Urban stormwater quality monitoring: From sampling to water quality analysis

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***Abstract*— This paper presents the outcomes of urban stormwater quality monitoring and research including associated activities in South East Queensland (SEQ). The issues associated with urban stormwater quality monitoring, ranging from automated field sampling to laboratory analysis of chemical, toxicological and microbiological constituents present in stormwater are elaborated. A medium density residential stormwater supply catchment of 290 hectares in northern Brisbane is presented as a case study and discussed in detail. Preliminary results indicate that the occurrence and concentration of chemical pollutants in urban stormwater runoff and the associated baseline toxicity is relatively low. However, the microbiological quality of stormwater may not be as good as initially perceived with high numbers of faecal indicator bacteria (FIB) detected during wet weather events. In addition, the polymerase chain reaction (PCR) detection of pathogens indicated the presence of human sewage contamination during wet weather events which might be due to potential sewer overflow events. Further monitoring will be conducted to further assess the stormwater quality before undertaking a comprehensive environmental and public health risk assessment.**

I. INTRODUCTION

Stormwater is one of the last major untapped urban water resources that can be exploited as an alternative water source. In South East Queensland (SEQ), it was estimated that the total urban water consumption during the 2009 transition year of high-to-medium level water restriction was 232 GL/annum [1]; whereas the unrestricted water consumption rate in 2004 was 450 GL/annum [2]. On the other hand, the estimated stormwater runoff from urban catchments in SEQ for 2007 is approximately 870 GL/annum [3]. This underlines the potential of stormwater harvesting for meeting the volumetric urban water demand to improve the security of water supply via the non-potable augmenting non-potable supply of traditional mains water demand. At present however, there have been very few prevalence cases in Australia where stormwater is harvested and treated for beneficial and higher value end-uses such as dual reticulation to local households for toilet flushing, cold water laundry use and other external uses.

One of the potential reasons for the limited exploitation of urban stormwater as a substitution water source is the lack of understanding of pollutant occurrence in the environment and the associated environmental and public health risks. Owing to the stochastic nature of hydrology and the source contributions from different anthropogenic activities and land uses, it has been reported that the concentrations of pollutants often exceed standard water quality guidelines [4]. A good understanding on the untreated quality of stormwater is essential as it allows for the development of a risk management framework to ensure water quality excursions are avoided, as well as making informed decisions on the design of "*fit-for-purpose*" water treatment processes. Duncan [5] reviewed the stormwater quality in Australia for suspended solids and nutrients, but not for other emerging pollutants of concern such as heavy metals, emission sourced organic chemicals (i.e. polycyclic aromatic hydrocarbons), pesticides, herbicides and other miscellaneous chemicals. The presence of chemical pollutants poses a long-term chronic health risk; whereas short-term acute health risks are related to pathogens found in urban runoff.

In Australia, the current stormwater quality guideline is the Australian Guidelines for Water Recycling: Stormwater Harvesting and Reuse which only encompasses a limited number of stormwater quality parameters [6]. Additional stormwater quality parameters such as the aforementioned chemicals as well as pathogens should be considered if stormwater is to be used for higher value end-uses (i.e. potential human contact, ingestion and cross-connection of stormwater pipeline). However, the monitoring of stormwater quality is usually severely constrained by the (1) limited monetary budget available for monitoring to enable a full suite of water quality analysis, (2) stochastic variations in rainfall and catchment hydrology and (3) uncertainties in pollutant occurrence in urban runoff. The combination of these factors normally results in an incomplete picture from a stormwater monitoring perspective, in terms of enumerating the potential chemical and microbiological risks in urban stormwater. Previously, a number of studies on analysing stormwater qualities were based on event mean concentration (EMC) to estimate the average pollutants concentrations and loads from rainfall events have been reported. No one study, however, has provided a sound methodological and comprehensive risk

assessment approach to accurately determine the potential environmental and public health risks in stormwater to enable a better risk management framework, water treatment and reuse scheme [7].

This study constitutes one of the first in Australia to concurrently investigate the potential chemical, toxicology and microbiological indicators of raw stormwater from urban catchments. A medium density residential stormwater supply catchment of 290 hectares (ha) in northern Brisbane was used as a case study in this study. A flow-proportional sampling method was used to capture the peak flows in the hydrograph before compositing to a final sample for EMC analysis. The composite sample was analysed using various advanced analytical techniques such as (1) UV and fluorescence spectroscopic methods for organic pollutants, (2) bioassays for different toxicological end-points and (3) standard membrane filtration technique and quantitative PCR for detection and quantification of faecal indicator bacteria (FIB) and pathogens, respectively. With this study, it is anticipated that a greater insight into pollutants in urban stormwater can be gained so that appropriate good management practices can be devised to minimise associated environmental and public health risks where possible.

II. Materials and Methods

A. Sampling Location

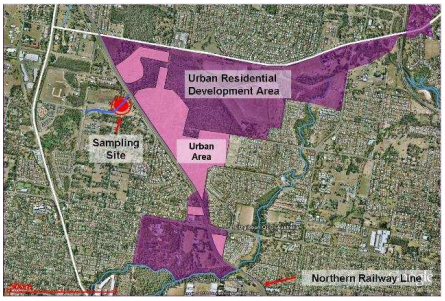

Fig. 1. Current location of the sampling site at a northern Brisbane suburb and the extent of urban and future urban residential development areas.

The studied catchment, Fitzgibbon, is located in the northern suburbs of Brisbane, Australia, and is a medium density residential stormwater supply catchment which covers a total area of 290 ha. The impervious surface coefficient was estimated by using an image classification and cadastral filtering of high-resolution visible aerial photography method and was determined to be 0.25-0.30. This stormwater catchment area is situated in a growing residential hub of northern Brisbane and consists of mixed residential dwellings, commercial centres and buildings, education facilities and semi-rural areas. At present, the Urban Land Development Authority (ULDA) is considering the harvesting of stormwater runoff from this supply catchment to serve as an alternative water source. The harvested stormwater sources will be treated and reticulated back to local households via a dual-reticulation scheme for non-potable uses (i.e. toilet cisterns, cold water laundry tap and general external uses such as car washing, garden watering and public open space irrigation). The sampling point for this study is located at a downstream urban stormwater drain where the proposed dual-reticulation scheme is planned. Fig. 1 shows the current location of the sampling site along with the existing and future urban residential development areas.

B. Sampling Strategy

Three automatic samplers (ISCO 6700 series) are being used in parallel for stormwater sample collection in this study [8]. These samplers were programmed to fill up to 24 x 20 L high density polyethylene containers (HDPE) (Food & Drug approved grade) during a storm event. In this instance, three automatic samplers were used to capture the dynamics of stormwater flow (i.e. peak flows) where the samplers were simultaneously triggered to give a sample volume of 20L for each preset flow threshold. This large sample volume was required for subsequent concentration and analysis of viral and protozoan pathogens. A submersible Argonaut Flow Doppler (Thermo Fisher Sci) was installed to measure the in-stream stormwater flow during the wet weather events, so as to trigger the automatic samplers for sample collection. The Argonaut Flow Doppler is capable of accurate measurement of depth and velocity, after a series of manual flow gauging and calibration procedures. A remote telemetry system was used to notify via SMS alert once the first 20L of samples was collected at the site. This system removes the need for sample refrigeration, as the research team can attend to the site for (almost) immediate sample collection and transfer back to the laboratory for subsequent analysis. Fig. 2 shows the automatic samplers setup with 24 x 20 L HDPE bottles in a custom-made shed. To avoid cross-contamination in the HDPE bottles, they were cleaned using sodium hypochlorite solution (10%) and rinsed with ultra-pure water (MilliQ system, Millipore) in the laboratory before replacing the used HDPE bottles at the field site.

Fig. 2. Automatic sampler (a.k.a. Octopus sampler) setup with 24 x 20 L HDPE bottles in a tailor-made shed.

C. Sample Analyses

CI. UV and Fluorescence Spectroscopic Analysis

UV analysis was performed using a UV spectrometer (Varian 50 Bio). The spectrophotometer was operated at bandwidth 1 nm, with quartz cell of 10 mm path length, wavelength of 190 to 400 nm and at a scanning speed of 190 nm/min (slow). In this instance, the photometric accuracy was 0.004 Abs at 1.0 Abs.

Three-dimensional fluorescence excitation-emission matrix (EEM) spectra were obtained using a spectrofluorometer (Perkin Elmer LS 55) with a wavelength range of 200 nm to 500 nm (for excitation); and 280 nm to 500 nm (for emission). The spectra were taken at an incremental wavelength of 5 nm in excitation; and 2 nm in emission. The EEM value of blank (MQ water) data was subtracted from the analysed samples for blank correction. The data obtained from EEM was analysed using Microsoft Excel®.

CII. Bioassay Analysis

A 30-min bioluminescence inhibition test with the marine bacterium *Vibrio fischeri* was performed according to the protocol of the International Standard Organization (ISO 11348–3) [9]. This test is also often known as the Microtox test. Freeze-dried bacteria were reconstituted in saline buffer containing 4 mM KCl, 10 mM $MgCl_2$, 10 mM MOPS β-[*N*-morpholino] pro-panesulfonic acid), and 346 mM NaCl with the pH adjusted to 7.0 ± 0.2 with HCl/NaOH. Exactly 500 µl of diluted bacterial suspension were mixed with 500 µl of sample in the saline buffer (plus a maximum 2.5% [v/v] of ethanol at the highest-exposure concentration) and incubated for 30 min at 288 ± 1 K. The luminescence output was then read with a LUMIStox 300 luminometer. Data evaluation was performed according to ISO 11348–3 (Eq. 1).

$$\text{bioluminescence inhibition (\%)} = \left(1 - \frac{\text{light intensity}_{\text{sample}}}{\text{light intensity}_{\text{control}}}\right) \cdot 100 \qquad (1)$$

Table 1. Summary of different bioanalytical test battery used for toxociological end-points measurement in stormwater [10].

Mode of action	Assay	Targeted chemicals
Baseline toxicity	Bioluminescence inhibition assay	All chemicals
Acetylcholinesterase (AChE) inhibition	AChE (neurotox)	Organophosphates, carbamate insecticides
Phytotoxicity, photosynthesis (PSII) inhibition	I-PAM (phytotox)	Triazine and phenylurea herbicides
Estrogenic effects	E-SCREEN	Estrogens, estrogenic industrial chemicals
Binding to Ah receptor	AhR CAFLUX	Polychlorinated dibenzodioxins/furans, PCB, PAH
Genotoxicity	umuC (genotox)	Aromatic amines, PAH

CIII. Quantification of FIB and Pathogens

Quantification of FIB (*E. coli* and *Enterococcus* spp.) was performed by standard membrane filtration technique. Briefly, 1 and 10mL samples were filtered through 0.45µm nitrocellulose (Millipore) filter (47mm) and placed on respective selective agar plates in triplicate. *E. coli* was enumerated on Chromocult™ coliform agar (Merck) and *Enterococci* on Chromocult™ enterococci agar (Merck). Plates were incubated at 37°C overnight and then typical colonies were counted to determine the average number of colony forming units (cfu $100mL^{-1}$). The remaining water sample was the concentrated using hollow fiber ultra-filtration using disposable hemoflow cartridges to less than 100 mL.

Nucleic acid was extracted from 200 µL of concentrated samples using the MoBio PowerSoil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA) and stored at -80°C prior to analysis. Duplex PCR was used to improve detection of pathogens from the concentrated water samples. Analysis for the enteric pathogens adenovirus, polyomavirus, *Salmonella enterica*, *Campylobacter spp*, *Giardia lamblia* and *Cryptosporidium parvum* was undertaken using published primer and probe sets. The human faecal contamination marker, *E. faecium esp* gene was detected by using previously published primer sets [11]. PCR was carried out after extraction of DNA from the enriched sample (500 µL). The human specific bacteriodes HF183 gene was detected in the DNA extracted from concentrated water samples with published primer sets [12]. Fresh primer and probe sets for real time PCR using Primer3 software were designed for *C. jejuni* and *C. coli* targeting mapA and ceuE genes, respectively (GenBank numbers X80135 and X88849). A homology search was performed against the GenBank database sequence similarity using BLAST program to check for primer and probe specificity to ensure the specificity of the primers and probe.

Standards for PCR amplification were prepared from the genomic DNA of standard cultures of *C. jejuni*, *C. coli, Salmonella enterica* serovar typhimurium, human adenovirus type 41, and human polyomavirus (JC). The concentration of the genomic DNA was measured by using a NanoDrop ND-1000 spectrophotometer. After calculation of genomic copy numbers, a serial 10-fold dilution (10^6 to 10^0 copies per µl of DNA) was prepared from the genomic DNA. To determine the potential presence of PCR inhibitory substances in the DNA extracted from water samples, a sample of cachou-ph extract was spiked with 10^3 gene copies of adenovirus. The cycle threshold (C_T) values obtained for stormwater samples spiked with adenovirus were compared to those of the control MilliQ water spiked with adenovirus DNA to determine the extent, if any, of PCR inhibition.

Quantitative PCR reactions were performed on Bio-Rad iQ5 (Bio-Rad Laboratories, California, USA), using iQ supermix (Bio-Rad) or So Fast™ EvaGreen® Supermix (Bio-Rad). Bovine serum albumin (BSA) was added to each reaction mixture to a final concentration of 0.2 µg μL^{-1} to relieve PCR inhibition [13]. For each PCR run, a corresponding positive (i.e., target DNA) and negative (sterile water) control were included.

III. Results and Discussion

A. Flow Proportional Sampling

The selection of sampling modes and frequencies was largely based on the prior knowledge of rainfall-runoff within the urban catchment. Typically, the sampling modes are highly catchment site and compound specific. Conventional water sampling was usually operated in common time- or flow-proportional modes [14]. In the case of urban stormwater sampling (i.e. increasing frequencies in peak flows with the impervious surface coefficient), time-proportional sampling might not be the appropriate choice as it requires a rigorous calibration between the rainfall-runoff relationships to inform the sample collection intervals. Flow-proportional sampling modes are easy to implement but still requires a valid scientific justification for the sampling intervals and frequencies. Automated samplers as shown in Fig. 2 are usually used to collect a number of discrete stormwater samples over the hydrograph, usually within a 24 h period. To minimize the analytical costs over the number of discrete stormwater samples (i.e. depending on sampling frequencies), the samples are usually composited to yield an EMC [14]. In previous studies, however, the number of EMC reported for a single catchment was usually quite limited, possibly constrained by available monetary budget, with only a few number of stormwater quality parameters being reported on [7, 8, 14]. This makes a complete stormwater quality risk assessment far from achievable and thus, defies the purpose of water quality monitoring.

In this study, a flow-proportional sampling mode was chosen to pool the discrete samples collected during the

course of a storm event into a composite sample for EMC analysis. Fig. 3 shows an example of an event hydrograph at the Fitzgibbon site where four discrete samples were collected over the storm period. Following this, the samples were pooled according to Eq. 2 to yield a composite sample for subsequent EMC analysis. Rapid spectroscopic methods as described in Section II were used to measure the occurrence of organic chemicals, followed by toxicity measurement as described in Section III. If in any case, the toxicity measurements showed a significant peak in certain classes of toxicity (as classified by their mode of action/targeted chemicals in Table 1), a comprehensive suite of chemical analysis was performed. The composite samples were also subjected to quantification of FIB and pathogens as discussed in Section IV. Other than the standard EMC measurements, currently we are also measuring the concentrations of chemicals and microbiological pollutants for each discrete sample collected during each storm event, in order to validate the robustness of our EMC approach.

$$EMC = \frac{\sum_{1}^{n}(Q_i \quad C_i)}{\sum_{1}^{n} Q_i} \qquad (2)$$

B. Physical and Chemical Analysis

UV spectroscopic method is well known for the qualitative and quantitative study of water quality. Several studies have shown the importance of UV absorption spectra as a qualitative tool to identify the nature and quantity of organic substances, their functionality, and sometimes their molecular weight [15, 16]. Thus far, the application of UV spectroscopic analysis in stormwater has not been used rigorously. The UV scan (absorbance recorded at various wavelengths) can be applied to correlate with several types of organics in the sample. Pollutants in stormwater depend on several factors such as rainfall intensity, antecedent dry weather period, and catchment characteristics. Thus, stormwater being dynamic will change its quality as well as quantity during storm events. Quality and quantity changes include both changes in the composition and concentration of organics in stormwater. The spectral information of the UV range helps explain the nature and possible changes in the composition of the organic matter whilst the absorbance may explain concentration variation.

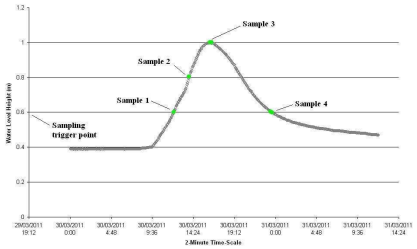

Fig. 3. A wet-weather event hydrograph measured at Fitzgibbon site, and the corresponding sampling regime.

In the past, a number of authors have discussed application of UV spectra and its correlation to the conventional parameters such as TOC, DOM, COD and BOD [17-19]. Aryal et al. [16] reviewed the application of spectroscopy for organic detection. Table 2 summarises key wavelengths and their associated organic properties, used for water quality assessment as reported in the literature.

Table 2. Organic compounds and key wavelength in UV [16].

Wavelength (nm)	Property	Reference
195	Proteins	Yabushita et al., 1987
210	Amino acids	Aitken and Learmonth, 2002
214	Peptides	Kuipper and Gruppen, 2007
230	Proteins	Liu et al., 2009
254	Aromaticity	Her et al., 2008
260	COD	Chevakidagaran, 2005
265	Relative abundance of functional groups	Chen et al, 2002
280	Proteins	Aitken and Learmonth, 2002
285	Humification index	Kalbitz et al., 2000
300	Characterisation of humic substances	Artinger et al., 2000
320	PAHs, Phenolics	Khorassani et al., 1998

Fig. 4 shows the UV spectra of stormwater samples collected from Fitzgibbon site and three other control catchments in SEQ. Urban stormwater samples from the two control catchments of Cabbage Tree Creek and Oxley Creek were very similar and their UV spectra trend reflected the domination of humic acid type [20, 21]. Although the Fitzgibbon site samples showed a similar trend of humic acid type, the slope of the UV spectra is shifted towards the right (indicated by the dotted line and arrow) indicating a dissimilarity in organic matter distribution from the two control catchments. The UniRoad sample showed a shoulder around 220 nm, indicating the presence of some other contaminants besides humic acid type substances. In this instance, it was hard to differentiate the type of organics that the shoulder represents due to the limited research work in stormwater.

Fluorescent spectra, commonly known as EEM has been widely used to identify the nature of organic substances in water and wastewater [22-25]. An advantage of EEM fluorescence spectroscopy is that information regarding the fluorescence characteristics of organics can be obtained by changing excitation wavelength and emission wavelength simultaneously without destroying the samples. EEM results from the spectra provide important information for studying the physical and chemical properties of the organics of various origins in stormwater. Based on the nature of organics and its origin, the spectra are generally divided into five groups. Fig. 5 provides information regarding organics and their appearance in the EEM spectra.

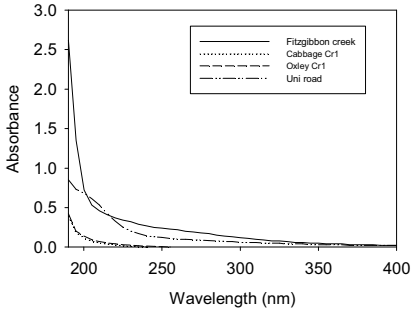

Fig. 4. UV spectra of stormwater collected from the Fitzgibbon catchment and its distinction to the UV spectra of quality from other stormwater catchments.

Fig. 6 shows the EEM spectra of dissolved organics from the Fitzgibbon site in comparison to a control urban catchment (UniRoad). Results in Fig. 6a show a strong occurrence of fulvic acid (Ex/Em: 200-260/380-500) and humic acid (Ex/Em: 280-380/380-500). When the EEM spectra is being compared with the control catchment, it is evident that the presence of organic regions as well as the fluorescent intensity percentage distribution profiles (i.e. relative organic concentrations) for each region is quite distinct. This indicates that the presence of organics in stormwater is quite catchment site specific and might be dominated by the land use characteristics, which determines the potential diffuse sources contribution to stormwater runoffs.

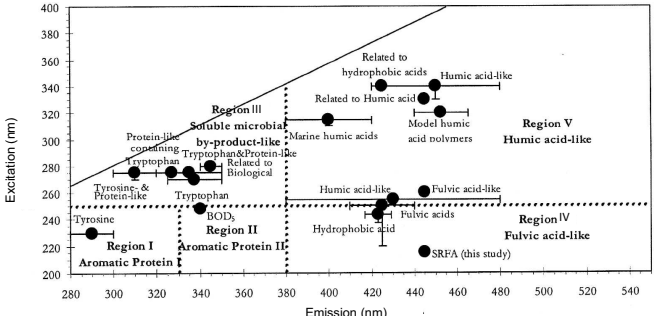

Fig.5. EEM spectra and organics position in the spectra [26].

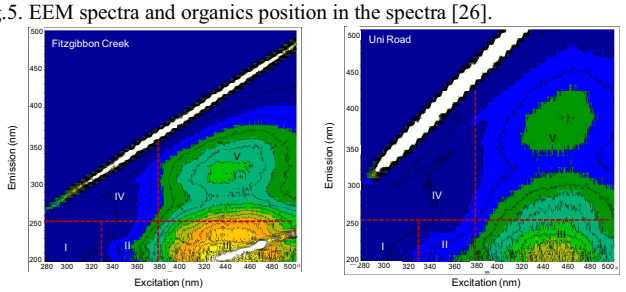

Fig. 6. EEM spectra of dissolved organics in (a) Fitzgibbon stormwater catchment and (b) a control urban catchment (UniRoad).

C. Bioassay Analysis

The major concern of stormwater for receiving water bodies is its toxicity. A previous study has shown the potential impact of stormwater on receiving water bodies [27]. Thus, the toxicity evaluation method may provide complementary information to the chemical analysis of the many pollutants present in stormwater. A number of bioanalytical techniques have appeared in recent research journals [10, 28]. These techniques target the group of chemicals of particular relevance to human and environmental health including genotoxicity, endocrine activity, neurotoxicity, dioxin-like activity and non-specific cell toxicity [10, 29, 30]. Table 1 gives an overview of the bioanalytical test battery used to study wastewater [10]. Application of these tools is relatively new to the stormwater field.

Fig. 7 shows the reduction in luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri* in the Microtox test. Preliminary results showed that the stormwater collected (wet weather events) at the Fitzgibbon site has 10 times less baseline toxicity equivalents than the raw sewage as reported in the Macova et al. [28] study. In comparison, the measured toxicity in stormwater sample from wet weather events has similar level of chemical mixture burden to the secondary treated sewage effluent. This suggests that stormwater is relatively clean and will require fewer polishing treatment stages for the removal of chemical contaminants to achieve a Class A^+ water source status for non-potable uses.

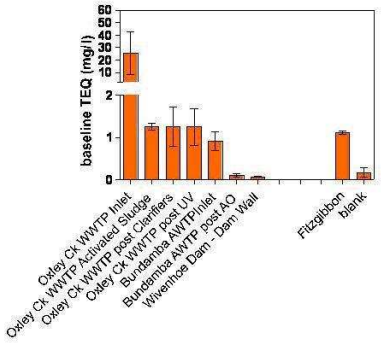

Fig. 7. Baseline toxicity measurement at Fitzgibbon site and its comparison to the baseline toxicity in wastewater [30].

D. Quantification of FIB and Pathogens

Table 3 shows the FIB numbers for stormwater samples collected from two dry weather flow and two wet weather flow events at the Fitzgibbon site. Preliminary results show that the *E. coli* and *Enterococcus* spp. numbers during the wet weather flow events were higher than the dry weather base flows. No significant difference was observed between *E. coli and Enterococcus* spp. numbers during the dry or wet weather flow events.

Table 3. Quantification of FIB in collected stormwater under dry and wet weather events in Fitzgibbon site.

Fitzgibbon Site (Event)	*E. coli* (CFU/100mL)	*Enterococcus* (CFU/100mL)
Dry Weather Flow E1	3.60×10^2	5.10×10^2
Dry Weather Flow E2	1.33×10^2	1.27×10^2
Wet Weather Flow E1	8.93×10^3	2.23×10^3
Wet Weather Flow E2	1.07×10^4	3.11×10^3

Table 4. PCR detection of pathogens in collected stormwater under dry and wet weather events in Fitzgibbon site.

Fitzgibbon Site (Event)	1	2	3	4	5	6	7	8
Dry Weather Flow E1	+	+	-	-	NT	NT	-	+
Dry Weather Flow E2	+	+	-	-	NT	NT	-	-
Wet Weather Flow E1	+	-	+	-	+	-	-	+
Wet Weather Flow E2	+	-	+	+	+	+	+	+

Note: 1: *Campylobacter spp*, 2: *C.jejuni*, 3: *C.coli*, 4: *Salmonella*, 5: *Adenovirus*, 6: *Polyomavirus*, 7: *Esp genes*, 8: *Bacteriodes HF183*. *N.T: Not Tested.*

Table 4 shows the summary of PCR detection of pathogens in stormwater samples from the Fitzgibbon site. All samples tested positive with the *Campylobacter* species primer set, which primarily detects *C. jejuni*, *C. coli* and *C. lari* [31]. After speciation primers analysis, the occurrences of *C.jejuni* and *C.coli* were only detected at certain events. Human specific adenovirus and polyomavirus were also detected in the collected water samples after storm events. The human-specific HF183 bacteriodes marker was widely found in the stormwater samples tested, suggesting the contamination from human sewage during the dry and wet flow events whereas, the *E. faecium esp* gene was found in only 1 wet weather event. Unlike HF183, the *esp* gene was absent in the water

samples containing even higher FIB counts (Table3) after storm events, suggesting its low prevalence in stormwater.

IV. Conclusion

Our research to date has suggested that the occurrence and concentration of chemical pollutants in urban stormwater runoff and the associated baseline toxicity is relatively low when compared to other alternative water sources such as wastewater. However, the microbiological quality of stormwater may not be as good as commonly perceived with high numbers of FIB were found during wet weather flow events. In addition, it was also found that there was potentially human sewage contamination present during wet weather events which suggests potential sewer overflow events. Further stormwater quality monitoring is required to increase the confidence in the potential environmental and public health risks of stormwater. The outcomes of this preliminary monitoring during a few events suggests that further stormwater treatment is required and more particularly, a disinfection treatment to reduce the microbiological pollutants in stormwater.

On-going and detailed monitoring data obtained from this study and other urban catchments throughout Australia will enable a more comprehensive and accurate health risk assessment to be undertaken, in order to determine how urban stormwater can be used for a wider range of end-uses (i.e. potable and non-potable) than currently permitted, as well as the necessary water treatment processes required to mitigate the associated risks in stormwater.

Acknowledgment

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