Faecal Source Investigations in Selected Southland Waterways

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1. INTRODUCTION

Faecal matter and urine from domestically farmed animals, including sheep, cattle, pigs and poultry, contributes to the microbial contamination of water, crops and food. This faecal and urine contamination represents a pathway through which human-relevant pathogens enter the environment. Riverbed sediment can act as a reservoir of microbial contamination by entrainment of microorganisms from the overlying water. These microorganisms can be remobilised to the water column during river bed disturbance including during recreational activity and high rainfall events. Understanding the source of microbial contamination is important for identifying activities that may lead to the degradation of surface waters and is therefore important for managing diffuse pollution.

Animal-specific viruses, mainly from the Adenoviridae and Polyomaviridae families have been suggested as faecal source tracking (FST) tools due to their host-specificity, resistance to many inactivation processes, high prevalence and common excretion in faeces or urine. Polyomaviruses were previously known to be shed by several vertebrate species including cattle (bovine) and humans, but until our recent work there had not been a polyomavirus known to infect sheep (ovine). We have developed an assay to detect a novel ovine Polyomavirus in sheep urine. This test has the potential to discriminate whether faecal and urine pollution originates from sheep and represents a new marker for the FST toolbox.

In our current study, based in Southland, FST analysis was carried out on 11 rivers, targeting a variety of dairying, sheep and urban catchments to demonstrate the ability of the assay to detect ovine pollution in NZ waters. We also determined the rivers' sediment particle size and *E. coli* concentration of the water and sediment to determine if correlations existed between the *E. coli* concentration of the water and sediment and virus particles and sediment particle size.

2. METHODOLOGY

2.1 Sampling Sites

Eighteen sediment samples were collected in the Mataura, Oreti, Otepuni, Waikawa, Waimea and Waituna river catchments (Table 1). Water samples were collected at these sites by Environment Southland staff and tested for *E.coli* amongst other water quality parameters. Eleven of the rivers which previously contained elevated *E. coli* concentrations were analysed by FST. Examples of two sampling locations, Winton Stream and Otamita Stream, are shown in Figure 1 and Figure 2. The relative position of each sampling location to other locations is shown in Figure 3.

Table 1 Locations and tests carried out at each site.

CMB Number	ES Number	Location	*E. coli* counts	Viral markers
CMB 130107	65	Waikawa River at Progress Valley	☑	☑
CMB 130108	64	Waikopikopiko Stream at Haldane	☑	
CMB 130109	152	Waitura - Carran Creek at Waitura Lagoon Rd	☑	☑
CMB 130110	154	Moffat Creek at Moffat Rd	☑	
CMB 130111	63	Waituna Creek at Marshall Rd	☑	☑
CMB 130112	150	Waituna Creek 1m u/s Waituna Rd	☑	
CMB 130113	32	Makarewa river at Wallacetown	☑	
CMB 130114	31	Winton stream at Lochiel	☑	☑
CMB 130115	120	Otapiri Stream at Otapiri Gorge	☑	☑
CMB 130116	135	Tussock Creek at Cooper Rd	☑	☑
CMB 130117	40	Waikiwi Tream at North Rd	☑	
CMB 130118	42	Otepuni Creek at Nith street	☑	☑
CMB 130131	52	Waikaia River u/s Piano Flat	☑	
CMB 130132	98	Waikaia River at Waikaia	☑	☑
CMB 130133	51	Waikaia River at Waipounamu Br	☑	☑

CMB 130134	58	Otamita Stream at Mandeville	☑	
CMB 130135	46	Mataura River at Otamita Br	☑	☑
CMB 130136	234	Sandstone Stream	☑	☑

Abbreviations used: ES = Environment Southland. u/s = upstream; Br = bridge.

Figure 1 Winton Stream.

Figure 2 Otamita Stream.

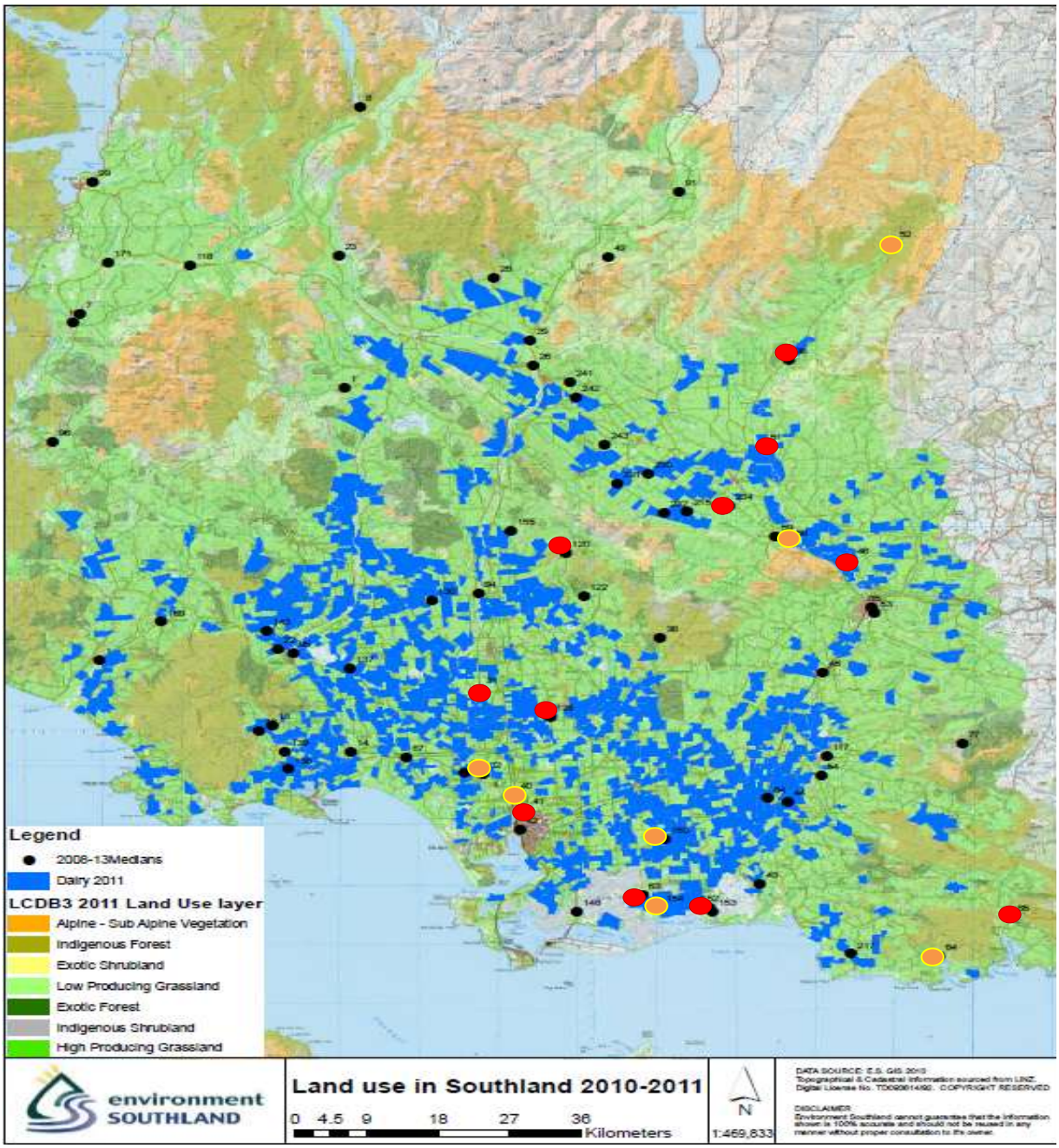

Figure 3 Sampling locations. Where the Red spots are the sampling sites tested for viruses and bacteria (MST), water and sediment samples, and the Orange spots are the sampling sites tested for *E. coli in* sediment samples.

Viruses tend to be present in freshwater samples in low concentrations, therefore a large volume of water (10 lt) needs to be analysed to determine their presence.

A scoop of river bed sediment was removed using a *Mighty Gripper* with a sterile 200ml pottle attached to it. The pottle was dragged along the riverbed to remove the top 1-2 cm of sediment. The sediment was stored chilled in the dark until processing (approximately 24 hrs). In the laboratory the sediment sample was mixed thoroughly and a 20 g sub-sample removed and placed in a sterile 250 mL bottle, to which 80 mL of ¼-strength Ringers solution was added. The bottle was shaken vigorously for two minutes, allowed to settle for about five minutes and 20 ml of supernatant was poured into a sterile 30 mL vial. The supernatant was serially diluted in 0.1% peptone water. Appropriate dilutions of the sediment suspension were enumerated for cultured *E. coli* in duplicate using pour plating of Chromo

2.2 Sediment processing

The total solids content of the sediment was determined according to Clesceri et al (1998). Briefly, a sample of sediment (approximately 15 g) was heated at 103°C until a stable weight was recorded on 3 consecutive days.

The sediment sizing was done using a number of different sized sieves and filter paper to determine the fraction of the sediment that corresponded to different sediment sizes. The particles sizes were then classified using the Wentworth scale (Figure 4).

Diameter (mm)	Diameter (phi)	Wentworth Size Class	
4096	-12		Boulder
256	-8	Gravel	Cobble
64	-6		Pebble
4	-2		Granule
2	-1		Very Coarse Sand
1	0		Coarse Sand
0.5	1	Sand	Medium Sand
0.25	2		Fine Sand
0.125	3		Very Fine Sand
0.0625	4		Coarse Silt
0.0313	5		Medium Silt
0.0156	6	Silt	Fine Silt
0.0078	7		Very Fine Silt
0.0039	8		Clay
0.00006	14	Mud	

Figure 4 Wentworth Scale for particle size classification.

3. RESULTS

3.1 Microbial concentrations

The concentration of *E. coli* in the river water and sediments showed a wide range from essentially no contamiantion at Waikaia River upstream of Piano Flat to quite high levels of 57,000 *E. coli* per 100 mL at Otamita Stream at Mandeville. A similar result, but at different places, was found for the sediments, with 1 cfu g^{-1} sediment at Winton stream at Lochiel and at Waikiwi Tream at North Road up to 18,286 cfu g^{-1} sediment at Waikaia River at Waikaia The FST, the human marker human adenovirus (HAdV) was found at one of the 11 sites, while the marker for sheep (ovine polyomavirus (OPyV)) was found at all 11 sites sampled (**Table 2**).

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Location	Bacteria		Viruses (particles/L water)		
	E. coli *per* 100 mL water	*E. coli* cfu/g dry weight sediment	Human (HAdV)	Bovine (BPyV)	Ovine (OPyV)
Waikawa River at Progress Valley	600	1068	nd	nd	3495
Waikopikopiko Stream at Haldane	300	307			
Waitura - Carran Creek at Waitura Lagoon Road	320	488	nd	nd	895
Moffat Creek at Moffat Road	160	791			
Waituna Creek at Marshall Road	320	667	nd	37	3419
Waituna Creek 1m u/s Waituna Road	430	3			
Makarewa river at Wallacetown	260	27			
Winton stream at Lochiel	1600	1	nd	nd	157
Otapiri Stream at Otapiri Gorge	1100	6	nd	nd	2693
Tussock Creek at Cooper Road	800	25	nd	87	43
Waikiwi Tream at North Road	120	1			
Otepuni Creek at Nith street	1600	205	12638	nd	44
Waikaia River u/s Piano Flat	<10	10			
Waikaia River at Waikaia	260	18286	nd	nd	139
Waikaia River at Waipounamu Br	100	75	nd	nd	384
Otamita Stream at Mandeville	57000	1632			
Mataura River at Otamita Br	170	5	nd	nd	1076
Sandstone Stream	300	3	nd	1202	976

Table 2 Enumeration of *E. coli* in the water and sediments for the 18 Southland sites sampled and the microbial source tracking markers (human, bovine and ovine) for the 11 Southland sites sampled.

Abbreviations used: HAdV = human adenovirus; BPyV = bovine polyomavirus; OPyV = ovine polyomavirus; u/s = upstream; nd= not detected; Br = bridge.

3.1 Sediment size

The main sediment sizes for the sites sampled ranged from colloidal clay at less than 45 μm to fine gravel at greater than 5 mm. These size fractions could be broadly divided into three general classes: clay/silts (<45 μm to 45 μm), sands (63 μm to 1 mm) and gravels (2 mm to >5 mm). Half the sites had sediments dominated by particle sizes of sands and half by gravels (Table 3).

There was no apparent relationship between the concentrations of *E. coli* in sediments and whether the sediment was classified as sands and gravels (Table 4). The sediment varied in size from predominately gravel (n=10) and (n=8) (Table 3). The concentration of *E .coli* per g of sediment dry weight ranged from 1 to 18,286.

Table 3 Sediment sizes (percentage of total fractions) at the sites samples, with the classifications according to the Wentworth scale (see Figure 4). Not all fraction sizes are shown in this table, with some of the very coarse material not included (i.e., the row may not sum to 100). The colour banding indicates the broad classifications of sediments as clay/silt, sand and gravel. The table continues on the next page.

Location	Total Solids (%)	Sediment size (percentage of total fraction) and Wentworth scale type								
		<45 µm	45µm	63µm	125µm	250µm	500µm	1mm	2 mm	>5 mm
		Clay colloid	Silt	Very fine sand	Fine sand	Medium sand	Coarse Sand	Very Coarse Sand	Very fine gravel	Fine Gravel
Waikawa River at Progress Valley	28	19.4	3.71	**22.51**	19.6	9.55	**25.2**	0	0	0
Waikopikopiko Stream at Haldane	54	7.81	7.41	17.3	**30.11**	**31.45**	5.05	0.2	0.66	0
Waitura - Carran Creek at Waitura Lagoon Rd	57	2.98		1.44	2.86	4.17	1.49	0.6	4.47	**81.99**
Moffat Creek at Moffat Rd	81	0.76	0.2	0.33	0.68	2.53	1.3	1.9	9.56	**63.24**
Waituna Creek at Marshall Rd	58	10.36	11.88	**57.26**	9.05	6.67	1.92	0.91	1.94	0
Waituna Creek 1m u/s Waituna Rd	85	**26.67**	4.0	16.78	**25.41**	11.78	0.19	0	0	0
Makarewa river at Wallacetown	72	8.64	10.16		19.15	**30.27**	3.34	3.71	12.63	12.50
Winton stream at Lochiel	78	0.48	0.04	0.09	0.21	4.36	16.74	**29.68**	26.6	0
Otapiri Stream at Otapiri Gorge	89	0.3	0.09		0.68	0.1	0.1	1.36	27.36	**70.03**
Tussock Creek at Cooper Rd	52	26.01	7.25	**29.69**	21.03	11.13	4.89			

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Location	Total Solids (%)	Sediment size and type								
		<45 µm	**45µm**	**63µm**	**125µm**	**250µm**	**500µm**	**1mm**	**2 mm**	**>5 mm**
		Clay colloid	**Silt**	**Very fine sand**	**Fine sand**	**Medium sand**	**Coarse Sand**	**Very Coarse Sand**	**Very fine gravel**	**Fine Gravel**
Waikiwi Tream at North Rd	76	17.41	9.85	17.0	6.86	4.47	2.19		6.31	**35.96**
Otepuni Creek at Nith Street	73	0.76	0.24	17.52		37.92	4.09	2.80	**36.66**	0
Waikaia River u/s Piano Flat	83	0.91	1.16	6.8		13.58	10.86	8.35	**29.63**	28.67
Waikaia River at Waikaia	41	4.81	6.97	37.1	**44.41**	6.49	0	0	0	0
Waikaia River at Waipounamu Br	83	0.75		0.55		1.78	4.98	33.66	**47.69**	10.57
Otamita Stream at Mandeville	77	1.07	0.21	2.84		5.43	15.36	16.16	**48.02**	10.9
Mataura River at Otamita Br	86	0.76	2.79		2.13		3.95		42.35	**48.0**
Sandstone Stream	85	1.42	0.53	2.11		6.23		4.79	24.79	**60.01**

Abbreviations used: u/s = upstream; Br = bridge.

3.1 Bacterial and virus relationships

The bacterial and virus concentration in the river water did not appear to be related, with no obvious increase of in virus concentration when *E. coli* was high (Table 4).

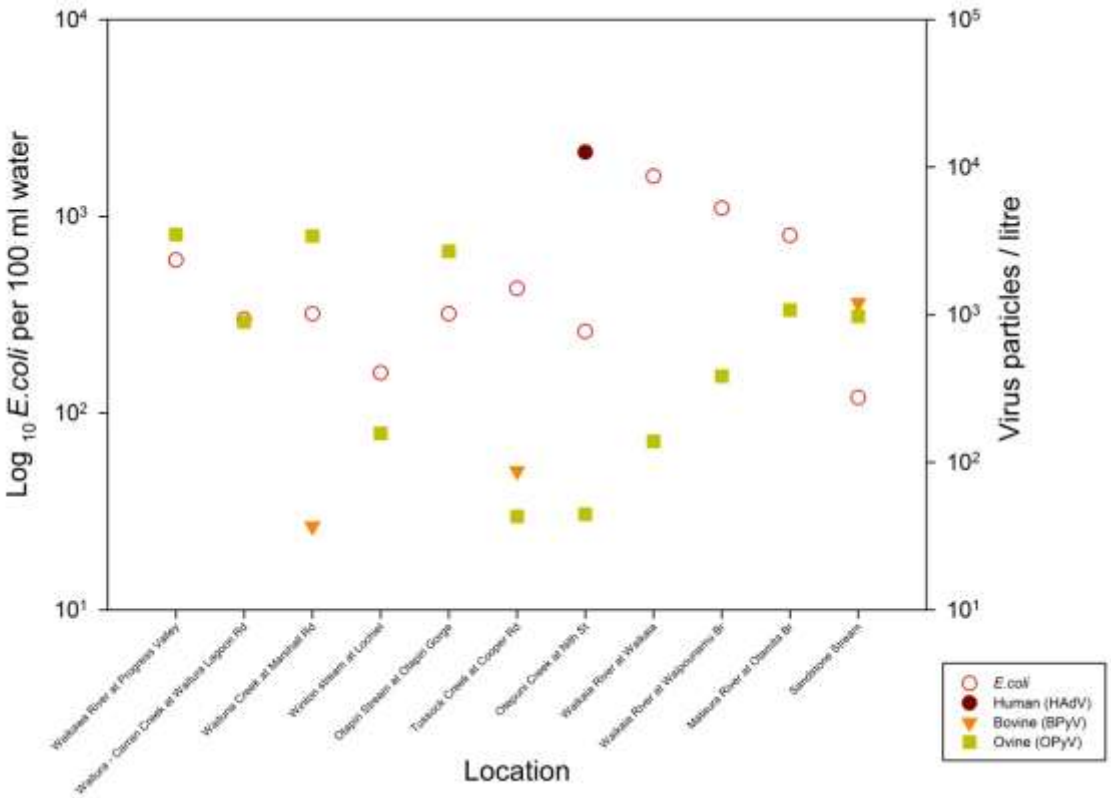

Figure 5 The relationship between *E. coli* and viruses in the water, where *E. coli* are per 10mL water and virus particles are per L water.

3.1 Recreational Water Quality Guidelines

There was no significant relationship between the concentration of *E. coli* in the sediments and the concentration of *E. coli* in the overlying water (Table 4**Error! Reference source not found.**). A comparison of the *E. coli* concentrations of the river water to the *Microbiological Water Quality Guidelines for Marine and freshwater Recreational Areas* (Ministry for the Environment 2003), the concentration of *E. coli* is at or exceeded the alert level of 260 *E. coli* per 100 mL thirteen times (Figure 6) .Of these exceedances, six also exceed the action level of 550 *E. coli* per 100 mL. Currently, there are no guidelines relating to the microbial quality of sediment.

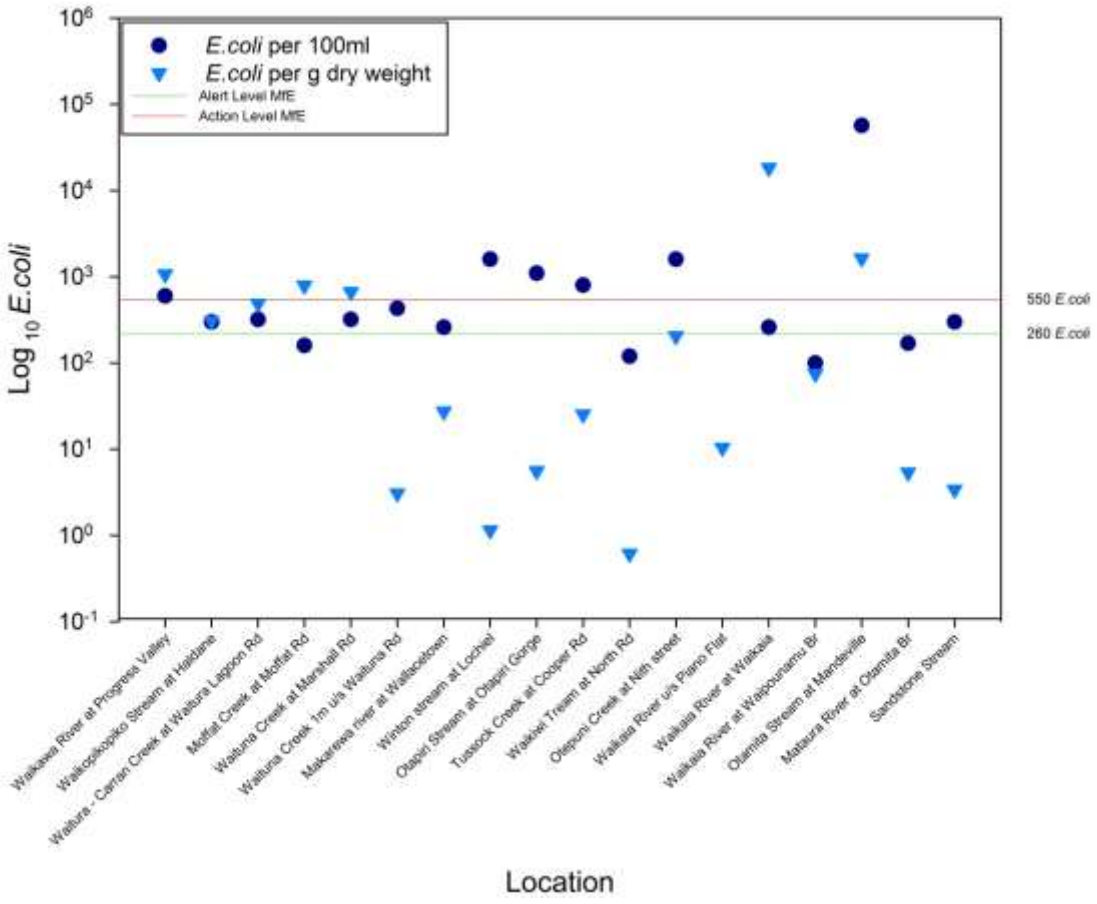

Figure 6 *E. coli* per 100 mL water and per gram sediment dry weight at each sampling location.

3.1 Correlation analysis

There was only one significant correlations between the different variables investigated; the concentration of *E. coli* in the sediments was negatively correlated with the percentage solids in the sediment (Table 4).

Table 4 Correlations between different variables investigated in the Southland study.

Variable 1	**Variable 2**	**Correlation**	**Explanation**
% Total solid	*E. coli* per g dry weight sediment	Negative (P=0.523)	As % solid increases, the concentration of *E. coli* decreases
E. coli in water	*E. coli* per g dry weight sediment	ns	(P > 0.050)
E. coli in water	Ovine marker in water	ns	(P > 0.050)
E. coli in water	Human marker in water	ns	(P > 0.050)
E. coli in water	Bovine marker in water	ns	(P > 0.050)
Sediment size	*E. coli* per g sediment	ns	(P > 0.050)
Sediment size	*E. coli* in water	ns	(P > 0.050)

Abbreviation: ns = not significant.

4. DISCUSSION

Human faecal pollution, as determined by the presence of HAdV, was detected at only one sampling site. This site was located in Invercargill city and had HAdV at greater than 10^4 viral particles (vp) per litre and a high *E. coli* concentration in the water (1,600 *E. coli* per 100 mL). Together these data indicate that urban wastewater is entering the local streams. Typically municipal wastewater has a mean HAdV concentration of 10^6 vp/L. Thus, assuming the Invercargill city population is shedding HAdV at an average rate similar to other communities, it would suggest that the human wastewater entering the river was being diluted approximately 100-fold by the river water.

Bovine faecal pollution was detected in three of the sampling sites; Waituna Creek, Tussock Creek and Sandstone at concentrations of $3.6x10^1$, $8.7x10^1$ and $1.2x10^3$ vp/L, respectively. These three sites are all in the vicinity of dairy farms and would suggest that these farms are likely sources of the contamination. Although the water exceeded recreational water guidelines for *E. coli* at each of the three sites, we did not find any correlation between virus particle concentration and *E. coli*. No human pollution was detected at these three bovine-impacted sites.

All river water samples tested positive for the ovine marker, indicating that sheep farming was the main source of faecal pollution into the Southland waterways. Waituna creek and Waitawa River were the most ovine-impacted waterways, whereas Tussock creek and Otepuni creek the least ovine-impacted.

The highest concentration of *E. coli* per gram of sediment was detected at the Waikaia River at Waikaia, where the sediment was classified as fine/very fine sand. Despite the high sediment loading of *E. coli* at the Waikaiar River site, the overlying water quality of was actually at the limit acceptable level for contact recreation (260 *E. coli* per 100 mL). This result suggests that when the sediment is disturbed by recreational activity, *E. coli* will be re-suspended in the water column. It is that likely that this will change the water from being acceptable for recreation contact to being unacceptable. The result also highlights the lack of correlation between river water and sediment quality during base flow. The Otamita Stream at Manderville had the highest concentration of *E. coli* in the water at 57,000 per 100 mL and the second highest *E. coli* per g of sediment (1,632 per g dry weight). This would be an interesting site to carry out faecal source tracking in the future.

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