et al.* 1985).

Buffer solution

A 0.01 M CaCl₂ solution was used in all experiments to prevent the breakdown of the soil aggregates which would otherwise reduce porosity and change flow conditions. It was also used as a diluent to prepare the bacterial inoculum. A salt diluent, 8,500 mg/L NaCl in 0.002 M CaCl₂, was used for the suspension of MS-2 phage during the leaching and adsorption experiments. The high ionic strengths of the leaching solutions represented what would be found in cesspools and septic tanks.

Methods

Assay of *E. coli*

The standard membrane filtration technique (*Standard Methods* 1999) was used to analyze for *E. coli* in liquid samples such as the column influent and column effluent. Soil from the columns was assayed for *E. coli* by eluting the soil with appropriate buffer and performing membrane filtration on the eluate. Most probable number (MPN), a multiple-tube fermentation method (*Standard Methods*

1999), was used for enumerating *E. coli* in soil from all columns treated with the surfactant LAS.

Assay of phage

For the assay and enumeration of bacteriophage MS-2, a modified double agar overlay method (Adams 1959) was used. In brief, the sample (0.2 mL) and the host bacteria (0.3 mL) were added to a tube containing 5 ml of melted agar (top agar). The contents were then mixed and gently poured over an agar plate (bottom agar) containing nutrients that support the growth of the host. The plate was incubated at 37°C for 24 hours. Plates containing 20 to 200 plaques (clear areas in the lawn of host bacterium) were used for phage enumeration, and the results were reported in plaque-forming units (PFU)/mL for effluent samples or PFU/g of dry soil. The host bacterium in this system, F(ampR) *E. coli* is resistant to ampicillin, which when incorporated into the bottom agar, limits the growth of other background bacteria. The bacterial host was prepared as previously described by Debartolomeis & Cabelli (1991). An additional enrichment procedure, as outlined in Wong (2001), was used to increase the sensitivity of the assay.

E. coli and phage viability in buffer and soil

The respective viabilities of *E. coli* and MS-2 were determined in buffer and soil to ensure their viability over the period of the experiment. Approximately 5×10^{10} CFU of *E. coli* were added to 500 ml of 0.01 M CaCl₂ or CaCl₂ with PAM and held for 5 days at room temperature (20°C). Samples were taken on days 1, 3, and 5 and analyzed for *E. coli* by membrane filtration. Approximately 5×10^{10} PFU of MS-2 phage were added to 500 ml of salt diluent or salt diluent with PAM and held at room temperature for 10 days. Samples were analyzed for MS-2 by the double agar overlay method. A total of 1×10^{10} CFU of *E. coli* or 1×10^{10} PFU of MS-2 phage were added to 500 ml of 0.01 M CaCl₂ with 25 mg/L of LAS and held at room temperature for 10 days. Samples were taken on days 1, 3, 5, and 10.

The viability of *E. coli* and MS-2 in soil was examined by mixing 300 g of plain soil with 90 ml of appropriate

buffer. For PAM-treated soil, 300 g of soil were mixed with 60 ml of appropriate buffer. The soil was inoculated with 1×10^8 colony forming units (CFU) or PFU bacteria or MS-2 per gram of soil. After thoroughly mixing the soil, two subsamples (about 10 to 20 g each) were taken to determine soil moisture content and for microbial analysis. The remainder of the soil was held at room temperature for 5 or 10 days. *E. coli*-inoculated soils were sampled on days 1, 3, and 5; whereas MS-2-inoculated soils were sampled on days 2, 4, 6, 8, and 10. The soil was eluted with an appropriate buffer and assayed for *E. coli* by membrane filtration or for MS-2 by the double agar overlay method.

Column experiments

Plexiglas columns, 5 cm in diameter and either 10 cm or 15 cm in length, were packed with sterile soil which had been passed through a 2 mm sieve. Before packing, the column cells and tubings were thoroughly disinfected with 10% chlorox solution, dechlorinated with 10% sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) solution, and rinsed several times with sterile distilled water. All columns were packed with soil to achieve a field bulk density between 1.01 and 1.03 g/cm^3 . Using a power sprayer, the PAM was applied to the top of the soil column at a dose of 10 kg/ha. The PAM-treated soil column was then allowed to dry overnight before being used. Water and inoculum solutions were uniformly applied over the surface of the column. The leaching experiments were run under unsaturated flow conditions. The bottom of the soil columns were connected to the vacuum chamber (see Figure 1). To obtain the desired flow and to minimize air entering the soil columns, a suction of 5 cm was applied to the 15 cm columns and 2 cm to the 10 cm columns. Solution was continuously applied to the top of the column with a multichannel syringe pump at flow rates of 17.50 cm/day for the 15 cm columns and 61.25 cm/day for the 10 cm columns. These flow rates, which are substantially higher than agronomic rates, were selected to increase the chances of breakthrough in relatively short periods of time. The viral transport experiments were run for 10 days, and the bacterial and the combination viral/bacterial transport tests were run for 5 days.

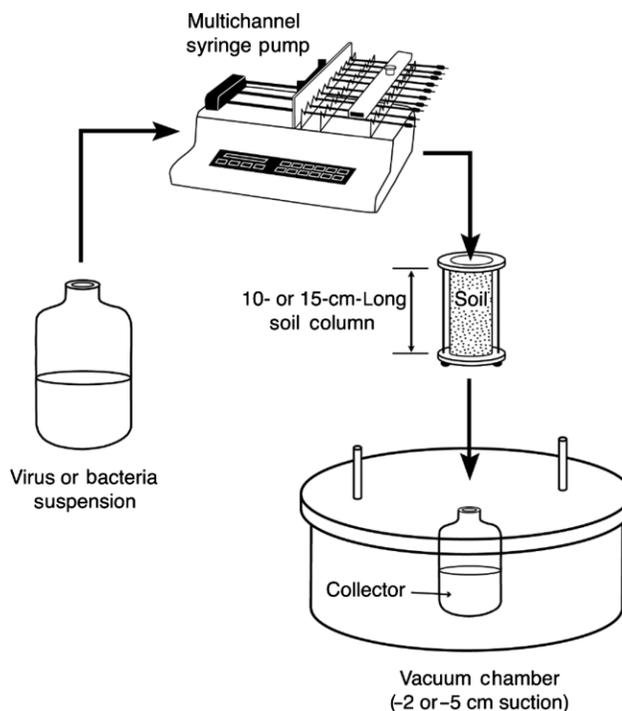


Figure 1 | Experimental setup for a leaching column. Leaching solutions are collected in sterile containers.

The feed solution contained approximately 1×10^8 CFU/mL of *E. coli* or 1×10^8 PFU/mL of MS-2 phage. Details of the preparation are presented in Wong (2001).

Adsorption experiments

Batch adsorption experiments were conducted to assess the sorption distribution coefficient (K_d) of *E. coli* or phage to the Wahiawa Oxisol, and to observe the effect of contact time on adsorption. Various amounts of air-dried soil were added to phage and *E. coli* suspensions and mixed until equilibration occurred. The slurry was centrifuged at 8,000 rpm for 20 minutes, and the supernatant was analyzed for phage. For bacterial enumeration, absolute quantification was not feasible because of sedimentation of cells from the supernatant. So, the bacterial samples were centrifuged at a lower speed (5,000 rpm) for 10 minutes only. The supernatant was analyzed for the unsettled (unadsorbed) bacteria. This provided an apparent bacterial density for the given conditions. *E. coli* and MS-2 phage were enumerated as previously described. The amount of bacteria or phage attached to the soil was obtained by

subtracting the amount free in the supernatant from the amount originally added.

RESULTS

Viability of *E. coli* and MS-2 phage

The *E. coli* and MS-2 phage suspensions were stable over the desired experimental period. The starting concentrations for *E. coli* and MS-2 were in the range 1×10^8 CFU/ml or PFU/mL in the salt diluent or in the CaCl₂ solution, respectively. The starting concentrations did not vary whether PAM was present or not. Their viability remained at that level for a period of 5 days for *E. coli* and 10 days for phage. Starting concentrations of *E. coli* and MS-2 for viability tests with LAS were at 1×10^7 PFU/mL and CFU/mL, respectively. There was a marginal reduction (< 1 log) for *E. coli* counts over a period of 5 days. However, the phage concentration remained at the initial level for the entire 10 days.

Unlike the water samples, there was a substantial reduction in recovery of both phage and bacteria from the soil as a function of time. Up to a 3-log (i.e., 1,000-fold) reduction in recovery was observed for phage at day 10 and up to a 1-log reduction in recovery was observed for *E. coli* at day 5.

MS-2 column leaching experiment: 10-cm-long column

No MS-2 was found in the breakthrough solution from the 10 cm long untreated soil column after passing 100 pore volumes of water containing about 1×10^8 PFU/mL. The total MS-2 input into the system was 1.18×10^{13} PFU. At the end of the experiment, the column was cut into three sections, each measuring 3.3 cm. The average concentrations of MS-2 recovered from the top, middle, and bottom portions were 8.90×10^8 , 1.90×10^9 , and 2.20×10^6 PFU/g, respectively, with a total recovery of less than 5% of the input.

MS-2 column leaching experiment: 15-cm-long column

Similar to the results for 10 cm long column leaching experiment, no MS-2 phage was found in the leachates of both 15 cm long soil columns (PAM-treated and control)

over a period of 10 days. During this time, approximately 18 pore volumes of water containing a total of 1.30×10^{12} PFU MS-2 passed through the soil column. The soil from each column was sliced into six 2.5 cm long sections to determine the phage movement in the column. The first two columns in Table 1 show that the downward movement of phage was somewhat enhanced in the PAM-treated soil column as compared to that in the control column. MS-2 was found in eluted soil samples down through the 5th section (10.0–12.5 cm depth zone) of the PAM-treated soil column but only through the 3rd section (5.0–7.5 cm depth zone) of the control soil column.

The percent recovery of MS-2 phage from soils during the elution procedures was about 5.0% from the PAM-treated soil and 17.5% from the control soil. Although, the phage moved slightly deeper in the PAM-treated soil than in the control soil, it proved difficult to elute phage particles from the PAM-treated soil. The low recovery was probably due to strong bonding between the soils and MS-2.

E. coli column leaching experiment: 15-cm-long column

Due to the potential for growth of *E. coli* in soil under laboratory conditions (Byappanahalli & Fujioka 1998), the leaching experiments were conducted for ~5 days, during which time about 9 pore volumes of water passed through the 15 cm long column. The resulting leachate samples from the treated and control columns contained *E. coli* in small numbers (less than 10 CFU/ml). These recoveries were perhaps due to bypass along the column walls and preferential flow. The soil from each column was again sliced to six equal 2.5 cm long sections. The *E. coli* eluted from the PAM-treated and control soil columns are presented in the last two columns of Table 1. *E. coli* was found in the depth range of 7.5–10.0 cm for both soil columns. The total *E. coli* input into the columns was 2.29×10^{11} CFU. The percent recovery during elution procedures was 4% from the PAM-treated soil and 18% from the control soil. This parallels the results found for MS-2 phage.

Leaching with LAS: 15-cm-long column

The leaching experiment to evaluate the impact of LAS on control and PAM-treated soil columns was run for 5 days.

Table 1 | Eluted concentrations of MS-2 phage and *E. coli* in PAM-treated and control (untreated) 15-cm soil columns. For MS-2, 18 pore volumes were displaced and for *E. coli*, 9 pore volumes were displaced

Depth zone (cm)	MS-2 phage		<i>E. coli</i>	
	PFU/g of dry soil	PFU from depth zone	CFU/g of dry soil	CFU from depth zone
PAM-treated column				
0–2.5	1.18×10^9	4.80×10^{10}	1.23×10^8	8.77×10^9
2.5–5.0	3.79×10^8	1.53×10^{10}	4.34×10^6	2.07×10^8
5.0–7.5	3.12×10^6	1.64×10^8	60.2	2.71×10^5
7.5–10.0	8.20×10^4	5.02×10^6	<1	27
10.5–12.5	8.08×10^5	5.87×10^7	<1	<1
12.5–15.0	<1	<1	<1	<1
Control column				
0–2.5	3.96×10^9	2.25×10^{11}	6.29×10^9	3.79×10^{10}
2.5–5.0	4.52×10^7	2.17×10^9	8.35×10^7	3.93×10^9
5.0–7.5	5.66×10^5	2.71×10^5	11.75	562
7.5–10.0	<1	<1	<1	31
10.5–12.5	<1	<1	<1	<1
12.5–15.0	<1	<1	<1	31

The total amount of influent applied to each of the two columns was 9 pore volumes. The influent contained 25 mg/L of LAS as well as *E. coli* or MS-2 at concentrations of 5×10^7 CFU or PFU/mL, for a total input of 9.1×10^{11} CFU or 9.1×10^{11} PFU, respectively. Continuous injection of the influent to the PAM-treated soil column resulted in no MS-2 in the effluent, but *E. coli* at a concentration of less than 10 CFU/ml began to appear in the effluent on day 1 and remained at that level for the rest of the period. For the control column (no PAM, but 25 mg/L LAS in solution), there was no MS-2 in the effluent, but *E. coli* began to appear at a concentration of 1 CFU/mL on day 1 and increased to 90 CFU/mL by day 5.

As before, the columns were cut into six 2.5 cm long sections, and the phage and the bacteria were eluted out of the soil sections. Table 2 shows the recovered amounts of

MS-2 phage and *E. coli* for the two treatments. The phage was limited to the top 5 cm (two sections) of the columns. Low concentrations of *E. coli*, however, were obtained from soils from all six sections of the columns, albeit the majority of the *E. coli* seemed to be concentrated in the top 2 sections.

Sorption experiments

There was no significant difference in the adsorption behavior between the untreated control and PAM-treated soil for MS-2. The 45 minute contact time experiment was not performed at the same time as the 30 minute and 4 hour contact time experiments. The input concentrations were prepared with the assumption that each gram of soil could adsorb 1×10^8 PFU of phage. In the control soil, as the

Table 2 | Concentrations of MS-2 phage and *E. coli* eluted from 15-cm soil columns treated with PAM and LAS. For each case, 9 pore volumes were displaced

Depth zone (cm)	Column treated with PAM and LAS		Column treated with LAS but no PAM	
	MS-2 (PFU/g of dry soil)	<i>E. coli</i> (CFU/g of dry soil)	MS-2 (PFU/g of dry soil)	<i>E. coli</i> (CFU/g of dry soil)
0–2.5	3.54×10^7	3.54×10^8	1.33×10^8	1.95×10^8
2.5–5.0	869	3.12×10^8	2.08×10^7	8.69×10^7
5.0–7.5	6.7	4.48×10^5	<1	4.50×10^3
7.5–10.0	<1	207	<1	908
10.5–12.5	<1	60	<1	225
12.5–15.0	<1	90	<1	641

contact time increased from 30 minutes to 45 minutes, the phage concentrations in the supernatant decreased by one log (from 2,900 PFU/mL to 253 PFU/mL). This decrease was much smaller for the PAM-treated soil.

When the contact time in the control soil was increased from 45 minutes to 4 hours, there was little difference in the concentration of the phage in the supernatant (253 PFU/mL at 45 minutes and 248 PFU/mL at 4 hours). This indicated that the control soil reached its equilibrium adsorption between 30 and 45 minutes. The concentration of the phage in the supernatant of the PAM-treated soil did not vary much as the contact time increased. The addition of PAM caused the soil to reach equilibrium adsorption faster. Under the conditions of the experiment, it takes approximately 800 minutes to pass one pore volume of water through the soil column; thus it is apparent that sorption is equilibrium-controlled during transport through the columns. As it was not feasible to get an absolute number of *E. coli* present in the supernatant after batch equilibration, the apparent concentrations, based on earlier specified centrifugation time and speed, were used to determine its sorption. The differences of sorption between 30 minutes and 4 hours were minimal, and PAM did not have any measurable effect on sorption.

When both control and PAM-treated soils were equilibrated with phage, both had the same maximum adsorption capacity in the range of 1×10^9 to 1×10^{10} PFU/g of dry soil. Such tests were not conducted for *E. coli* since its removal from the supernatant due to centrifugal force was not quantifiable.

DISCUSSION

In previous studies (e.g., Teo *et al.* 2006), PAM-treated soils showed higher rates of infiltration relative to control soils. PAM was also found to aid in coagulation and flocculation of several soils. It was noticed that when the PAM-treated soil column was cut, it was more intact than the control soil column. It is speculated that maintenance of large pores and possibly bypass flows might have contributed to a somewhat deeper migration of phage in the PAM-treated columns. The impacts of the soil as well as the chemicals on transport mechanisms are discussed below.

Impact of soil properties

As presented in the “Materials” section, the soil used for the experiments is rich in metal oxides. Their concentrations are typically large compared to that in soils in temperate climates. In fresh stream sediments, DeCarlo & Spencer (1995) reported percentages of iron and aluminum oxides as high as 10%. We hypothesize that loss of silica fractions due to weathering can increase the fraction of iron and aluminum oxides in the residuum. Grain-size analysis of the soil samples indicated that 75% of the soil minerals were less than 0.01 mm in size and over 55% were even smaller than 0.001 mm (Figure 2). Kaolinite is the predominant clay mineral found in Oxisols. The 1:1 clay is composed of one layer of silica and one layer of alumina.

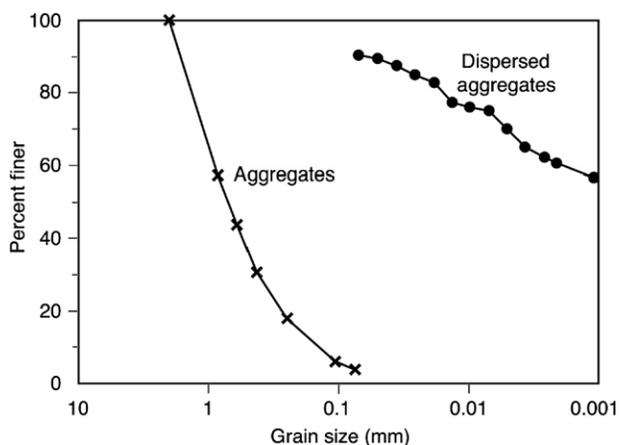


Figure 2 | Grain-size distribution of intact and dispersed aggregates in water. The plot to the left is determined by conducting a sieve of aggregated soil in a nest of sieves and that to the right is for dispersed aggregates in water.

The role of metallic oxides on virus retention is clear (Pieper *et al.* 1997; Zhuang & Jin 2003). Oxides or oxyhydroxides of iron and aluminum present in soil have been shown to enhance adsorption of pathogens. High clay content (Carlson *et al.* 1968; Bitton 1975) and iron oxide (Warren *et al.* 1966) are known to enhance adsorption of phage to soil.

Unsaturated flow conditions (Lance & Gerba 1984; Powelson *et al.* 1990) favor pathogen adsorption. Decreasing water content reduces the thickness of water films around the soil particles, which enhance the adsorption process. In our experiment, unsaturated transport conditions most likely promoted sorption of phage to the soil solids, causing no breakthrough in columns.

Lower solution pH favors adsorption of microorganisms to soil particles (Bitton 1975; Gerba *et al.* 1981). The isoelectric point (pI) is used to express the change in surface charge of bacteria and viruses due to changes in solution pH. The pI is that pH where the net charge on the bacteria or virus surface is zero. The pI of bacteria varies in a narrow range from 2.5 to 3.5, whereas that for viruses varies from 2.5 to 8.2 (Gerba 1984; Ackerman & Michael 1987). A microbe will have a net positive charge when the pH of the soil solution is lower than the pI.

Phage/bacterial elutriation from the columns was minimal in most cases, indicating irreversible sorption. Without the benefit of further investigations, it is unclear at this time what conditions in the environment would desorb the adsorbed phage and whether the desorbed phage would still have the viability to infect the host. Although our soil

had a pH of about 5.7 (above the pI of bacteria and MS-2 phage), it is below the neutral point (pH 7). There is an abundance of H^+ ions compared to OH^- ions. Low pH conditions favor adsorption.

PAM and LAS impact

As discussed earlier, soil in PAM-treated columns was more intact when removed, indicating a preservation of structure. Intact structure provides stable pores which help in enhancing/maintaining infiltration over long periods of time. With the enhancement of infiltration in the soil, microorganisms can move faster through the soil columns. The pH of the PAM solution in water was slightly higher than the solution pH of the soil. This could have also impacted microbial transport to some extent. PAM has a net negative charge, which might have competed with viruses for adsorption sites, thus allowing the phage to move deeper in the soil column. However, the same was not true for the bacteria. This poses questions on the surface properties of MS-2 phage versus that of *E. coli*. Because the relative size of *E. coli* is approximately 100 times greater than that of MS-2 phage, their surfaces and attachment behaviors are expected to be different.

Surface-active chemicals, such as LAS, can coat the metal oxides and reduce their sorption potential. However, the concentration used in the study (25 mg/L) was probably not sufficient to coat all mineral surfaces. Use of LAS at a higher concentration was not representative of wastewater and was considered potentially toxic to the microorganisms. Other studies which have used surfactants (Pieper *et al.* 1997) observed a longer travel distance of bacteria and viruses in field settings, such as at Cape Cod, Mass. The presence of organic matter in sewage along with the surfactant and low mass fraction of iron oxides/oxyhydroxides in the sediments may have contributed to farther migration of the injected bacteria and viruses in the sewage-contaminated areas.

Finally, the ionic strength of the leaching solution (0.01 M $CaCl_2$) was moderately high. For the MS-2 phage experiment, a salt diluent was used. High ionic strength favors deposition of colloidal particles such as the viruses and bacteria onto the collector (soils). However, the ionic strength of the leaching solution was expected to not be

higher than that of the brackish waters. Future work on other soil types and variable ionic strengths may indicate if the mobilization of the pathogen indicators will be higher.

CONCLUSIONS

The experimental data showed that PAM appears to have a slight effect on transporting MS-2 phage in soil but no measurable effect on *E. coli* movement in soil under similar conditions. It is possible that the surface charge of the soils and the MS-2 phage might have been affected differently due to the presence of PAM. LAS did not appear to have any significant effect on MS-2 phage mobility in control soils. However, there was slight enhancement of *E. coli* mobility in LAS-treated columns. When PAM was added to control and LAS-treated columns, there was a reduction in mobility for MS-2 phage and a slightly enhanced downward mobility for *E. coli*. Since there was no breakthrough of MS-2 phage or *E. coli*, comprehensive modeling was deemed infeasible.

Bacterial/phage sorption was generally complete in 30 to 45 minutes. The oxide-rich soil appeared to have tremendous sorption capacity for both *E. coli* and MS-2 phage. Removal of sorbed bacteria or phage was not completely reversible. It was further observed that with the addition of PAM or PAM with LAS, removal of *E. coli* and MS-2 phage became more difficult.

Based on these findings, our initial assessment is that highly weathered tropical soils, such as Oxisols, can act as a potential sink for the microbes associated with land-applied wastewater. However, we must use caution to extrapolate the data to all settings as the experiment used relatively higher ionic strength of the leaching solution and the particular soil used for the experiment contained a large percentage of metal oxides and submicron size clay. Future work should elucidate more into the mechanisms of pathogen indicator transport in a variety of settings.

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REFERENCES

- Aase, J. K., Bjorneberg, D. L. & Sojika, R. E. 1998 Sprinkler irrigation runoff and erosion control with polyacrylamide – Laboratory tests. *Soil Sci. Soc. Am. J.* **62**, 1681–1687.
- Ackermann, H. W. & Michael, S. D. 1987 *Viruses of Prokaryotes*. CRC Press, Boca Raton, FL.
- Adams, M. H. 1959 *Bacteriophages*. Interscience Publishers, New York, NY, USA.
- Ben-Hur, M. & Keren, R. 1997 Polymer effects on water infiltration and soil aggregation. *Soil Sci. Soc. Am. J.* **61**, 565–570.
- Bitton, G. 1975 Adsorption of viruses onto surfaces in soil and water. *Water Res.* **9**, 473–484.
- Byappanahalli, M. N. & Fujioka, R. S. 1998 Evidence that tropical soil environment can support the growth of *Escherichia coli*. *Water Sci. Technol.* **38**, 171–174.
- Carlson, G. F. Jr., Woodard, F. E., Wentworth, D. F. & Sproul, O. J. 1968 Virus inactivation on clay particles in natural waters. *J. WPCF* **40**, 89–106.
- Chang, S. Y. K. & Young, R. H. F. 1977 *An investigation into environmental effects of reuse of sewage effluent at the Kaneohe Marine Corps Air Station Klipper Golf Course*. Tech. Memo. Report No. 53. Water Resources Research Center, Univ. of Hawaii, Honolulu, HI, USA.
- Debartolomeis, J. & Cabelli, V. J. 1991 Evaluation of an *Escherichia coli* host strain for enumeration of F male-specific bacteriophages. *Appl. Environ. Microbiol.* **57**(5), 1301–1305.
- DeCarlo, E. H. & Spencer, K. J. 1995 Sedimentary records of anthropogenic inputs of heavy metals to Ala Wai, a small man-made estuary in Honolulu, Hawaii. *Pac. Sci.* **49**, 471–491.
- Gardiner, D. 1996 *Gypsum and polyacrylamide soil amendments used with high sodium wastewater*. Tech. Rep. 174. Texas Water Resources Inst., Texas A&M Univ., College Station, Texas, USA.
- Gerba, C. P. 1984 Applied and theoretical aspects of virus adsorption to surfaces. *Adv. Appl. Microbiol.* **30**, 133–168.
- Gerba, C. P., Goyal, S. M., Cech, I. & Bogdan, G. F. 1981 Quantitative assessment of the adsorptive behavior of viruses to soils. *Environ. Sci. Tech.* **15**, 940–944.
- Hori, D. H., Burbank, N. C. Jr., Young, R. H. F., Lau, L. S. & Klemmer, H. W. 1970 *Migration of poliovirus type 2 in percolating water through selected Oahu soils*. Tech. Rep. No. 36. Water Resources Research Center, Univ. of Hawaii, Honolulu, HI, USA.
- Lance, J. C. & Gerba, C. P. 1984 Virus movement in soil during saturated and unsaturated flow. *Appl. Environ. Microbiol.* **47**, 335–337.

- Lau, L. S., Dugan, G. L., Ekern, P. C., Young, R. H. F. & Burbank, N. C. Jr. 1975 *Water recycling of sewage effluent by irrigation: A field study on Oahu - Final progress report for August 1971 to June 1975. Tech. Rep. No. 94.* Water Resources Research Center, Univ. of Hawaii, Honolulu, HI, USA.
- Miller, M. E. 1987 *Hydrogeologic Characteristics of Central Oahu Subsoil and Saprolite: Implication for Solute Transport*, M.S. Thesis, Dept. Geology and Geophysics, University of Hawaii at Manoa, Honolulu.
- Pieper, A. P., Ryan, J. N., Harvey, R. W., Amy, G. L., Illangasekare, T. H. & Metge, D. W. 1997 **Transport and recovery of bacteriophage PRD1 in a sand and gravel aquifer: Effect of sewage-derived organic matter.** *Environ. Sci. Technol.* **31**, 1163–1170.
- Powelson, D. K., Simpson, J. R. & Gerba, C. P. 1990 Virus transport and survival in saturated and unsaturated flow through soil columns. *J. Environ. Qual.* **19**, 396–401.
- Rapaport, R. A. & Eckhoff, W. S. 1990 **Monitoring linear alkyl benzene sulfonates in the environment: 1973–1986.** *Environ. Toxicol. Chem.* **9**, 1245–1257.
- Sojka, R. E., Lentz, R. D., Trout, T. J., Ross, C. W., Bjorneberg, D. L. & Aase, J. K. 1998 Polyacrylamide effects on infiltration in irrigated agriculture. *J. Soil Water Conserv.* **53**, 325–331.
- Standard Methods for the Examination of Water and Wastewater* 1999 20th edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.
- Tanimoto, R. M., Burbank, N. C., Jr., Young, R. H. F. & Lau, L. S. 1968 *Migration of bacteriophage T₄ in percolating water through selected Oahu soils. Tech. Rep. No. 20.* Water Resources Research Center, Univ. of Hawaii, Honolulu, HI, USA.
- Teo, J. A., Ray, C. & El-Swaify, S. A. 2006 **Screening of polymers on selected Hawaii soils for erosion reduction and particle settling.** *Hydrol. Processes* **20**, 109–125.
- Trout, T. J., Sojka, R. E. & Lentz, R. D. 1995 Polyacrylamide effect on furrow erosion and infiltration. *Trans. Am. Soc. Agric. Eng.* **38**, 761–765.
- van Duin, J. 1988 Single-stranded RNA bacteriophages. In *The Bacteriophages* (ed. R. Calendar). Plenum Press, New York, USA. pp. 117–167.
- Vanotti, M. B., Hunt, P. G. & Szögi, A. A. 1996 *Solids and nutrient removal from swine wastewater using polyacrylamides.* An ASAE meeting presentation, paper no. 96–4052, Am. Soc. Agric. Engineers, St. Joseph, MI, USA.
- Warren, J., Neal, A. & Rennels, D. 1966 Adsorption of myxoviruses on magnetic iron oxides. *Proc. Soc. Exp. Biol. Med.* **12**, 1250–1253.
- Wong, T. P. 2001 *Polyacrylamide (PAM) Effects on Viruses and Bacteria Transport in an Unsaturated Oxisol*, M.S. Thesis (Civil Engineering), University of Hawaii at Manoa, Honolulu, HI, USA.
- Zerda, K. S. 1982 *Adsorption of Viruses to Charge-modified Silica.* Ph.D. thesis, Baylor College of Medicine, Houston, TX, USA.
- Zerda, K. S., Gerba, C. P., Hou, K. C. & Goyal, S. M. 1985 Adsorption of viruses to charge-modified silica. *Appl. Environ. Microbiol.* **49**, 91–95.
- Zhang, X. C. & Miller, W. P. 1996 Polyacrylamide effect on infiltration and erosion in furrows. *Soil Sci. Soc. Am. J.* **60**, 866–872.
- Zhuang, J. & Jin, Y. 2003 **Virus retention and transport through Al-oxide coated sand columns: Effect of ionic strength and composition.** *J. Contam. Hydrol.* **60**(3–4), 193–209.

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