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Bacteriophage Technique for Assessing Viral Removal in Constructed Wetland and Detention Pond Systems

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ABSRACT

Constructed wetland and detention pond as a treatment system was applied for stormwater management in two adjacent areas in western Sydney. F-specific RNA and somatic coliphages were used as a model for assessing two systems for removal of viral pollution, fate, behavior and survival of viruses in the sediment. Water samples were collected weekly in sterile containers and sediment samples were collected three times using a box dredge sampler via a boat at the inlet, middle and outlet areas of the systems. F-specific RNA coliphages were enumerated using the double layer plaque assay (ISO 1995) with *Salmonella typhimurium* WG49 as a host. Survival test continued 28 d for each sub-sample. Viral removal in constructed wetland was more effective than the detention pond system. Survival of somatic coliphages in the inlet and middle of the systems was similar. Slope of declining for outlet of two systems was very slow and completely stable in whole of test duration. Constructed wetland may offer an attractive alternative to stormwater management for reducing the load of disease-causing viruses to the receiving waters.

Keywords: *Constructed wetland, Detention pond, Somatic coliphages, F-specific RNA, Survival analysis, Western Sydney*

INTRODUCTION

Constructed wetlands and detention ponds are increasingly being employed in Australia and worldwide to reduce pollutant loads carried by urban stormwater. Wetlands and ponds provide a combination of physical, chemical and biological processes that contribute to the removal or transformation of pollutants from a range of waters. The removal of indicator and pathogenic bacteria from municipal wastewater by constructed wetlands are well-documented (Gersberg et al., 1989) but it has been repeatedly stated in the literature that the mechanisms of removal are not well understood (Kadlec, 1995; Decamp et al., 1998; Perkins et al., 1999). A recent epidemiological study has shown an increased risk of viral infection by swimming in recreational waters polluted with stormwater (Haile et al., 1999). Few studies have examined the efficiency of constructed wetlands and ponds to remove viruses. Certainly the few virological studies that have been carried out have focused on the treatment of municipal and industrial wastewater (Gersberg et al., 1987; Chendorain et al., 1998) and have not considered stormwater. The paucity of data on the fate of viruses in aquatic environments is due in part to the lack of reliable, simple and economical methods for virus detection. Suspended particles appear to be a dominant natu-

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ral vehicle and survival aid for viruses in water. It is estimated that about 77% of viruses and 65% of coliphages are associated with suspended particles in natural waters (Payment et al., 1988). A number of researchers have reported that bacteria and viruses adsorb preferentially to particles less than 2 µm in size. It is also reported that ponds and wetlands differ in their abilities to remove particles of different sizes from stormwater (Davis et al., 1999).

The study will complement previous research conducted at the Water Research Laboratory (WRL), on the removal of faecal bacteria, suspended solid, and nutrients by wetland and pond stormwater treatment systems. It will allow the development of techniques by the WRL for the use of bacteriophages as surrogates for human enteric viruses in the environment which will have application for a wide-range of investigations and provide a basis for attracting external funding in the future.

MATERIALS AND METHODS

Site Characteristics The investigation conducted at the Plumpton Park constructed wetland and the Woodcroft Estate detention pond. Both of them are close to Blacktown approximately 40 km northwest of Sydney, New South Wales, Australia. Area of Plumpton Park Wetland System and Woodcroft Pond System are 0.45 ha, and 1.5 ha, respectively. Plampton Park Wetland System was built in 1994. Constructed wetland system was completed within the existing 75 ha residential catchments of Plumpton Park consisting of a Gross Pollutant Trap to remove coarse sediment, and a trash-rack, followed by a wetland planted extensively with indigenous macrophytes (Fig. 1). The wetland consists of five cells, each approximately 40 m long separated by loose rock weirs 400 mm high. The minimum and maximum water depths are 200 and 600 mm, respectively. Stormwater enters the system via two inlets (PI1 and PI2) and there is a single outlet (PO). Sampling locations for inflow and outflow samples and for sediment samples are indicated in Fig. 1. A detention pond system is located within a 53 ha residential catchments and has an approximate storage volume of 23000-39000 m^3 (Fig. 2). The average depth is 2.5 m in pond system and 0.2-0.6 m in wetland system. Woodcroft Catchment's area is 53 ha. In the pond system emergent macrophytes occur around the perimeter of the pond. The pond has a single inlet (WI), and a single outlet (WO). The soil landscape for each of the systems is typified by hard setting clays that are slightly saline and acidic with occurrences of soil which has a high potential for erosion along the watercourses (Hunter et al., 1997)

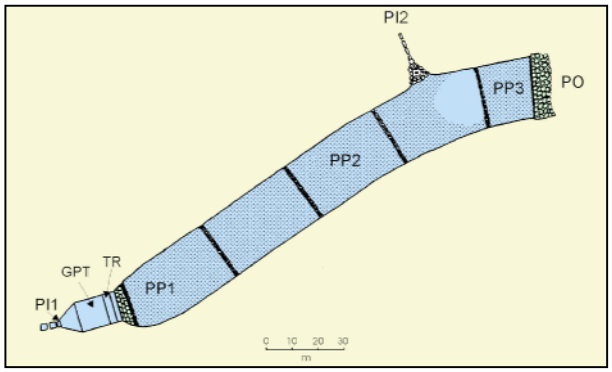

Fig. 1: Plumpton Park Wetland System

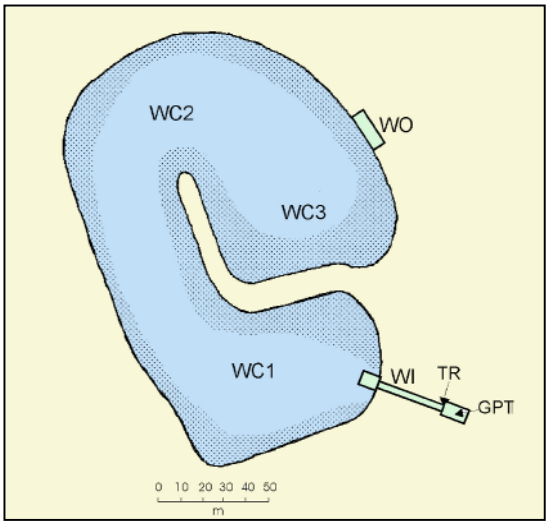

Fig. 2: Woodcroft Detention Pond System

SAMPLING

Water sampling Discrete inflow and outflow water samples was collected weekly in sterile containers from Plumpton Park wetland and Woodcroft pond over a period of 5 months.

Sediment sampling Sediment samples were collected using a box dredge sampler via a boat at the inlet, middle and outlet areas of the systems. Sediments were taken three times manually and were collected from Woodcroft ponds using a box dredge via a boat.

Total daily rainfall data for the sampling period was obtained from a weather station located within 8 km of Plumpton Park and within 5 km of Woodcroft at St Marys Sewage Treatment Plant, NSW, and Australia.

Sediment characteristics Sediment samples were collected from each of the systems and used in laboratory experiments to examine: i) the adsorption of bacteriophages to particles of different sizes, and ii) the persistence of the bacteriophages in the sediment using laboratory microcosms. The pipette method, which is based on the settling properties of different sized particles, used to separate the different particle size fractions. The use of laboratory microcosms (closed bottle systems) allowed the persistence of bacteria withdrawn periodically (weekly) from the microcosms and concentrations of bacteriophages in the subsamples determined. These experiments provided an estimation of the potential for accumulation of pathogenic viruses in wetland and pond sediments.

Particle size analysis The particle size distribution of three sediment samples for each system was determined in duplicate using the pipette. Settling times for particles <2, 2-5, 5-10, 10-20, 20-62, and >62 μm were 0 s, 26 s, 4′/10s, 16′/ 40 s, 68′/ 40 s, and 416′/ 40 s, respectively. The sediments (100 g) were mixed with distilled water and the suspensions allowed settling in 1L cylinders. At the determined settling times, 25 ml of sediment suspension was removed from a depth of 10 cm below the surface and dried at 105°C for 24 h in a preweighed crucible. The dried fractions were analyzed for organic material (at 550°C for 10 h). Simultaneously, the concentrations of somatic coliphages remaining suspended in the top 10 cm were determined from an additional subsample at each of the sampling times.

Survival analysis Sediment composite samples were collected from inlet, middle and outlet of the systems. Three of 300 grams of sediment (Sub-sample) was weighed out into 3 bottles sterile and the sediment remaining in the microcosm was covered with 100 ml of sterilized pond or wetland water (equilibrated to 25° C). At zero time a sub-sample (50 g) of each bottle was weighed out into a 50 ml buffer sterile especial for recovery of phages and then placed in a Basket in adjacent of ice (at 4° C). These were shaken by a shaker in 100 1/min for two hours. Then with dispenser (sterile tips) the mixture was transferred into many centrifuge tube and centrifuged for 5 min at 2500 rpm. The supernatant was transferred to a sterile bottle and used for bacteriophages analysis (Somatic coliphages). Weekly sub-samples of sediment (50 g) were withdrawn from the microcosms by aseptically pipetting off the overlying water, taking care not to resuspend any of sediment to determine concentration of bacteriophages. This analysis continued for 28 d.

Water analysis

Bacteriophage analysis *Salmonella typhimurium* WG49 was used for our analysis to act as host strain. Cultures to be used for the bacteriophages analysis were selected from those lactose-positive colonies on MacConkey agar plate (overnight cultured). These colonies by aseptically was uniculumed in Trypton Soy Broth (TSB) plus kanamycine and glocuse/ cacl2 solution for 3.5–6 h. After 3.5 h, reading absorbance of host culture at 620 nm was performed periodically (each 0.5 h) by pipetting off 3 ml. Getting the absorbency to a pleasant turbidity (in base of Pre-Test), host culture was used for bacteriophages assay.

Bacteriophages were enumerated by the double layer plaque assays (ISO, 1995).

Data analysis Analysis of variance and Student's t-tests were carried out using Microsoft Excel 2000, Data Analysis Software.

RESULTS

Table 1 shows the particle size distribution of sediments samples taken at three different points in each systems.

Table 1: Particle Size Distribution of the wetland and pond sediments

		Particle Size Distribution (%)					
Sample sites	**% moisture**	**<2**	**2-5**	**5-10**	**10-20**	**20-62**	**>62**
Plumpton Park inlet	57.7	10.3	6	11.3	11.7	30	30.7
Plumpton Park middle	65.1	17	15	15.2	15.6	30.8	6.4
Plumpton Park outlet	81.5	7.5	7.1	9.8	5.9	40.6	29.1
Woodcroft inlet	41.5	28.7	19.5	1.2	14.6	18.6	17.4
Woodcroft middle	74.5	18.9	10.4	13.2	10.8	42.9	3.8
Woodcroft outlet	50.7	26.3	15.5	11.2	10.9	16.1	20
Woodcroft outlet	74.3	28.1	17.4	17.4	28.9	3.3	4.9

Figs. 1-3 show the particle size distribution and somatic-phage concentrations for sediment collected at three different points respectively in inlet, middle and outlet of Plumpton Park wetland system. Concentration of somatic coliphages is based on log10 PFU/100g of dry sediment. Concentration of somatic coliphages in inlet point of the system varied from 4.1 to 5.4 (log10 PFU/100g), in middle point from 4.1 to 5.3 and in outlet point from 4.6 to 5.4.

Figs. 4-7 show particle size distribution and somatic coliphages concentrations for sediment collected at three different points, respectively in inlet, middle and outlet of Woodcraft detention pond system. Concentration of somatic coliphages in inlet point of the system varied from 1.7 to 2.2 (log10 PFU/100g), in middle point from 1.6 to 2.8 and in outlet point from 1.5 to 2.5 in A sampling point and from 4.3 to 5.3 in B sampling point. Difference between these two sites of outlet was due to adjacent of A to the trees and entering of bird's wastes into this site that clearly considered in sampling time, although difference of particles in range of >10 micron was significant (P<0.05). Presence of particle size in range of 5-10 micron in inlet point was significantly different from the other sites of the system (P<0.05). Also as before figures, concentration of phages in fine particle size was higher than that of the coarse particle.

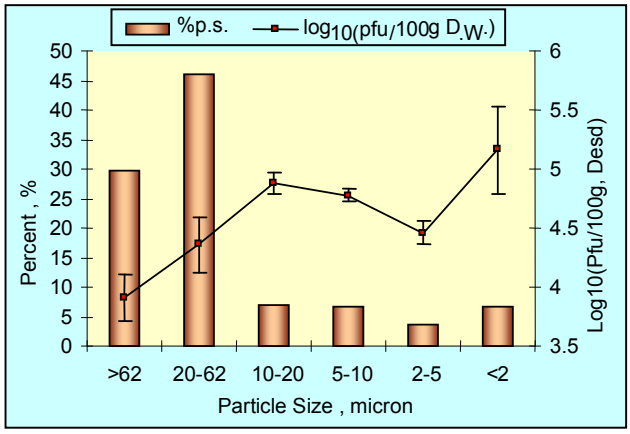

Fig. 1: Conc. of S-Phage in PPWS (inlet)

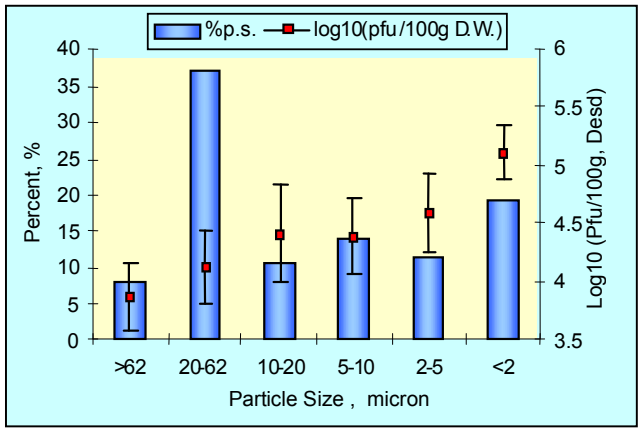

Fig. 2: Conc. of S-Phage in PPWS (Center)

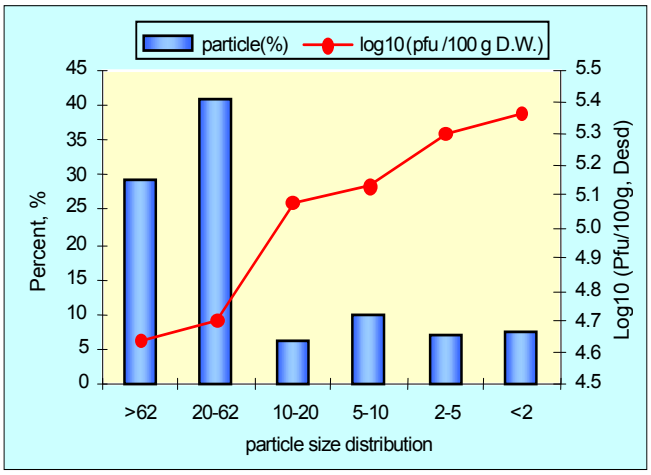

Fig. 3: Conc. of S-iphage in sed for PPWS (Out)

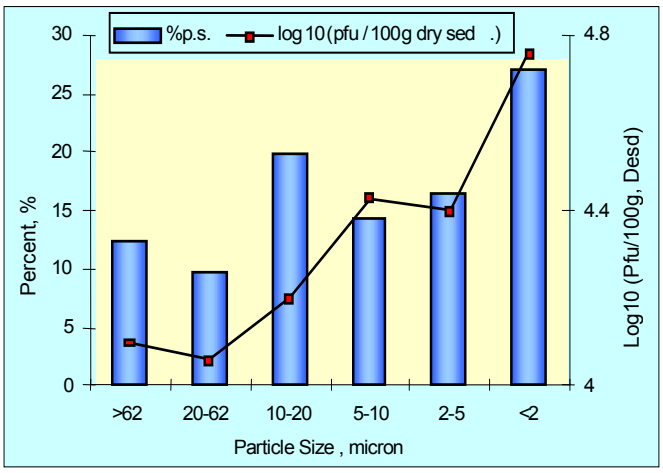

Fig. 6: Conc. of Somatic-Phage in WCPS (Outlet)

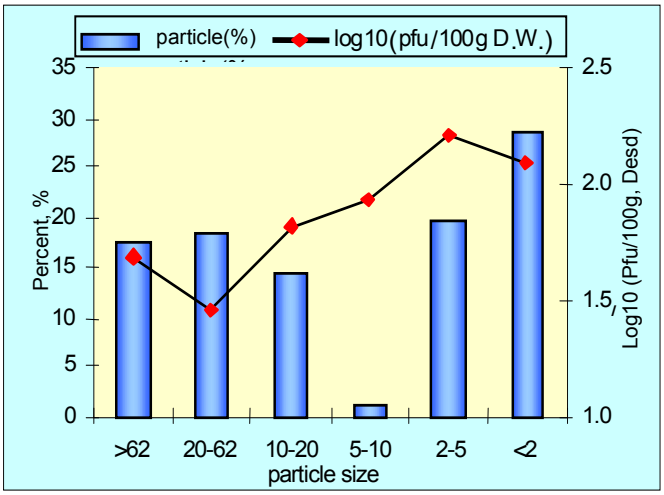

Fig. 4: Conc. of S- Phage in sed for WPS (inlet)

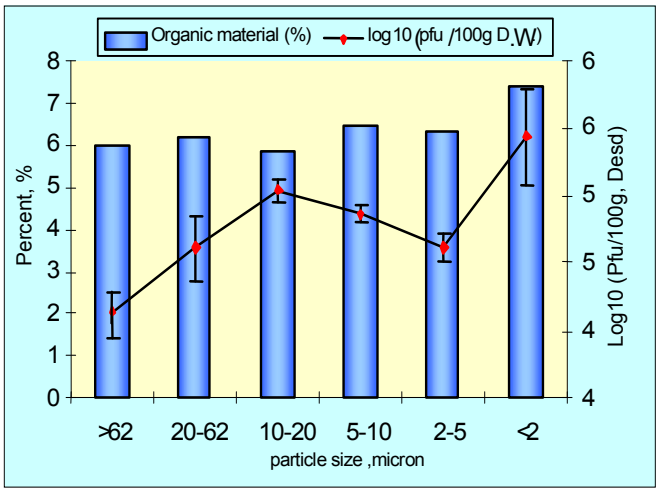

Fig. 7: Conc. of S- Phage vs sed.org Material for PPWS (inlet)

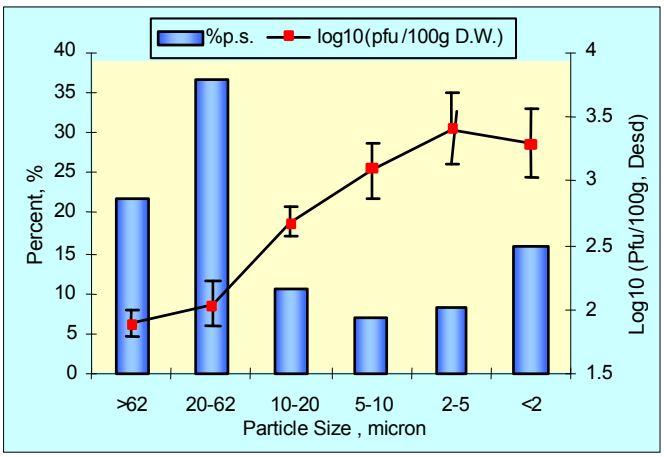

Fig. 5: Conc. of Somatic-Phage in WCPS (Center)

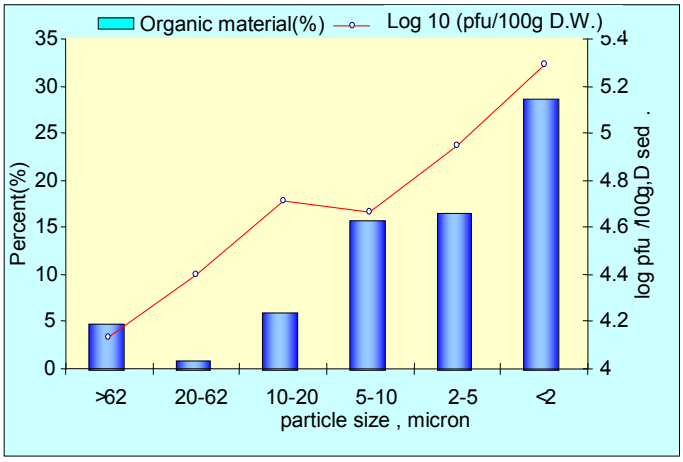

Fig. 8: Conc. Of S- Phage vs sed.org Material for PPWS (Center)

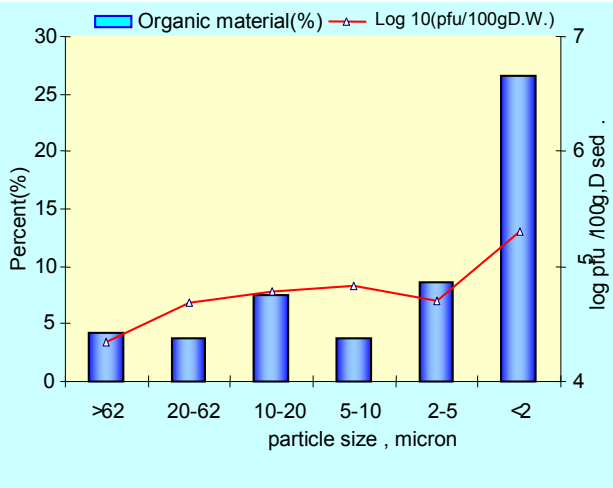

Fig. 9: Conc. of S- Phage vs sed.org Material for WCPS

Figs. 7-9 show the concentration of somatic coliphages vs sediment organic material distribution for three different points of wetland system and outlet of pond system. Difference between presence of organic material in inlet and outlet of wetland system was not significant, but the difference between presence of organic materials for middle point in range of <2-10 micron and>10 micron is significant. There was significant difference between<2 micron and>2 micron at outlet point of pond system (P<0.05).

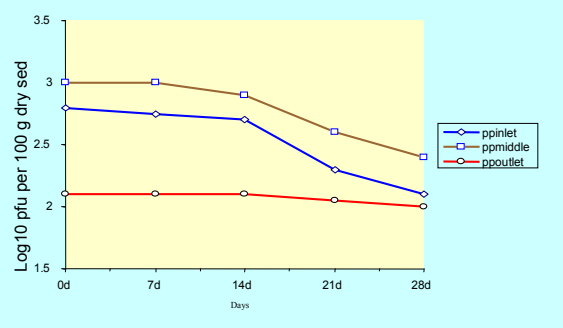

Fig. 10: Survival of S-Phage in PPWS Sediment

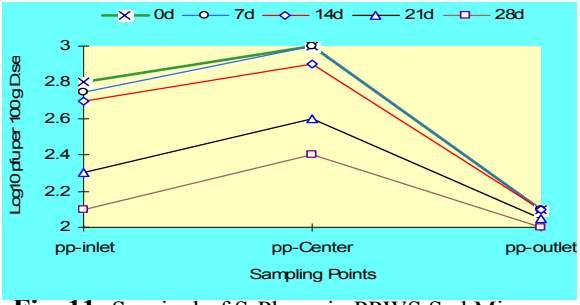

Fig. 11: Survival of S-Phage in PPWS Sed.Microcosms

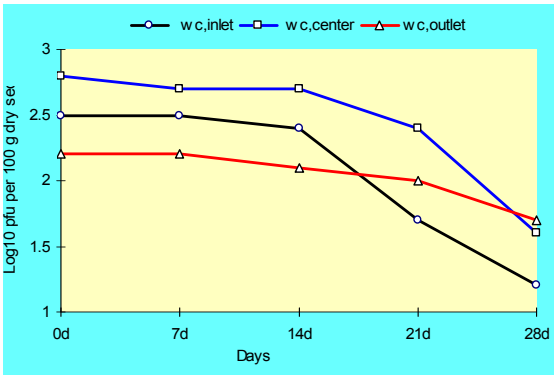

Fig. 12: Survival of S-Phage in WPS Sediment Microcosms

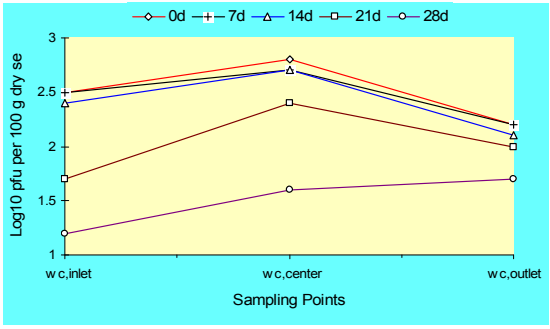

Fig. 13: Survival of S-Phage in WPS Sediment Microcosms

Figs. 10-13 give presentative data from somatic coliphages survival test on sediment microcosms for each system. Monitoring of the sediment microcosms over a period of 28 d indicated stable condition throughout the two week test period, which this stability was continued at outlet point of each system.

DISCUSSION

The difference in sediment particle size distribution in the two systems was most likely due to the different particle size inputs based on activities in catchments and presence of plants in wetland that affect the hydraulic characteristic of sediment microcosm and type of soil bed in two systems. Residential development within the wetland catchments has been established for several years and the soil has been stabilized to

some extent. In contrast, construction work in the catchments of the detention pond and consequently existence of large areas of disturbed and exposed clay, which may be easily mobilized and transported in storm water, were the reasons of this difference.

This result confirmed previous researches (Chendorain et al., 1980; Havelaar et al., 1984). Also these figures showed that the presence of particle in the range of 20-60 micron was significantly higher than the others (P<0.05). Concentration of phages in fine particle size was higher than that of coarse particle (Figs. 1-3) that is due to more effective attachment of bacteriophages to fine particle size. Although there was little rainfall during the sampling period, concentration of F-specific RNA in the stormwater entering to Wetland system in wet weather was different with dry condition, however, there were too few positive samples to obtain a meaningful correlation. Concentration of F-specific RNA in the first time of raining condition or heavy raining was higher than that of dry or light raining condition. This was clearly showing that in time of a heavy raining (27 Sep. 2000), concentration of F-specific RNA gets up very high. So this result is a reason for looking at to these bacteriophages as an adequate model for the look over of the fate and transport of enteric viruses through surface water constructed wetland and detention pond systems or stormwater management.

The concentration of F-specific RNA in inflow and outflow of detention pond system indicating dependency of the concentration of F-specific RNA to dry or raining condition also showed that the efficiency of detention pond system was not pleasant for removal of the bacteriophages, when compared with wetland systems. So the wetland systems is preferable than the pond for water pollution control in stormwater management. This result confirms the research which has been reported that wetlands are more effective in removing fine particles than ponds (Wong et al., 1999).

Somatic coliphages and F-specific RNA bacteriophages in stormwater were largely associated with the heavy raining. This research was suggested that wetlands are more effective in removing enteric viruses and the pollutants associated with stormwater than the detention pond systems. These observations have implications in the selection of wetland for urban stormwater management. In certain situations water pollution control ponds may not be the appropriate choice of stormwater treatment system, particularly if soils in the catchments area have high clay content and potentially easily mobilize by storm activity. Of course, the soils in the vicinity of the pond, where construction work is still in progress, may be easily mobilized during storm events.

This study showed extended survival of somatic coliphages in wetland and detention pond sediments. These results support the findings of the other studies indicating that the resistance of viruses in sediment microcosms is higher than that of bacteria (Gersberg et al., 1987; Chendorain et al., 1998). There was a general decline in concentration of somatic coliphages with time, in each microcosm, indicating Die-off. Concentration of somatic coliphages in the middle point of each system was higher than that of inlet and outlet points. Also the results show that the rate of die-off of somatic coliphages in inlet and middle points of each system is more rapid than that of outlet point.

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