ABSTRACT
At present major challenges in stormwater harvesting and reuse remain on the characterization of non-point source pollutants, especially microbial pathogens, and the associated public health risks due to the intrinsic short-term acute health risks. Due to the negative surface charge of most microbes, their transport in urban stormwater often relies on the attachment to particulate matter, suspended solids and organic matter. In this study, the transport behavior of two microbes, *Escherichia coli* and *Enterococcus spp.* and their relationship with dissolved organic matter present in stormwater were investigated over five stormwater runoff events collected from a medium density residential urban catchment in South East Queensland. Analyses on organic matter included hydrophobic and hydrophilic fractions. The hydrophillic substances were further fractionated into humic substances, building blocks and low molecular weight organics. *Escherichia coli* numbers of $3.6 \times 10^3$ cfu 100mL$^{-1}$ were detected in the stormwater runoff, while *Enterococcus* spp. numbers as high as $3 \times 10^4$ cfu 100mL$^{-1}$ were detected. The mean dissolved organic carbon in the stormwater samples was 5.1±1.9 mg/L in which hydrophilic constituted the highest mass fraction (60-80%). Results showed that microbial transport was hindered by an increase of the hydrophilic organics fraction, especially the humic fraction.

KEYWORDS
Stormwater; Dissolved organic matter; Microbes.

INTRODUCTION
Stormwater harvesting and reuse in urban areas has been identified as a viable strategy for reducing water demand for both potable and non potable water supplies. However, a wide range of pollutants from non-point sources are the major challenge in its treatment and reuse. These pollutants include pathogens and chemicals and pose a potential human health risk. Besides, elevated concentration of pollutants in stormwater may impair ecological health of receiving water bodies.

Microbes are a major challenge in stormwater recycling and reuse due to their acute toxic effects. They are present in stormwater in both free phase and particle-bound phase. The density (mass per volume) of the pathogens is often very similar to that of water (Bratbak and Dundas, 1984). So, the transport behavior of the microbes is largely influenced by their interactive behavior with the surrounding constituents such as dissolved organic matter present in water. Microbes are usually negatively charged at neutral pH (Fletcher and Marshall 1982, Richardson et al., 2000). Dissolved organic matter (DOM) or dissolved organic carbon (DOC), which is defined as the organic fraction that passes through a 0.45 μm filter, contains both hydrophilic and hydrophobic fractions (Leenheer 1981; Provenzano et al. 2008). The hydrophilic fraction is composed of humic materials, fulvic
materials, polysaccharides, polyphenols, proteins, lipids and other heterogeneous organic molecules (Provenzano et al., 2008) and may possess negative or positive charge based on the functional groups present in the molecules.

Anesio et al., (2004) observed a significant correlation between bacterial abundance and dissolved organic carbon (DOC). Some researchers have documented that interaction occurs between two surface properties: the charge and hydrophobicity/ hydrophilicity of the microbes and dissolved organic matter to be important for transport of microbes (Scholl et al. 1990; Richardson et al., 2000; Mozes et al., 1987). However, as the characterization of dissolved organic matter is complex, studies on correlating organic matter fractions and microbes abundance are so far limited to either hydrophilic/hydrophobic or humic materials.

Every catchment possesses unique landscape, geochemistry, vegetation and climate characteristics. The composite characteristics may make the transport of microbe complex in stormwater runoff. One of the pragmatic approaches to manage and reduce human health risk is to understand transport of microbes during stormwater runoff and design treatment strategy accordingly. The aim of this research is to measure two faecal indicator bacteria (FIB), E. coli and Enterococcus spp. and correlate them to dissolved organic matter and their fractions in multiple samples taken throughout five separate storm events. The results may provide transport behaviour of FIB under the influence of different DOM fractions present in stormwater.

METHODS

Sample collection
Stormwater samples were collected in between March-May 2011 from Fitzgibbon drain over five events from a medium density residential urban catchment in South East Queensland, shown in Figure 1. Dry weather sample (7 March 2011) was collected by grab sampling method whereas wet weather samples were collected in using three automatic samplers (ISCO 6700 series) in ambient temperature. The auto-samplers were programmed to fill up to 24 x 20 L high density polyethylene (HDPE) container (Food & Drug approved grade) during the storm duration. A submersible Argonaut Flow Doppler (Thermo Fisher Sci.) was installed to measure the stormwater flow. The autosamplers were programmed to trigger simultaneously to collect 20 L volume in each step. A remote telemetry system was used to notify the sampling in each container via SMS. A flow-proportional sampling mode was chosen to pool the discrete samples collected over a storm event into a composite sample for event mean concentration (EMC) analysis. Total four wet weather events (31 March, 4 April, 18 April and 30 May) were captured. Collected stormwater samples were transported to laboratory and analysed for faecal indicator bacteria within 10 hours of collection. A portion of collected samples was stored at 4ºC and chemical analysis was carried out within a week. To avoid contamination to the sampling containers, they were cleaned using sodium hypochlorite solution (10%) and rinsed with ultra-pure water (MilliQ system, Millipore) in the laboratory before replacing the filled HDPE bottles at the site.

E. coli and Enterococcus spp.
Quantification of faecal indicating bacteria (FIB) (E. coli and Enterococcus spp.) was performed by standard membrane filtration method (APHA et al., 1998). Briefly, 10 and 1mL of collected samples were filtered through 0.45µm nitrocellulose (Millipore) filters (47mm) and placed on respective selective agar plates in triplicate. E. coli was enumerated on Chromocult™ coliform agar (Merck) and Enterococcus spp. on Chromocult™ Enterococci agar (Merck). Plates were incubated at 37ºC overnight and then typical colonies were counted to determine the average number of colony forming units (cfu 100mL⁻¹).
**Dissolved organic matter**

Samples collected from the site were transferred to the laboratory and immediately filtered through 0.45 µm glass fibre filters. The sample was analysed in UV spectrophotometer (Varian 50 Bio) by scanning between 190-400 nm and its absorbance was recorded at an interval of 1 nm and fluorescence spectrometer (Varian Eclipse) by scanning between excitation 200-500 nm and emission 280-500 nm at 5 nm interval. The filtered samples were also analysed by liquid size exclusion chromatography with organic carbon detection (LC-OCD, DOC Labor, Dr. Huber) to determine dissolved organic fractions. The instrument measured hydrophilic and hydrophobic fractions. In the hydrophilic fraction it distinguishes biopolymers, humic substances, building blocks and low molecular weight organics.

The LC-OCD system consists of a HW-50S column (GROM Analytik + HPLC GmbH, Herrenberg, Germany) and an organic carbon detector based on the Grantzel thin film reactor. 1 mL stormwater samples were injected into the instrument and eluted with the eluent mixture containing 2.5 g L\(^{-1}\) KH\(_2\)PO\(_4\) and 1.5 g L\(^{-1}\) Na\(_2\)HPO\(_4\).2H\(_2\)O at flow rate of 1.1 mL min\(^{-1}\). Suwannee River Humic Acids Standard II (Catalog # 2S101H) and Suwannee River Fulvic Acids Standard II (Catalog # 2S101F) were analyzed as a reference standard. Potassium nitrate and potassium hydrogen cyanide were used to calibrate the detectors.

![Figure 1: Queensland, Australia map and sampling site Fitzgibbon.](image)

**RESULTS AND DISCUSSION**

**Microbes**

Collected samples were analysed for FIB numbers and results were plotted after log\(_{10}\) transformation of raw data (Figure 2). Both *E. coli* and *Enterococcus* spp. numbers were significantly higher during the wet period than the dry period. The *E. coli* numbers varied between \(10^2\) to \(10^3\) 100 cfu mL\(^{-1}\) during the dry and wet periods whereas, the corresponding numbers for *Enterococcus* spp. varied between \(10^2\) to \(10^4\) cfu 100mL\(^{-1}\). *Escherichia coli* numbers as high as 3.6x10\(^3\) cfu 100mL\(^{-1}\) were detected in the stormwater runoff, while *Enterococcus* spp.numbers were one log higher (3x 10\(^3\) cfu 100mL\(^{-1}\)) during the same event. McCarthy observed up to 240,000 MPN/100 mL E.Coli in stormwater first flush in Victoria, Australia. Sauer et al., 2011 presented a wide range of *Ecoli* (1.1x10\(^3\)-3.410\(^6\) cfu per 100mL) and *Enterococcus* spp. (3.4x10\(^3\)-6.1x10\(^6\) cfu per 100mL) in stormwater in Milwaukee, USA.
Dissolved organic matter concentration

Figure 3 shows UV absorbance spectra of stormwater samples. The spectra show a shoulder between 200-220 nm (marked by an arrow). The UV measured at 254 nm showed a range of 0.1-0.06 with standard deviation of 0.04. The UV$_{254}$ nm usually refers to the aromatic organics present in the sample (Yigit et al., 2009; Liu and Fitzpatrick 2010; Philibert et al., 2011). The data show some differences in the absorbance in five events indicating variation in the concentration of dissolved organic matter. Among the five samples the dry weather one (7 March) showed the highest absorbance indicating a higher concentration of organics in the sample. The stagnant water in the drainage seemed to help to release/decompose organics present in the bed sediment (Spellman 2009). Other samples showed a decrease in concentration from every runoff event to the next. The absorbance trend was similar to the trend for humic acid (Korshin et al., 1999; Vaillant et al., 2002) overall but closer observation reveals that there is a weak shoulder between 200-230 nm and after that a gradual tailing of the absorbance above the wavelength of 230 nm. In a previous work, Aryal et al. (2011) related the peak 200-230 nm to amino acids, peptides and proteins in wastewater. Besides, other inorganic peaks (nitrate) also appear in the region (Vaillant et al. 2002).

![Figure 2: E. coli and Enterococcus spp. numbers in stormwater samples](image)

![Figure 3: UV spectra of dissolved organics present in stormwater collected at five samples.](image)

Figure 4 shows LCOCD chromatogram for six samples. The line in Figure 4 show organic carbon detection (OCD). The peaks at 43 min, 50 min, 54 min and 68 min shows humic, building blocks, low molecular weight acids (LMW acids) and low molecular weight neutrals (LMW neutrals). The results show that there is a higher concentration of humics and other organics in the sample on 7 March 2011 (dry event), whereas the lowest concentration occurs in the sample collected on 4 April 2011. We observed a significant difference in chromatogram patterns for each sample (Figure 4).
The different chromatogram patterns in collected samples showed that there was a variation in proportion of organic matter (humics, building blocks, LMW acids and LMW neutrals) between events. Reasons behind variation in organics (hydrology and dry weather period) are not discussed here as it is not within the scope of this paper. Table 1 provides a summary of proportion of organics present in the stormwater samples obtained from LCOCD analysis.

Table 1: Dissolved organic carbon and fractions present in stormwater

<table>
<thead>
<tr>
<th>Sample No</th>
<th>DOC (µg/L)</th>
<th>Bio-polymers</th>
<th>Humics</th>
<th>Building Blocks</th>
<th>*LMW Neutrals</th>
<th>*LMW Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 March (dry)</td>
<td>7779</td>
<td>1508</td>
<td>6273</td>
<td>nq</td>
<td>4184</td>
<td>1445</td>
</tr>
<tr>
<td>31 March (wet)</td>
<td>5274</td>
<td>789</td>
<td>3484</td>
<td>nq</td>
<td>2398</td>
<td>768</td>
</tr>
<tr>
<td>4 April (wet)</td>
<td>2190</td>
<td>802</td>
<td>1388</td>
<td>nq</td>
<td>643</td>
<td>479</td>
</tr>
<tr>
<td>18 April (wet)</td>
<td>5892</td>
<td>1229</td>
<td>4663</td>
<td>87</td>
<td>2695</td>
<td>984</td>
</tr>
<tr>
<td>30 May (wet)</td>
<td>5938</td>
<td>1427</td>
<td>4511</td>
<td>84</td>
<td>2606</td>
<td>952</td>
</tr>
</tbody>
</table>

*Note: DOC=dissolved organic carbon, HOC=hydrophobic organic carbon, CDOC, hydrophilic organic carbon, LMW neutral=low molecular weight neutrals and LMW acids=low molecular weight acids

Figure 5 shows fluorescence spectra of dissolved organic (also known as EEM) on these two events. High spectral intensity on 7 March sample compares to 4 April shows that there is a significant difference in organics. Interpretation of organics in EEM spectra (region I to V) can be found elsewhere (Chen et al. 2003).
Faecal indicator bacteria are mostly particle bound and may compete for adsorption sites to colloidal surface with dissolved organic matter such as humics (Schillinger and Gannon, 1985; Characklis et al, 2005). Since organic matter are mostly negatively charged, the repulsion would be expected as a result of interaction between organic matter and the FIB (Fletcher and Marshall 1982; Mozes et al. 1987) since both of them are negatively charged. A relationship between *E.coli*, *Enterococcus* spp. and organic matter was calculated using regression analysis (Figure 6). In both cases decrease of organic concentration favoured increase in FIB numbers. The correlation coefficient found between FIB and DOC, HOC, CDOC, humics and building blocks is shown below in Table 2. The correlation coefficient shows that hydrophobic fraction may have less influence on mobility of *E.coli* and *Enterococcus* spp. compared to hydrophilic fraction. Hydrophobic fraction being less polar, they mainly interact with microbes via adsorption (Baughman and Paris 1981; Magnusson 1982; Kefford and Marshall 1986). Among the hydrophilic fractions, humic substances showed negative correlation indicating that their presence may hinder the microbes transport. Increase of humic substances possibly altered the surface of colloids and reduced the attachment of FIB (Krometis et al., 2007; Foppen et al, 2008). We also observed a good correlation between *E. coli* and *Enterococcus* spp (R= 0.80). This preliminary result suggests that further studies are necessary to understand the microbes and organic matter’s interrelationship in the stormwater runoff.

Figure 5: Fluorescence spectra of dissolved organic of sample taken on 7 March (left) and 4 April (right).

![Fluorescence spectra](image)

Figure 6: Relationship between *E.coli* (top), *Enterococcus* spp. (bottom) and dissolved organic carbon in stormwater

![Relationship between E.coli and Enterococcus spp](image)
Table 2: Relationship between dissolved organic matter fractions and FIB (number of sample =5) using correlation coefficient (R).

<table>
<thead>
<tr>
<th></th>
<th>HOC</th>
<th>CDOC</th>
<th>Humic acid</th>
<th>Building blocks</th>
<th>LMW neutrals</th>
<th>LMW acids</th>
<th>E. coli</th>
<th>Enterococcus spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC</td>
<td>0.90</td>
<td>1.00</td>
<td>0.97</td>
<td>0.98</td>
<td>-0.11</td>
<td>0.29</td>
<td>-0.74</td>
<td>-0.87</td>
</tr>
<tr>
<td>HOC</td>
<td>0.86</td>
<td>0.79</td>
<td>0.85</td>
<td>0.85</td>
<td>-0.36</td>
<td>0.11</td>
<td>-0.38</td>
<td>-0.72</td>
</tr>
<tr>
<td>CDOC</td>
<td></td>
<td>0.98</td>
<td>0.98</td>
<td>-0.06</td>
<td>0.32</td>
<td>-0.79</td>
<td>-0.88</td>
<td></td>
</tr>
<tr>
<td>Humic acid</td>
<td>0.98</td>
<td>0.98</td>
<td>-0.07</td>
<td>0.33</td>
<td>-0.87</td>
<td>-0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Building blocks</td>
<td>-0.17</td>
<td>0.14</td>
<td>-0.76</td>
<td>-0.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMW neutrals</td>
<td></td>
<td>0.53</td>
<td>0.53</td>
<td>-0.20</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMW acids</td>
<td></td>
<td></td>
<td></td>
<td>0.53</td>
<td>-0.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.80</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSIONS
Stormwater samples collected from a medium residential urban catchment in South East Queensland were analyzed for faecal indicator bacteria and their transport relation with dissolved organic matter for five storm events. The FIB numbers as high as 3x10^4 cfu 100mL^-1 for *Enterococcus* spp. and 3.6x10^3 cfu 100mL^-1 for *Escherichia coli* was observed. The mean dissolved organic carbon in the stormwater samples was 5.1±1.9 mg/L in which hydrophilic constituted the highest mass fraction (60-80%). Results showed a negative correlation between the microbes and dissolved organic carbon especially hydrophilic organic. Within the hydrophilic fraction humics substances hindered the FIB transport more than the other organics. The result suggests further research is necessary to understand the influence of dissolved organic matter in microbes transport in stormwater in different catchments.

ACKNOWLEDGEMENT
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