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Leaching Assessment of Pathogen Indicators in the Tropical Soils of Hawaii

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ABSTRACT: Ground water contamination from human pathogens is a concern in tropical islands where ground water is the source of drinking water. Understanding the processes of pathogen behavior, especially in unsaturated tropical soils, is important in preventing groundwater contamination. Laboratory column experiments (100 to 150 mm long columns) were conducted to study the effects of an anionic polyacrylamide (PAM) polymer on the movement of *Escherichia coli* and the FRNA phage MS-2 under unsaturated conditions. The study was designed to evaluate if PAM would enhance the mobility of human pathogens in tropical soils under unsaturated conditions. Soil in the columns was kept under unsaturated conditions by applying a small vacuum at the bottom of the columns. *E. coli* or MS-2 phage in the feed solution was pumped into the top of the columns. In addition, the surfactant, Linear Alkylbenzene Sulfonate (LAS), was used in low amounts to simulate a concentration that is present in sewage. The soil used for the column experiment was an Oxisol from the island of Oahu, Hawaii. No breakthrough of phage was observed in a 100 mm column after passing 100 pore volumes of solution containing 1×10^8 plaque-forming units (PFU)/mL. In later experiments, after passing 10 to 20 pore volumes of solution containing 1×10^8 viral particles or bacterial cells per milliliter through 150 mm columns, the soil columns were sliced and the organisms eluted from the soil. Neither phage nor *E. coli* breakthrough occurred at the bottom of the columns with either PAM or PAM + LAS. Phage progressed slightly further 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 during the leaching experiments, and relatively high ionic strengths of the leaching solution contributed to significant retention of the indicators. The impact of PAM and LAS on the mobility of *E. coli* or MS-2 phage in the chosen soils was not significant under the conditions of the study. Additional studies were conducted for two other soils to evaluate the mobility of MS-2 under conditions of variable pH and solution salinity.

INTRODUCTION

Transport of soil and land-based pollutants such as pesticides, chemicals, nutrients, pathogens, and other toxins that enter the receiving waters as well as leaching of these constituents to ground water are major water pollution problems in regions that receive significant rainfall. This is especially relevant for islands, coastal states and other regions that receive heavy rainfall or have a significant acreage under irrigated agriculture. A recent management strategy to reduce soil erosion problems is to apply anionic high molecular weight polymers such as polyacrylamides (PAM) to soil to retain the structure of the soil and to prevent soil erosion and subsequent environmental problems (Sojka *et al.*, 1998).

Agricultural irrigation is recognized as a cause of surface and ground water pollution. Pesticides, nutrients (nitrate and phosphate), bacteria and viruses are associated with agriculture and wastes produced from agriculture. Animal wastes and composts have traditionally been used for agricultural purposes throughout the world. Disposal of biosolids and secondary-treated effluent from wastewater treatment plants either on agricultural or on non-agricultural areas such as golf course and forested areas, is being actively pursued as a mode of reusing the wastes. Excessive loading of these wastes often leads to serious pollution problems. Some areas even use treated wastewater for groundwater recharge.

Enhanced infiltration in soils treated with wastes that contain pathogenic organisms may cause a faster movement of pathogenic micro-organisms through the soil and finally to aquifers. Thus, the use of polymers on agricultural land for erosion control and in wastewater treatment to improve coagulation of suspended matter, may increase the potential for ground water contamination. Fecal micro-organisms, including pathogenic human enteric viruses are prevalent in wastewater as well as in animal wastes. Because of their small size, they are likely to be transported through the soil matrix to contaminate groundwater supplies and to cause water-borne diseases in human.

The objective of this study is to examine how the addition of PAM and a common sewage derived surfactant, would affect the transport of pathogen indicators *E. coli* and bacteriophage MS-2 in a Hawaii soil.

MATERIALS AND METHODS

The soil used for this study is an Oxisol collected from the Poamoho Agricultural Experiment Station of the University of Hawaii, located in central Oahu. The soil was collected from a depth range of 0.25 to 0.6 m. Much of the surficial organic matter was excluded by sampling at this depth. The average annual rainfall in the area is around 1,500 mm. The soils are highly weathered and have lost most of their silica over time. As a result, they are heavily enriched with oxides of Al, Fe, and Mn. Also, the soil is highly aggregated with water-stable particles. Abundance of iron oxides gives a distinct reddish color to this soil. When the soil particles are fully dispersed, more than 80% of the material can be of clay size < 2 μm, (see Wong *et al.*, 2008).

Two chemicals were used for this study: (a) anionic polyacrylamide (PAM, brand name—Superfloc A-836) and (b) sodium dodecylbenzene sulfonate, a linear alkylbenzene sulfonate (LAS). The PAM was obtained as a sample from Cytec Industries in Stamford, CT (USA) and the LAS was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The molecular weight of the PAM used was about 20 mg/mole. A stock solution was prepared with a concentration of 1000 mg/L. This was used to apply to the soil surface at the rate of 10 kg/ha. The LAS was used at 25 mg/L for the experiment.

Pathogen indicator *E. coli* (strain 25922 from American Type Culture Collection) and male-specific bacteriophage MS-2 were used in the experiment. *E. coli* HS(pFamp) was used as the host for MS-2 phage. MS-2 is an RNA coliphage that has diamater between 26 and 27 nm and an isoelectric point at a pH value of 3.9 (Zerda, 1982; Zerda *et al.,* 1985).

All experiments were conducted using 10 mM $CaCl_2$ solution to prevent the breakdown of soil aggregated during the leaching studies. Aggregate breakdown can cause a reduction of porosity and flow rate through the column. The MS-2 phage was suspended in a salt diluent that contained 0.145M NaCl and 2 mM $CaCl_2$.

E.coli was assayed in liquid samples following membrane filtration (see APHA, AWWA, and WEF, 1999). Also, the Most Probable Number (MPN) method (see APHA, AWWA, and WEF, 1999) was followed to enumerate *E.coli* in soil samples, particularly those in the LAS study. MS-2 phage was assayed and enumerated using a modified double agar overlay method (Adams, 1959). The bacterial host was prepared following the method of Debartolomeis and Cabelli (1991). In order to improve the sensitivity of the assay, an additional enrichment technique, as described in Wong (2001), was followed.

The viability of *E. coli* and MS-2 phage was tested in buffer solution and soil for periods ranging from 5 to 10 days. The procedures are detailed in Wong *et al.* (2008). In short, *E. coli* or phage at given concentrations were mixed with soil samples (separately) and the soil was incubated for 5 days at room temperature for *E. coli* enumeration and 10 days for phage enumeration. Samples for *E. coli* were taken on days 1, 3, and 5, whereas phage enumeration was done on days 2, 4, 6, 8, and 10. The moisture content of the soils was similar to that near field-capacity water content. For testing viability in water samples, 100 million *E. coli* or phage per mL of solution in a 500 mL bottle were held in room temperature. Phage samples were analyzed on days 1, 3, 5, and 10 and *E. coli* were analyzed on days 1, 3, and 5.

Soil column leaching experiments were conducted in acrylic columns that were 50 mm in diameter and with a length of either 100 mm or 150 mm. The soils to be packed in the columns were air dried. The fraction that passed through a 2 mm sieve was used to achieve field bulk density (between 1 and 1100 kg/m^3). For PAM treatment, the top of the soil in the column was sprayed with a PAM solution to have an effective dose of 10 kg/ha. This was allowed to dry before conducting any experiment. Before conducting the leaching experiment, the columns, tubing, and PAM solution were sterilized (chemically or by autoclaving). Experiments were conducted under unsaturated conditions so that the column bottom remained constantly at a suction pressure of either -2 or -5 cm inside a vacuum chamber. Initially, the columns were fed with the buffer solutions followed by a leaching experiment with Br^- ion. For the 100 mm long columns, the flow rate was 0.61 m/day and for the 150 mm column, it was 0.175 m/day. The feed solution was then spiked with solution containing 100 million *E. coli* per mL or 100 million phage per mL. The effluent was collected at the bottom and enumerated at regular intervals. Figure 1 shows a leaching column setup where the column effluent was being collected inside a vacuum chamber.

The initial experiments were conducted using the short (100 mm) column until 100 pore volumes of solution containing phage was pumped through the soil. However, the effluent did not contain any phage. At the end, the column was sliced into three equal sections and the adsorbed phage was washed into a buffer solution. More than 90% of the recovered phage was from the top third of the column. In subsequent experiments, the longer (150 mm) columns were used. Leaching was conducted for 5 days for *E. coli* and 9 to 10 days for phage. At the end, the columns were sliced into six equal sections (each 25 mm long) and the phage adsorbed to each section was eluted.

Adsorption experiments were conducted to examine the capacity of soil to sorb phage or *E. coli* and to determine the time needed to reach equilibration in batch sorption experiments. The solution used contained 100 million MS-2 phage per mL or 100 million *E. coli* per mL. Wong (2001) provides details of the experimental conditions. For phage, the suspension was centrifuged at 3000 g for 20 minutes whereas the bacterial suspension was centrifuged at 2000 g for 10 minutes. The bacteria being larger in size were likely to settle under higher gravitational forces.

Modeling of pathogen transport in the soil based on the batch sorption and column leaching experiments was considered. The sorption distribution coefficient values obtained from batch studies are used to calculate the retardation factor, which is normally used in transport modeling through columns or soils. Transport of viruses or bacteria follows the advection-dispersion equation, with reaction terms accounting for sorption, inactivation, and colloidal filtration. Both equilibrium and kinetic adsorption processes have been considered in modeling (for summary, see Schijven, 2000). The CXTFIT code (Toride *et al.*, 1995) was used to fit the bromide breakthrough data obtained from the 150 mm long column that contained untreated soil. The code was used to obtain the dispersion parameters for transport. Colloidal filtration is another mechanism contributing to the retardation of viruses in porous media. Yao *et al.* (1971) use a term called single collector efficiency (η) to explain the attachment efficiency of colloids to the collector (i.e., sand grains in their case). It is assumed that η is dependent upon Brownian diffusion, interception, and gravity settling. However, Penrod *et al.* (1996) show that the effect of interception and gravity settling can be negligible for viruses because of their small size.

For filtration, comparisons were for packed soil, dispersed soil, and filter sand, each with different median diameters.

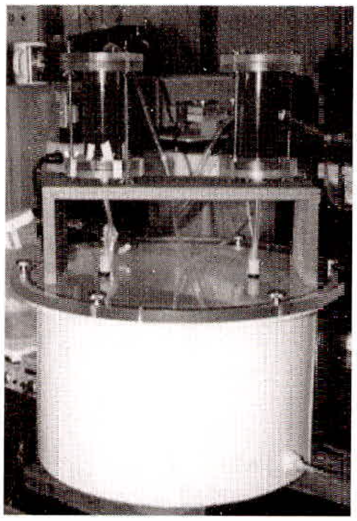

Fig. 1: Leaching apparatus in which the columns are setup above a vacuum chamber

RESULTS

Batch adsorption results for the MS-2 phage with and without PAM amendments are shown in Table 1. As shown in this table, by $t = 45$ minutes, the plain and the amended soils have reached their sorption capacity. There is no significant difference in sorption between the two treatments considering the dilution factors. PAM addition did not measurably change the sorption capacity of the soil.

However, centrifugation of the bacterial suspension reduced the bacterial count. Table 2 shows that nearly 37% of suspended bacteria settled in 10 minutes at a 2000 × g force. For this reason, we do not put much emphasis on the results obtained for the sorption capacity of the soils for *E. coli*. For both the untreated and amended soils, it was estimated that the maximum sorption capacity for MS-2 phage varied between 10^9 to 10^{10} PFU/g of dry soil.

The results of the recovered phage or *E.coli* for the control and PAM treated columns are presented in Figures 2 and 3, respectively. The recovered phage in the control column was mostly limited to the top 75 mm (Figure 2). However, phage was recovered up to a depth of 125 mm from the PAM-treated column. It is possible that the addition of PAM enhanced the mobility of MS-2 which was later recovered from lower depths. It is uncertain if PAM had any effect on the recovery of phage from the soil.

PAM did not seem to have any impact in increasing the mobility of *E. coli*, in the Poamoho soil. In fact, the recovery of *E. coli* from the PAM-treated soil was lower than the untreated soil in the first 50 mm depth.

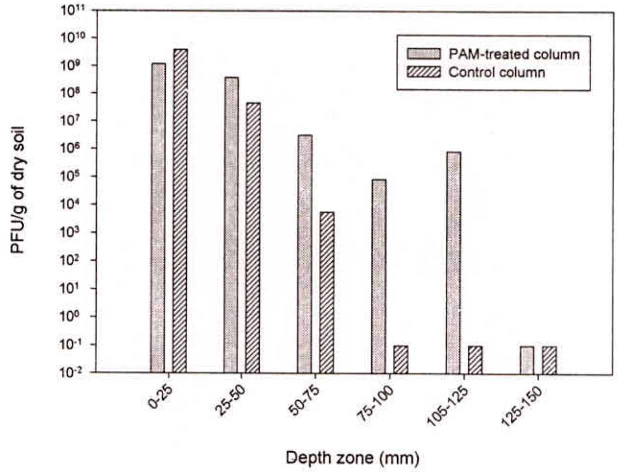

Fig. 2: Recovery of MS-2 phage from untreated and PAM-amended soil columns

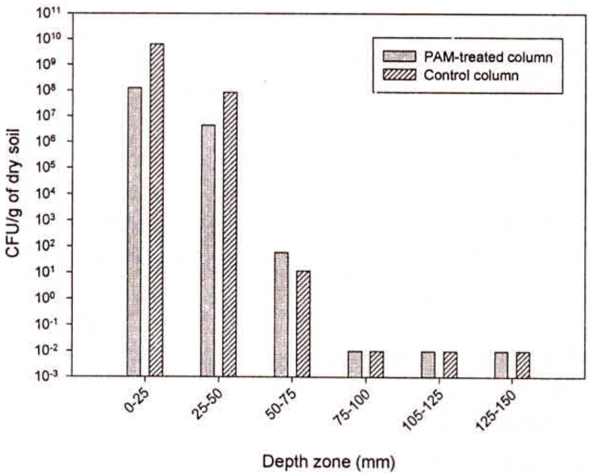

Fig. 3: Recovery of *E. coli* from untreated and PAM-amended soil columns

Table 1: MS-2 Adsorption Data for the Untreated Soil and PAM Amended Soil

	30 minutes	*45 minutes*	*4 hours*
Untreated soil			
Initial (PFU/mL)	1.31×10^8	1.66×10^8	1.31×10^8
Supernatant (PFU/mL)	2900	253	248
Adsorbed (PFU/g)	3.08×10^8	4.00×10^8	3.08×10^8
PAM Amended soil			
Initial (PFU/mL)	1.31×10^8	1.66×10^8	1.31×10^8
Supernatant (PFU/mL)	775	393	290
Adsorbed (PFU/g)	3.49×10^8	5.31×10^8	3.49×10^8

Table 2: Reduction of Bacteria Numbers Due to Centrifugation

Initial (CFU/mL)	*Sample Supernatant$_1$ (CFU/mL)*	*Sample Supernatant$_2$ (CFU/mL)*	*Average (CFU/mL)*	*Percent Reduction*
1.49E + 09	8.78E + 08	8.42E + 08	8.60E + 08	38.26%
1.47E + 07	9.18E + 06	9.58E + 06	9.38E + 06	36.19%

The affects of adding LAS to the leaching solution, with or without PAM are shown in Figures 4 and 5 for MS-2 phage and *E. coli*, respectively. As shown here, LAS slightly enhanced the recovery of phage compared to the column to which PAM alone was added. As LAS is an organic surfactant, it probably competed for sorption sites with the phage. There was no difference in recovery of *E. coli*, between the columns treated with LAS alone or with a combination of LAS and PAM. However, the *E. coli* moved deeper into the soil profile compared to phage.

DISCUSSIONS

Impact of Soil Properties

The Oxisols are known for their high iron oxide content (red color). Triplicate soil samples were analyzed for their mineral composition using X-ray fluorescence. The average respective percentages of Fe_2O_3, Al_2O_3, and MnO were 18.7, 32.5, and 1.1. In addition, TiO_2 in the soil averaged around 3%. Compared to soils in the continental United States (a temperate region), this volcanic tropical soil appears to be enriched with a significant amount of metallic oxides. It is possible that some of these oxides may be present in higher amounts than their actual concentrations because the samples are heated to about 900°C prior to analysis, thus combusting organics. In fresh sediments, DeCarlo and Spencer (1995) reported the percentages of iron and aluminum oxides each 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 residum compared to fresh sediments. Particle size analysis of the soil

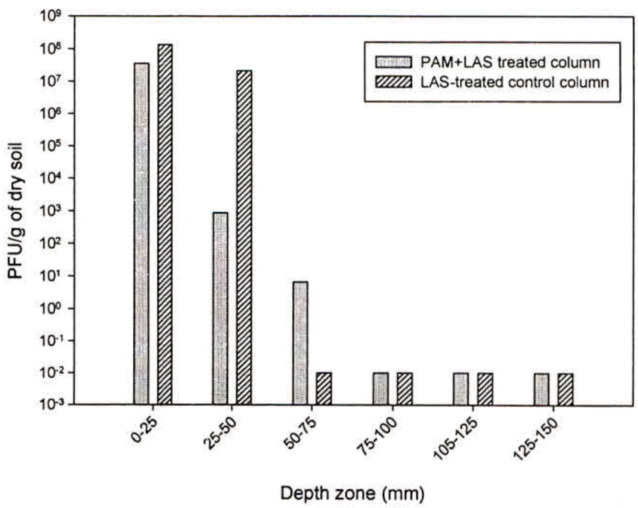

Fig. 4: Recovery of MS-2 phage from LAS and LAS + PAM treated columns

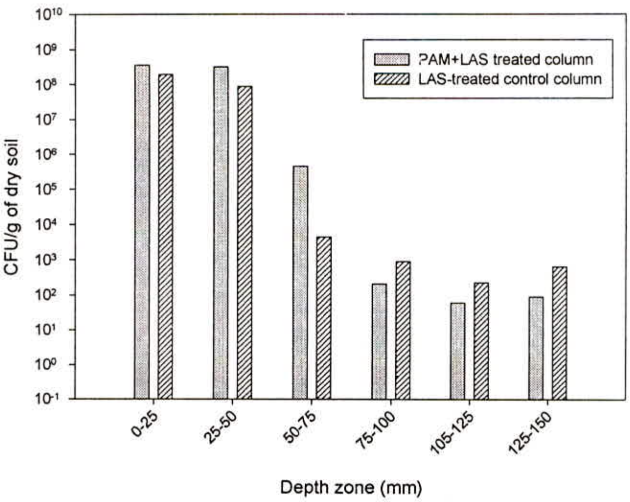

Fig. 5: Recovery of MS-2 phage from LAS and LAS + PAM treated columns

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. 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. Soil solution pH with 50% water and 50% soil was on the order of 5.5. Unsaturated conditions prevailed throughout the experiment.

The role of metallic oxides in virus retention is clear (Pieper *et al.,* 1997; Zhuang and Jin, 2003). Oxides or oxyhydroxides of iron and aluminum have been shown to influence sorption. High clay content (Carlson *et al.,* 1968; Bitton, 1975) and iron oxide (Warren *et al.,* 1966) are known to enhance sorption of phage to soil surfaces. In addition, low soil pH (Bitton, 1975; Gerba *et al.,* 1981) and unsaturated flow conditions (Lance and Gerba, 1984; Powelson *et al.,* 1990) favor adsorption. Decreasing water content reduces the thickness of water films around the soil particles, enhancing the adsorption process. In our experiment, all these factors most likely promoted sorption of phage to the soil solids, resulting in no breakthrough from the columns. Elution from columns was minimal (<10–12%) 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 a host.

Lower solution pH favors adsorption of microorganism to soil particles. 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 and Michael, 1987). A microbe will have a net positive charge when the pH of the soil solution is lower than the pI.

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 in the soil. The PAM solution pH 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 further 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 those of *E. coli*. Because the *E. coli* is two log orders larger than MS-2 phage, their surfaces and retention 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 would not have been representative of wastewater, and was considered potentially toxic to the microorganisms. Other studies that used surfactants (Pieper *et al.,* 1997) have observed longer travel distance of bacteria and viruses in field settings, such as 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 injected bacteria and viruses in sewage-contaminated areas.

Modeling Issues

As expected, the bromide breakthrough curve (not shown here) reached 50% of initial concentration after 1 pore volume of solution displacement under continuous injection. From the fitted data, the average pore-water velocity was determined to be on the order of 0.27 m/day, with a dispersivity of 4 mm for the column. It took approximately 800 minutes (0.55 day) for a tracer particle such as bromide to pass through the 150 mm long soil column. In 10 days, a tracer particle would have traveled 2.70 m. However, from the recovery analysis of phage from plain soil columns, the sorbed phage count dropped from 9 logs in the top 25 mm of the soil to 7 logs in the 25–50 mm depth range. If the 50% concentration is assumed to occur around 20 mm depth (which could even be less), the retardation factor for phage in plain soil is on the order of 135. For the PAM-treated soil, the corresponding retardation factor can be somewhat lower, probably around 100 or less. Since the viability of MS-2 was not affected in the feed solution over 10 days, it is likely that most of the retention of MS-2 resulted from sorption and colloidal filtration rather than inactivation.

For the plain soil, a linear isotherm (having an n value of 1.08) was used. For a bulk density of nearly 1050 kg/m^3 and a volumetric water content of the column that averaged about 0.35, we calculated a retardation factor of close to 164,600 (for K_d = 54,869 mL/g). For the PAM-treated soil, a Freundlich isotherm was found more appropriate with K_d = 991,700 and n = 0.69. However, the retardation factor calculated from batch sorption alone significantly exceeded that observed from residual concentration in the solid phase. Thus, the use of a distribution coefficient from batch sorption transport modeling is questionable (see Schijven, 2000).

The columns used in this study were packed with aggregated soil that passed through a 2 mm sieve. The median size of the dispersed particles in water is assumed to be 0.001 mm. Our experiment could not measure particles smaller than 0.001 mm (i.e., 1 μm), and 55% of the particles were smaller than this size. For the packed aggregates, the median size barely falls below 0.1 mm. Using these data, the single collector efficiency values (following Tobiason and O'Melia, 1988) for larger and smaller particles as well as for representative rapid-sand filter media (particle diameter 0.7 mm) are presented in Figure 6. In this figure, the assumed environmental conditions considered were a water temperature of 295°K and a virus density of 1080 kg/m^3. The average diameter of an MS-2 phage particle is 26 nm. From these curves, it is clear that the collection efficiency is relatively low for filter sand and the aggregates but higher for the dispersed particles. However, the calculation of single collector efficiency for the dispersed particles is estimated to be high since the mean diameter is much smaller. But this value is not realistic because flow rate in a porous medium with median particle size of 0.001 mm would be quite slow.

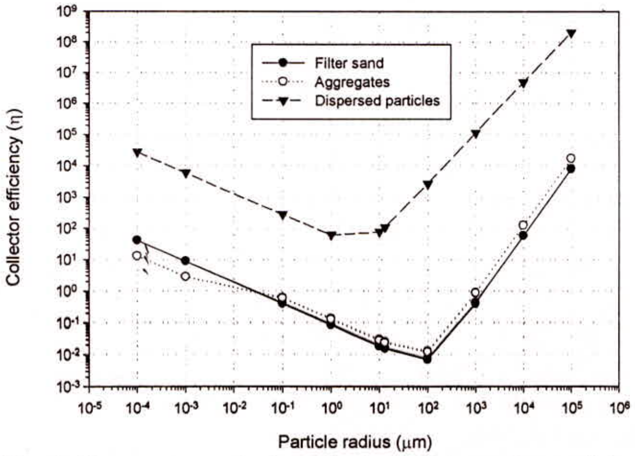

Fig. 6: Comparison single collector efficiency for particles of different size

Yao *et al.* (1971) also used another term called collision efficiency (α) to explain the role of physico-chemical factors that affect adsorption. Since we did not obtain a breakthrough, we resort to calculating collision efficiency from batch sorption experiments using an alternate approach given by Schijven (2000). Using this approach, the expected collision efficiency is 0.277/η. For an η value of 0.01 (low value for aggregates in Figure 6), the estimated α is 27.7. However, these numbers seem to be extremely high compared to those obtained by Harvey and Garabedian (1991) in their field experiment near Cape Cod, Mass (α values between 5.4 × 10^{-3} and 9.7 × 10^{-3}). The use of batch sorption data and retardation due to colloidal filtration poses a challenge to fit the measured data from the column experiments. First, the batch sorption is overestimated. Second, the eluted viral or bacterial data may not have provided the mid-point of the relative breakthrough curve accurately. Thus too numerous uncertainties exist to conduct any comprehensive modeling.

SUMMARY

The experimental data showed that PAM appears to have a slight effect on transporting MS-2 phage in soil at a faster rate than under control conditions, 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 in plain 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 mobility of *E. coli*. Since there was no breakthrough of MS-2 phage or *E. coli*, comprehensive modeling was deemed infeasible.

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. Sorbtion of bacteria or phage was not completely reversible. It was observed that with the addition of PAM or PAM and LAS, removal became more difficult.

Observing the behavior of *E. coli* and MS-2 phage in the aggregated Oxisols of Oahu, one may draw a preliminary conclusion that these soils can act as tremendous sinks for these micro-organisms. However, this study has not addressed the issue of bypass flow through fractures and macropores, particularly under saturated conditions. Although the potential of ground water contamination appears to be low, the effects of macropores and fractures cannot be ignored.

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REFERENCES

Adams, M.H. (1959). Bacteriophages, Interscience Publishers, New York, N.Y.

American Public Health Association, American Water Works Association, and Water Environment Federation. (1999). Standard methods for the examination of water and wastewater, 20th Ed., Washington, D.C.

Bitton, G. (1975). "Adsorption of viruses onto surfaces in soil and water." Water Res., 9, 473–484.

Carlson, G.F., Jr., Woodard, F.E., Wentworth, D.F. and Sproul, O.J. (1968). "Virus inactivation on clay particles in natural waters." J. WPCF, 40, 89–106.

Gerba, C.P. (1984). "Applied and theoretical aspects of virus adsorption to surfaces." Adv. Appl. Microbiol., 30, 133–168.

Harvey, R.W. and Garabedian, S.P. (1991). "Use of colloid filtration theory in modeling movement of bacteria through a contaminated sandy aquifer." Environ. Sci. Tech., 25, 178–185.

Lance, J.C. and Gerba, C.P. (1984). "Virus movement in soil during saturated and unsaturated flow." Appl. Environ. Microbiol., 47, 335–337.

Penrod, S.L., Olson, T.M. and Grant, S.B. (1996). "Deposition kinetics of two viruses in packed beds of quartz granular media." Langmuir, 12, 5576–5587.

Pieper, A.P., Ryan, J.N., Harvey, R.W., Amy, G.L., Illangasekare, T.H. and 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. and Gerba, C.P. (1990). "Virus transport and survival in saturated and unsaturated flow through soil columns." J. Environ. Qual., 19, 396–401.

Schaub, S.A. and Sorber, C.A. (1977). "Virus and bacteria removal from wastewater by rapid infiltration through soil." Appl. Environ. Microbiol., 33, 609–619.

Schijven, J.F. (2000). "Virus removal from groundwater by soil passage: Modeling, field, and laboratory experiments." Technical Univ. of Delft, The Netherlands.

Sojka, R.E., Lentz, R.D., Trout, T.J., Ross, C.W., Bjorneberg, D.L. and Aase, J.K. (1998). "Polyacrylamide effects on infiltration in irrigated agriculture." J. Soil Water Conserv., 53, 325–331.

Tobiason, J.E. and O'Melia, C.R. (1988). "Physico-chemical aspects of particle removal in depth filtraiton." Jour. AWWA. 80(12): 54–64.

Toride, N. Leij, F.J. and Van Genuchten, M. Th. (1995). "The CXTFIT code for estimating transport parameters from laboratory of field tracer experiments, version 2.0." Res. Rep. 137, U.S. Salinity Laboratory, Riverside, Calif.

Warren, J., Neal, A. and 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 Virus and Bacteria Transport in an Unsaturated Oxisol, M.S. Thesis, Department of Civil Engineering, University of Hawaii at Manoa, Honolulu, Hawaii.

Wong, T.P., Byappanahalli, M., Yoneyama, B. and Ray, C. (2008). An evaluation of the mobility of pathogen indicators Escherichia coli and bateriophage MS-2, in a highly weathered tropical soil under unsaturated conditions, Jour. Water & Health, 6(1): 131–140.

Yao, K.M., Habibian, M.T. and O'Melia, C.R. (1971). "Water and wastewater filtration: Concepts and applications." Environ. Sci. Tech., 5, 1105–1112.

Zerda, K.S. (1982). "Adsorption of viruses to charge-modified silica." Ph.D. thesis, Baylor College of Medicine, Houston, Tex.

Zerda, K.S., Gerba, C.P., Hou, K.C. and Goyal, S.M. (1985). "Adsorption of viruses to charge-modified silica." Appl. Environ. Microbiol., 49, 91–95.

Zhuang, J. and 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.