Changes in the Microbial Quality of a Tropical Freshwater Reservoir Before and After Rain Events

RAJNI KAUSHIK$^{1,2}$ and RAJASEKHAR BALASUBRAMANIAN$^{1,2,*}$

$^{1}$Singapore-Delft Water Alliance, National University of Singapore, Singapore 117576, Singapore

$^{2}$Division of Environmental Science & Engineering, National University of Singapore, Singapore 117576, Singapore

*Corresponding author address:
Associate Professor Rajasekhar Balasubramanian
Department of Civil and Environmental Engineering, National University of Singapore, 117576, Singapore
E-mail: ceerbala@nus.edu.sg Tel: +65 65165135; fax: +65 67791936

ABSTRACT
The impact of microbial loads of fresh rainwater on tropical reservoir water quality upon deposition was studied. Chemical and microbial water quality of fresh rainwater and reservoir water was assessed at four sites of a tropical reservoir in Singapore. The microbial composition of a tropical freshwater reservoir, together with that of the fresh rainwater, was assessed for four months. The study collected water samples during the northeast monsoon period in Singapore. The microbial quality was assessed based on the counts for the microbial indicators Escherichia coli, Total coliforms and Enterococci bacteria by the USEPA approved methods. The levels of E.coli ranged from 0 cfu/100 ml – 75 cfu/100 ml for rainwater. Baseline E.coli levels for reservoir water were 10- 94 cfu/100ml. Site 3 which was influenced by highway and land use showed the maximum counts for all the indicators assessed. However, their was no major increase in the microbial numbers of reservoir due to rainevents. The results of this study suggested that rainfall events did not have any significant impact on the reservoir water quality due to the processes within the reservoir that didn’t sustained the incoming loads.

Keywords: Microbial quality, Indicator bacteria, Reservoir water, Rainwater
1. INTRODUCTION

One out of every five deaths under the age of five worldwide is due to a water-related disease. Water is essential for all living beings and has become a scarce resource today. There is an increasing demand on water resources for development whilst maintaining healthy ecosystems, which put water resources under pressure. As water demand has increased over the last half-century, signs of water shortages have become commonplace (Matondo et al., 2005; Kaldellis and Kondili, 2007). Thus it’s essential to look for sustainable water management methods that incorporate all the possible uses (environment, agriculture, domestic and industry) by promoting ecosystems management, resource efficiency and climate change adaptation.

Rainwater is an alternative water resource as highlighted by the recent studies for it’s significant economic, social and environmental benefits (Sturm et al., 2009). Additional benefits from extensive domestic harvesting of rainwater may also be expected via reductions in the energy demand and greenhouse gas emissions associated with the treatment and distribution of water from centralized supplies (Coombes, 2007). Rainwater harvesting and utilization are good alternatives only in the absence of contaminants and pollution, as the purity of harvested rainwater compared to surface or reservoir water is still a controversial issue (Zhu et al., 2004). Two factors contribute to this controversy. The first being the apparently frequent non-compliance of roof-harvested rainwater with drinking water standards based on the presence of indicator organisms (Gould 1999; Lye 2002) — albeit that the actual health risks associated with non-compliant roof water are yet to be adequately demonstrated. The other is a limited knowledge of the processes at work within a rainwater storage tank, and their impact on end-product quality, for example, impacts of (1) the cleanliness and age of catchments, storage tanks, pipes and gutters and (2) atmospheric conditions each contribute to harvested rainwater quality (Chang et al., 2004; Zhu et al., 2004). Research has been conducted to investigate the prevalence of microorganisms in roof-harvested rainwater (Fujioka et al., 1991) that is exposed to external contaminants as mentioned above. Martin et al. (2010) have also recently reported an increase in bacterial numbers in storage systems following rain events. To the best of our knowledge, no studies have been conducted yet to determine the levels of bacterial pathogens in fresh rainwater prior to its collection and storage in order to assess its possible impact on the quality of aquatic systems upon deposition. Therefore, there is a strong need for quantifying the levels of bacterial pathogens in both fresh rainwater and reservoir water following their simultaneous collection at the same sampling site of the reservoir.

The objectives of this study were to 1) examine the potential of fresh rainwater as an alternative water resource by quantifying the microbial indicators in fresh rainwater prior to its collection and storage in roof –top tanks, 2) study the impact of microbial loads of rainwater on tropical reservoir water quality upon deposition.
2. MATERIALS AND METHODS

Sampling

Singapore is located at the southern tip of the Malayan Peninsula, between latitudes 1°09’N and 1°29’N and longitudes 103°36’E and 104°25’E. The state of Singapore comprises one major island and about 60 smaller ones; it is separated from mainland Malaysia by the Straits of Johor and from the Indonesian Islands by the Straits of Singapore. The main island measures 42 km from east to west and 23 km north to south. Because of its geographic location and maritime exposure, Singapore’s climate is characterized by uniform temperature and pressure, high humidity, and abundant rainfall.

The temperatures range from the minimum 23–26°C to maximum 31–34°C with the daily mean humidity of 84% and an annual rainfall of about 2400 mm. There are no distinct wet or dry seasons as rain falls every month of the year. The two main seasons, based on the prevailing dominant winds, are the Northeast monsoon season (from late November to March), and the Southwest monsoon season (from late May to September). April to early May and October to early November are generally the transitional months separating the monsoons. December is usually the wettest month with an average rainfall of 280 mm.

Reservoir water samples were taken from four different sites. Rainwater samples were also collected at these four sites (No. 1, 2, 3 and 4). Site 1 was located at the centre of the reservoir with no land use. Site 2 was located at the corner of the reservoir with influences from drainage system. Site 3 was situated near the highway and site 4 was near the golf course, having influences from these land use types. The fresh rainwater sample was collected in sterilized glass beakers placed at all the four sites of reservoir four sites of sampling. Dipping the collection tubes attached to sampler collected the reservoir rainwater samples at all the four sites. Both types of samples from all the four sites were transferred to 2 L sterilized bottles for microbiological and chemical analysis. Collected rainwater samples were transported to the laboratory in a chilled-cold box and processed within two hours of collection.

Chemical Analysis

For the chemical analyses, pH, conductivity, turbidity and conductivity were measured for all the samples. All these parameters were analyzed immediately upon arrival at the laboratory. The pH was measured with a combination electrode and meter. Conductivity and turbidity were measured with a conductivity meter and turbidity meter. Samples for, ammonia, phosphorous, nitrate and nitrite analysis were stored at 4°C and analyzed within 48 hrs. The samples were tested for the Ammonia, Nitrate, Nitrite and phosphorus content according to Standard Methods (APHA, 1995).

Microbiological Analysis

Concentrations of total coliform bacteria and E. coli were determined using the m-ColiBlue24® membrane filtration system (Millipore, Cat #M00PMCB24, Bedford, Massachusetts). 100 milliliters of sample was filtered onto cellulose esters membranes using vacuum filtration. The membranes were then incubated for 24 hours in sterile petri dishes.
containing absorbent pads soaked with 2 mL of m-ColiBlue24® broth at 37°C. The colonies in blue color were indicative of E. coli, while total coliforms were enumerated by considering colonies in red color. The average cfu /100ml values obtained for E. coli and Total coliforms are shown in Fig. 1 and Fig.2.

Total heterotrophic plate counts (HPC) and Enterococci counts were also determined for all the samples collected. Briefly, for HPC enumeration, one milliliter of each sample was aseptically plated in replicate onto Plate count agar (Sigma-Aldrich, USA) and incubated at 37°C for a maximum of 48 hrs. The average values obtained for all the samples are represented in Fig. 4. For Enterococci enumeration was performed as per USEPA, Method 1600 (USEPA, 2002). In brief, 100 ml of sample was filtered onto cellulose esters membranes using vacuum filtration. The membranes were then placed on top of mEI Agar incubated for 24 hours at 41 ± 0.5°C. Colonies with a blue halo, regardless of color, were enumerated as Enterococci. The average cfu /100ml values obtained for Enterococci are shown in Fig. 3.

3. RESULTS AND DISCUSSION

Table.1 shows the pH, conductivity and turbidity of the two types of water samples, namely fresh rainwater and reservoir water. The reservoir water (median pH 7.2 and conductivity 267 μS cm⁻¹) exhibited higher values than fresh rainwater (median pH 4.2 and conductivity 25 μS cm⁻¹), latter is a typical acidic value. pH of individual precipitation events, ranged from 3.51 to 5.20 . The pH of all the rainwater samples was below pH 5.6, the value of unpolluted water equilibrated with atmospheric CO₂. According to previous studies, the naturally existing CO₂, NOx and SO₂ will dissolve into the clouds and droplets and result in pH values of the rain in the clean atmosphere to be between 5.0 and 5.6 (Charlson and Rodhe, 1982; Galloway et al., 1993). pH values more than 5.6 indicate the presence of alkaline substances in the rainwater, values less than 4.8 suggest the influence of anthropogenic sources. Thus the pH of 4.2 through the study period reflects a strong impact of anthropogenically derived pollutants on rainfall quality in Singapore.

Microbiological quality of fresh rainwater and reservoir water

Microbial quality is usually assessed by measuring ‘faecal indicator bacteria’ (also referred to as fecal indicator organisms or FIOs). Thermotolerant coliforms (also termed fecal coliforms), Escherichia coli and intestinal enterococci (also termed fecal streptococci) are generally harmless bacteria that are present in high numbers in fecal material and are the most commonly examined FIOs. Their presence in water samples is used to indicate the presence of faecal pollution and the possibility that faecally associated pathogens may also be present. Fig. 1-3 shows the microbial indicators for both fresh rainwater and reservoir water over the four months of sampling.

E. coli are considered the best indicators of faecal contamination in water. They are present in faeces in high numbers. The presence of Thermotolerant Coliforms/ E. coli in water is unacceptable and indicates that a major health risk exists. Levels of Thermotolerant Coliforms/ E. coli are expressed as colony forming units per 100 millilitres (cfu/100ml). The levels of E. coli were found to be 0 cfu/100 ml – 75 cfu/100 ml for fresh rainwater and 10-
94 cfu/100ml in reservoir water (Fig.1). WHO guidelines for drinking water recommend that levels of *E. coli* should be less than 1 cfu/100ml.

Total coliforms were previously considered indicators of faecal contamination. The NHMRC and AWRC “Guidelines for Drinking Water Quality in Australia” do not consider total coliforms useful indicators of faecal contamination in the absence of *E. coli*, and have not proposed any guideline value for Total coliforms. Like HPC, total coliforms can be used as an indicator for the effectiveness of any treatment program. The total coliforms were found in the range of 10 cfu/100ml - 220 cfu/100ml in reservoir water as compared to 10 cfu/100ml - 139 cfu/100ml (Fig.2). The highest counts were obtained from site 3 in both rainwater and reservoir water which is near the highway and gets influenced from the run off from the land use.

*Enterococci* are a specific group of bacteria that are found in high numbers in both human and animal faeces and are therefore a valuable indicator for determining the extent of faecal contamination of a water source. *Enterococci* was found to be in the range of 0 – 11 cfu/100ml in fresh rainwater and was 0 – 35 cfu/100ml in the reservoir water. All the microbial indicators were found to be highest at site 3 in both types of samples. Thus, the results of our microbiological indicator analysis suggested that both the harvested rainwater and the reservoir water samples would be unsafe as drinking water without any treatment.

Heterotrophic plate counts of the reservoir water samples were two log higher than the rainwater. The baseline levels for the reservoir water ranged from 330 -7.9x10^4 cfu/ml, as compared to 280 - 7.2x10^2 cfu/ml. The reason for reservoir water having higher magnitudes of Heterotrophic bacteria and slightly higher number of total coliforms could be the sediments. These are carried by runoff into the reservoir from highland agricultural areas during intensive rainfall events, especially during the monsoon season. This may lead to an inflow of high nutrient concentrations as well as higher loads of microbes (Sargaonkar, 2006; Merz et al., 2006).

It was observed that despite the influx of continued rainfall events, microbial indicators proved incapable of establishing itself as a resident member of the reservoir bacterial community. These results suggested that the reservoir water did not provide a sustainable environment for the propagation of the introduced loads of microbial indicators from continuous rainwater. In fact, the load of viable bacterial contaminants appeared to be quickly eliminated via a decay process involving either removal from the water body (sedimentation or cell death) or conversion to non-culturable forms of bacteria. The net outcome would appear to represent an equilibration of microbial communities within the reservoir to maintain a viable stasis under oligotrophic conditions. Presumably the microbial indicators (*E.coli*, Totoal coliforms and *Enterococci*) were less well adapted to grow under the nutrient limited conditions than the resident heterotrophic bacteria.

Based on the microbial indicator numbers, there was no statistical significant difference in rainwater and reservoir water quality for *E.coli* and *Enterococci*. The microbial quality for rainwater and reservoir was significantly different (p ≤ 0.05) for Heterophic counts and total coliforms.
4. CONCLUSION

In this study, rainwater and reservoir water quality were compared in order to understand the impact of rainwater on the aquatic ecosystems. The results of this study suggest that inspite of presence of microbes or microbial pathogens in rainwater, there were no significant additional of microbial loads to the reservoir water. Reservoir water achieves a stable microbial quality without any impact from the rainwater as the survivability of incoming bacterial loads from rainwater entering the reservoir may be less due to the processes that may act to regulate the survival of incoming bacteria like competitive exclusion and nutrient change. Thus, despite of presence of microbial pathogens, Rainwater harvesting is an extremely promising alternative water resource as artificial reservoirs or catchment.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support and contributions to Singapore-Delft Water Alliance, National University of Singapore (NUS) for the financial support provided to support the pursuit of this study. Rajni Kaushik thanks SDWA for supporting her Ph.D. study. The technical assistance provided by Arun Mahadevan and Chen Sijing is gratefully acknowledged.

REFERENCES


TITLES AND LEGENDS TO FIGURES

Figure 1. Levels of the microbial indicator *E.coli* from both rainwater samples and reservoir samples collected from four sites.

Figure 2. Total coliform counts from both rainwater samples and reservoir samples collected at four sites.

Figure 3. *Enterococcus* counts from both rainwater samples and reservoir samples collected at four sites.

Figure 4. Total heterotrophic plate counts from both rainwater samples and reservoir samples collected at four sites.

TABLE CAPTIONS

Table 1. Summary of chemical analyses of Fresh rainwater and reservoir water quality

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rainwater</td>
</tr>
<tr>
<td>pH</td>
<td>4.2</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>0.91</td>
</tr>
<tr>
<td>Ammonium (mg/liter)</td>
<td>0.52</td>
</tr>
<tr>
<td>Nitrite (mg/liter)</td>
<td>0.04</td>
</tr>
<tr>
<td>Nitrate (mg/liter)</td>
<td>0.90</td>
</tr>
<tr>
<td>Phosphate (mg/liter)</td>
<td>0.54</td>
</tr>
<tr>
<td>Conductivity (μS/cm)</td>
<td>25.33</td>
</tr>
</tbody>
</table>
Fig. 1

![Graph showing E. coli CFU/100ml for rainwater and reservoir water across months.]

Fig. 2

![Graph showing total coliforms (CFU/100ml) for rainwater and reservoir water across sampling period.]

Legend:
- Site 1
- Site 2
- Site 3
- Site 4
Fig. 3

Rainwater Reservoir water

Enterococcus (CFU/100 ml) vs. Sampling Period

Fig. 4

Rainwater Reservoir water

Heterotrophic plate counts (CFU/ml) vs. Sampling Period