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Fecal Coliforms Increase in a Storm Drain Fed Pond After Rain Events

Veronica Albrecht
Department of Biology
Eastern Washington University
526 5th Street
Cheney, WA 99004 USA

Faculty Advisor: Dr. Andrea Castillo

Abstract

Cannon Hill Park Pond (CHPP), Spokane, WA is a residential pond that has historically been maintained by the continuous input of potable water (~14 million gallons/year, City of Spokane Water Quality Report Cannon Hill). In 2010, as part of the Lincoln Street Spokane Urban Runoff Greenways Ecosystem project, a vegetated bio-filtration cell (storm garden) was designed to capture and filter storm water and direct its flow to CHPP via a storm drain. It was meant to mitigate storm water and sanitary sewage overflow during storm events and contribute to CHPP water levels (estimated 315,000 gallons/typical year). While the City of Spokane has conducted some chemical analyses of CHPP, they have yet to conduct any fecal coliform (fc) testing. Our objectives were to compare fc levels in CHPP to levels recommended by the Washington State Department of Ecology (WA-DOE) and to determine if fc levels increased with rain events due to the storm water input from the storm drain. To address these objectives, The membrane filtration method and cultured filters on mFC agar were used to identify fc bacteria. Samples were taken weekly for 15 weeks (10 non-rain events, 5 rain events) from three pond sites: directly in front of the storm drain, from the potable water spigot, and at an off-shore point >10m from the storm drain. Fc levels at the storm drain and at the off-shore site were significantly different (p=0.0498) and both exceeded WA-DOE recommended levels (10% samples exceed 400 fc/100 ml). Additionally, there was a significant increase in fc detected at the storm drain (p=0.007), but not at the off-shore site (p=0.13), after rain events. Fc were never detected in our potable water samples.

Keywords: Fecal Coliforms, Rain Water, Washington

1. Introduction

Water is a common source of human and animal illness due to the presence of pathogenic microorganisms (microbes), which can survive suspended in water for an extended period of time[1]. Both potable water and non-potable water can be a source of pathogenic microbe infection, as waterborne infections may occur through ingestion, contact with contaminated water, or through an airborne route[1]. Non-potable contaminated water sources can include recreational water and water used for agricultural or residential irrigation. Contaminated water can have a variety of pathogens, from viruses and bacteria to protozoa[1]. For example, the hepatitis A virus which causes hepatitis and the bacterium *Salmonella typhi* which causes typhoid fever can both be transmitted through water and usually enter the water through domestic or wild animal feces[1]. Yearly more than 3.4 million people die from water related illnesses, and this is not only a threat in developing countries[2]. In the United States approximately 900,000 waterborne illnesses, are reported each year[1]. Understanding and controlling the sources of contamination and monitoring water is key to limiting infections.

One source of water contamination is by wild animals or pets defecating in or near the water[3]. This can be a problem for surface water like lakes and ponds[4] Migrating birds can also contribute to water contamination as they travel from contaminated bodies of water with high concentration of pathogens to a body of water with low levels or no contamination[5]. Birds can carry a variety of pathogens, including the protozoan *Cryptosporidium*, which can survive in water as hardy oocysts[5]. Migratory birds can also pick up pathogens when foraging for food through eating undigested plant material from cow waste or from contaminated garbage[3].

Recreational water sources and potable drinking water can become contaminated from storm water runoff in urban areas because as storm water flows through urban areas, the water picks up pollutants from impermeable surfaces[6]. Pollutants include heavy metals, oils, and microbial pathogens from soil or feces left by birds, insects, and animals[4]. Storm water runoff can transport microbial pathogens into receiving waters and create elevated concentrations of microbes[7]. In urban areas, impenetrable surfaces such as roads and sidewalks allow pathogens to be transported significant distances[8]. Cities have realized this problem and have undertaken measures to address it. Some cities have built storm gardens and catchment ponds to mitigate storm water run-off and its associated pollutants. Storm gardens are shallow water collection systems filled with layers of soils, sands, clays, and plants that catch, contain and filter pollutants[6]. Quality control ponds similarly catch and retain excess storm water to prevent it from reaching downstream bodies of water[6]. These methods have proven successful but can fail, and therefore routine monitoring should be implemented to protect the public from pollutant and pathogen exposure.

Monitoring water quality is an effective way to prevent infectious disease transmission via contaminated water, however, which pathogen should be monitored is not always clear, considering more than 1400 species of pathogens can survive in water[1,9,10]. Monitoring all common water pathogens would be cost prohibitive and time consuming for municipalities. Identifying pathogenic protozoans is difficult and costly, making them unsuitable for frequent monitoring[11]. Consequently, it is challenging for cities and water municipalities to determine the risk of contamination[11]. Since human and animal fecal matter is a significant source of contamination, fecal indicator microbes are ideal read-outs for water quality monitoring[1]. For a microbe to be a good indicator of fecal contamination, they need to be consistently present in fecal matter, not replicate faithfully outside of their host, and subject to simple and inexpensive detection methods[1]. Fecal coliform bacteria meet these criteria as they are abundant in feces, can survive in water for an extended period, and identified inexpensively and effectively by routine laboratory methods. Fecal coliforms are Gram negative, rod shaped, thermotolerant bacteria that are always found in mammalian feces[12]. In the lab, they can be easily identified by their ability to grow and ferment lactose at 44°C. As much as 95% of the thermotolerant fecal coliforms isolated from mammalian feces are *Escherichia coli*[12].

In this study, fecal coliforms were monitored in Cannon Hill Park Pond (CHPP) in Spokane, Washington. CHPP began receiving storm water overflow via a storm garden built in 2010 as part of the Lincoln Street Spokane Urban Runoff Greenways Ecosystem project[13]. The pond continually loses water because it is naturally porous and has historically been maintained through the input of potable water[13]. The Lincoln Street project was meant to reduce the amount of potable water required to maintain CHPP and to limit sewage overflow into the Spokane River[13]. A fecal coliform analysis has yet to be conducted at CHPP. Our study objectives included determining the fecal coliform levels in CHPP to see how they compared to the Washington State Department of Ecology (WA-DOE) limits, and testing the hypothesis that storm water input would increase the fecal coliform levels in the pond.

2. Methods and Materials

2.1 Site And Collection Description

The Lincoln Street storm garden is located along Lincoln Street, in Spokane, WA 99203, and from 29th to Shoshone Street drains into Cannon Hill Park Pond (Fig. 1a). Water samples were collected from three CHPP sites using Environmental Protection Agency guidelines[14]. The first site was directly in front of the storm drain where the rain water from the storm garden enters the pond (Fig. 1b). The second site was from the spigot that dumps potable water into the pond (Fig. 1b). The third site was from an off-shore point southeast and more than ten meters from the storm drain (Fig. 1b).

The samples were collected in autoclaved 500 ml polypropylene bottles with screw top lids[14]. Each week, six samples were taken, two from each site and the temperature of the pond was recorded. The bottles were filled to within an inch of the top to allow for mixing at the lab and placed in a cooler with ice for transport[14]. All samples were analyzed within 4 hours of collection[14].

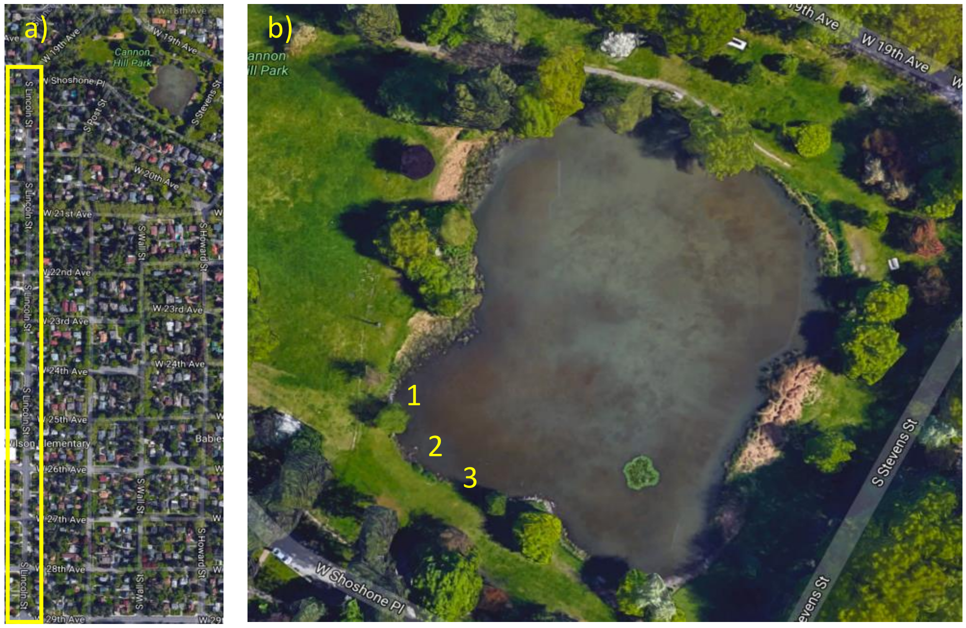

Figure 1. Storm garden and site location. A) The storm garden location along Lincoln Street that drains into CHPP, from 29th to Shoshone Street, yellow box. B) CHPP with sample site locations; storm drain, spigot and offshore site indicated by 1, 2, 3, respectively.

2.2 Fecal Coliform Analysis

Fecal coliform levels in CHPP were determined using the membrane filtration method in conjunction with mFC selective and differential culture media (Becton Dickinson BBL^{TM})[15]. Bacteria were isolated from our water samples by placing a membrane of pore size 0.45μm on a glass filtration apparatus that was sterilized before use and between each sample with an ethanol rinse followed by a sterile water rinse. Water samples were diluted 1:10 and then 10 ml of the sample was drawn through the membrane. Our dilution and volume filtered were based on preliminary experiments that gave countable colony forming units and a sufficient filterable volume. Membranes containing bacteria were placed onto mFC culture media and incubated at 44ºC[15]. A total of twelve samples were analyzed each sampling, two samples per bottle, two bottles per site for three sites. mFC culture media identifies fecal coliform bacteria based on growth and colony color after incubation at 44ºC[15]. Fecal coliform bacteria produce blue colonies when cultured on mFC media due to fermentation of lactose. All cultures were incubated for 18-24 hours.

2.3 Data Analysis

Following incubation of our samples, the number of blue colonies, representing fecal coliform bacteria, and colorless colonies, representing non-fecal coliform bacteria, were counted. An API 20E test kit for enteric bacterial identification (bioMerieux API) was used to verify that the blue colonies were in fact a fecal coliform bacterium, *E. coli.* The API 20E test kit was also used on two of the colorless colonies to verify they were not fecal coliform bacteria. Colony forming units/ml (CFU/ml) or colony forming units/100 ml (CFU/100 ml) were calculated for the rain events versus non-rain events (measurable precipitation within 24 hours of sampling) and WA-DOE comparison samples, respectively. Geometric means for bacteria types were calculated using the four CFUs/ml or CFUs/100 ml per site. A regression was performed with the geometric means and water temperature to determine if there was a correlation between the temperature and bacteria type present. Two tailed t-tests were performed to compare geometric means of the storm drain and off-shore site samples and to compare geometric means of samples taken during rain events and non-rain events.

3. Results

3.1 Assessing Fecal Coliform Levels In CHPP

Fecal coliform levels were monitored in Cannon Hill Park Pond (CHPP, Spokane, WA) to compare them to the Washington State Department of Ecology (WA-DOE) recommended limits for secondary contact recreation water. Three pond sites were sampled in our study, one in front of the storm drain that inputs water from the storm garden, a second at the spigot potable water input and a third at an off-shore site that is greater than 10m from the storm drain. Each site was sampled fifteen times, approximately weekly over a seven-month period from July (2016) to February (2017). The samples were analyzed for fecal coliform bacteria using the membrane filtration method and culture of samples on selective differential mFC media at 44ºC. The CFU/100 ml of fecal coliforms was determined by counting blue colonies present on mFC media; only fecal coliform bacteria grow and ferment lactose (blue colonies) when cultured at 44ºC. Geometric means of CFU/100 ml per site were calculated for each sampling, using data from replicates of the two samples (4 total, two samples x two replicates/sample) collected at each site. The WA-DOE recommended fecal coliform limit in secondary contact recreation water is no more than 10% of samples should exceed 400 CFU/100 ml[16]. Forty percent of the storm drain samples and 27% of the off-shore samples exceeded this limit (Fig. 2).

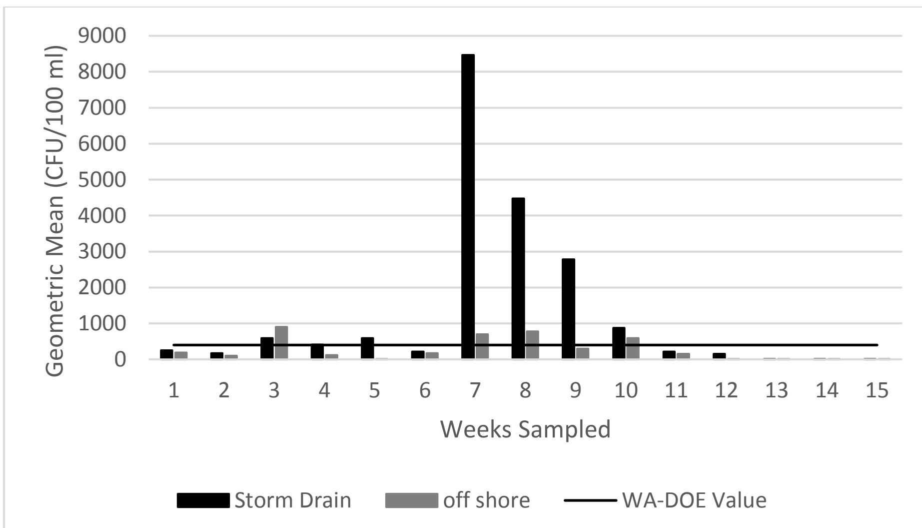

Figure 2. CHPP fecal coliform levels exceed WA-DOE recommended limits for secondary contact recreation water. Geometric means of colony forming units/100ml are presented for each sampling at the storm drain (black bars) and off-shore (gray bars) sites. More than 10% of the storm drain (40%, weeks 3, 5, 7, 8, 9, 10) and off-shore (27%, weeks, 3, 7, 8, 10) site samples exceed 400 CFU/100 ml, above the limit recommended by the WA-DOE for secondary contact recreation water.

3.2 Basis For High Fecal Coliform Levels

The basis for high fecal coliform levels in CHPP was investigated. To determine if temperature might impact fecal coliform levels, a regression was conducted between fecal coliform levels and pond temperature; CHPP temperature was recorded each time water samples were collected (Table 1). The regression analysis indicated there was no significant correlation between these variables at the storm drain ($p= 0.40$) or off-shore ($p=0.47$) sites. A regression analysis was also conducted between non-fecal coliform bacteria and pond temperature. In this case, a significant correlation was detected at both the storm drain ($p= 0.0008$) and off-shore sites ($p=0.0014$).

The analysis above indicated that more storm drain (40%) than off-shore (27%) samples exceeded the WA-DOE recommended fecal coliform levels for secondary contact recreation water. When compared directly, fecal coliform levels at the storm drain (19.5+/-25.8) site were significantly higher ($p = 0.0498$) than at the off-shore (9.9+/-17.8) site. This supported that storm water input via the storm garden/storm drain might contribute to the high fecal coliform levels in the pond. This was further explored by comparing samples taken after rain events with those taken after non-rain events (Table 2). Rain events were defined as any precipitation within 24 hours of sampling; five of the samplings followed rain events and ten followed non-rain events (Fig. 3). Fecal coliform levels detected at the storm drain were significantly higher following rain events than non-rain events (p=0.007). This was not the case for fecal coliform levels detected at the off-shore site (p=0.13). The non-fecal coliforms were not compared after rain events and non-rain events due to their correlation with temperature.

Table 1. Pond temperature measured in ^{o}C

Week sampled	Pond Temperature (°C)
1	18.1
2	23.3
3	ND
4	18.4
5	20.1
6	18.1
7	10.4
8	9.4
9	8.9
10	9.9
11	7.6
12	5.2
13	ND
14	0.8
15	0.6

Table 2. Fecal coliform levels (geometric means, CFU/ml) detected following rain events and non-rain events.

Site	Rain Event Geometric Means (CFU/ml)	Non-rain Geometric Means (CFU/ml)
Storm Drain	84.7	2.5
	44.8	1.7
	27.8	5.9
	1.6	4.1
	0	5.9
		2.2
		8.7
		2.2
		0
		0
Off-shore	0	1.9
	7	1
	7.8	1.2
	3	0
	0	1.7
		5.9
		1.6
		0
		0
		5.1

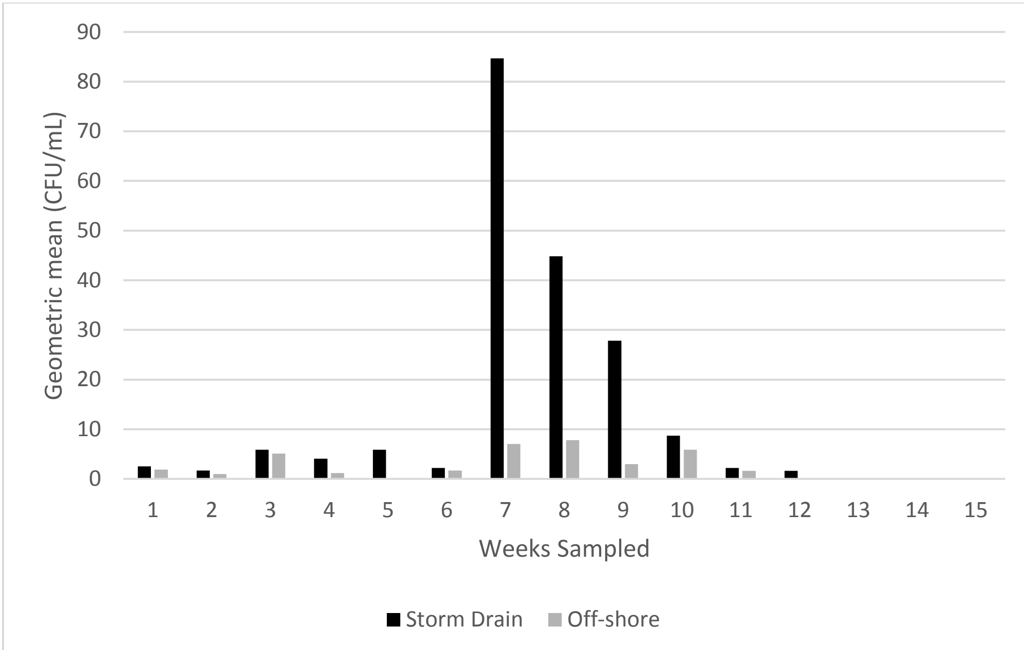

Figure 3. Fecal coliforms significantly increased at the storm drain following rain events. The relationship between weeks sampled and the geometric mean in CFU/ml of fecal coliforms at the storm drain (black bars) and off-shore (grey bars). The sampling days that followed rain events are 7, 8, 9, 12, and 15.

4. Discussion

CHPP is a residential pond designed in 1907 by the Olmsted Brothers to replace a brickyard previously at this site; prior to the construction of a storm garden through the Lincoln Street Spokane Urban Runoff Greenways Ecosystem project (SURGE, Spokane, WA), CHPP water levels were maintained through continuous input of potable water. The objectives of this study were to investigate the overall fecal coliform levels in CHPP and how storm water runoff via the SURGE storm garden would impact CHPP fecal coliform levels.

Fecal coliform levels were above the levels recommended by the WA-DOE for secondary contact recreation water (10% samples greater than 400 CFU/100 ml) at both the storm drain and off-shore sites. This data is consistent with the relatively large number of Washington State water bodies (629) being impaired with bacteria (WA-DOE 303d list)[17]. The sources of fecal coliforms detected in CHPP is likely from both waterfowl that inhabit the pond throughout the year and storm water runoff input via the storm garden. Waterfowl are known to be significant contributors of fecal coliforms to water sources and can harbor enteric pathogens[3]. For example, in a study conducted by Hussong *et al.* (1979) fecal coliform levels increased from a most probable number of 1/100ml to 2,400/100ml in Lake Shore Pond (Madison, WI) following the arrival of a migratory bird population. Additionally, 9.3% of the *E. coli* tested in this study harbored diarrheal disease-causing enterotoxin[18]. We hypothesize that the low level of fecal coliforms detected in CHPP during non-rain events is due to the local waterfowl population and that storm water input from the storm garden is responsible for pushing the fecal coliform levels in CHPP above the WA-DOE recommended limit. In support of this is the significantly higher fecal coliform levels at the storm drain compared to the off-shore site (p=0.0498). Additionally, half of the samples above the WA-DOE recommended fecal coliform limit occurred following rain events when storm water had recently entered (within 24 hours of sampling) CHPP. When only non-rain event fecal coliform data was analyzed, levels at the storm drain and off-shore site were no longer significantly different (p=0.197). This supports that elevated levels of fecal coliforms detected at the storm drain is likely due to storm water input.

Storm water contributes high levels of fecal coliforms at CHPP, but the high levels were not maintained nor equally distributed throughout CHPP. When samples following rain events and non-rain events were compared, fecal coliform levels at the storm drain, but not the off-shore site, were significantly higher (p=0.007 and p=0.13, respectively). There would not be a significant difference between rain event and non-rain event samples if high fecal coliform levels were maintained for longer than the week sampling period, nor a significant difference in storm drain and off-shore fecal coliform levels if fecal coliforms diffused equally throughout the pond. It is likely that some of the fecal coliform entering CHPP via the storm drain was removed from the water column through sedimentation. Approximately 15-30% of fecal coliform adheres to larger suspended particles in storm water and can be removed from the water column by sedimentation[19]. Settling rates calculated by Auer and Nehaus (1993) for attached and unattached fecal coliform suggest that 90% of bacteria will settle out of the water column in approximately two days[20]. Environmental factors, such as exposure to sunlight in the ultraviolet range (254nm), likely also reduced fecal coliform levels due to its bactericidal activity. Lastly, it is possible that the potable water input located between the storm drain and offshore site served to dilute the fecal coliforms entering via the storm drain; detection of significantly lower fecal coliform levels ($p=0.0498$) at the offshore site support this.

The Lincoln Street SURGE project permitted transmission into CHPP fecal coliform levels above the WA-DOE recommended limits for secondary contact recreation water. The SURGE project is a storm garden housed as vegetated curb extensions from 29th to Shoshone along Lincoln Street; it is meant to capture and remove pollutants from storm water and direct it toward CHPP, away from the combined sewer system (CSO basin 24A)[13] Since our study did not sample untreated storm water at a storm garden inlet, we cannot comment on the effectiveness of fecal coliform removal by the storm garden. However, other field studies suggest that fecal coliform removal by storm gardens is highly variable, ranging from 0-100% concentration reduction[21,22,23]. Laboratory experiments are being conducted to identify environmental and storm garden components that influence the efficacy of fecal coliform removal by storm gardens[24]. These types of studies will improve storm garden construction to better remove fecal coliforms.

The pattern of fecal coliform levels in CHPP that was observed during this study was consistent with the "first flush" effect. Fecal coliform levels were highest in four of the five samplings that followed rain events (Fig. 2, weeks 7, 8, 9 and 10) and the levels decreased with each subsequent post rain event sample. The rationale is that pollutants and fecal coliforms accumulate on exposed surfaces in the absence of precipitation and then become dislodged by the rainfall-runoff process[25]. Concentrations of pollutants and fecal coliforms are highest in runoff from the first rain event in a series or the initial part of a long rain event after a period without runoff[25]. Spokane, WA received very little precipitation, an average of 0.15" precipitation/month (April-September, 2016), in the six months preceding our rain event samplings. Flow data collected at the base of the storm garden (Post and Shoshone, Fig. 1) by the city of Spokane supports that no storm runoff entered the CHPP between July 9th and October 3th, 2017[26]. The sample collected on October 5th, following the October 4th rain event, contained the highest fecal coliform levels, 8564 CFU/100mL (Fig. 2).

This study serves as a good baseline for fecal coliform levels in CHPP and supports that high levels of fecal coliforms enter the pond via the storm drain. Although the measured fecal coliform levels in CHPP were above the limit recommended by the WA-DOE for secondary contact recreation water, public risk of infection is likely low; elevated fecal coliforms were detected after rain events most of the time (80%, Fig. 3). During our sampling period, the rain events occurred during the months of October and November when average high temperatures are 58.5°F and 42°F respectively. These temperatures typically discourage pond entry. To eliminate all risk, we recommend the city of Spokane post no swimming signs for people or pets.

5. Acknowledgements

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