



## Case Report

# Detection of Coliform Bacteria from Water and Soil at Recreation Park in Malaysia

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## Abstract

**Background and Objective:** Polluted water in a natural recreation river can cause an adverse effect on human health if it is mistakenly consumed by the users. Bacteria presence with the group of Enterobacteriaceae, like an *E. coli* survived well in the environment and is often used as an indicator for water contamination. The main aim of this study was to determine the presence of *E. coli* bacteria in a famous water recreation Park in Malaysia via soil and water sampling. **Materials and Methods:** The study was carried out during a student's practical visit in May, 2019 at a recreation waterpark at Hulu Langat, Selangor. Both water sample and soil samples were collected from a few regions at the recreation park for *E. coli* investigation. Next, test like BOD estimation and colilert test was carried out in the university laboratory to detect the presence of *E. coli* bacteria. **Results:** The entire water sample collected from the upper, mid and lower regions was positive in colilert test, which indicated the presence of *E. coli* bacteria. The BOD reading was above 3, which showed high pollution rate. For the soil testing, an *E. coli* bacterium was found in the EMB agar. **Conclusion:** Therefore, awareness of the potential risk associated with this recreation water park needs to be communicated with the public and users should not contaminate the water and surrounding areas while visiting.

**Key words:** Recreation water, *E. coli*, water pollution, soil pollution, natural park, colilert, soil pollution

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Water in the natural recreation river has higher chance to become polluted as it is not treated and is filled with local campers and tourists most of the time. Polluted recreation water contains an essential microbe that causes travelers' diarrhea and waterborne illnesses<sup>1</sup>. Water pollution caused by fecal contamination contains *Enterobacteriaceae* which can invade hosts as they are motile, possess intestine colonizing ability and to have endotoxin/enterotoxin that can cause harmful illness to human<sup>2</sup>. Clinical symptoms for example acute gastrointestinal were observed previously among children who accidentally swallowed recreational water<sup>3</sup>. Since screening for water at all ends is impossible and the number of pathogens is small, the presence of coliform *E. coli* bacteria is used as an indicator to identify water contamination. Human activities such as in industries, agriculture, untreated waste dumping can also contribute to water pollution. The degradation in water quality is a widely discussed issue in developing tropical countries, where the treatment of effluents before being discharged into the water bodies is of low priority<sup>4</sup>.

Sungai Tekala Recreational Forest (Hutan Lipur Sungai Tekala) is located 43 km from Kuala Lumpur City in the Hulu Langat area, close to Semenyih Dam. It forms part of the Sungai Lalang Forest Reserve which in turn is part of State Park Forest, also known as Taman Warisan Negeri Selangor, Malaysia. This area is popular among the locals to enjoy recreational activities at the scenic river. Therefore, it is considered aptly significant to investigate and determine the potential risks of bacteria presence which may lead to waterborne illness<sup>1</sup>. Thus, this study aimed to investigate the presence of indicator microorganisms that can cause water-borne diseases in the study area.

## MATERIALS AND METHODS

**Study area:** The study was carried out at a natural recreation park at Hulu Langat, Selangor, Malaysia during a student's practical visit in May 2019. The recreation park was Sg. Tekala waterfall and the experimental procedures were carried out at Environmental Health Laboratory at UniKL, MESTECH.

**Sample collection:** Water samples were collected from Sg. Tekala (GPS Coordinate: 3.058982, 101.873135) in the month of May, 2019 and the surrounding temperature was around 22-27°C. Water samples were collected from three different locations of the waterfall, upper site, middle site and lower

site. For each site, three replicates of water were collected by immersing a sterile 1000 mL of polypropylene bottle. The samples were kept in the 4°C icebox during transportation to the lab. For soil samples, around 3 grams of soil were collected at the depth of 3 cm from the upper site, middle site and lower site and kept in sterile poly bags in the icebox before transporting to the laboratory.

**Detection of coliform bacteria:** Total coliform determination in water samples was carried out using the USEPA method 9223B (Colilert-18/Quanti-Tray 2000). Colilert reagents were applied to detect the presence of *Escherichia coli* (*E. coli*) in the water sample. Those water samples were then observed under the light of the UV chamber. The positive result for *E. coli* for this test would appear yellow in color and fluorescent under the UV chamber. Next, the samples were streaked on Eosin Methylene Blue (EMB) agar. The samples were also being incubated in lactose broth and with this test, one can observe the ability of the samples to ferment lactose. Additionally, an API test was being carried out to determine the species of bacteria.

**Biochemical Oxygen Demand (BOD) estimation:** Biochemical Oxygen Demand or Biological Oxygen Demand is a measurement of the amount of Dissolved Oxygen (DO) that is used by aerobic microorganisms when decomposing organic matter in water. Basically, for BOD measurement, the collected water was transferred into a 2 L bottle and shaken vigorously. Using a Dissolved Oxygen Sensor that has just been calibrated, the DO was measured and recorded.

**Detection of bacteria from soil:** To isolate bacteria from the soil samples, 1 g of the soil from the upper site, middle site and lower site were serially diluted in a solution of 100 mL distilled water. Total 1 mL of the solution in the bottle is transferred to the tube labeled  $10^{-1}$  using a new pipette and swirled gently until the solution is well mixed. The method is repeated to transfer the solution from the  $10^{-1}$  tube to the  $10^{-6}$  tube. By using a new pipette, the solution is also streaked in Eosin Methylene Blue (EMB) agar. The results obtained from the Petri plate were then taken for the gram staining process.

## RESULT AND DISCUSSION

For the water samples, it was observed that all samples turned yellow within 24 hrs of incubation and under UV light, the water sample became fluorescent (Fig. 1). This indicated

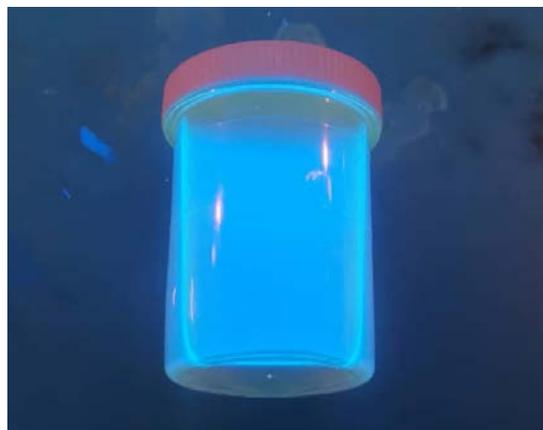


Fig. 1: Colilert test under UV light, the water (from the upper, middle and lower site) became fluorescent within 24 hrs of incubations at 37°C

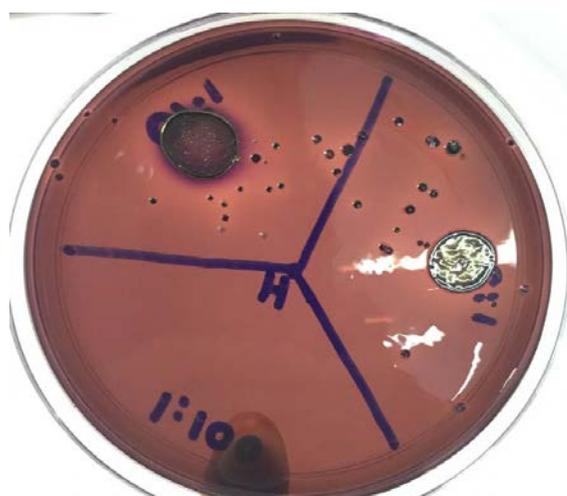


Fig. 2: Single colonies on the eosin methylene blue (EMB) agar

Table 1: Reading of BOD from various site

Site	BOD (mg L <sup>-1</sup> )
Upper	6.21
Middle	7.88
Lower	7.99

No conflict of interest reported

that the water sample has a presence of *E. coli* bacteria and possible contamination. Next, for the BOD measurement, all sites had BOD above 3 (scale for recreation use with contact body) which indicated poor water quality (Table 1). For confirmation of bacteria from soil and water, both plates had the presence of metallic green colonies which indicated the presence of *Escherichia coli* bacteria (Fig. 2).

This brief study via sampling of water and soil has indicated that Sg. Tekala has a water pollution issue.

Despite it's mild to a strong flow of running water, we found the presence of *E. coli* bacteria in all the sites, upper, middle and lower. Gram staining for water and soil showed rod shape crystal violet colonies as well as gas production, was observed in lactose broth incubated with the water sample. The presence of the same bacteria in the water could be due to water surface touch to the soil and fecal of animals like cats, dogs and monkeys littering around the recreation park<sup>1,5</sup>.

Despite no official outbreak reported, the presence of *E. coli* in the recreational river may pose a health risk to holiday makers and precautions need to be taken while in contact with water to avoid gastrointestinal related diseases<sup>2</sup>. Aside from stray animals, it is speculated that the water could have fecal contamination from nearby

aborigine villages as the stream runs through a few other villages<sup>1</sup>. The presence of *E. coli* was also reported previously in another Malaysia river in Baram, Sarawak. They also found a few other strains of Enterobacteriaceae like *Enterobacter*, *Klebsiella*, *Citrobacter*, *Pantoea* and *Serratia*<sup>6</sup>.

It is understood that to eradicate *E. coli* in natural water is impossible due to environmental and human factors<sup>5,6</sup>. However, notices of information such as signboards and pamphlets can be displayed or set up and some informational fliers may be distributed to members of the public at the recreational park to create awareness on the potential risks of bacterial infection.

### CONCLUSION

This preliminary study confirms the presence of *E. coli* bacteria in soil and water at Sungai Tekala Recreational Forest thus indicating a certain degree of water pollution, despite no official outbreak reported. As a precaution, it is recommended for the municipal health authority to inform the general public as in informative/ visual displays of precautions at the waterfall area.

### SIGNIFICANCE STATEMENT

This study shows the presence of fecal coliform bacteria which indicates water contamination in a well-known natural recreation water fall in Malaysia. Hence, the public authorities must take prompt action in notifying visitors of the health and safety conditions at waterfall areas.

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