Detection of E-coli & total coliforms at the Twelvemile Ohio Creek in different climatic conditions

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Abstract

The Ohio River is a large and diverse biome of great importance. With access to 200 communities, and around 6 million people, it has a direct impact on culture, tourism, commercial transportation and people's lives. Thanks to its significance, there must have a constant report of its health. Lately there has been an increase in pollution and bacterial contamination, in its streams, creeks and interiors specifically regarding coliform type bacteria.

During this study, the bacteriological quality of the water of the Twelvemile Ohio Creek was analyzed, since it is suspected that the nearby communities, along with waste from septic tanks, a not appropriate wastewater treatment plants, and agricultural production generate a direct point of contamination to the stream. The quality of the water from the Twelvemile Ohio Creek was determined and compared in different weather conditions, taking as the main aspect the number of coliform colonies 1) total and 2) fecal during the months of June-July 2021: also, there were different physicochemical aspects of water measured. Water samples were taken from five determined study sites, with priority being the areas with the greatest anthropogenic influence: where three of the five study sites were located within the stream while the remaining two were located on the outskirts of it. For the identification of the presence of bacterial communities, the ¨Coliscan EasyGel¨ technique was used, while the physicochemical parameters were determined using the ¨YSI¨ instrument.

The results showed a difference in the bacterial concentration in both climatic conditions. A clear increase in the concentration of fecal coliforms was noted at stations SS4 and mainly in SS5, possibly due to the low water flow present in the twelve-mile Ohio creek compared to the other stations studied in addition to their closeness to human settlements.

Introduction

The objective of this study is to measure the bacterial quality of the twelve-mile Ohio creek under different weather conditions: specifically, the concentration of total and fecal coliform type bacteria.

The presence of *E-coli* indicates fecal contamination in water, since this microorganism is a normal inhabitant of the digestive tract of warm-blooded animals and is rarely found in water or soil that has not suffered some type of fecal contamination, therefore it is considered as universal indicator. This microorganism generates an alert to any water supply system since its presence alone can generate gastroenteritis and cause death as in the case of the *E-coli* strain or it can suggest the presence of other highly pathogenic microorganisms such as *Salmonella*, *Shigella* , *Klebsiella*, *Listeria*.

The identification of total coliforms is more difficult since they can come from soil, and from other various sources, so they are not always intestinal. The presence of Coliforms suggests flaws in the efficacy of the water body treatment.

Table 1: Level of E-coli permitted for different types of water (ADEQ, 2010 and EPA, 2009)

Purpose	Level of *E-coli*
Drinking water	Zero
Surface Water Full Body Contact (swimming)	235 cfu/100 mL
Surface Water Partial Body Contact (fishing, boating, etc…)	575 cfu/100 mL
Wastewater (irrigation or discharge)	<2.2cfu&100 mL <1.0cfu&100 mL

To differentiate E.coli type bacteria from total coliforms and verify the safety of the system, the Coliscan Easy Gel technique was used.

Sample Site

The five study sites(SS) are located at the inside and closeness of the twelve-mile Ohio creek. Twelve-mile Creek is a tributary to the Ohio River that is in Campbell County, where it founds itself surrounded by almost 2,600 households. The SS5 is deep inside the creek, the SS4 is located mid creek, and the SS3 is located on the creek's mouth. Differently the SS2 is downstream outside the creek, and the SS1 is located upstream outside the creek.

Figure 1: Location of study sites at the Twelvemile Ohio Creek

Methods

The Coliscan EasyGel formula contains a sugar linked to a dye that, when it acts on the enzyme *B-galactosidase*, turns the colony a pink color. Similarly, there is a second sugar linked to a different dye that produces a blue-green color when the enzyme *B-glucuronidase* acts. Because fecal coliform-type bacteria produce both enzymes, *E-coli* colonies will grow purple (pink + blue-green).

1. **In the field Sample Collection:**

- Collect the water samples of the five study sites.

- Record sample ID on each vile tube for every study site and take water samples back to the lab in a period of 1 hour. Take physic-chemical parameters at each Study site.

2. **Inside Plating:**

- Deposit 3mL of water sample in the Coliscan Easy Gel bottle. Repeat for the second duplicate sample.

- Record on the bottom of the petri dish the site ID, date, the time, the volume of the sample and the name of the sample (#1 or #2).

- Pour the Coliscan EasyGel medium bottles with the water sample into the petri dish making sure that the entire bottom is coated. Leave the petri dish on a leveled surface until it solidifies.

3. **Incubation Period:** After solidification.

- Store petri dishes on an inverted position. Leave inverted petri dishes at room temperature (20°C - 24°C) for 48+ hours.

- After formation, allow 24-30+ hours for the maturation: do not surpass the 72-hour limit or colonies will reproduce.

4. **Counting and identification:**

- Record the number of purple and pink colonies for each sample.

- Divide 100 by the sample size (3 mL) and multiply this dilution factor by the counted colonies.

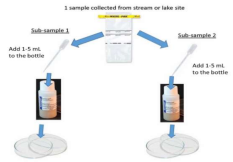


Figure 2: General steps to E-coli sampling (Utah water watch Coliscan Easygel)

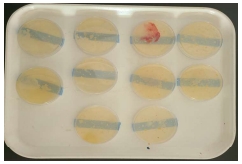


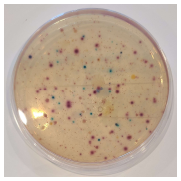
Figure 3: Inverted coliform samples, start of incubation.

Results

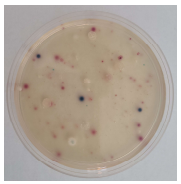
The results of the microbiological parameters evaluated in the twelve-mile Ohio creek showed variations in the two climatic seasons. Both counts of total coliforms and fecal coliforms were higher during the wet climatic conditions compared to dry at the SS4 and SS5 study sites, respectively. Likewise, the lowest levels of total and fecal coliforms were presented in stations SS3, SS2 and SS1, showing similar values.

Total coliform colonies at study sites SS5 and SS4 were higher in both climatic conditions. In wet conditions, the study sites SS5 and SS4 showed to have a value of (3846.15cfu / 100mL) and (3846.15cfu / 100mL) respectively: these being the highest values. The study sites SS3, SS2 and SS1 were shown to have a value of (2214.45cfu / 100mL), (1531.8cfu / 100mL) and (2231.1cfu / 100mL) respectively, these being the lowest values. Under dry conditions, the study sites SS5 and SS4 presented values of (3646.35cfu / 100mL) and (1448.75cfu / 100mL). The study sites SS3, SS2 and SS1 presented values of (1348.65cfu / 100mL), (1531.81cfu / 100mL) and (687.65cfu / 100mL).

The colonies of fecal coliforms in the study sites SS5 and SS4 were higher in both climatic conditions equally. In wet conditions, the SS5 and SS4 sites showed a value of (1615.05cfu / 100mL) and (1481.85cfu / 100mL) respectively. The study sites SS3, SS2 and SS1 showed a value of (965.7cfu / 100mL), (432.9cfu / 100mL) and (532.8cfu / 100mL) respectively. Under dry conditions, the study sites SS5 and SS4 presented values of (882.45cfu / 100mL) and (349.65cfu / 100mL). The study sites SS3, SS2 and SS1 presented values of (299.7cfu / 100mL), (183.15cfu / 100mL) and (133.2cfu / 100mL).



Figures 4: Developed coliform sample from SS2 at wet conditions



Figures 5: Developed coliform sample from SS2 at dry conditions

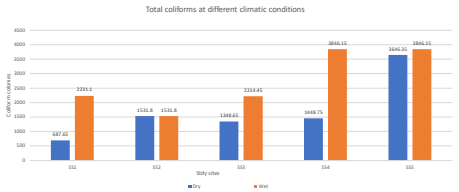


Figure 6: Distribution of total coliforms at both climatic conditions.

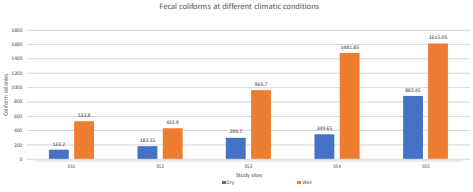


Figure 7: Distribution of fecal coliforms at different climatic conditions

Discussion

It is speculated that the high concentrations of fecal and total coliforms in the Twelvemile Ohio Creek during the wet conditions are attributed to rural-type human settlements as it is not known if they possess a solid waste collection plan and sewage treatment systems for the water that corresponds to this type of discharge in flood situations.

Sites SS4 and SS5 had a higher bacteriological concentration, possibly due to the little water flow present in the creek as opposed to the river: in addition to the closeness to human settlements.

The sites that had a lower bacteriological concentration (SS3, SS2 and SS1), was possibly due to the principle of low influence of anthropic activities related to agriculture, livestock and surrounding populations, in addition to the higher water flow thanks to the proximity of the River.

The study sites SS1, SS2, SS3 and SS4 in dry conditions had a lower *E-coli* concentration than (575 cfu / 100mL), required for the development of recreational activities, however the SS5 site had a higher concentration, disabling it for full and partial contact recreational activities.

In wet conditions, sites SS1 and SS2 had lower *E-coli* concentration levels than (575 cfu / 100mL), required for the development of recreational activities. Contrary to the sites SS3, SS4 and SS5 which are higher, disabling them for consumption and recreational use.

Conclusions

The presence of bacteria indicative of fecal contamination (fecal coliforms) and potential pathogens is reported for the study sites in the twelve-mile Ohio creek: the possible impact of discharges of domestic and agricultural sewage, human settlements and low vegetation cover were seen in the study sites SS4 and SS5 in greater proportion. Even though the creek cannot be used for direct contact activities and consumption in wet situations, it can be used for agricultural preservation of fauna and flora.

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For Further Information

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