The Influences of Green Infrastructure on Levels of Somatic and Male-Specific Coliphages at Shadyside Park

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Abstract

Microbial contamination is still considered one of the major causes of diminished function and quality of urban rivers and streams in Michigan. Floodplains, a type of green infrastructure used to reduce flooding after large amounts of rainfall, were reported to have variable effects of reducing levels of stormwater runoff within these bodies of water (Hubbart et al., 2011). Viral indicators of fecal contamination in bodies of water include male-specific and somatic coliphages. Water samples from Plaster Creek were collected before and after the floodplain in order to identify the levels of somatic and male specific coliphages during dry and wet days (no rainfall vs. rainfall events). Phages were then isolated by using the EPA method 1602, followed by an RNA extraction using reverse-transcriptase PCR in order to identify sources of microbial contamination, such as human and animal fecal matter. This study evaluated the levels of coliphage at Shadyside Park, while also observing if a green infrastructure, a matured floodplain, has reduced these levels compared to past years. Ultimately, we found no significant differences in phage levels between sampling sites surrounding the floodplain and that it shows a variable effect in controlling and reducing coliphage levels in Shadyside Park.

Introduction

The One Health paradigm describes the interconnected relationship between animal, human, and environmental health (Gebreyes et al., 2014). There are many current examples that highlight the importance and severity of these relationships, including environmental justice issues focusing on microbial contamination. Microbial contamination is considered one of the leading causes of impairment in urban rivers and streams in Michigan ("Michigan Water Quality Assessment Report"). Types of microbial contamination include *Escherichia coli* (E. coli), *Salmonella spp*, and human enteric viruses (Ekaterine et al., 2013). Microbial contamination in bodies of water can lead to diseases such as cholera or other bacterial infections ("FAQs: Microbial Contamination"). The source of these contaminants usually come from agricultural runoff, erosion, and flooding that eventually wash down into rivers and streams (Ekaterine et al., 2013). Specifically, flooding events have been shown to increase the amount of microbial contamination (Castro-Ibáñez et al., 2014). In addition, bacteriophages, a type of microbial contaminant, can be used as indicators of fecal contamination in urban rivers and streams (Dias et al., 2018) (Lee et al.). Monitoring these bacteriophages, specifically male specific coliphages, can allow for the planning of preventive measures such as green infrastructure implementation to improve the quality of water.

Best management practices (BMP) are methods that assist in the removal or decrease presence of various pollutants (Collins et al. 2010). Bioswales, green infrastructures, and wet ponds are examples of BMPs that are used to reduce and prevent contamination in bodies of water. It remains unclear whether or not BMPs have any effect on microbial contamination (Ahmed et al., 2019); however, there have been some studies that display that they do assist in the decreased presence of coliphages used for fecal indicators (Karim et al., 2004). Green infrastructure, a type of BMP, is a method to manage water in a way that protects and restores the environment by imitating the natural water cycle. Green infrastructure is a very efficient and practical method of improving the quality of life and safety of animals, humans, and environmental health (Felappi et al., 2020). Specifically, green infrastructure can function by being designed as a preventive measure of high levels of flooding after a significant amount of rainfall (Deeb et

al., 2018). Floodplains, a type of green infrastructure, are used to decrease the amount of stormwater runoff and increase biodiversity with the use of native plant species (Hubbart et al., 2011).

Plaster Creek, an urban watershed located in Grand Rapids, Michigan, is an example of a highly polluted watershed that traverses a number of land use areas, including agricultural, residential, and industrial (Warners et al. 2021). Plaster Creek has also served as an important study system for evaluating the effects of urbanization on biodiversity and evaluating for the efficacy of watershed management plans (Warners et al. 2021). In Plaster Creek-specific watershed management plans, urban runoff and microbial contamination have been identified as problems contributing to the creek's pollution; however, it is still unknown if green infrastructure has the ability to eliminate or reduce microbial contamination in the creek. During the summer of 2019, Plaster Creek Stewards executed a green infrastructure project that proceeded with development of a floodplain at Shadyside park with the purpose to reduce the flow rate of Plaster Creek and prevent the occurrence of erosion from both heavy rainfall and flooding (Reynolds et al., 2016). In addition, microbial contamination (both bacterial and viral) continues to have a significant impact on Plaster Creek both before and after the installation of the floodplain.

Understanding the effects that implementing green infrastructure has on phage levels, specifically fecal bacteria indicator -somatic and male-specific coliphages, was the main objective of this study because these coliphages have been measured previously in high amounts in Plaster Creek (Meer et al., 2021). The goal of this study was to observe whether the matured floodplain at Shadyside Park has influenced the levels of coliphage in Plaster Creek. Studying this influence will specifically identify if the floodplain has any effect on coliphage levels in both dry and wet conditions. This case study tested the hypothesis that there is a relationship between implementing a green infrastructure and phage levels. We predicted that both somatic and male-specifc phage levels were affected by the matured green infrastructure in Plaster Creek. Both coliphage levels were predicted to be low during dry conditions and high during wet conditions due to the increased amount of urban runoff after heavy rainfall (Stallard et al., 2021) (Jiang et al., 2001). However, coliphage samples taken in wet conditions after the floodplain was implemed were predicted to have lower phage levels as compared to before the floodplain was implemented. This relationship was expected due to floodplains being used to decrease the amount of stormwater runoff (Hubbart et al., 2011). Overall, this study will inform the installation of green infrastructures in order to improve water quality of urban rivers and streams just like Plaster Creek.

Materials and Methods

Water Sampling

In order to identify any significant changes, 10 water samples, 5 wet days when rain fall has occurred and 5 dry days where no rainfall occurred, were collected over a course of a ten week period from Shadyside Park in Dutton, Michigan. Wet and dry samples were differentiated by whether or not a large amount of rainfall occurred. Both the inflow and outflow of Plaster Creek at Shadyside Park were chosen as the two sampling sites. Samples were collected and analyzed within 24 hours upon collection. At each site a qualitative assessment, by taking a picture, was taken of the surroundings, specifically looking at the quality of the water and its surroundings (e.g. environmental factors or nearby roads) (Fig. 3). Water samples were then taken in triplicates each from two sites (SS1 and SS2) within Shadyside (Fig. 1), having a total of 6 water samples. Samples were immediately put in ice and were later transported to

storage at 4 degrees Celsius. Water chemistry measurements, including pH, ppm, conductivity (µS), and other measurements such as temperature (ºC) and depth (cm) were taken (Fig. 3).

Phage Counts

Within 24 hours, phages were isolated using the U.S. Environmental Protection Agency Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure, 2007. Positive controls included MS2 (ATCC 15597-B1) with F^{amp} *E. coli* and PhiX174 (ATCC 13706-B1) with CN-13 *E. Coli*, while negative controls included sterile regent water. Positive and negative controls were performed alongside each set of samples. After 16-24 hours of incubation at 37 degrees Celsius , clear visible plaques were counted (Fig. 2) and used to calculate the Plaque Forming Unit per 100 mL (PFU/100 mL) for each sample site and coliphage. A Spot Test was used to verify the plaques on the microbial plates.

RNA Extraction

RNA was isolated by using SIGMA-ALDRICH TRI Reagent LS for fluid samples, RNA Isolation (T3934). The exact amount of RNA extracted was found using the nanogram (Table 4) (RNA Isolation).

RT-PCR

RT: These RNA were reverse-transcribed using OneScript Plus Reverse Transcriptase Protocol. 4 µL if 5X RT buffer, 1 µL of dNTP (10 mM), 1 µL of primer mix, ~1 µg of extracted RNA, 1 µL of One-Script Plus Rtase, as well as ~1-8 of DEPC-treated water was added to a 20 µL optical cap; specific amounts are shown in Table 4. Put in a thermal cycler with a sequence shown in Table 2 with the lid at 42 °C (OneScript).

PCR: AccuPower Taq PCR PreMix was used with cDNA from each water sample as a template. Made a 1:100 dilution of subgroup 1, 1:50 dilution of subgroup 2 and 3, and 1:10 dilution for subgroup 4 for both forward and reverse primers. In a 20 µL optical cap 4 µL of genomic DNA, 2 µL of diluted forward primer, 2 µL of diluted primer, 12 µL of DEPC-treated water was added. Then add Put in a thermal cycler with a sequence shown in Table 3 with the lid at 105 °C. Then put in a -20 °C freezer for storage (AccuPower) (Kirs et al., 2007).

Controls: Procedure was repeated exactly for controls however, for the negative control no RNA extract was added and 13 µL of DEPC-treated water was added to a separate 20 µL optical cap. As well as for the positive control, 4 µL of known RNA was extracted, while 12 µL of DEPC-treated water was added to a separate 20 µL optical cap.

PCR Gel

Made a PCR gel using 2% agarose gel, with 3 comb holders. Once the gel had hardened, 1 L of 1X TBE was added. 10 µL of 100 bp and samples were added to each well. Gel ran at 145 V for 45 minutes, and was put under UV light to view results (Fig. 5).

Statistical Tests

A T-Test: paired two sample means was administered via Excel. Potential significance observed between weather condition and coliphage type across were identified at p=0.05* (Table 1).

Results & Discussion

Significant values were obtained via Excel, by organizing the data to the appropriate categories: all coliphages in wet conditions, somatic coliphage SS1 vs. SS2 in wet conditions, male-specific coliphage SS1 vs. SS2 in wet conditions, and somatic vs male-specific coliphage (repeated these categories but for dry condition). Then a paired two sample means test was administered comparing those categories. Results of the t-test are shown in Table 1. Coliphage counts in PFU/100 mL of both weather conditions are shown in Fig. 4. Viewing these results, there appears to be a greater amount of somatic coliphages within site one than in site two during both weather conditions (Fig. 4). However, the difference between somatic coliphage compared to both weather conditions did not reveal any significant difference, as displayed in Table 1. ($p= 0.121$, $p= 0.477$). There also appears to be a greater amount of male-specific coliphages at SS2 than at SS1 after the fifth time of sampling (Fig 3). Yet, there is no significant difference between male-specifc coliphage compared to both weather conditions, as displayed in Table 1. ($p= 0.203$, $p=0.207$). Furthermore, there is a significant difference between somatic and male-specific coliphages in wet conditions. There are significantly more somatic coliphages than male-specifc after rainfall, as displayed in Table 1. ($p=0.0420$). There doesn't appear to be a significant difference between all the coliphages in SS1 vs. SS2 any weather conditions as shown in Table 1. ($p=0.135$, $p=0.447$), displaying that there is significance when comparing between wet and dry conditions. In addition, the identification of sources of fecal contamination was not completed due to complications with the DNA Gel (Fig. 5). Complications included not a lot of DNA being present (Fig. 5, Attempt 1), inefficient amount of forward and reverse primers being used (Fig. 5, Attempt 1), and a problem with the positive control group (Fig. 5, Attempt 2).

Green infrastructures, in this case floodplains, are used to reduce the amount of stormwater runoff (Hubbart et al., 2011). Our results somewhat display a change in the amount of both coliphage levels and portray that the floodplain did have some effect on reducing the amount of stormwater runoff and an effect on coliphage levels in Shadyside Park. Specifically, somatic coliphage levels in SS1 decreased when looking at levels in SS2 (Fig. 4). The null hypothesis that there is no significant difference when comparing coliphage levels and weather conditions would be accepted, because there is a no significant value when viewing all coliphages in both weather conditions. In addition, the initial hypothesis of there being lower levels of coliphages after the installation of the floodplain in both weather conditions were not statistically supported by the data in its entirety. Specifically, on some days male-specific coliphages displayed greater levels in dry conditions. Explanations for this result would most likely be due to the delaying flow rate after rainfall. Delay in flow rate or "lag time" usually occurs when there has been an inefficient amount of time for an installed BMP to mature (Meals et al., 2010). This research could be extended by investigating how flow rate influences coliphage levels of communities surrounding the Plaster Creek watershed, by the use of level loggers as well as to recollect data after a couple of years.

Conclusions

Overall, over this ten week period, the data shows that the floodplain displays no clear effect of controlling phage levels in Shadyside Park. The data collected provided little significant evidence to support our initial prediction that both coliphage levels would be low in dry conditions and high during wet conditions, and that both coliphages in wet conditions have lower phage levels as compared to before the floodplain was implemented. This data demonstrates that the floodplain's effect on bacterial

contamination at Shadyside is not fully understood and further research must be conducted. One specific complication that could factor in these results is that male-specific coliphages were extremely difficult to count, even after implementing a Spot Test. In addition, the flow rate of the creek was variable specifically during periods after a significant amount of rainfall had occurred. A couple days after heavy rainfall, somatic-coliphage levels specifically in SS2 showed a significant increase on a dry sampling day. This displays that flow rate after heavy rain probably has an effect on coliphage levels and further research must be implemented looking at this relationship.

Acknowledgments

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Tables and Figures

Figure 1. The area surrounding Shadyside Park as well as the specific sample locations of Shadyside 1, Shadyside 2, and flow direction. Arrows display the direction of water flow and "X" portray the site of sample collection.

Figure 2. An example of plaques in a wet sample of somatic coliphage from Shadyside Park.

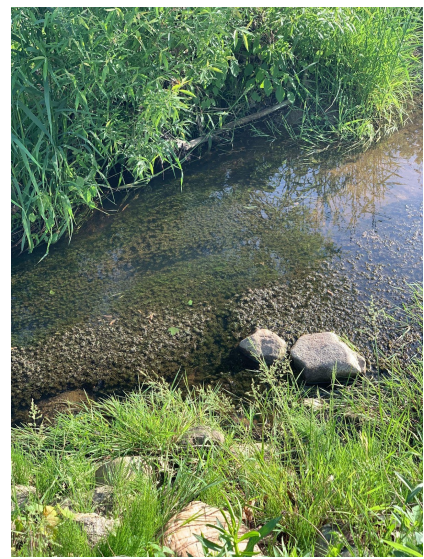| 5/24/2021-Wet	SS1	SS2	5/27/2021-Dry	SS1	SS2				
Conductivty (µS)	766	752	Conductivty (µS)	713	722				
pH	6.82	6.78	pH	7.06	7.12				
Temperature (℃)	15.6	18.1	Temperature (℃)	13.4	12.9				
ppm	396	375	ppm	355	362				
Depth (cm)	20	37	Depth (cm)	21/4.5	42				
6/3/2021-Wet	SS1	SS2	6/8/2021-Wet	SS1	SS2				
	771	764		771	772				
	6.62	6.77		6.6	6.92				
	14.5	15.5		17.8	16.2				
	384	382		384	387				
	12.5	32		5.5 or 6.5	39				
6/10/2021-Dry	SS1	SS2	6/15/2021-Wet	SS1	SS2				
	689	697		735	732				
	6.79	6.95		6.21	6.59				
	19.2	19.1		18.4	20.5				
	345	348		367	365				
	15.5	39		24	40				
6/20/2021-Wet	SS1	SS2	7/1/2021-Dry	SS1	SS2				
Conductivty (µS)	695	697	Conductivty (µS)	534	557				
pH	6.18	6.55	pH	6.16	6.34				
Temperature (℃)	17.8	19.1	Temperature (℃)	18.7	18.7				
ppm	350	348	ppm	268	379				
Depth (cm)	14	39	Depth (cm)	28	48.5				
7/6/2021-Wet	SS1	SS2	7/8/2021-Wet	SS1	SS2				
	656	375		695	X				
	6.08	6.29		7.16	X				
	21.1	22.7		19	X				
	324	340		347	X				
	14.4	47		23	X				
			(Samples taken from more upstream in SS2 due water being too contaminated after storm)						
7/13/2021-Wet	SS1	SS2	7/20/2021-Dry	SS1	SS2				
	720	730		756	750				
	7.62	7.46		7.96	7.72				
	16.7	16.9		17.3	17.5				
	360	363		376	369				
	23	29		13	20				
7/22/2021-Dry	SS1	SS2							
Conductivty (µS)	758	756							
pH	8.86	8.41							
Temperature (℃)	16.2	15.9							
ppm	377	380							
Depth (cm)	13.5	10							

SS2

SS1

SS1

Figure 3. Both qualitative as well as water chemistry measurements, including pH, ppm, conductivity (μS), and other measurements such as temperature (ºC) and depth (cm) of both SS1 and SS2 at different sample collection days.
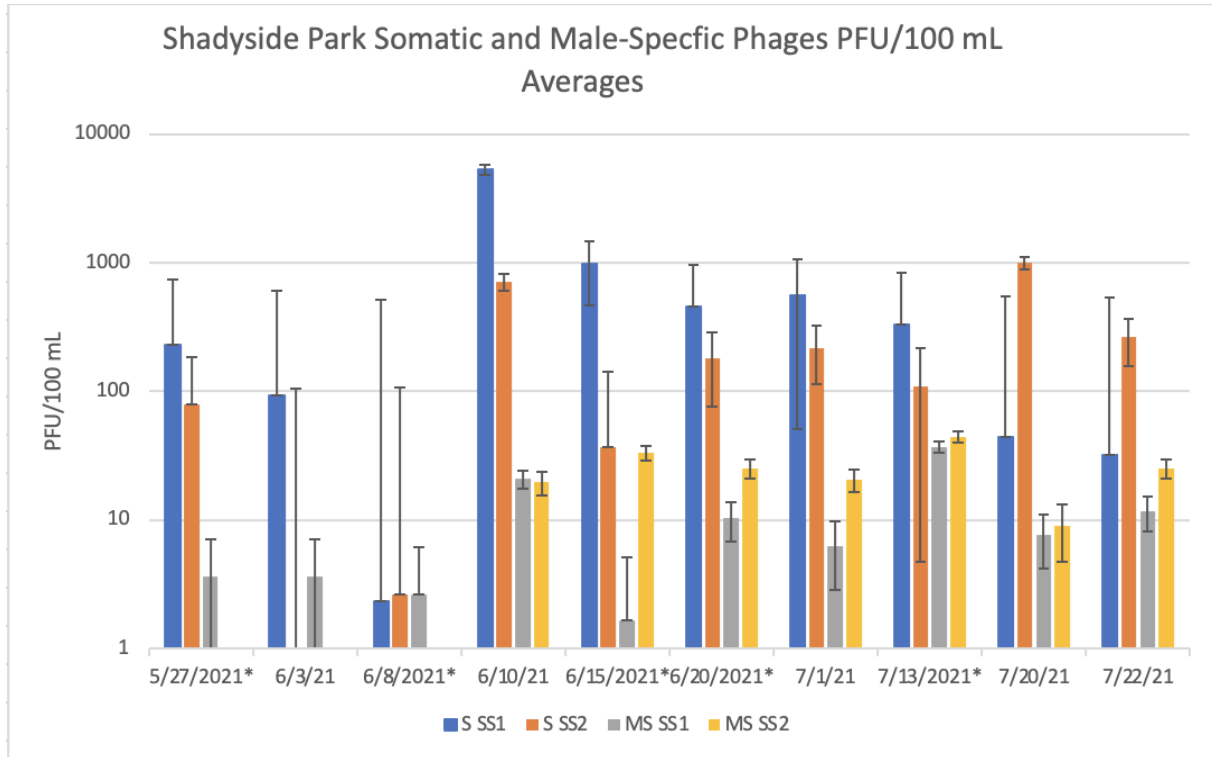

Figure 4. This figure demonstrates the phage levels of both somatic (S) and male-specific (MS) coliphages in PFU/100 mL in both Shadyside Park sites, SS1 and SS2. " * " at the end of the date signifies a wet day.
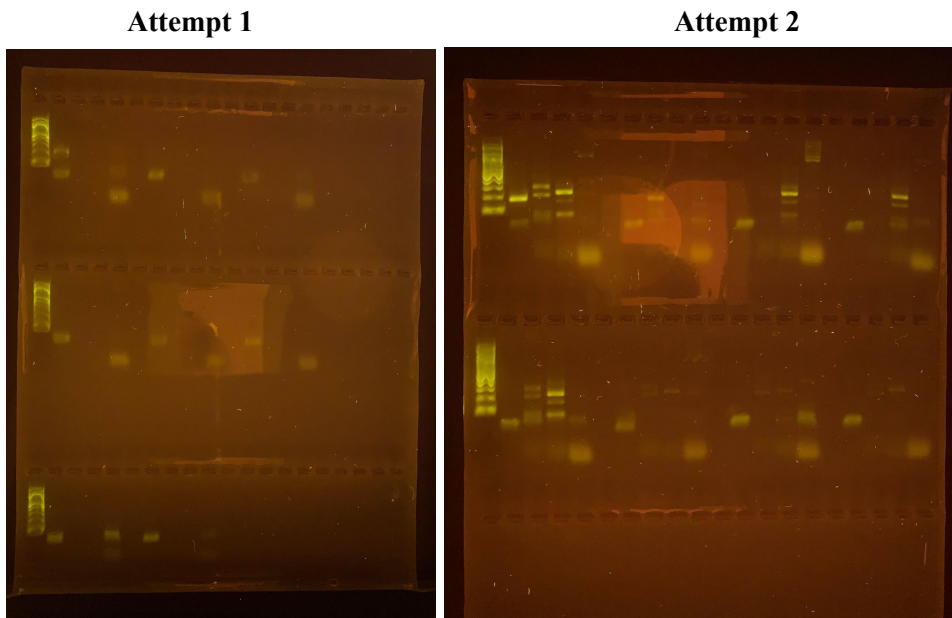
Figure 5. This figure demonstrates the identification of sources of fecal contamination via a DNA Gel.

Table 1. This table shows the p-values for Shadyside Creek PFU/100mL between the weather condition, coliphage type, and site location (SS1 and SS2) with significant values bolded.

Condition	All Coliphages SS1 vs. SS2	Somatic SS1 vs SS2	Male-Specific SS1 vs SS2	Somatic vs. Male-Specific
Wet	0.135	0.121	0.203	**0.0420**
Dry	0.447	0.477	0.207	0.144

Table 2. This table displays the thermal cycler sequence for the OneScript Plus Reverse Transcriptase Protocol with the lid at 42 °C.

Temperature (°C)	Time
50 °C	15 min
85 °C	5 min
4 °C	∞

Table 3. This table displays the thermal cycler sequence for the AccuPower Taq PCR PreMix with the lid at 105 °C.

Temperature (°C)	Time	Number of Repeated Cycles
95 °C	1 min	None
95 °C	30 sec	34 cycles
60 °C	30 sec	34 cycles
72 °C	1 min	34 cycles
72 °C	5 min	None

Table 4. This table shows the exact amount of extracted RNA, the amount of RNA added, and the amount of DEPC-Treated Water added for the OneScript Plus Reverse Transcriptase Protocol.

Amount of Extracted RNA	Amount of Extracted RNA	Amount of DEPC-Treated

from Nanogram (ng)	added to a 20 µL optical cap (µL)	Water added to a 20 µL optical cap (µL)
0 ng (negative control)	0 µL	13 µL
218.5 ng (positive control)	4 µL	12 µL
71.4 ng	12 µL	1 µL
195.7 ng	5 µL	8 µL
195.4 ng	5 µL	8 µL
188.4 ng	5 µL	8 µL
120.0 ng	8 µL	5 µL
133.6 ng	7 µL	9 µL