APPLICATION OF FAECAL SOURCE TRACKING TOOLS TO UNDERSTANDING THE SOURCES AND RISKS OF MICROBIAL CONTAMINATION OF STORMWATER

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ABSTRACT

Stormwater has been recognised as one of the major sources of diffuse pollution to the aquatic environments. Several studies have shown that stormwater can contribute to the deterioration of water and sediment quality of a waterway. To correctly manage the pollution sources in stormwater it is beneficial to understand the main sources during dry and wet weather events. Faecal Source Tracking methods can be used to determine the sources of pollution in Stormwater. This allows for suitable mitigation options to be put in place to manage the source. Details of two case studies involving Stormwater are discussed as well as the impact of structural best management practices on the microbial loading of Stormwater.

KEYWORDS

Faecal Source Tracking, Bacteria, Microbiology, *E. coli*

PRESENTER PROFILE

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1 INTRODUCTION

Stormwater is described as excess rainwater that is unable to infiltrate into the ground. Urbanisation 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 including litter, oils, heavy metals, sediments, nutrients, organic matter and microorganisms. Stormwater has been recognised as one of the major sources of diffuse pollution to the aquatic environments (Davies & Bavor 2000). Several studies have shown that stormwater can contribute to the deterioration of water and sediment quality of a waterway. Stormwater can contain a number of chemical and biological contaminants as well as sediments. These may come from a number of different sources including urban and rural environments as well as industrial areas. Studies from monitoring programmes around the United States have shown regardless of the type of land use in the watershed, bacteria concentrations were well above primary contact recreational standards (Clary et al. 2002).

Studies have shown that faecal indicator organisms attach to sediment particles with stormwater. Once attached, they may settle to the bottom of a water column. Here they may experience a more favourable chemical and biological microenvironment and prolong their survival. This may pose a risk for potential recontamination of a water column if resuspension occurs prior to die-off. Therefore the quality of the water and the sediment needs to be considered if stormwater is being discharged to the environment (Jeng et al. 2005). These sediment bound bacteria can be re-suspended in low flow stormwater events during dry weather overflow events. This may result in elevated levels of indicator bacteria present in the stormwater. The health risk from this stormwater may be quite a lot lower than that of a wet weather event.

Several stormwater treatment and best management practice (BMP) strategies are in place to reduce stormwater volumes. A database has been established known as the BMP database The International Stormwater BMP Database has recently completed a comprehensive stormwater BMP performance analysis based on data within the dataset. The database contains results of stormwater BMP studies independently conducted and provided by researchers worldwide. Although over 500 studies are entered in the database, very few studies contain microbial data. The study concluded that the majority of conventional stormwater BMP in the database do not appear to be effective at reducing faecal indicator bacteria concentrations to recreational contact. They report that wet ponds and media filters help reduce microbial loadings of stormwater. Grass swales/strips and dry detension basins do not appear to provide meaningful reduction in bacterial concentrations, and often show higher concentrations in the effluent compared with the influent (Jones et al. 2012).

Areas with widely different land-use practices, including agricultural, commercial, rural or residential, can contribute stormwater to environmental waters. The possibility also exists of cross-connections from sewer pipes, or leakage from sewer or septic systems delivering human sewage to the stormwater conveyance system. Both human health risk and strategies for remediation of microbial pollution from stormwater are influenced by the host source of microorganisms, but measurement of indicator bacteria alone does not provide information on this important parameter. Also in order to correctly mange the pollution sources in stormwater it is beneficial to understand the main sources during dry and wet weather events. The benefit of this is that is allows for practices such as illegal sewer connections, leaking sewer pipes, incorrect disposal of pet waste, wildfowl sources to be established. Once these sources are established management steps can be put in place to target them specifically. These include education of the public on correct disposal of pet faeces and the provision of bags and bins to assist with this and repair of damaged pipes.

Currently, water quality criteria do not differentiate risks to human health due to sources of faecal indicator bacteria. Expert panels convened by US Environmental Protection Agency (EPA) and the Water Environment Research Foundation have generally agreed that human sources of bacteria are expected to pose a greater health risk than animals and environmental sources, but have also recommended additional research to better quantify this risk. In Review of Zoonotic Pathogens in Ambient Waters, EPA (2009) concludes "Contamination of recreational waters with faeces from warm-blooded animals poses a risk of zoonotic infection of humans with some of the pathogens in those waters. Although the risk and severity of human illness due to contamination with animal faeces and zoonotic pathogens is most likely lower than the risk and severity of illness from treated or untreated human sewage, currently available data are insufficient to quantify the differences" (Wright Water Engineers 2010). Consequently, recreational water quality guidelines in NZ require both natural and human-caused sources of faecal indicator bacteria to be addressed.

Understanding sources of bacteria is important in selecting appropriate BMPs targeted to these sources. Several methods, known collectively as Faecal Source Tracking (FST), are available to determine the source of biological pollution in stormwater. These methods rely on chemical and biological profiles in the stormwater being determined and attributing a source to these profiles. These methods allow for non point sources of pollution within a stormwater network to be established.

ESR has been commissioned to carry out investigations of elevated bacterial levels in environmental water by several Councils and Regional Councils. The results of two Case studies involving Stormwater are discussed in this paper.

2 FAECAL SOURCE TRACKING

Understanding sources of bacteria is important in selecting appropriate BMPs targeted to these sources. Several methods, known collectively as Faecal Source Tracking (FST), are available to determine the source of biological pollution in stormwater. These methods rely on chemical and biological profiles in the stormwater being determined and attributing a source to these profiles. These methods allow for non point sources of pollution within a stormwater network to be established.

There are an increasingly large number of methods available which can be used to identify the possible sources of faecal pollution. These include molecular markers, faecal sterols and fluorescent whitening agents.

2.1 FAECAL STEROLS

Faecal sterols are a group of C27-, C28- and C29- cholestane-based sterols found mainly in animal faeces. The sterol profile of faeces depends on the interaction of three factors. Firstly, the animal's diet determines the relative quantities of sterol precursors (cholesterol, 24-ethylcholesterol, 24-methylcholesterol, and/or stigmasterol) entering the digestive system. Secondly, animals differ in their endogenous biosynthesis of sterols (for example, human beings on a low cholesterol diet synthesise cholesterol). Third and perhaps most importantly, is that the anaerobic bacteria in the animal gut biohydrogenate sterols to stanols of various isomeric configurations.

The sterol, cholesterol, can be hydrogenated to one or more of four possible stanols. In human beings, cholesterol is preferentially reduced to coprostanol, whereas in the environment cholesterol is predominately reduced to cholestanol. Similarly, plant-derived 24-ethylcholesterol is reduced to 24-ethylcoprostanol and 24-ethylepicoprostanol in the gut of herbivores, whereas in the environment it is primarily reduced to 24-ethylcholestanol. As a consequence, analysis of the sterol composition of animal faeces can generate a sterol fingerprint, which can be quite distinctive from one species to another. Coprostanol is the principal human biomarker. High relative amounts indicate fresh human faecal material. Coprostanol constitutes 60% of the total sterols found in human faeces, while dogs and birds have either no coprostanol or only trace amounts, present in their faeces.

Faecal sterols analysis is performed by filtering 4 litres of river water onto glass fibre filters. Filters can then be stored frozen until analysis. Each sample id derivatised, a system monitoring compound added, and analysed by gas chromatography with mass spectrometric detection. Each sterol and stanol detected is expressed as parts per trillion (ppt).

Interpretation of the sterol is based on comparisons of ratios of key sterols. The ratios typically used used include: two indicators of faecal pollution, three indicators of human faecal source, two of herbivore and two of avian sources and one ratio suggesting plant decay.

2.2 Molecular Markers

There are a range of microorganisms other than faecal coliforms, *Escherichia coli* and enterococci present in the faeces, which are specific to animal hosts. Difficulties in culturing and identifying these organisms have previously limited their useful application to faecal source identification. An alternative approach is to extract total DNA from a water sample and examine the sample using the polymerase chain reaction (PCR) for DNA from source-specific organisms. Four main classes of assays are used in environmental monitoring. The first targets Bacteroidales bacteria which are indicative of general faecal pollution (Siefring et al. 2008). The second targets human-specific Bacteroidales (Shanks et al., 2009), the third also targets Bacteroidales, but in this case indicates an animal specific, canine dominant source (referred to as a dog marker). The final marker is a wildfowl specific E2 marker and is referred to as a duck marker. This marker is common in duck faeces, and has also been detected in geese, seagulls and swans (Devane et al. 2007).

2.3 FLUORSCENT WHITENING AGENTS

FWAs are common constituents of washing powders that adsorb to fabric and brighten clothing. There is a range of FWAs, but only one (4,4′-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)-amino]stilbene-2,2′-disulfonate) is used in New Zealand. Most household plumbing mixes effluent from toilets with "grey water" from washing machines and, as a consequence FWAs are usually associated with human faecal contamination in both septic tanks and community wastewater systems.

FWAs are extracted from 50 mL water samples and analysed by High Pressure Liquid Chromotography (HPLC) (Glipin et al. 2002). Results are expressed in parts per billion (ppb) equivalent to g/litre. Detection of 0.1 ppb of FWA is suggestive of human faecal pollution and a level of 0.2 ppb is indicative of human faecal pollution in a waterway.

At ESR we have applied these techniques to stormwater to determine the sources of elevated bacterial loadings. In this paper we will present three case studies of their application to stormwater in New Zealand.

3 CASE STUDY 1

Previous sampling of the stormwater and river undertaken by the Local Council revealed elevated levels of *E. coli* in. ESR were commissioned to undertake sampling during dry weather events to establish the source of the pollution. Samples of the stormwater and river water were taken from four locations on five occasions over a 30 day period (N=20).

Site 1: Upstream site 1

Samples were taken from the river, on the upstream side of the road. This is a rural area with cows visible from the sampling site. There may be farm inputs to the river further upstream.

Figure 1 Site 1

Site 2: Upstream site 2

Samples were taken from narrow part of the river approximately 200 m upstream of the Site 3 stormwater drain. There is another stormwater drain input upstream of this site.

Site 3: Stormwater outlet

This stormwater outlet drains the stormwater from the surrounding urban area

Site 4: Downstream of Stormwater Drain

Samples were taken adjacent to the bridge on the opposite side of the river, approximately 100 metres downstream from the stormwater outlet.

Figure 2 Sampling Site 4

3.1 RESULTS

3.1.1 WATER MICROBIOLOGY

All samples collected were tested for the concentration of *Escherichia coli* (*E. coli*) present in the water sample. At Site 1 a mean of 264 *E. coli*/100mL were detected, with a peak of 458 *E. coli*/100mL. Even at the minimum level detected this water would not be acceptable for fresh water bathing.

Site 2 contained a mean level of 649 *E. coli*/100mL, with a peak level of 1000 *E. coli*/100mL. Site 3 at the Stormwater outlet contained a mean of 156,00 *E. coli*/100mL, with a range from 13,000 to 580,000 *E. coli*/100mL. At Site 4, a mean of 1,300 *E. coli*/100mL was detected with a range from 400 to 3,400 *E. coli/*100mL. This water contained seawater, and measurements of enterococci are the current recommended standard for marine waters. A mean level of 106 enterococci/100mL was detected at this site. As a running median this would exceed the New Zealand marine bathing guidelines acceptable level of less than 35 enterococci/100mL, and two of the samples exceeded 136 enterococci/100mL. Under this water would be in Alert/Amber Mode II which would require:

- Increase sampling to daily
- Undertake a sanitary survey, identify sources of contamination

3.1.2 FAECAL SOURCE TRACKING

Faecal source tracking was undertaken of the samples to help determine the source of the pollution. Fluorescent Whitening Agents (FWA) concentrations and faecal sterol profiles were determined for the samples.

FWA's were detected in all samples except for one sample at Site 1, and one sample at the Site 2. The levels at Site 1 and Site 2 were all close to the detection limit (0.01 g/100ml) and are probably insignificant. In contrast the detected levels of FWA's in the Stormwater outlet ranged from 1.66 to 26.29 g/100ml. These levels clearly indicate the presence of "grey water". Three of the five Site 4 samples contained significantly elevated FWA level. One sample contained 0.14 g / 100ml and this sample also contained the highest concentration of faecal coliforms of all samples tested.

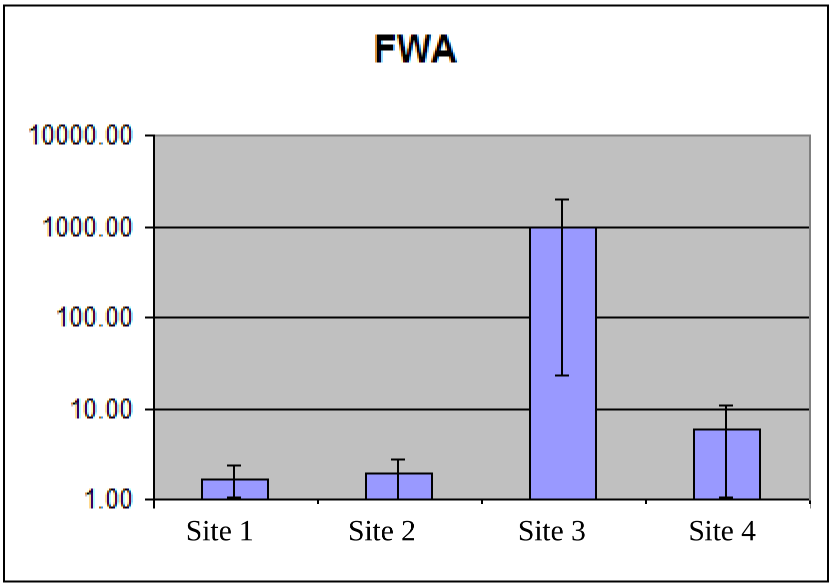

Figure 3 FWA concentrations

The levels of faecal sterols were elevated in the stormwater samples in line with the traditional microbial indicators. Comparison of the ratios of the sterols and stanols all support human faecal contamination at the stormwater drain.

Coprostanol:24-ethylcoprostanol: This ratio in stormwater sample was consistently elevated indicating human faecal pollution.

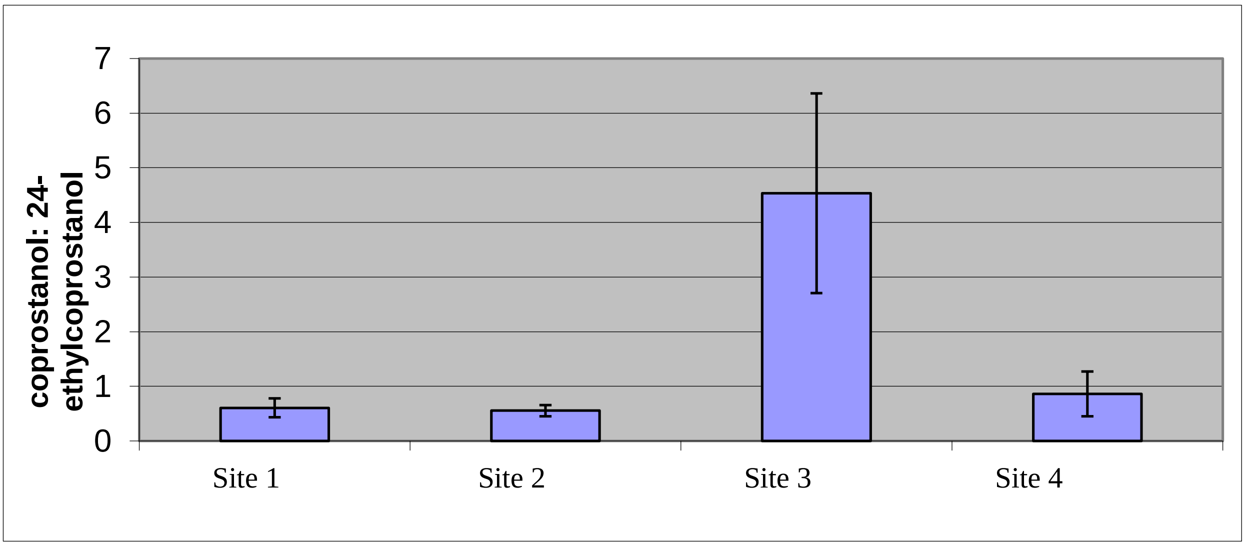

Figure 4 Coprostanol:24-ethylcoprostanol

Coprostanol:epicoprostanol: High ratio suggesting fresh human faecal contamination in the stormwater sample.

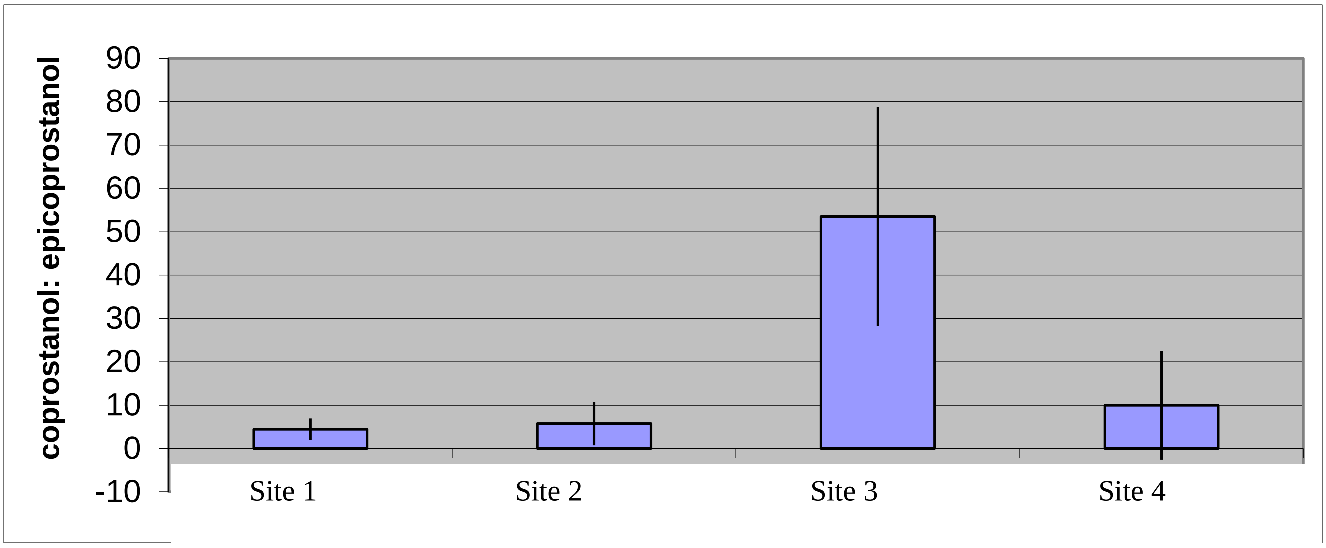

Figure 5 Coprostanol:epicoprostanol

Coprostanol:cholestanol: The ratio of coprostanol:cholestanol in most of the samples were above 0.5 can indicating that the coprostanol present was of faecal origin, and present as a result of preferential reduction from sterols by gut microbiota. The low levels at Site 1, whereas ratio less than 0.3 may suggest environmental reduction by for example anaerobic bacteria in sediments.

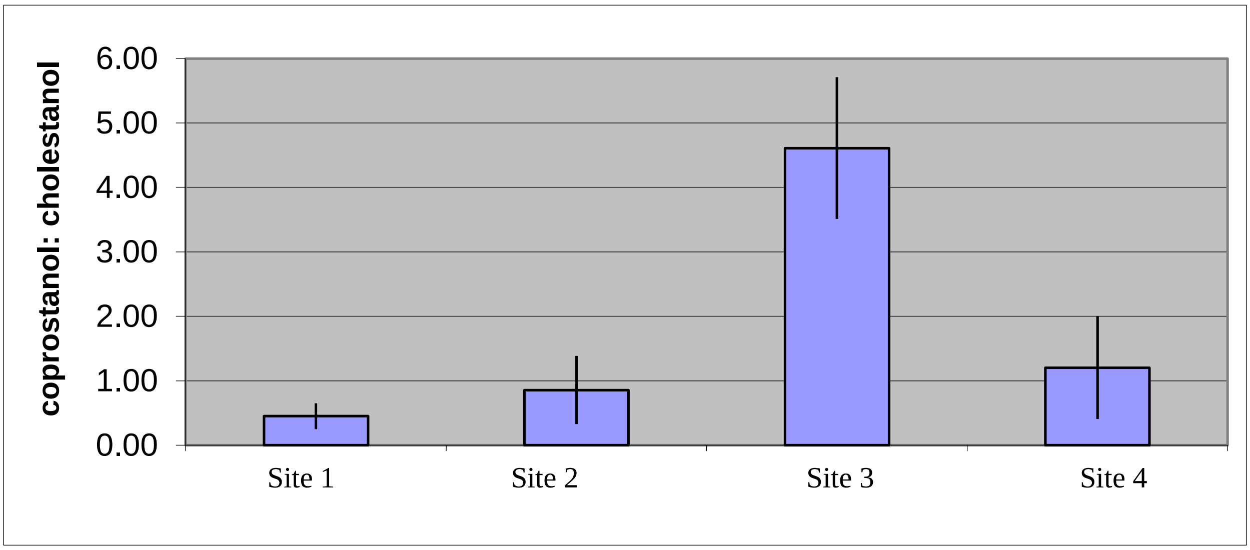

Figure 6 Coprostanol:cholesterol

Coprostanol:cholesterol and 24-ethylcoprostanol: Stigmasterol and 24-ethylcoprostanol:24-ethylcholesterol

Cholesterol levels increased dramatically in stormwater drain, but ratio to Coprostanol showed even more significant increase.

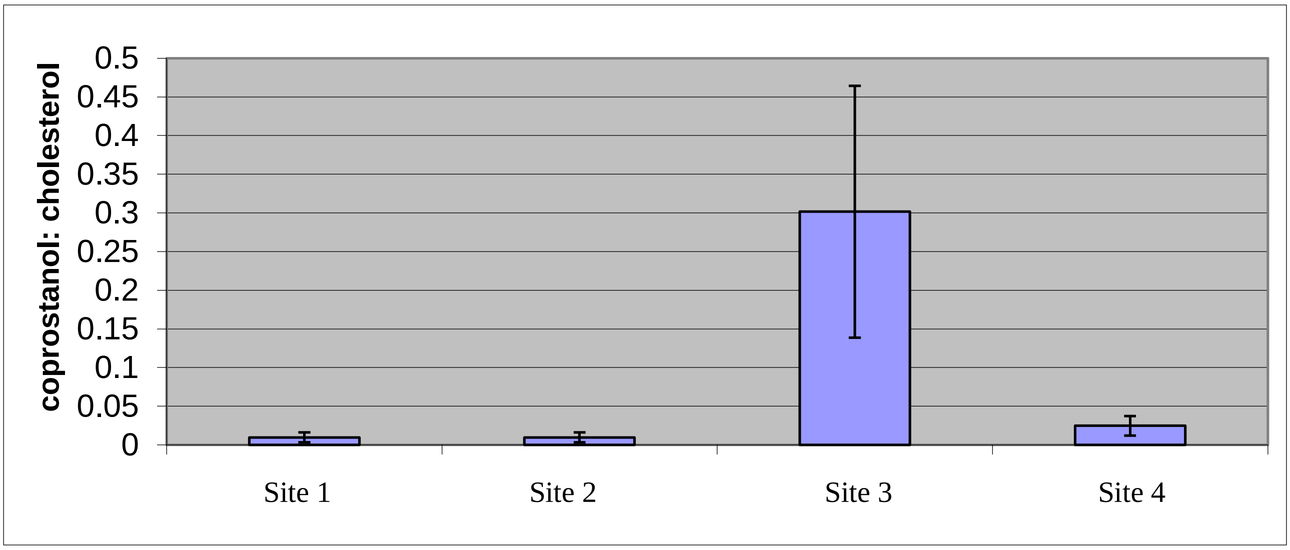

Figure 7 coprostanol:cholesterol

Coprostanol/epicoprostanol to coprostanol/24-ethylcoprostanol

When the ratios of coprostanol/epicoprostanol to coprostanol/24-ethylcoprostanol are plotted alongside previously analysed human and animal effluent, a separation of the sampling sites is clearly evident.

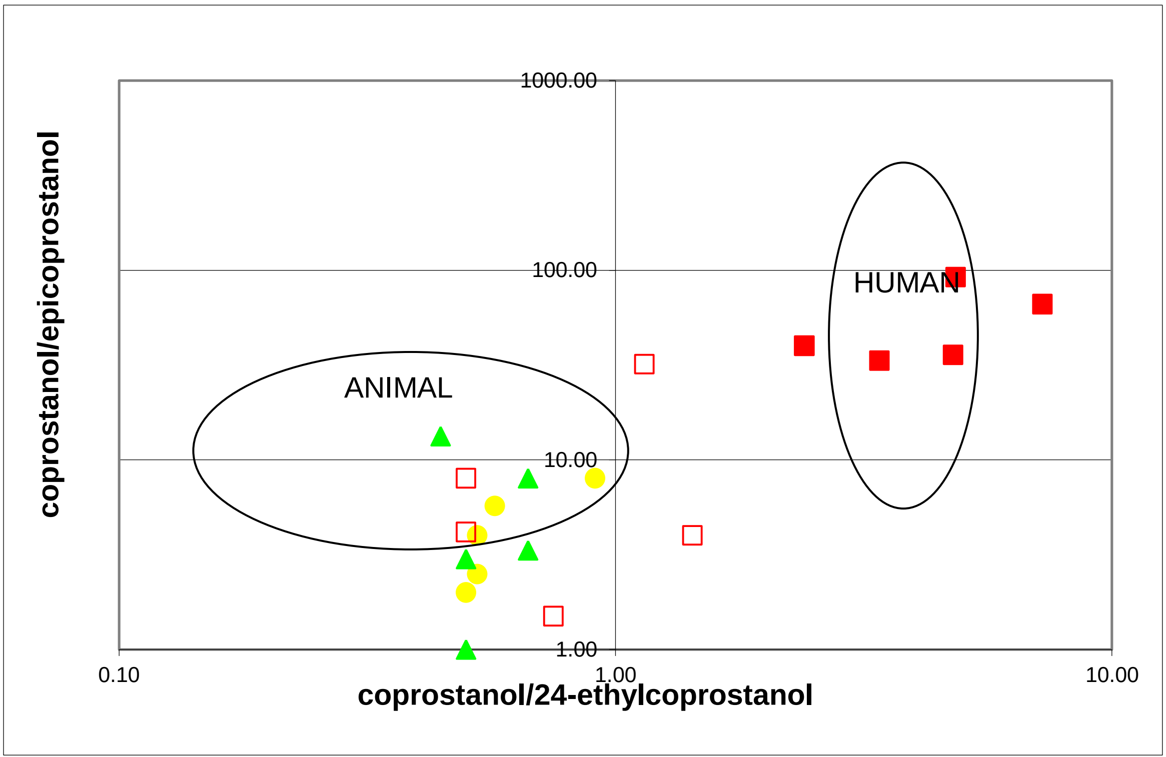

Figure 8 coprostanol:24-ethylcoprostanol

Site 1 Site 2 Site 3 Site 4

The circles indicate the clustering points of previously analysed animal effluent (beef and sheep meat works), and human effluent (septic tanks, wastewater processing plants).

The stormwater samples (■) all cluster with human effluent. The Site 1 (●) and Site 2 (▲) all cluster closer to the previous animal effluent. Three of the Site 4 samples cluster with or close to the animal samples (□), while two samples fall between animal and human.

3.2 CONCLUSIONS

The evidence examined suggests that the increase in traditional microbiological faecal indicators in the storm water drain is due to human faecal input.

Key support for this statement is:

- Significant levels of FWAs in stormwater at levels of at least 100-fold greater than upstream samples. FWAs are man-made chemicals with no known environmental sources, used in washing powders.
- Faecal sterol analysis in gross amount, and ratios, all support human faecal contamination. Stormwater samples cluster with previously analysed human effluent in terms of faecal sterol characteristics.

4 CASE STUDY 2

A series of open concrete channels, into which stormwater feeds had shown, elevated levels of *E. coli*. ESR were commissioned to determine the source of the elevated microbial levels in the stormwater system. A sampling plan was devised involving sampling along the system and at points of entry of stormwater.

Table 1 Sampling sites

Sample no.	Description
B1	Downstream of Stormwater Drain
B2	Tributary Channel
B3	Just by stormwater
B4	Upstream of Stormwater

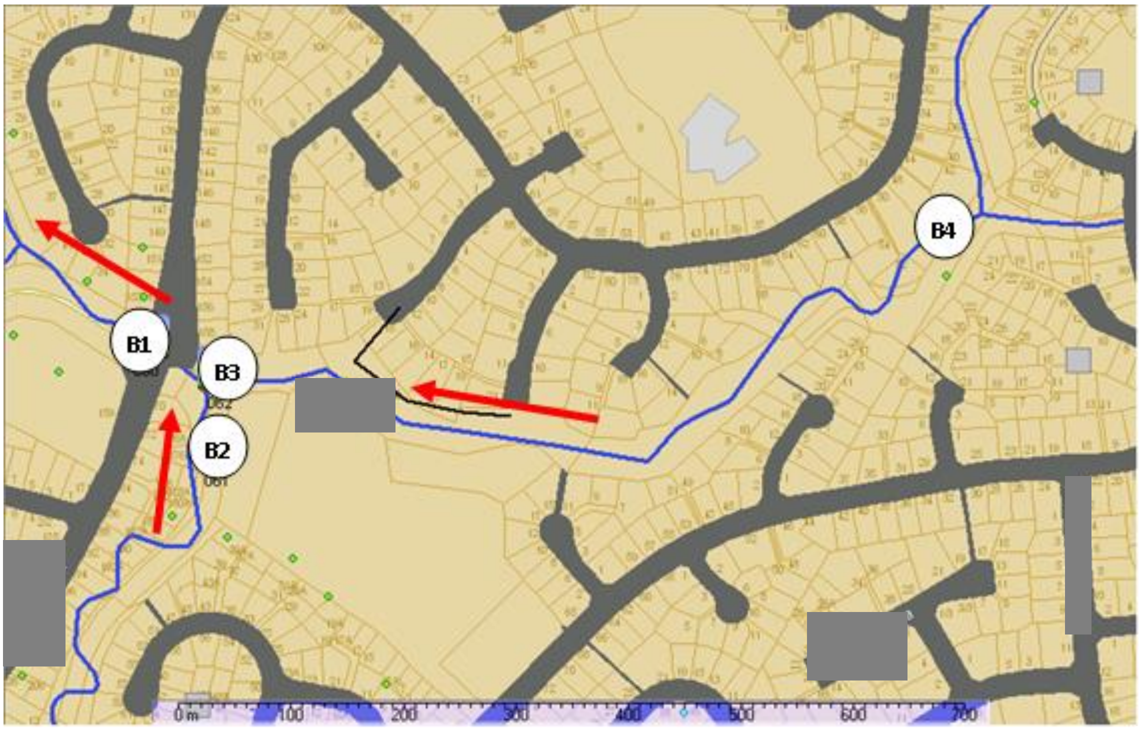

Figure 9 Sampling sites. Arrow indicates the flow of direction

Figure 10 Sample collection

4.1 RESULTS

Analysis of *E. coli* levels indicated very high levels of faecal contamination in samples B1, B3 and B4. The faecal source indicators all showed very strong evidence of human faecal pollution at site B4. Sorbitol fermenting bifidobacteria, and FWA levels are similar concentrations to that found in dilute sewage. *B. adolescentis* marker was only detected in B4, and has lower survival and/or sensitivity than the Human Bacteroides marker, and is indicative of more recent human faecal pollution. The faecal sterol levels in B4 were also very high (2,860 ppt), with a high ratio of coprostanol:cholestanol indicating the sterols were faecally derived, and the ratio of coprostanol:24-ethylcoprostanol significantly above 1.0 suggesting primarily a human source of sterols present.

Sites B1 and B3, 900 metres downstream of B4 contained 20-fold lower levels of FWAs, but still indicative of human faecal pollution. This was supported by the human *Bacteroides* marker, and faecal sterol results. The faecal sterol levels were lower, but still significant, and the ratios support human pollution. Site B2 in contrast contained no significant human faecal source indicators.

Table 2. Measured levels of microbial indicators, FWAs and DNA markers

	Faecal indicators		Human effluent indicators			Non-human indicators	
Sample	**Total coliforms**[a]	***E. coli***[a]	**FWAs**[b]	**Human *Bacteriodes***	**Human *B. adolescentis***	**Herbivore *Bacteriodes***	**Avian E2**
B1	52,000	6,600	0.3	**Positive**	Negative	negative	negative
B2	21,000	300	0.02	negative	Negative	negative	negative
B3	61,000	18,000	0.31	**Positive**	Negative	negative	negative
B4	65,000	6,200	5.84	**Positive**	**Positive**	negative	negative

[a]MPN/100ml; [b]parts per billion (ppb) equivalent to μg/litre

Table 3 Sterol results

Sterol/stanol (ppt)[a]	B1	B2	B3	B4
Coprostanol	965	30	1285	2860
Epicoprostanol	35	<10	45	60
Cholesterol	11095	2095	8775	10205
Cholestanol	890	220	960	925
2,4 Ethylcoprostanol	300	15	405	950
2,4 Methylcholesterol	1835	870	2675	4965
2,4 Ethylepicoprostanol	<10	<10	<10	15
Stigmasterol	1165	895	1275	1485
2,4 Ethylcholesterol	4385	2315	5510	4685
2,4 Ethylcholestanol	155	65	160	205
coprostanol:cholestanol[b]	1.08	0.14	1.34	3.09
coprostanol:cholesterol	0.09	0.01	0.15	0.28
24 ethylcop:24 ethylcholestanol	1.9	0.23	2.53	4.63
coprostanol:24-ethylcoprostanol[c]	3.22	2.00	3.17	3.01
coprostanol:epicoprostanol[d]	27.57		28.56	47.67
Estimate % human sterols	100.00	0	100.00	100.00

[a]Results all parts per trillion.

Coprostanol (principal human stanol), 24-ethylcoprostanol (principal herbivore stanol), Epicoprostanol (minor human stanol), Cholesterol (precursor), cholestanol (stable sterol formed from coprostanol),
[b]ratio >0.5 indicates faecal source of sterols, ratio <0.3 suggests environmental source; [c]ratio >1.0 indicates human faecal pollution; [d]High ratio suggests fresh faecal pollution.

4.2 CONCLUSIONS

The results of the study indicate high levels of human sewage at point B4. This appears to be diluted and degraded as the stream flows down to site B1. The B2 branch contained no significant levels of human source indicators at the time sampled. From the results it appears that sewage is entering the stormwater system.

From these two studies the benefit of Faecal Source Tracking applied to elevated levels of *E. coli* within a stormwater system can be seen. Equally it has benefits when looking at a stormwater system with several possible inputs such as agricultural run-off and wildfowl. Targeted measures can be put in place on the land to mitigate against or reduce the volume of agricultural run-off entering the system. Monitoring can be put in place prior to and post mitigation.

5 DISCUSSION

The microbial profile of Stormwater is a reflection of both the area it originated from and contamination it received while in transit such as leaking sewer pipes and wildfowl inputs. By establishing the sources of contamination in the stormwater in dry and wet weather events suitable mitigations can be put in place. Faecal source tracking can play an important role in this and a useful tool to use in conjunction with routine microbial analysis of stormwater. Although the International Stormwater BMP Database holds information on over 500 studies they determined that less than 50 were found suitable for use to determine the effectiveness of BMP to reduce microbial loadings. Further research is needed on the temporal variation in microbial composition of Stormwater and the influence BMP have on the composition and the FST profile of the Stormwater

REFERENCES

Clary J, Urbonas B, Jones J, Strecker E, Quigley M, O'Brien J. Developing, evaluating and maintaining a standardized stormwater BMP effectiveness database. *Water Sci Technol* 45: 65-73 2002.

Davies CM, Bavor HJ. The fate of stormwater-associated bacteria in constructed wetland and water pollution control pond systems. *J Appl Microbiol* 89: 349-60 2000.

Devane ML, Robson B, Nourozi F, Scholes P, Gilpin BJ. A PCR marker for detection in surface waters of faecal pollution derived from ducks. *Water Res* 41: 3553-60 2007.

Glipin BJ, Gregor JE, Savill MG. Identification of the source of faecal pollution in contaminated rivers. *Water Sci Technol* 46: 9-15 2002.

Jeng HC, England AJ, Bradford HB. Indicator organisms associated with stormwater suspended particles and estuarine sediment. *J Environ Sci Health A Tox Hazard Subst Environ Eng* 40: 779-91 2005.

Jones JE, Clary J, Strecker E, Quigley M, Moeller J 2012 BMP Effectiveness for Nutrients, Bacteria, Solids, Metals, and Runoff Volume. Stormwater.

Siefring S, Varma M, Atikovic E, Wymer L, Haugland RA. Improved real-time PCR assays for the detection of fecal indicator bacteria in surface waters with different instrument and reagent systems. *J Water Health* 6: 225-37 2008.

Wright Water Engineers 2010. International Stormwater Best Management Practices (BMP) Database . Pollutant Category Summary: Fecal Indicator Bacteria 31 p.