ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ UNIVERSITY OF PATRAS

TRANSPORT OF VIRAL FAECAL INDICATORS IN SATURATED POROUS MEDIA

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INTRODUCTION

Enteric viruses are important pathogens that may have severe impact on health. They are shed in enormous quantities in feces and have an infectious dose from 10^1 to 10^2. Groundwater may be contaminated with infective human enteric viruses through wastewater discharges, sanitary landfills, septic tanks, and agricultural practices or by artificial groundwater recharge. Viruses persist for several months in soils and groundwater when temperatures are low and soils are moist, and they are less efficiently eliminated during soil passage than other microorganisms, due to their small sizes and long survival. A large number of studies focusing on various factors (i.e. pore water solution chemistry, fluid velocity, moisture content, temperature, and grain size) that affect viral transport have been published over the past two decades. To predict the presence of pathogenic viruses in water and wastewater, microorganisms known as indicator organisms (e.g. coliphages MS2 and ΦX174 and human adenoviruses) are monitored. Most viral transport studies, focus on coliphages, included MS2 and phiX174. Adenoviruses have been suggested as index organisms for viral pathogens because they fit most criteria for an ideal indicator. Both coliphages and adenoviruses are widespread in the environment and are found in marine, river, ground, drinking, recreational and wastewaters. Quantitative detection of hAdVs as indicators of human fecal contamination may provide a means of better understanding the risk associated with contaminated water. Nevertheless, there is still very little literature reporting the mobility of hAdVs under porous media filtration and the relative transport behavior of hAdVs and coliphages.

OBJECTIVES

Despite research on virus transport, our understanding of how hAdV contamination occurs in groundwater is far from complete and additional investigation is needed. A thorough understanding of the key processes governing the transport of viral faecal indicators in the natural environment is essential for public health protection through the development of effective regulations and disinfection strategies. The main objective of this study was to evaluate the effect of pore water velocity on MS2, ΦX174 and hAdV, transport in water saturated laboratory-scale columns.

METHODS & MATERIALS

Coliphages and assay

The bacteriophage MS2 has been recommended as a surrogate for poliovirus due to similarities in size, and has been employed as a conservative tracer for enteric virus transports. The bacteriophage ΦX174 has been recommended as a surrogate for norovirus due to similarities in size. Both bacteriophages were assayed by the double-layer overlay method (Syngouna and Chrysikopoulos, 2011).

Human Adenovirus QPCR assay

Human Adenovirus serotype 35 (hAdV35) was cultivated in human lung carcinoma cell line A549. The qPCR assay for the detection and quantification of hAdV35 with the inclusion of uracil N-glycosylase (UNG) was performed. All samples were tested both neat and at a 10-fold dilution, in duplicate (Kokkinos et al., 2015).

Column Experiments

All flow through experiments were conducted using a 30 cm long glass column with 2.5 cm diameter, which was packed with 2 mm diameter glass beads. Glass beads were used for the packing of the columns in order to eliminate possible experimental difficulties associated with real soil, which may provide numerous uncertainties that can considerably complicate the analysis of the experimental data. Following previous procedure (Syngouna and Chrysikopoulos, 2013), the glass beads were purified to remove surface impurities. The column was packed with glass beads understanding sterile low ionic strength buffer solution to minimize air entrapment. The dry bulk density was estimated to be ρ_b=1.61 g/cm3, and the porosity θ=0.42. The column was placed horizontally to minimize gravity effects. A fresh column was packed for each experiment. Also, 3 pore volumes (PVs) of sterile buffer solution were passed through the column prior to each transport experiment. The entire packed column and glassware used for the experiments were sterilized in an autoclave at 121 °C for 20 min. Constant flow of buffer solution at three volumetric discharge rates of Q=2.5, 1.5, and 0.8 mL/min, corresponding to specific discharge or approach velocities of q=0.51, 0.31, and 0.16 cm/min, and pore water velocities of $U=q/\theta$=1.22±0.02, 0.75±0.02, and 0.39±0.01 cm/min, respectively, was maintained through the packed column with a peristaltic pump (Masterflex L/S, Cole-Palmer). For each experiment, the virus suspensions were injected into the packed column for 3 PVs, followed by 3 PVs of buffer solution. All experiments were carried out at room temperature (~25 °C).

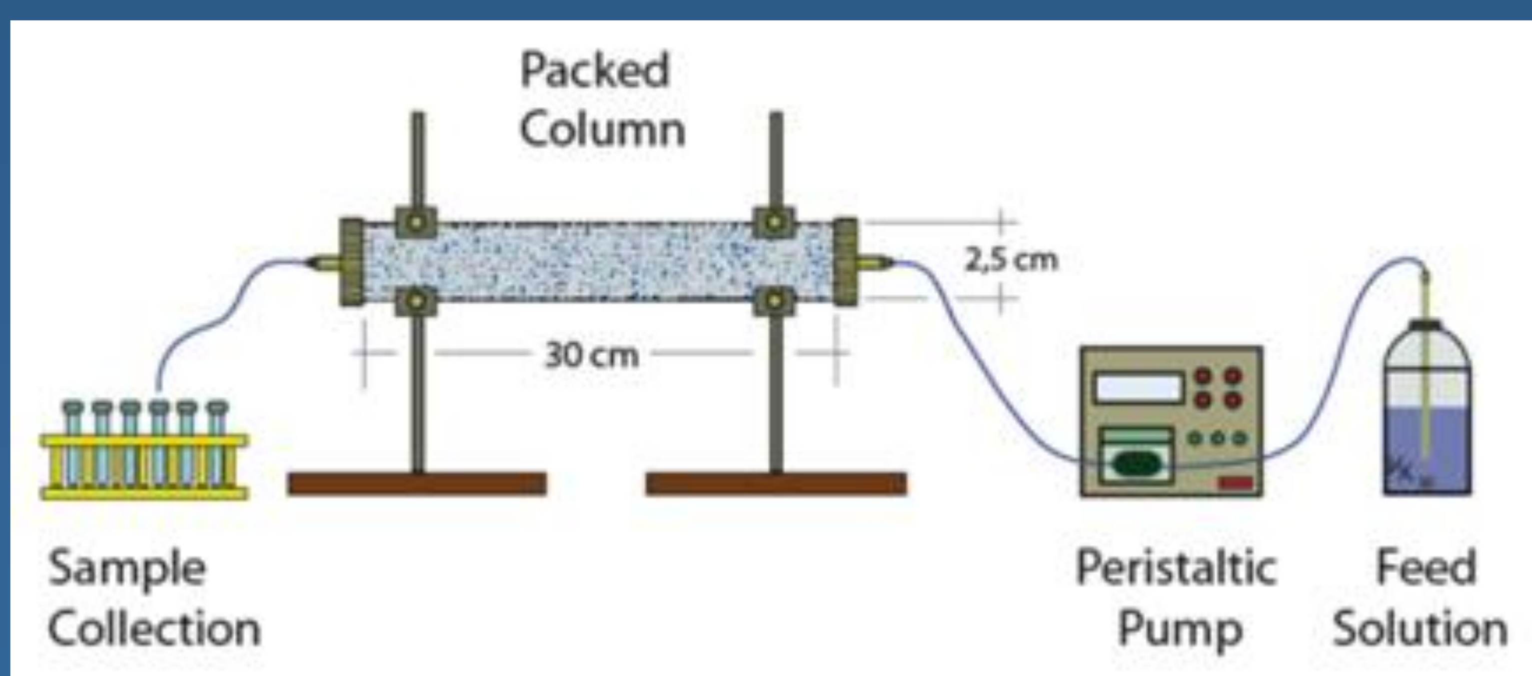

Figure 1. Schematic illustration of the experimental apparatus.

RESULTS

Table 1. Data analysis for the transport experiments.

Exp No.		U (cm/min)	C_0 PFU/mL (coliphages), GC/mL (HAdV	$M_{r(v)}$ (%)	α
	Q=2.5 mL/min				
1	ΦX174	1.22	3185	100	0.00026
2	MS2	1.22	282000	74.2	0.075
3	HAdV35	1.22	2130734	25.89	0.752
	Q=1.5 mL/min				
4	ΦX174	0.75	8867	100	0.00018
5	MS2	0.75	1350	70.1	0.063
6	HAdV35	0.75	483231	32.82	0.522
	Q=0.8 mL/min				
7	ΦX174	0.39	12300	96.2	0.0044
8	MS2	0.39	6000	49.7	0.077
9	HAdV35	0.39	1094559	24.02	0.378

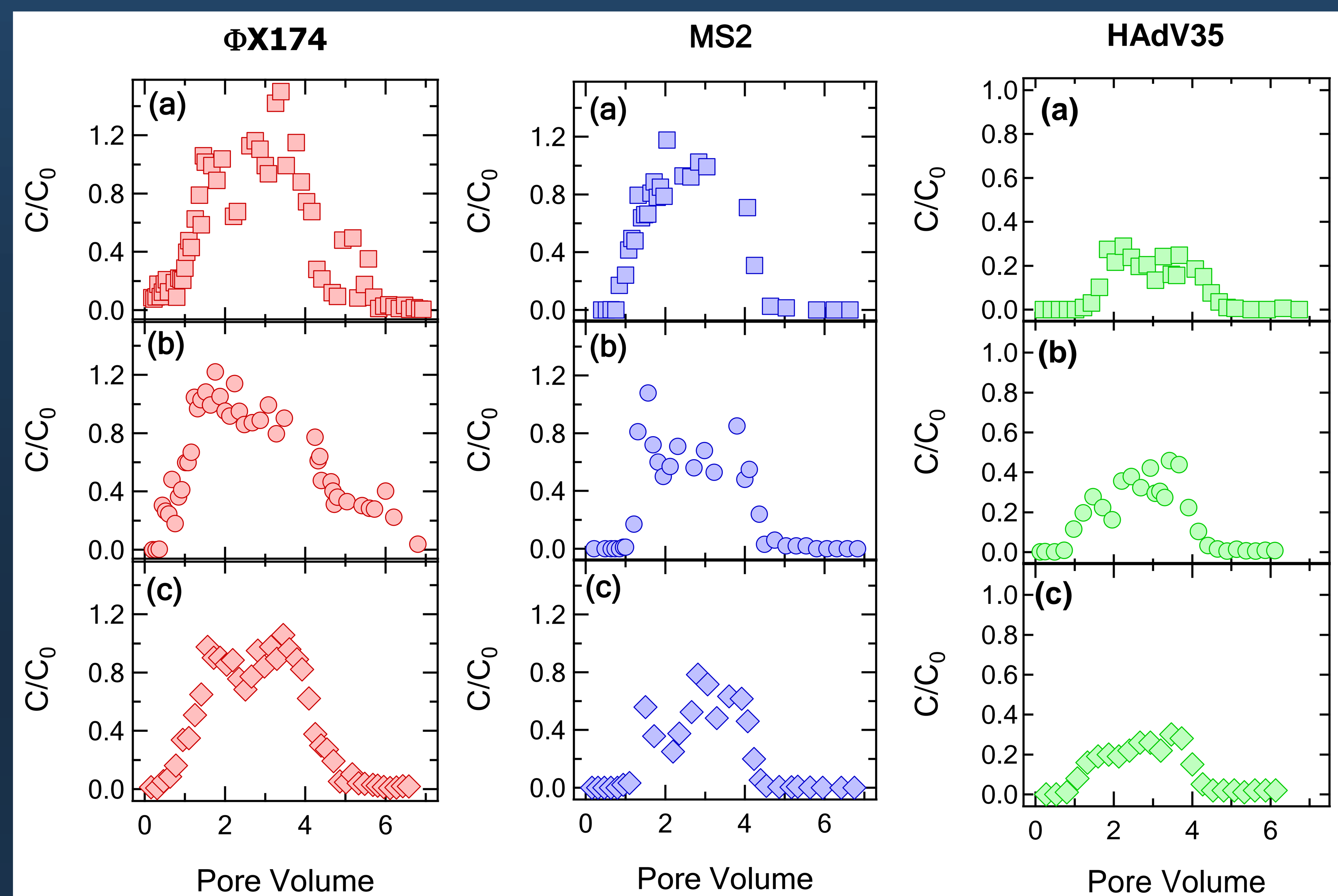

Figure 2. Experimental data for transport of ΦX174 with U equal to: (a) 1.22 (squares), (b) 0.75 (circles), and (c) 0.39 (diamonds) cm/min.

Figure 3. Experimental data for transport of MS2 with U equal to (a) 1.22 (squares), 0.75 (circles), and (c) 0.39 cm/min.

Figure 4. Experimental data for transport of HAdV35 with U equal to: (a) 1.22 (squares), 0.75 (circles), and (c) 0.39 cm/min.

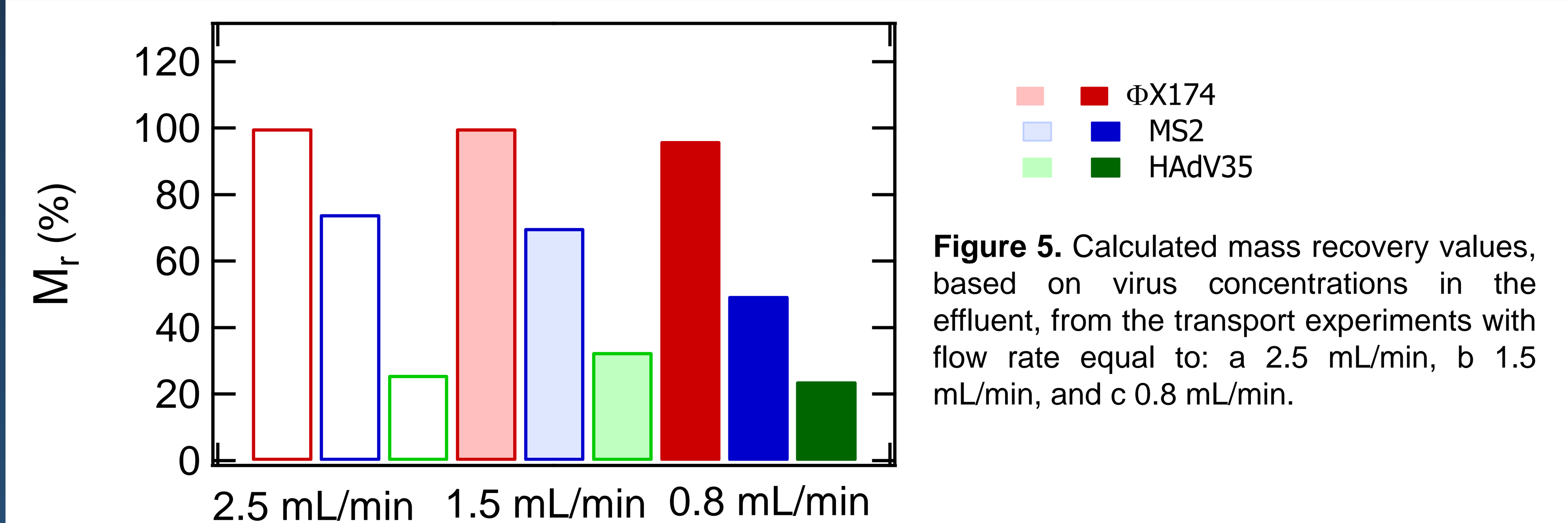

Figure 5. Calculated mass recovery values, based on virus concentrations in the effluent, from the transport experiments with flow rate equal to: a 2.5 mL/min, b 1.5 mL/min, and c 0.8 mL/min.

CONCLUSIONS

The results of this study indicated that M_r values of coliphages decrease with decreasing pore water velocity; whereas, for the hAdV35 mass recovery no clear trend could be determined. With no exception, all estimated Mr values for MS2 were lower than those of ΦX174 while the estimated average M_r values for hAdV35 were similar for all interstitial velocities (U=1.22, 0.75, 0.39 cm/min). High hAdV35 retention was observed for all cases examined. With no exception, all estimated average Mr values for hAdV35 were lower than those of MS2 and ΦX174 under the same experimental conditions. Similarly, lower mass recovery values were observed for hAdV type 41 (VR-930 strain) than MS2. Note that hAdV35 is less negatively charged than coliphages MS2 and ΦX174 at the experimental conditions of this study. Therefore, hAdV35 was expected to attach onto the solid matrix more than coliphages. However, other factors (e.g. collector surface heterogeneity) may have contributed to the observed hAdV35 retention. Note that, the average experimental collision efficiency, α values decreased with decreasing flow rate Q and pore water velocity U only for hAdV35 transport.

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