SHORT COMMUNICATION

E. coli contamination in hot springs at Hulu Selangor

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Abstract:

Recreational use of water can have benefits to health and well-being However it also poses a risk to health through exposure to chemical and microbiological organisms as well as physical risks such as drowning and injury if the water is polluted and unsafe. There are many well-known hot springs in Malaysia that are serves as tourist attractions. The purpose of this study was to assess the recreational water quality at selected hot springs in Hulu Selangor. A total of three samples of water were taken from each of the three hot springs in Hulu Selangor which are Kuala Kubu Bharu, Batang Kali and Kerling. The study was repeated twice. Physical parameters (pH, temperature and turbidity) were measured *in-situ* using Hanna Multiparameter and turbidity meter. Biological parameter (*E. coli*) was measured *ex-situ* used colilert method. All physical parameters for all three hot springs complied with the standard but the number of *E. coli* exceeded the acceptable level from the standard in all of the hot springs. An independent t-test showed that there was a significant difference ($p<0.05$) for turbidity during high peak day and low peak day between hot springs but there are no significant difference for pH, temperature and *E. coli*. There are no significant correlation ($p>0.05$) between the presence of the *E. coli* with turbidity. The findings in this study shows that increased users may pollute the hot spring and pose a risk to the users. Thus, this study can be used to formulate strategies to overcome the issues.

Keywords: *E. coli,* hot springs, recreational water, water quality,

1. INTRODUCTION

Natural spring water originate from groundwater and are usually rich in salts and minerals such as calcium, magnesium, potassium, sulphur and sulphate [1]. These areas are attractive places for both locals and foreigners either for excursion or for medical purposes as Hot spring water is believed to have benefits to the human body, therefore the reasons people have used for cosy bathing, therapy and hhealth purposes [2].

However, bacteria can contaminate spring waters and care should be taken to prevent illness or adverse health events such as Recreational Water Illness (RWIs). RWIs consist of a wide variety of infections, including gastrointestinal, skin, ear, respiratory, eye, neurologic and wound infections. The most commonly reported RWI is diarrhoea. It can be caused by exposure to *Giardia* sp., *Shigella* sp., norovirus and *E. coli* through swallowing or contact with contaminated water in swimming pools, hot tubs, water parks, water play areas, interactive fountains, lakes, rivers, or oceans [3]. *E. coli* is a better indicator of fecal pollution than fecal coliform for the purposes of evaluating ambient fresh water quality and in many cases *E. coli* coexists with pathogenic organisms; thus, its presence may reflect the presence of enteric pathogens [4].

Recreational water standards is important as when we have direct contact with water, we will be exposed to the risk and health hazard. In Malaysia, there are no strict and specific regulations for recreational water quality, but the quality of recreational water is monitored using the Malaysian National Water Quality Standards (NWQS) and falls under Class IIB which is for recreational use with body contact [5]. Typically, people will spend half a day at the hot springs. Due to the high number of people in the same place at the same time, there is a probability for visitors to be affected with either physical, chemical or biological hazards which can be dangerous. Thus, the aim of this study was to conduct water quality assessment using physical and biological parameters at hot springs in Hulu Selangor.

2. MATERIALS AND METHODS

2.1 Sample collection

Three sampling points were selected randomly at each hot springs. In each location, three samples were taken twice during the study period which are in the weekday and weekend (n=18). Water samples were taken randomly at a depth of 20 cm from the surface for microbiological analysis in sterilized 500 ml amber Schott bottles.

2.2 *In-situ* measurement

Physical parameters were measured at the site. pH and temperature were recorded using Hanna Multiparameter and turbidity was recorded using the turbidity meter according to standard procedures.

2.3 *Ex-situ* measurement

Colilert method was used for the biological analysis to assess the presence of *E. coli*. 100 ml of water sample was combined with a pack of Colilert reagent and shaken until dissolved. The sample-reagent mixture was poured onto a Quanti-Tray 2000 and sealed in an IDEXX Quanti-Tray Sealer. The sealed tray was placed in a 35±0.5°C incubator for 24 hours. The number of positive wells were counted and referred to the MPN table provided with the trays to obtain a Most Probable Number. When no yellow shading appears after incubation it indicates that the result is negative for both total coliforms and *E. coli*. In contrast, when the tray is put under fluorescence with a 6-watt, 365-nm UV light within 5 inches of the sample in a dark environment and the wells becomes fluorescent in indicates the presence of *E. coli* [6].

3. RESULTS AND DISCUSSION

3.1. Water quality status of hot springs

The NWQS designate water into five different classes and each class is described according to its utilization. In this study, the Class IIB is used as it is categorised as water for recreational use with body contact.

A summary of the water quality parameters of the hot springs is depicted in Table 1. Based on the results obtained, all pH values complied with the standards at 8.92, 8.91 and 8.89 for Kuala Kubu Bharu hot spring, Batang Kali hot spring and Kerling hot spring, respectively. Hot springs need to maintain its pH to maintain disinfection levels and bather's comfort. The lower the pH level, the more acidic the hot spring and microbes that can found in acidic hot springs are typically thermoacidophilic archaea and bacteria [7]. The acceptable value for pH according to the NWQS for water used for recreational body contact is in the range of 6-9.

The average value of temperature of the three hot springs were 40.8 °C, 42.8 °C and 42.1 °C for the Kuala Kubu Bharu hot spring, Batang Kali hot spring and Kerling hot spring respectively. There is no specific value to define the standard for temperature, as different types of natural water bodies have different temperatures based on the weather and environmental conditions. Additionally, even hot springs have different temperatures depending on the geography and other characteristics. Nonetheless, for all hot springs the water temperature is suitable for body contact without causing any burns or scalds though care should be taken for the total duration that is spent immersed in the water.

E. coli can stand in a temperature up to 53°C, thus is an ideal faecal indicator in the hot spring as most hot springs used for bathing have a temperature range from 36.5°C to 50°C [8]. The presence of *E. coli* varied among each hot springs was assessed against the standard by the World Health Organisation [9], whereby the acceptable limit of *E. coli* should be less than 1 in 100 ml of sample. However the presence of *E. coli* was found in all water samples of all hot springs in values exceeding the standard. Since microorganism can be found everywhere and may cause infections to humans, it is essential for the hot spring operator and also the users to implement good hygiene practices to avoid any recreational water-related illness.

Table 1. Water quality parameters

Parameters	Hot spring	Mean (SD)	Standard
Turbidity (NTU)	Kuala Kubu Bharu	0.7 (0.14)	<50 NTU
	Batang Kali	1.15 (0.35)	
	Kerling	2.23 (1.63)	
Temperature (°C)	Kuala Kubu Bharu	40.8(0.2)	-
	Batang Kali	42.8(0.3)	
	Kerling	42.1(0.1)	
pH	Kuala Kubu Bharu	8.92 (0.03)	6 – 9
	Batang Kali	8.91 (0.02)	
	Kerling	8.89 (0.02)	
E. coli	Kuala Kubu Bharu	125 (100)	Weekly (<1/100ml)
	Batang Kali	6.8 (2.8)	
	Kerling	3.9 (2.8)	

3.2. Comparison during peak days and non-peak days

Non-peak days is classified as weekdays namely Mondays to Fridays whereas, peak days is classified as weekends corresponding to Saturdays and Sundays. The comparison of the physicochemical parameters between hot springs is analysed and the results for pH and temperature values have no significant difference on peak and non-peak days (Figure 1), probably due to fact that the number of people in the water do not have any impact to the characteristics of the water. The increased number of people during peak days cannot increase the temperature and pH of the hot springs as that depends on climatic and environmental conditions.

However, turbidity shows a significant difference between peak and non-peak days ($p<0.05$). The mean values show that the readings are higher during the peaks days compared to non-peak days (Figure 1). The increasing number of visitors during the weekend holidays cause the turbidity levels to increase. More people affect the turbidity through activities such as walking when bathing in the hot springs. This causes the sediment at the base of hot spring to be suspended and increases the turbidity [10]. It is essential for any use of water body for recreational purposes to have an ideal level of turbidity complying with the standards to ensure the safety and wellness of the user.

Additionally, the mean values of *E. coli* also show a significant difference ($p<0.05$) between peak and non-peak days at 14.8 and 75.8 respectively. Most people prefer to be involved in recreational activities during the weekend compared to weekdays as they have more free time. Due to the increase in numbers, the increase in bacteria can pollute the water by the bowel movement from humans or animals. *E. coli* is a type of faecal coliform bacteria that can be usually found in the intestines of animals and humans. Thus, the number of people in the water can influence the numbers of *E. coli*. Additionally, all hot springs received different numbers of visitors due to the characteristics of the hot springs. The water level at the Kerling hot spring is at ankle height and is not suitable for users to bath in it whereas the Batang Kali hot spring has a fixed operational hour and entrance fees, in contrast the Kuala Kubu Baru hot spring can be accessed anytime as it is located in an open space.

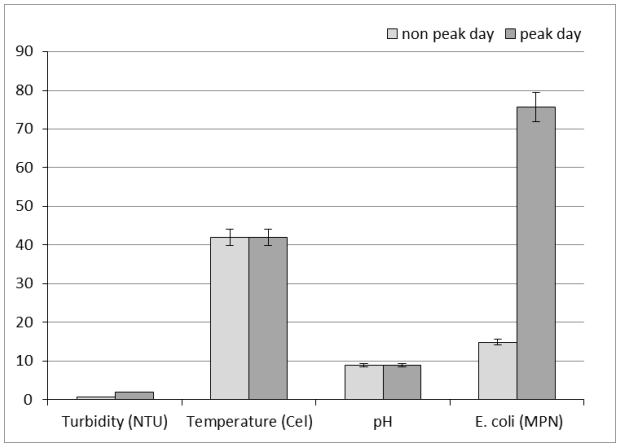

Figure 1: Comparison of parameters between peak and non-peak days

3.3. Correlation between turbidity and E. coli

There was no correlation between the presences of *E. coli* with the turbidity of the hot springs. Generally, there is a correlation between *E. coli* and turbidity, even if the association is not linear [11]. But in this study the result shows otherwise. This could be due the concentration of dissolved oxygen that can influence the growth of microbes. The introduction of higher concentration of oxygen to the water causes oxidative stress to *E. coli* cells associated with reduced or inhibited growth [12]. In this study, dissolved oxygen was not tested hence it is unclear whether the influence of dissolved oxygen towards *E. coli* might have had an effect on *E. coli*. Additionally the small (n=18) sample size may contribute to this negative result.

4. CONCLUSION

Based on this study results, all of the hot springs did not comply with the acceptable standard range for *E. coli* content during all days of the week. Peak days had a higher presence of E. coli in the water due to increased number of visitors. All of the hot springs need to be monitored regularly in terms of their water quality especially on microbiological parameters to ensure it can be used safely by the public and free from causing any potential recreational water illnesses.

REFERENCES

[1] Leclerc, H., Moreau, A., "Microbiological safety of natural mineral water", *FEMS Microbiol. Reviews.* 26**:** 207–222, 2002.

[2] Zaini, H, *et al.*, "Determination of hot springs physico-chemical water quality potentially use for balneotherapy", *Malaysian Journal of Analytical Sciences*, 17(3): 436-444, 2013.

[3] Centre for Disease Control, "Healthy Swimming", (2016, May 04). Retrieved November 15, 2017, from https://www.cdc.gov/healthywater/swimming/swimmers/index.html

[4] USEPA, "Ambient water quality criteria for bacteria-1986", #EP*A440-584-002*, U.S. EPA, Washington, DC, USA, 1986.

[5] DOE. *Water Quality Criteria and Standard for Malaysia*. Department of Environment, Ministry of Science and Technology, Malaysia. (1995).

[6] IDEXX. *Colilert test kit: instruction manual*. Westbrook, ME: 2002.

[7] Haring, M., *et al*., "Viral diversity in hot springs of Pozzuoli, Italy, and characterization of a unique archaeal virus, acidianus Bottle-Shaped Virus, from a new family, the Ampullaviridae", *Journal of Virology*. 79:9904-9911, 2005.

[8] Fotadar, U., *et al*., "Growth of *Escherichia coli* at elevated temperatures", *Journal of Basic Microbiology*, 45: 403–404, 2005.

[9] World Health Organization. Water sampling and analysis. *Guidelines for Drinking-Water Quality, Jan/Feb 19*, 51–72, 1997.

[10] Almeida. C., "A recreational water quality index using chemical, physical and microbiological parameters", *Environmental Science and Pollution Research*, 19(8): 3400-3411, 2012.

[11] Smith, R., *et al*., "Short communication: Turbidity as an indicator of escherichia coli presence in water troughs on cattle farms", *Journal of Dairy Science*, 91(5): 2082-2085, 2008.

[12] Baez, A., Shiloach, J., "*Escherichia coli* avoids high dissolved oxygen stress by activation of SoxRS and manganese-superoxide dismutase. *Microbial Cell Factories*, 12: 23, 2013.