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The fate of stormwater-associated bacteria in constructed wetland and water pollution control pond systems

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C.M. DAVIES AND H.J. BAVOR. 2000. The performances of a constructed wetland and a water pollution control pond were compared in terms of their abilities to reduce stormwater bacterial loads to recreational waters. Concentrations of thermotolerant coliforms, enterococci and heterotrophic bacteria were determined in inflow and outflow samples collected from each system over a 6-month period. Bacterial removal was significantly less effective in the water pollution control pond than in the constructed wetland. This was attributed to the inability of the pond system to retain the fine clay particles ($< 2\,\mu$m) to which the bacteria were predominantly adsorbed. Sediment microcosm survival studies showed that the persistence of thermotolerant coliforms was greater in the pond sediments than in the wetland sediments, and that predation was a major factor influencing bacterial survival. The key to greater bacterial longevity in the pond sediments appeared to be the adsorption of bacteria to fine particles, which protected them from predators. These observations may significantly affect the choice of treatment system for effective stormwater management.

INTRODUCTION

Stormwater refers to the excess rainwater that is unable to infiltrate into the ground. Urbanization leads to an increase in areas of impermeable surfaces such as roads, driveways and parking areas, and a decrease in areas that are available for percolation and infiltration of stormwater. Urban stormwater carries significant quantities of debris and pollutants that include litter, oils, heavy metals, sediment, nutrients, organic matter and micro-organisms, and has been recognized as one of the major sources of diffuse pollution to the aquatic environment (Yu and Nawang 1993). The quantity and range of pollutants carried and the volumes of stormwater generated are influenced by the natural and built character of the catchment and the degree of contamination by non-stormwater inputs (Field *et al.* 1993).

The presence of micro-organisms of faecal origin in stormwater can be attributed to septic tank seepage, sewer leakage and overflow, and domestic animal faeces. Recent epidemiological evidence has suggested that there is an increased risk of adverse health associated with swimming in recreational waters that are contaminated with untreated urban stormwater (Haile *et al.* 1999).

Constructed wetlands and water pollution control ponds are increasingly being used worldwide to reduce pollutant loads carried by stormwater in urban areas. Basically, the main differences between wetland and pond systems are their macrophyte cover and density, and their depth. Constructed wetlands are shallow detention systems that fill and drain, and are extensively vegetated with emergent plants. Water quality control ponds have a small range of water level fluctuation in which emergent plants are generally restricted to the edges due to water depth (Wong *et al.* 1999). Submerged plants may also be present. Wetlands and ponds provide a combination of physical, chemical and biological processes that contribute to the removal or transformation of pollutants.

The removal of faecal indicator bacteria from wastewater by constructed wetlands is well documented (Bavor *et al.* 1987; Gersberg *et al.* 1987; Ottová *et al.* 1997; Perkins and Hunter 1999). Reported removal efficiencies for coliforms generally exceed 90% (Kadlec and Knight 1996) with significantly higher removal in extensively vegetated systems compared with unvegetated systems (Gersberg *et al.* 1987; Garcia and Bécares 1997). Removal efficiencies for faecal streptococci by wetlands generally exceed 80% (Kadlec and

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Knight 1996). Processes believed to be responsible for bacterial removal in constructed wetlands include filtration, solar irradiation, sedimentation, aggregation, oxidation, antibiosis, predation and competition (Gersberg *et al.* 1987). However, few quantitative studies have been carried out to determine the relative importance of various mechanisms for the removal of allochthonous bacteria by wetlands and ponds, and consequently these are poorly understood (Kadlec 1995; Perkins and Hunter 1999). The work presented here focuses on the fate of stormwater-associated bacteria in constructed wetland and water pollution control pond systems, and was part of an extensive investigation to compare the effectiveness of the two treatment systems for stormwater management.

MATERIALS AND METHODS

Study sites

Plumpton and Woodcroft Estate are two recently established residential developments approximately 40 km north-west of Sydney, New South Wales, Australia, which produce large volumes of stormwater with high suspended solids and nutrient concentrations during storm events (Hunter and Claus 1995). Stormwater from these developments flows via a system of creeks, into the Hawkesbury River (Fig. 1), further increasing the pollutant load on a river that is already degraded and prone to algal blooms due to the discharge of nutrients and other pollutants from the catchment. Stretches of the river are extensively used for recreational purposes involving primary and secondary contact.

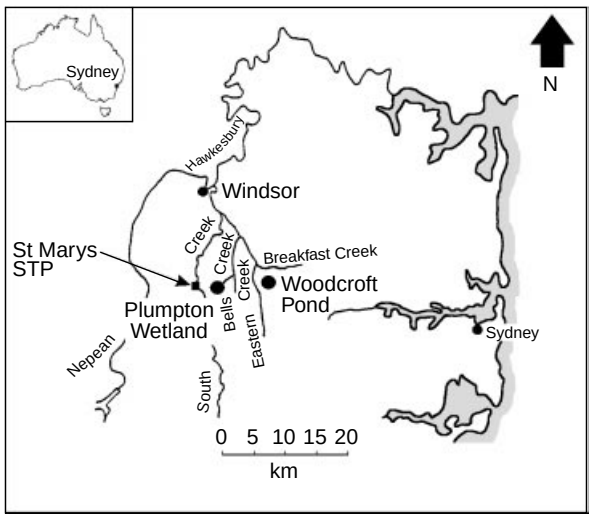

Fig. 1 Location of study area

The 0·45 ha constructed wetland system at Plumpton Park was completed in 1994 within the existing 75 ha residential catchment. It consists of a gross pollutant trap to remove coarse sediment, a trashrack, and a wetland planted extensively with emergent indigenous macrophytes (Fig. 2a). The wetland is separated into five cells, each approximately 40 m long separated by loose rock weirs 400 mm high. The minimum and maximum water depths are 200

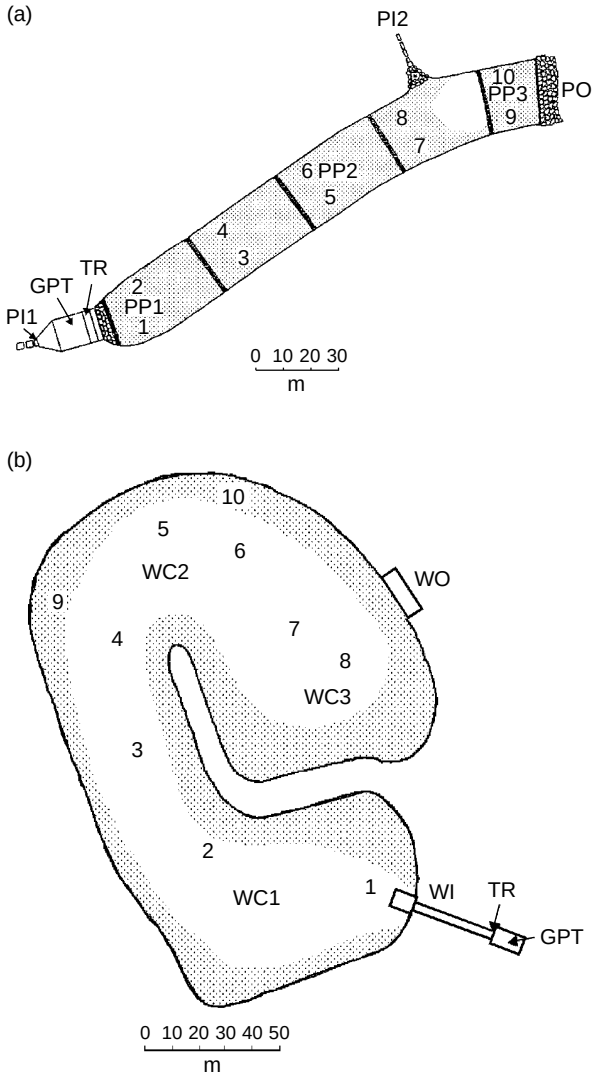

Fig. 2 Schematic plan of (a) Plumpton Park wetland and (b) Woodcroft water pollution control pond systems indicating water and sediment sampling sites. PI1 main wetland inlet, PI2 secondary wetland inlet, PO wetland outlet, WI pond inlet, WO pond outlet. 1–10 water column and sediment samples. PP1-PP3 and WC1-WC3 sediments for microcosms. Shading indicates vegetated areas. GPT gross pollutant trap, TR trashrack

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 and water column samples are indicated in Fig. 2a.

The 1·5-ha water pollution control pond system at Woodcroft Estate (Fig. 2b) was completed approximately 12 months after Plumpton Park wetland, during the early stages of residential development of the area. Active construction work in the vicinity of the pond is presently still in progress. The catchment size is 53 ha. The storage volume of the pond ranges from 23 to 39 ML. The pond consists of a gross pollutant trap, a trashrack and three cells of approximately 2·5 m in depth with an intervening ridge depth of 1 m. Emergent indigenous macrophytes are present around the periphery of the pond. The pond has a single inlet (WI) and a single outlet (WO). The outflowing water is discharged into an artificial lake, 3·2 ha in size. Sampling locations for inflow and outflow samples, and for sediment and water column samples are indicated in Fig. 2b.

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 and Constandopoulos 1997).

Sampling

Discrete inflow and outflow water samples were collected weekly in sterile containers from Plumpton Park wetland and Woodcroft pond during the period July to December 1998.

Sediment and water column samples were collected on a single occasion during January 1999. Sediments from Plumpton Park wetland were collected using Perspex cylinders (length 30 cm, diameter 8 cm), by penetrating areas of undisturbed sediment with the cylinder and capping both ends with plastic caps. The overlying water was removed using a sterile disposable syringe. Sediment samples were collected from Woodcroft pond using a 2·5-m corer (diameter 6 cm). The top 5 cm of each sediment core was transferred using a sterile spatula into a sterile polycarbonate container. Samples of water overlying the sediment were collected simultaneously and the *in situ* pH, temperature, turbidity and dissolved oxygen determined for each sample. A box dredge sampler was used to collect sediment for microcosm studies and sediment characterization from the inlet end, middle and outlet end of each system.

Total daily rainfall data for the sampling period were obtained from a pluviometer located approximately 5 km from Plumpton Park and 8 km from Woodcroft at St Mary's Sewage Treatment Plant (NSW, Australia).

Desorption of bacteria from sediments

Sediment samples were mixed thoroughly using a sterile spatula. Ten grams of sediment was weighed out into 90 ml sterile phosphate-buffered saline (PBS) and shaken by hand for 2 min. These were allowed to stand undisturbed for 10 min to enable coarser solids to settle out, after which the top 25 ml of the supernatant was transferred to a sterile bottle and used for bacteriological analysis. Previous work had shown that there was no significant difference between bacterial numbers desorbed from the sediments using chemical agents such as sodium dodecyl sulphate, Tween 80 and Triton X 100, or sonication, and bacterial numbers desorbed by handshaking in PBS (not shown).

Bacteriological analysis

Presumptive thermotolerant coliforms (TTC) and enterococci (ENT) were enumerated using standard membrane filtration techniques. TTC were enumerated using mem Faecal Coliform Agar (AM 124, Amyl Media Pty Ltd, Dandenong, Vic., Australia) without rosolic acid. The plates were incubated at $44{\cdot}5 \pm 0{\cdot}2\,^{\circ}C$ for 24 h (APHA 1998). ENT were enumerated using mem Enterococcus Agar (AM 54, Amyl) (Anonymous 1982). The plates were incubated at $44{\cdot}5\,^{\circ}C$ for 48 h. Concentrations of total heterotrophic bacteria were determined by the spread plate technique using standard plate count agar (CM463 Oxoid). The plates were incubated at $25\,^{\circ}C$ for 5 d (APHA 1998).

Clostridium perfringens spores were enumerated in a heat-shocked portion of each sample ($75\,^{\circ}C$ for 20 min) by membrane filtration using Perfringens agar base (AM 147, Amyl) supplemented with tryptose sulphite cycloserine (SR 88, Oxoid). Incubation of the plates was in an anaerobic environment at $35\,^{\circ}C$ for 18–24 h. Presumptive *Cl. perfringens* were determined by counting the numbers of black and grey colonies.

All dilutions were prepared in PBS. Bacterial counts were expressed as colony forming units (cfu) per 100 ml or 100 g dry sediment, except for microcosm and settlement experiments in which they were expressed as cfu 100 g wet sediment^{-1}.

Sediment microcosms

Sediment samples from the inlet and outlet ends of each system (PP1, PP3, WC1 and WC3) were used for sediment microcosms. For each sample, 100 g of well-mixed sediment was weighed into six sterile 500-ml Pyrex bottles containing sterile magnetic stirrer bars to allow mixing. Cycloheximide was added to three of the containers to give a final concentration of 1 g 100 g sediment^{-1} and mixed well. A sub-sample (10 g) was withdrawn from each con-

tainer using a sterile spatula and diluted in 90 ml of sterile PBS. This was shaken by hand for 2 min and analysed for TTC and ENT as described above. Filter-sterilized (0·2-μm pore size) pond or wetland water (100 ml) was used to overlay the sediment in the microcosms which were then incubated in the dark at 25 °C for 28 d. Weekly sub-samples of sediment were withdrawn from the microcosms by aseptically pipetting off the overlying water, taking care not to resuspend any of the sediment. The sediment was mixed and a 10-g portion withdrawn using a sterile spatula. The sediment remaining in the microcosm was covered with 100 ml of filter-sterilized pond or wetland water (equilibrated to 25 °C). The concentrations of TTC and ENT were determined in the sub-sampled sediments.

Sediment characteristics

The particle size distribution of three sediment samples (inlet, middle, outlet) for each system was determined in duplicate using the pipette method (Palmer and Troeh 1995) as follows: the settling velocities at 25 °C for particles ranging in size from < 2 to $> 62\ \mu$m were calculated using a modified version of Stoke's Law, $V = kd^2$, where k is a constant combining density, gravity and viscosity, V is the velocity of fall of the particles, and d is the diameter of particles. The settling velocities were used to calculate sampling times for each size fraction at a depth of 10 cm from the surface. The sediments (100 g) were mixed with sterile distilled water and the suspensions allowed to settle in 1-l cylinders. At the determined sampling times, 25 ml sediment suspension was removed from a depth 10 cm below the surface and dried at 105 °C for 24 h in a preweighed crucible. Dispersive agents were not used nor was organic matter removed before settling. Simultaneously, the concentrations of TTC and ENT remaining suspended in the top 10 cm were determined from an additional sub-sample at each of the sampling times.

The moisture contents of the sediment samples were determined in duplicate by oven-drying 5–10 g of the sediment in preweighed crucibles at 105 °C for 24 h. The dried sediments were then ashed in a muffle furnace at 550 °C for 24 h to estimate the organic matter content (Palmer and Troeh 1995).

Data analysis

Linear regression, correlation analyses and analysis of variance were performed using Minitab Release 7·1 Data Analysis Software (Mintab Inc., State College, PA, USA).

RESULTS

The geometric means and ranges of inflow and outflow bacterial concentrations to the two systems over the period July to December (mid winter to early summer in Australia) are given in Table 1. Simultaneous sampling of the two inlets (PI1 and PI2 data combined) and the outlet in the wetland showed that outflow concentrations of TTC, ENT and heterotrophic bacteria were generally lower than inflow concentrations, often by an order of magnitude. Mean removal efficiencies for the wetland were 79, 85 and 87% for TTC, ENT and heterotrophic bacteria, respectively. However, the difference between inflow and outflow concentrations of bacteria was generally much less in the pond, with outflow bacterial concentrations often exceeding inflow bacterial concentrations. Mean bacterial removal efficiencies for the pond were −2·5, 23, and 22% for TTC, ENT and heterotrophic bacteria, respectively.

The total daily rainfall for each 24-h period preceding sample collection ranged from 0 to 28·5 mm (not shown).

Table 1 Weekly stormwater inflow and outflow bacterial concentrations at Plumpton Park wetland and Woodcroft water pollution control pond

	Plumpton Park wetland		Woodcroft pond	
	Inflow concentration* (cfu 100 ml^{-1})	Outflow concentration* (cfu 100 ml^{-1})	Inflow concentration* (cfu 100 ml^{-1})	Outflow concentration* (cfu 100 ml^{-1})
Thermotolerant coliforms	$1{\cdot}7 \times 10^4$	$3{\cdot}6 \times 10^3$	$7{\cdot}9 \times 10^3$	$8{\cdot}1 \times 10^3$
	$3{\cdot}6 \times 10^2$–$3{\cdot}6 \times 10^5$	$2{\cdot}0 \times 10^2$–$1{\cdot}2 \times 10^5$	$1{\cdot}0 \times 10^2$–$1{\cdot}1 \times 10^6$	89–$7{\cdot}1 \times 10^4$
Enterococci	$6{\cdot}1 \times 10^3$	$9{\cdot}0 \times 10^2$	$1{\cdot}2 \times 10^3$	$9{\cdot}2 \times 10^2$
	76–$8{\cdot}5 \times 10^4$	8–$2{\cdot}4 \times 10^4$	76–$2{\cdot}7 \times 10^4$	89–$2{\cdot}6 \times 10^4$
Heterotrophic bacteria	$2{\cdot}3 \times 10^7$	$3{\cdot}0 \times 10^6$	$6{\cdot}3 \times 10^6$	$4{\cdot}9 \times 10^6$
	$1{\cdot}6 \times 10^6$–$9{\cdot}1 \times 10^7$	$6{\cdot}8 \times 10^4$–$1{\cdot}3 \times 10^8$	$5{\cdot}5 \times 10^5$–$2{\cdot}3 \times 10^8$	$3{\cdot}5 \times 10^5$–$6{\cdot}8 \times 10^7$

*Geometric mean and range for 24 samples.

Table 2 Correlation of stormwater inflow and outflow bacterial concentrations with total daily rainfall measurements for the preceding 24-h period

Sample	Bacteria	Correlation coefficient, *r*	
		Plumpton Park wetland	Woodcroft pond
Inflow	Thermotolerant coliforms	0·365*	0·261
	Enterococci	0·622*	0·690*
	Heterotrophic bacteria	0·182	0·602*
Outflow	Thermotolerant coliforms	0·548*	0·442*
	Enterococci	0·615*	0·805*
	Heterotrophic bacteria	0·749*	0·529*

*Correlation significant ($P = 0{\cdot}05$)

The rainfall data was analysed for correlation with the log-transformed inflow and outflow concentrations of each bacterial indicator. The Pearson coefficients of correlation *r* are given in Table 2. Total daily rainfall was significantly correlated ($P < 0{\cdot}05$) with inflow and outflow ENT concentrations for both the wetland and the pond, with outflow TTC and heterotrophic bacterial concentrations for the wetland, and with inflow and outflow concentrations of heterotrophic bacteria for the pond.

Physical and chemical characteristics for the water column samples collected at the time of sediment sampling are given in Table 3. The turbidities of the pond water column samples were much higher than those of the wetland water column samples. The water column and sediment bacterial concentrations for the wetland and pond are given, respectively, in Figs 3 and 4. The concentrations of bacteria in sediments were generally higher than the water column concentrations, often by several orders of magnitude. This difference was most pronounced for *Cl. perfringens* spores, the concentrations of which ranged from < 1 to 40 per 100 ml in the water column and 10^4 to 10^7 per 100 g dry weight in the sediment. Table 4 shows the particle size distributions for sediments collected at three different points in each system (PP1, PP2, PP3, WC1, WC2 and WC3). The pond sediments had significantly higher proportions of particles that were < 2 μm and 2–5 μm in size

Table 3 *In* situ physicochemical characteristics of water column samples

Water column sample	Temperature (°C)	pH	Dissolved oxygen (mg l^{-1})	Turbidity (NTU)
Plumpton 1	25·4	7·57	9·6	100
Plumpton 7	25·5	7·04	4·7	–
Plumpton 9	21·8	6·92	1·0	84
Woodcroft 1	20·5	6·54	2·0	600

Table 4 Sediment characteristics

Sediment†	Moisture content (%)	Organic matter content (%)	Particle size distribution (%)*					
			< 2 μm	2–5 μm	5–10 μm	10–20 μm	20–62 μm	> 62 μm
PP1	65 ± 3	13 ± 1	7 ± 1	7 ± 1	8 ± 1	8 ± 0	44 ± 1	26 ± 1
PP2	57 ± 2	11 ± 1	3 ± 4	1 ± 11	0 ± 6	48 ± 1	12 ± 4	37 ± 3
PP3	64 ± 4	10 ± 0	5 ± 1	7 ± 2	14 ± 1	0 ± 10	35 ± 7	52 ± 18
WC1	48 ± 1	7 ± 0	19 ± 0	11 ± 1	7 ± 0	10 ± 2	30 ± 6	23 ± 7
WC2	48 ± 2	7 ± 1	34 ± 1	14 ± 1	23 ± 2	8 ± 4	18 ± 3	3 ± 6
WC3	55 ± 0	9 ± 0	28 ± 1	17 ± 1	9 ± 13	25 ± 28	9 ± 3	12 ± 1

*Mean of two determinations ± S.D. settlement times for particle size fractions were 0, 26 s, 4 min 10 s, 16 min 40 s, 68 min 40 s, 416 min 40 s.

†PP Plumpton Park wetland, WC Woodcroft water pollution control pond.

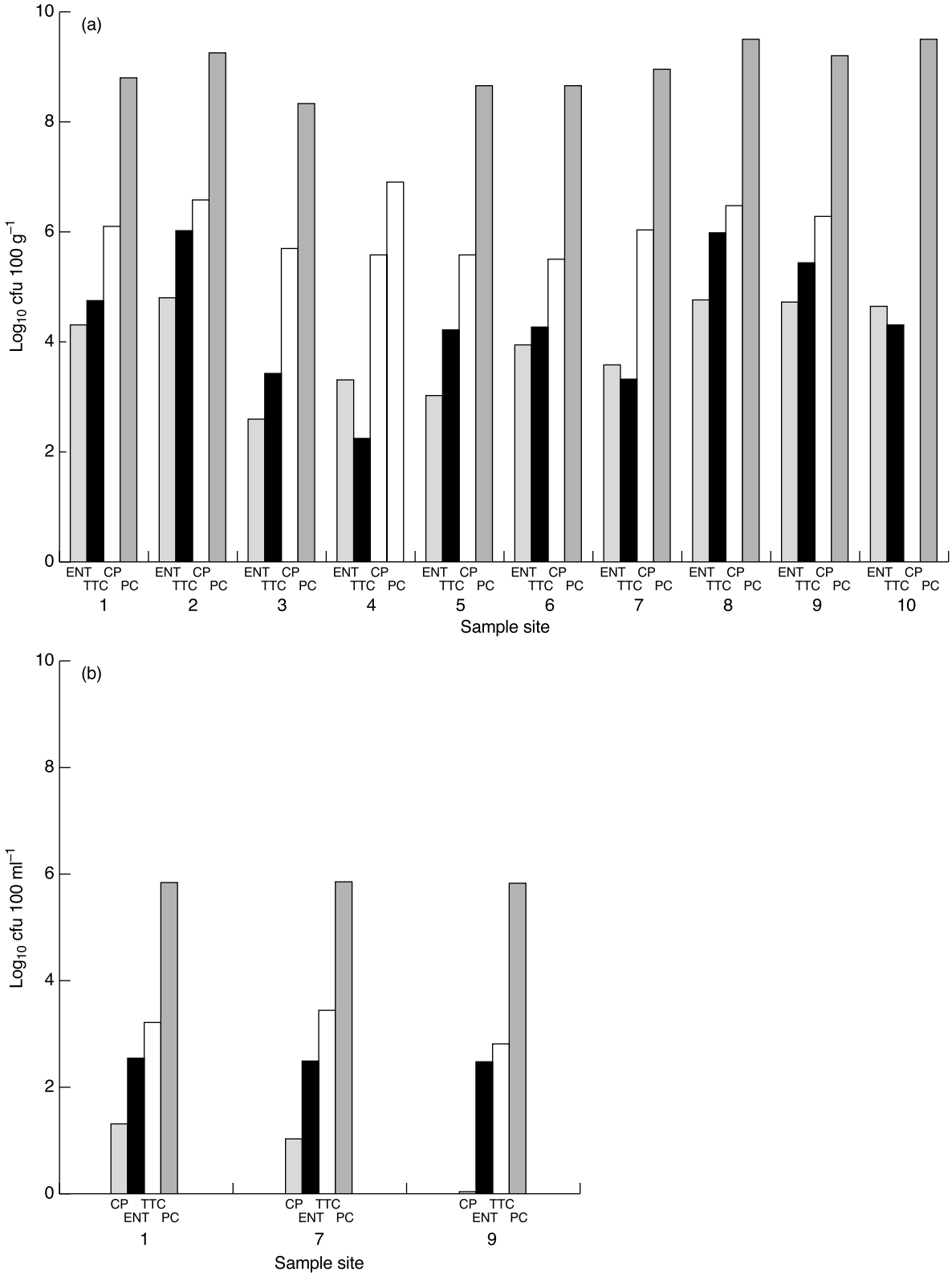

Fig. 3 Concentrations of indicator bacteria in (a) sediment and (b) water column samples (1–10) from Plumpton Park wetland, per g dry weight of sediment. TTC thermotolerant coliforms; ENT enterococci; CP *Clostridium perfringens*; PC heterotrophic plate count

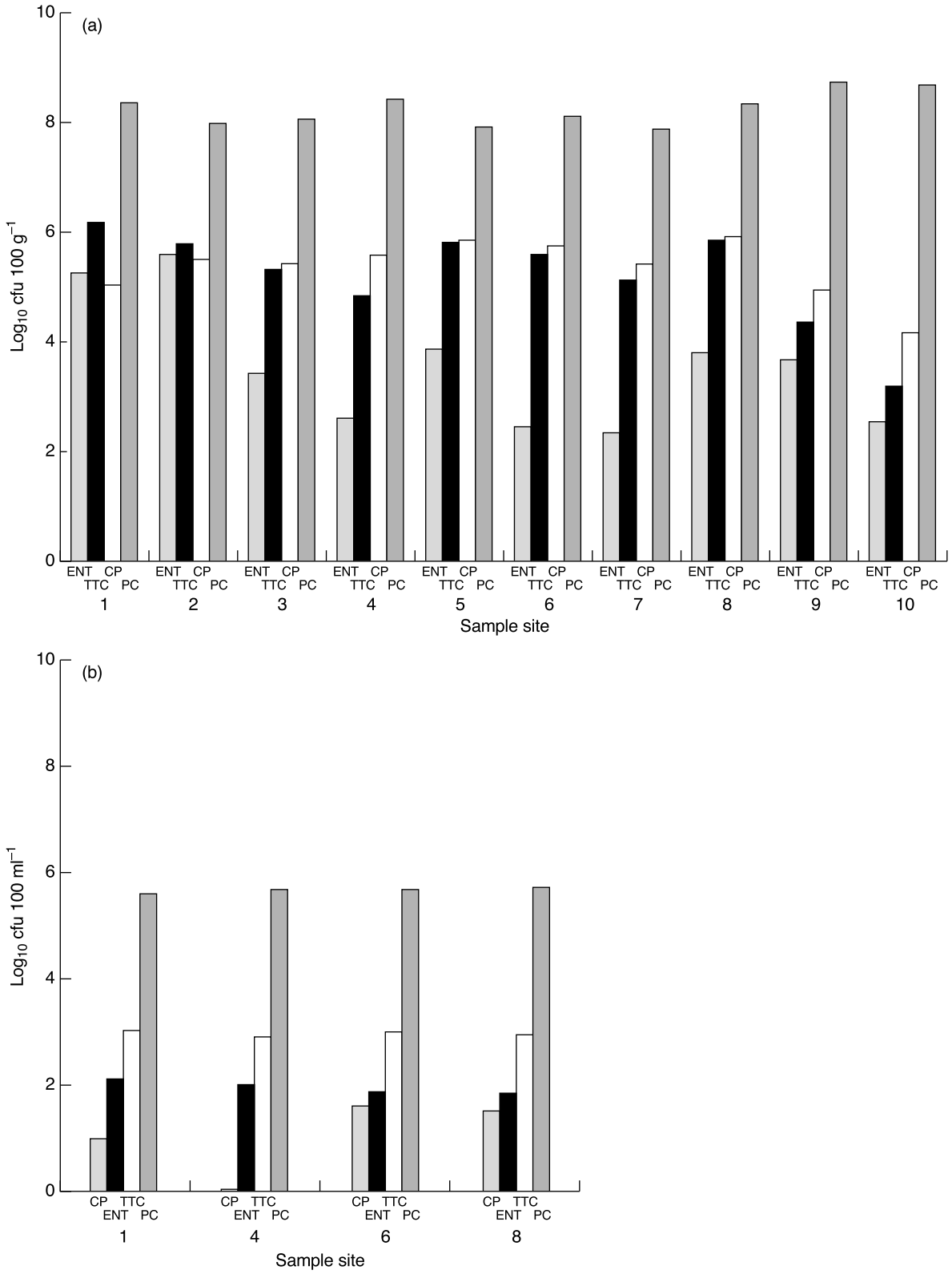

Fig. 4 Concentrations of indicator bacteria in (a) sediment and (b) water column samples (1–10) from Woodcroft water pollution control pond, per g dry weight of sediment. TTC thermotolerant coliforms; ENT enterococci; CP *Clostridium perfringens*; PC heterotrophic plate count

than the wetland sediments ($P < 0{\cdot}05$), whereas the wetland sediments had significantly higher proportions of particles that were $> 62\,\mu$m in size ($P < 0{\cdot}05$). Although the pipette method for particle size analysis is not generally recommended for particles greater in size than $62\,\mu$m which settle out rapidly, it was possible to overcome this problem using a magnetic stirrer to keep the particles suspended whilst withdrawing the initial fraction.

Figure 5(a,b) shows the concentrations of TTC and ENT, respectively, present in the top 10 cm of the sediment suspension over the duration of settlement (416 min 40 s). The bacterial concentrations in the top 10 cm remained relatively constant with time. This suggests that the bacteria were almost exclusively associated with the smaller particles ($< 2\,\mu$m) that remained suspended throughout the duration of the settling experiment, and not attached to the larger particles that settled out within the duration.

The survival of TTC and ENT in closed-bottle sediment microcosms over a period of 28 d is shown in Figs 6 and 7. In each microcosm there was a significant general decline in concentration of both TTC and ENT with time, indicating mortality. Assuming that bacterial mortality may be predicted by a first order exponential decay model, the following equation was used to calculate mortality rate constants for the bacteria in the sediments: $\log_{10}(N/N_o) = -kt$, where N is the bacterial concentration at time t, N_o is the

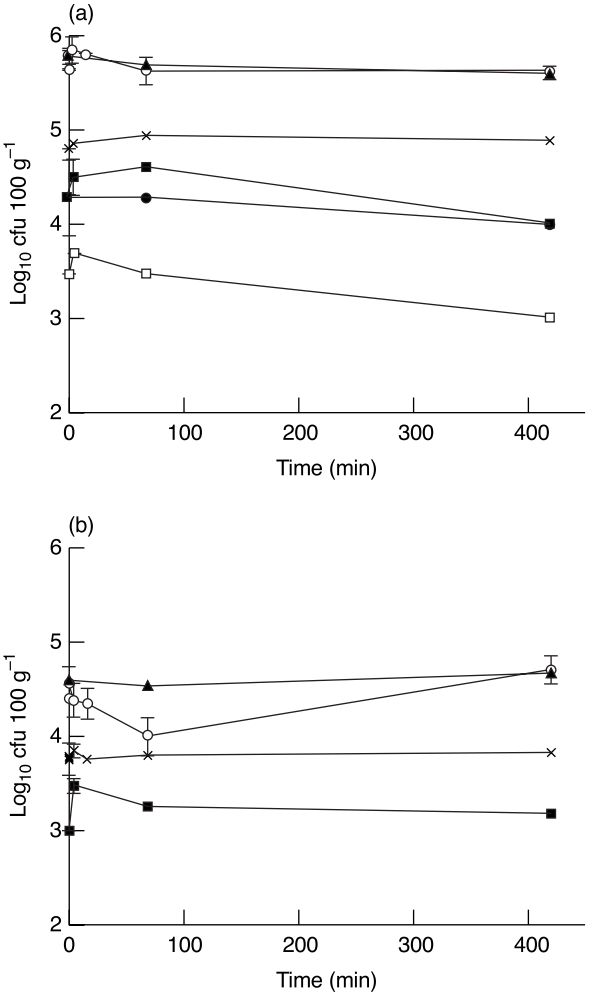

Fig. 5 Concentrations of (a) thermotolerant coliforms and (b) enterococci remaining suspended in the top 10 cm during settlement of sediments, per gram wet weight of sediment. Error bars represent the S.D. (×) PP1; (□) PP2; (●) PP3; (○) WC1; (■) WC2; (▲) WC3

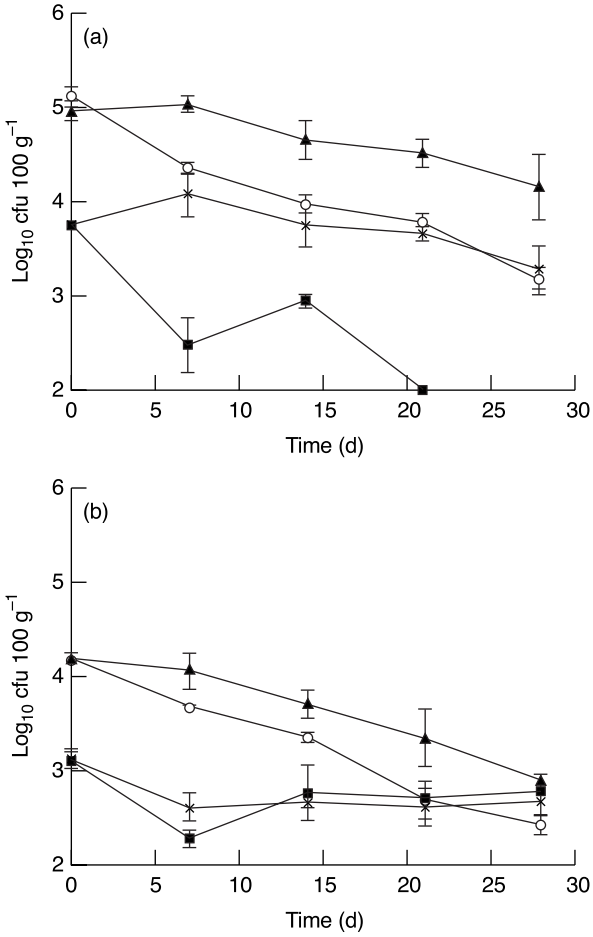

Fig. 6 Survival of thermotolerant coliforms and enterococci in wetland sediment microcosms (a) inlet sediment (PP1) and (b) outlet sediment (PP3), per g wet weight of sediment. Error bars represent the S.D. of three replicate microcosms. (○) TTC; (▲) TTC + cycloheximide; (■) ENT; (×) ENT + cycloheximide

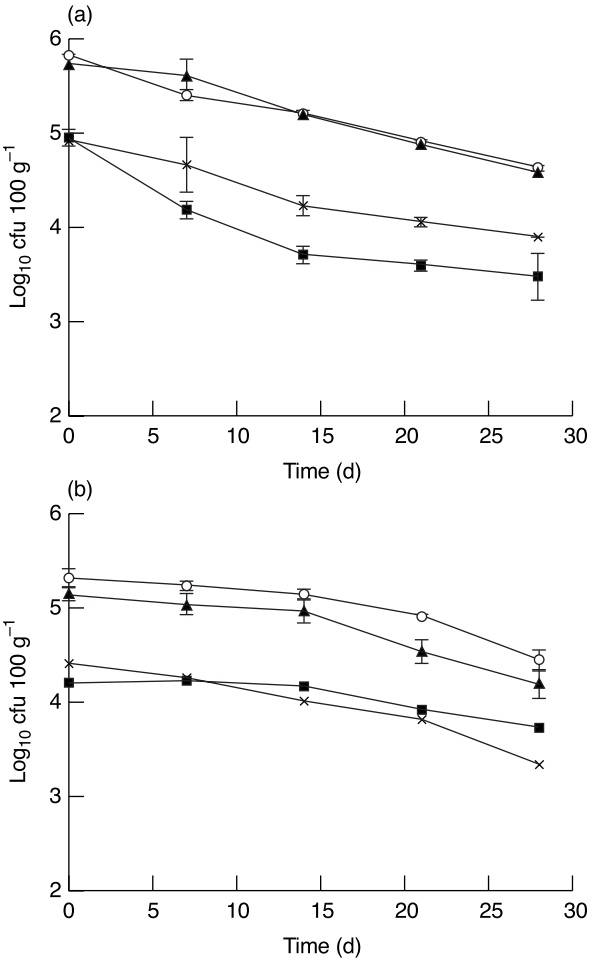

Fig. 7 Survival of thermotolerant coliforms and enterococci in pond sediment microcosms (a) inlet sediment (WC1) and (b) outlet sediment (WC3), per g wet weight of sediment. Error bars represent the S.D. of three replicate microcosms. (○) TTC; (▲) TTC + cycloheximide; (■) ENT; (×) ENT + cycloheximide

concentration at time 0, and k is the mortality rate constant. The mortality rates for TTC and ENT in the sediments are given in Table 5. The r^2 values for the linear regressions indicate that the exponential decay equation adequately described bacterial mortality in each of the microcosms, with the exception of ENT in outlet wetland sediment, in which the mortality rates were very low. The lower detection limit for determining concentrations of bacteria in sediment using the procedure described above was approximately 1×10^2 100 g wet weight^{-1} and therefore mortality of the bacteria below this concentration could not be determined.

One-way analysis of variance was used to determine if the mortality rates were significantly greater in the absence of cycloheximide compared with in the presence of cycloheximide for the replicate microcosms and, hence, if predation was occurring. The mortality rates of TTC in pond sediments were not significantly different in the presence or absence of cycloheximide, whereas in wetland sediments the mortality rates were significantly greater in the absence of cycloheximide ($P < 0{\cdot}05$). The mortality rates of ENT were significantly greater in the absence of cycloheximide ($P < 0{\cdot}05$) for the inlet wetland sediment but not for the outlet wetland sediment or for either of the two pond sediments.

DISCUSSION

In natural aquatic systems the adsorption of allochthonous micro-organisms to sand, silt and clay particles which then undergo physical sedimentation facilitates their removal from the water column and leads to their accumulation in sediments. Many wastewater treatment systems use this process to remove bacteria of faecal origin and other particle-bound pollutants from wastewaters.

Due to the adsorption of bacteria preferentially to fine particles (Dale 1974), the effectiveness of treatment systems for the removal of bacteria is related to the rate at which fine particles settle out in the system. It has been reported that efficient sedimentation of coarse to medium-sized solids occurs in water pollution control ponds and that fine particles are less effectively removed. In contrast, the extensive vegetation in wetlands impedes the water flow and enhances the sedimentation of fine particles as well as coarse and medium-sized particles (Wong *et al.* 1999). The findings of the present study are consistent with these observations. Bacterial concentrations in stormwater were significantly reduced by the wetland system but not by the pond system. The TTC removal efficiencies for the wetland, however, were somewhat lower than values previously reported which usually exceed 90%. However, most previous microbiological studies have focused on the assessment of wetlands for the treatment of municipal and industrial wastewater rather than for the treatment of stormwater. Stormwaters may contain higher proportions of fine particles ($< 2\ \mu$m) than municipal wastewaters.

It could be reasoned that the proportions of fine particles should be higher in the wetland sediments than in the pond sediments, due to the more effective settlement of clay particles in wetlands. However, greater proportions of fine particles were found in the pond sediments despite evidence to suggest that the pond was not effectively retaining particle-bound bacteria. This may be explained by differences in particle size inputs to the two systems. Residential development within the wetland catchment has been established for several years and the soil has been stabilized to some extent by turfing and planting by residents and by

Table 5 Mortality rates for thermotolerant coliforms and enterococci in Plumpton Park wetland and Woodcroft water pollution control pond sediments

	Mortality rate constant, k *			
	Thermotolerant coliforms		Enterococci	
Sediment†	No cycloheximide	With cycloheximide	No cycloheximide	With cycloheximide
PP1	0·063	0·031	0·069	0·020
	(0·967)	(0·901)	(0·686)	(0·580)
PP3	0·064	0·047	0·012	0·002
	(0·973)	(0·987)	(0·378)	(0·007)
WC1	0·041	0·044	0·050	0·038
	(0·989)	(0·988)	(0·861)	(0·968)
WC3	0·029	0·034	0·018	0·037
	(0·873)	(0·908)	(0·845)	(0·958)

*Values in parentheses are r^2 values for the linear regression.
† PP Plumpton Park wetland, WC Woodcroft water pollution control pond.

the importation of loamy top soil, which may reduce mobilization of the clay particles. In contrast, construction work in the catchment of the water pollution control pond was still in progress at the time of the study and consequently there were large areas of disturbed and exposed clay, which may be easily mobilized and transported in stormwater. The input of clay particles to the pond system was therefore likely to be much greater than for the wetland system. However, particle size determinations on the stormwater inputs to each system are required in order to confirm this.

It has been shown that the process of bacterial adsorption to particles increases bacterial persistence in aquatic environments by protecting them from environmental pressures that may otherwise be responsible for their mortality, e.g. solar radiation, starvation and attack by bacteriophages (Roper and Marshall 1974; Gerba and McLeod 1976). In addition, several workers have found a significant relationship between sediment bacterial mortality rates and sediment particle size. TTC mortality rates were shown to be significantly lower in sediment with predominantly clay-sized particles than in coarser sediments (Howell *et al.* 1996). Burton *et al.* (1987) found that particle size was the only sediment characteristic that was related to the survival of *Escherichia coli* and *Salmonella newport*, both of which survived significantly longer in sediments containing at least 25% clay. In addition, there is evidence of adsorption of viruses to clay particles (Gerba and Schaiberger 1975; Rao 1987)

Several factors could be responsible for the observed difference in persistence of TTC in the pond and wetland sediments. The bactericidal substances reportedly produced by macrophytes in wetlands (Seidel 1976) are likely to be absent in the pond sediment which is sparsely vegetated. Additionally, higher nutrient concentrations have been found to be associated with smaller sediment particles (Chan *et al.* 1979). Therefore, nutrient concentrations in the pond sediments may be higher and because the pond sediments are more likely to be anoxic, the nutrients may be more bioavailable. However, as TTC mortality rates were not significantly different in the wetland and pond sediments in the absence of predators, it appears that predation was the determining factor. In the presence of predators the mortality of TTC was greater in the wetland sediments than in the pond sediments. A possible explanation for this is that the higher proportions of clay particles in the pond sediments protect the bacteria from predators (Heijnen *et al.* 1991). Previous workers have suggested that the location of soil bacteria in small pores, from which the predators were excluded due to their larger size, provided the bacteria with significant protection from predation (Wright *et al.* 1995; Decamp and Warren 2000).

The greater effect of predation on TTC compared with ENT concentrations may be related to the hydrophobic properties of streptococci which enable them to bind more efficiently than coliforms to clay particles (Huysman and Verstraete 1993). Consequently, ENT may be protected from predators to a greater degree. Additionally, it is possible the protozoa may preferentially prey upon coliform bacteria over ENT (Gonzalez *et al.* 1990). According to Decamp and Warren (1998), predation by ciliate protozoa could account for the total removal of *E. coli* from wastewaters treated by constructed wetlands. Cycloheximide, an

inhibitor of protein synthesis in eukaryotes, has been used previously to study protozoan predation of bacteria in stormwater (Marino and Gannon 1991). The use of cycloheximide as a predator inhibitor, however, may underestimate the significance of biotic factors on bacterial mortality as it does not inhibit lytic bacteria and bacteriophages. In addition, although effective against flagellate protozoa, cycloheximide is only partially effective against ciliate protozoa (Sherr *et al*. 1986).

The persistence of micro-organisms in wetland and pond sediments suggest that the sediments may act as reservoirs of viable bacteria. It has been shown that sediment-bound bacteria may be resuspended back into the water column by storm activity, thereby resulting in a deterioration in the quality of the overlying water (Crabill *et al*. 1999). Constructed wetlands are generally much shallower than water pollution control ponds but the higher density of macrophytes in wetlands may stabilize the sediments thereby reducing turbation by storm activity.

It is suggested that water pollution control ponds are less effective than constructed wetlands in removing micro-organisms which bind to fine clay particles. The use of water pollution control ponds may not be appropriate, therefore, for the treatment of stormwater in situations where the receiving waters are used for recreational purposes, particularly if soils in the catchment area have a high clay content and are potentially easily mobilized by storm activity. Constructed wetlands may offer a more effective, low technology approach for reducing stormwater bacterial loads to recreational waters.

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