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RESEARCH ARTICLE

Escherichia coli POLLUTION IN COASTAL LAGOON AND DAM RESERVOIR: REPERCUSSIONS ON PUBLIC HEALTH AND AQUACULTURE

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ABSTRACT

Escherichia coli are rod-shaped gram-negative bacteria naturally present in the lower intestinal tracts of warm-blooded organisms including humans and are often released into the environment through faecal material and wastewater effluent. The presence of *E. coli* in waters has long been regarded as an indicator for recent faecal contamination, and possible presence of other more harmful enteropathogens. This study examined the total coliform load and occurrence of *E. coli* to determine the microbial water quality of Merchang Lagoon on Terengganu coast facing the South China Sea, and Raban Lake, part of the Chenderoh Hydroelectric Dam reservoir in the Lenggong Valley, Perak. Water samples were collected from three sites in the lagoon, and two sites in the lake. Total coliform load in water was enumerated by most probable number (MPN) method, and *E. coli* isolation was carried out using eosin methylene blue (EMB) agar. The results showed greatly varied total coliform counts from site to site in both the lagoon (site 1: 7; site 2: 220; site 3: 920 CFU/100 mL) and lake (site 1: 220; site 2: 1600 CFU/100 mL). All sites showed presence of *E. coli*. The results suggest faecal pollution, and the impact of water stagnation and human activities on the coliform load. The impacts of faecal pollution on public health and aquaculture in the investigated areas are discussed. Long-term monitoring of faecal pollution constitutes the essential part of environmental conservation and preservation against the risk of waterborne faecal pathogens in recreational and commercial activities.

KEYWORDS

Faecal pollution, microbial pollution, human health, Merchang Lagoon, Raban Lake.

1. INTRODUCTION

Water is one of the most essential natural resources for existence of life including mankind. The growth of human population and economic activities such as manufacturing and agricultural productions has led to various types of environmental pollutions. Many rivers in Asia are known to be highly polluted with domestic waste (Leong et al., 2018). Industrialisation, urbanisation and other economic activities degrade and pollute the natural resources worldwide (Nallathiga, 2011). Microbial pollution in water may risk disseminating pathogens that could directly or indirectly affect human health and aquatic biodiversity in the waterbodies. Enteric pathogens are known to be primarily responsible for waterborne illnesses. Annually, it is estimated that 1.7 billion diarrhoea cases claimed 525,000 human lives in childhood (WHO, 2017). Various microorganisms such as viruses, bacteria and protozoa are able to enter the body of fish if reared in polluted water, which likely have food safety implication (Nadirah et al., 2012; Cortes-Sanchez et al., 2021).

Faecal coliforms are rod-shaped gram-negative bacteria naturally present in the lower intestines of warm-blooded organisms including humans. Faecal coliforms are a more faecal-specific group or subset of total coliforms and are often discharged into the environment through faecal waste and wastewater effluent. *Escherichia coli* is the key faecal coliform species long used as a faecal indicator bacterium (FIB) for recent faecal contamination in water, and possible presence of other more harmful enteropathogens such as *Cryptosporidium*, *Giardia*, *Shigella*, and norovirus. Faecal coliforms are regarded as indicators for human faecal pollution in lakes, rivers, coastal waters, estuaries and within aquatic animals such as fishes (Ashbolt et al., 2011; APHA, 2006).

Merchang Lagoon is a coastal lagoon located in the Marang District in Terengganu on the coast of South China Sea. The lagoon serves as a natural seaport for the coastal fishing communities, and also a site for cage aquaculture and recreation. Because of the natural accumulation of sediments from the inflowing rivers, runoff from the residential and agricultural areas, as well as the incoming sediment through tidal inlets, the lagoon habitat is threatened by diverse forms of eutrophication and pollution and is potentially a reservoir for microorganisms of public health concern such as those of faecal origin. Raban Lake is situated in the Hulu Perak District in the state of Perak, near the UNESCO World Heritage Site (archeology) of Lenggong Valley. Raban Lake is part of the reservoir of Chenderoh Hydroelectric Dam on the Perak River.

The lake is an attraction for ecotourism, especially sport fishing because of its richness in fish species. Other economic activities here include cage aquaculture and *pekasam* fermented fish industry (Desa and Aidi, 2013). The reservoir habitat is also subjected to degradation of terrestrial and anthropogenic origins. Pollutants from human activities (recreational, residential, agricultural and industrial) expose the reservoir to ecological alteration, and also put the recreational water quality at risk. The present work examines the total coliform load and presence of *E. coli* in Merchang Lagoon and Raban Lake to determine the microbial quality of the waters,

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and assesses the resulting impacts from the perspectives of public health and aquaculture.

2. Materials and Methods

2.1 Study Area

Three sites in Merchang Lagoon (Figure 1) and two sites in Raban Lake (Figure 2) were selected for water sampling to analyse the total coliform load and presence of *E. coli*.

2.2 Water Sampling and Water Quality Measurement

Water samples were collected from each site in Merchang Lagoon (during low tide) and Raban Lake using sterile 1L flasks for most probable number (MPN) analysis. Water temperature, dissolved oxygen, and salinity were measured in-situ using YSI Professional Plus Handheld Multiparameter Meter.

2.3 Bacteriological Analysis

Presumptive, confirmatory and completed tests of MPN were conducted as previously described (Ahmed et al., 2013). Eosin methylene blue (EMB) agar was used for selective and differential isolation of faecal coliform from the positive MPN tubes. Metallic green colonies on EMB agar were subjected to identification by standard biochemical tests (Cappuccino and Sherman, 1996; Alfrad and Bensons, 2007).

3. Results and Discussion

3.1 Total Coliform Count

Merchang Lagoon showed notable variations in total coliform count at sites 1 (7 CFU/100 mL), 2 (220 CFU/100 mL) and 3 (920 CFU/100 mL), where sites 2 and 3 exceeded the DOE Malaysia Class I water quality standard for total coliform (100 CFU/100 mL) (DOE, 2021). According to WHO, total coliform bacteria must not be detectable in any 100-mL of treated water entering the distribution system, and also water in the distribution system (World Health Organization and International Programme on Chemical Safety, 1996). On the other hand, Raban Lake also showed great variations in total coliform count between site 1 (220 CFU/100 mL) and site 2 (1600 CFU/100 mL), and both exceeded the reference standards (Table 1). The low DO (3.97 ± 0.01 mg/L) at site 2 suggests water stagnation in the boat docking area. All the water samples showed growth of metallic green colonies on EMB agar, and identified as *E. coli* (Figure 3), which indicates faecal contamination.

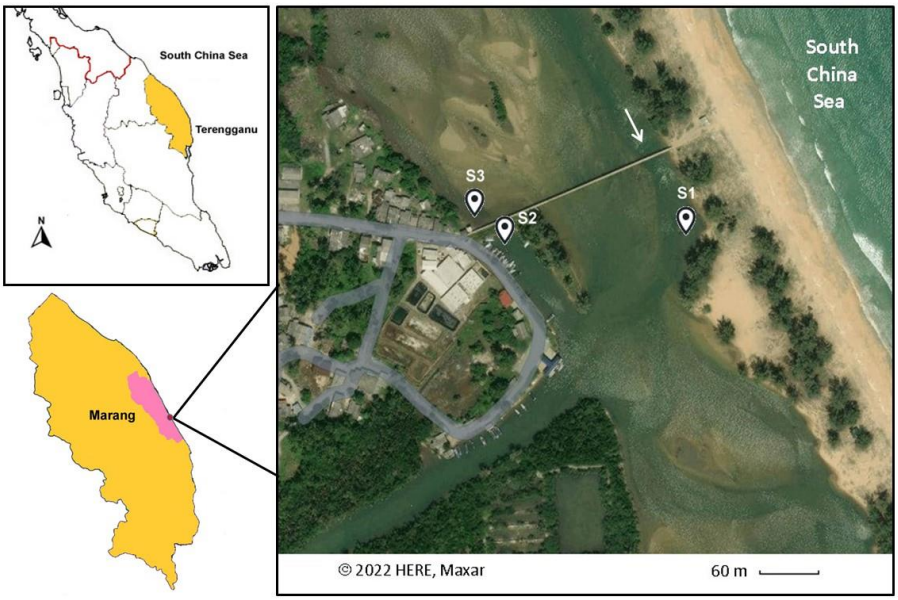

Figure 1: Location map of Merchang Lagoon in the district of Marang, Terengganu, and satellite imagery (© 2022 HERE, Maxar) of the sampling sites with different water flow conditions. S1: waterway; S2: boat docking site and water outlet of a marine hatchery nearby; S3: hatchery water inlet. The arrow shows direction of water flow under the bridge during low tide.

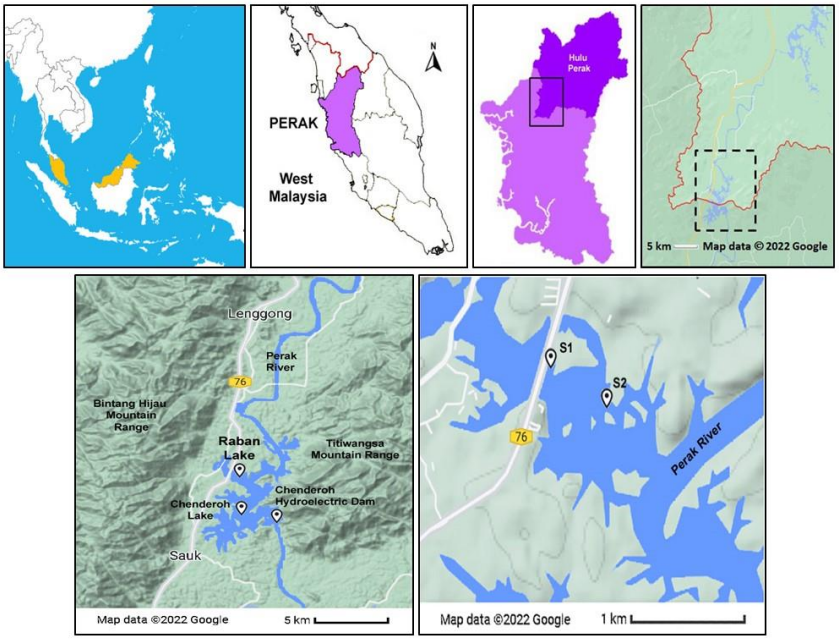

Figure 2: Location map of the sampling sites (S1, S2) in Raban Lake in the Hulu Perak District, Perak. The terrain map shows the valley landscape where the Perak River flows through between the Bintang Hijau and Titiwangsa Mountain Ranges. The Raban Lake and Chenderoh Lake compose the reservoir of Chenderoh Hydroelectric Dam. Imagery © 2022 CNES/ Airbus, Maxar Technologies, map data © 2022 Google.

Table 1: Sampling Sites, MPN-Based Total Coliform Counts for Merchang Lagoon and Raban Lake

Location	Site	Temp (°C)	DO mg/L	Salinity	MPN index CFU/ 100 mL	*E. coli*	Total coliform reference standards (CFU/100 mL)	
Merchang Lagoon	S1 N 05°02.227² E 103°17.863² (lagoon waterway)	29.8 ±0.1	5.00 ±0.01	24.00 ±0.01	7	+	DOE MY 100 Class I: Conservation of natural environment. Water supply I – Practically no treatment necessary. Fishery I – Very sensitive aquatic species. 5000 Class IIA: Water supply II – conventional treatment required. Fishery II – sensitive aquatic species.	WHO Must not be detectable in any 100-ml sample Treated water entering the distribution system, and in the distribution system
	S2 N 05°02.137² E 103°17.454² (boat docking site and hatchery water outlet site)	31.2 ±0.1	7.30 ±0.01	25.00 ±0.01	220	+		
	S3 N 05°02.143² E 103°17.444² (hatchery water inlet site)	33.6 ±0.1	3.00 ±0.01	25.00 ±0.01	920	+		
Raban Lake	S1 N 4°59'52.9" E 100°57'05.7"	31.2 ±0.1	7.11 ±0.01	0.02 ±0.01	220	+		
	S2 N 4°59'42.5" E 100°57'21.8"	29.6 ±0.1	3.97 ±0.01	0.02 ±0.01	1600	+		

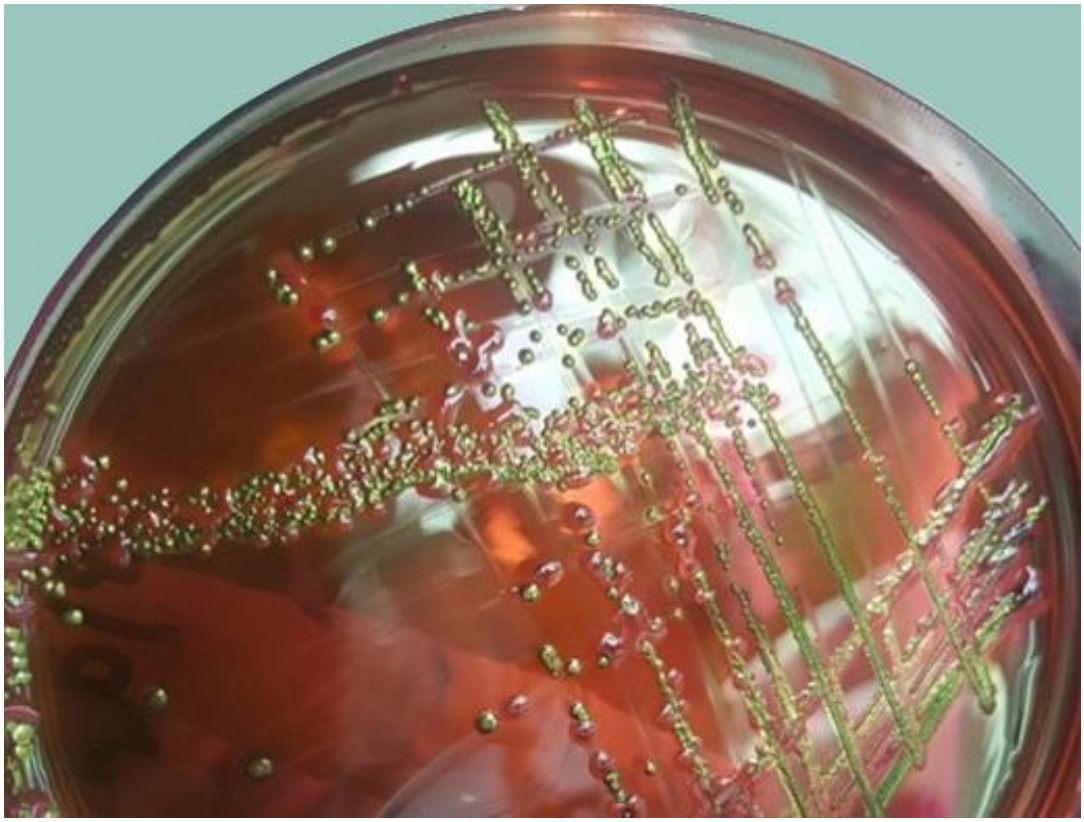

Figure 3: Metallic green *E. coli* colonies with dark centres on EMB agar.

The lowest total coliform count (7 CFU/100 mL) in the lagoon waterway (site 1) that has good flow of water and exchange during low tide and high tide suggest the positive effect of water flow on lowering the total coliform load in the water. Water exchange at site 2 (boat dock) is relatively limited during low tide because of the docking site structure. Site 2, however, receives continuous discharge of water from a marine hatchery nearby. The DO at site 2 was the highest (7.30 ± 0.01 mg/L) compared with site 1 (5.00 ±0.01 mg/L) and 3 (3.00 ±0.01 mg/L). This could be a result of the discharge of aerated water from the hatchery. The total coliform level at site 1 was within the DOE Malaysia Class I standard (100 CFU/100 mL) for conservation of natural environment.

The highest total coliform load at site 3 (920 CFU/100 mL) was very likely due to water stagnation caused by the earthen bank of docking site structure that largely limits flow and exchange of water and promotes accumulation of coliforms originated from the smallholder ruminant farming and human activities in the area. Coliform count is used to determine the hygiene quality of seawater environment (Ryan et al., 2004). Their presence and load can reflect the degree of pollution by domestic and industrial sewage. The presence of *E.coli* at all three sites in Merchang Lagoon suggests faecal contamination of animal or human origin, or both, and is an indicator for potential public health risk for individuals in contact with the waterbody, especially during water sport activity such as kayaking.

Faecal coliforms may occur in ambient water due to overflow of domestic

sewage system or nonpoint sources of human and animal wastes. Overloaded household sewage system can cause flow of untreated human waste into the drainage system and nearby waters. Agricultural activities in the Merchang River basin such as livestock waste disposal and cleaning, and livestock ranching might have also contributed to faecal contamination in the water. *Escherichia coli* has been associated with 19% faecal pollution. Contamination of water with faecal matter could be the major cause of transmission of many clinically important pathogens to humans (Ekhaise and Omoigberale, 2011).

3.2 Impacts on Public Health and Aquaculture

The presence of *E. coli* in Merchang Lagoon and Raban Lake requires further investigation for public health implications because there could be pathogenic strains besides the commensal ones. Intestinal pathotypes such as enteropathogenic *E. coli* (EPEC) and Shiga toxin-producing *E. coli* (STEC) are often associated with waterborne outbreaks globally. The recent case (June 2022) of tourists contracting illness after swimming in the waters off Perhentian Island (Kecil) believed to be polluted with untreated sewage, serves as a warning against unsatisfactory sewage management. The follow-up joint survey by the national water and sewage service agencies found *E. coli* in the well water along the coast (The Sun Daily, 2022). Globally, recreational waters have been well documented as a route of transmission for infectious diseases, including *E. coli* O157 outbreak associated with swimming pool of leisure center (Verma et al., 2007).

It has also been demonstrated that *E. coli* can persist for prolonged periods of time in the secondary habitat such as macroalgae that potentially support their growth, and the abundance of macroalgae-associated *E. coli* varies with tidal regime of lagoon with higher loads typically observed during low tide (Quero et al., 2015). The survival and distribution of *E. coli* in aquatic environments depend on a complex interplay of abiotic and biotic factors. The abiotic factors include temperature, salinity, nutrient concentration, pH and UV radiation, while protozoan grazing, microbial competition and viral infection are among the biotic factors (Barcina et al., 1997; Fernandez-Astorga et al., 1992; Byappanahalli et al., 2006; Petersen and Hubbart, 2020; Roszak and Colwell, 1987). Rainfall has an important effect on the presence and distribution of microbes in the environment by spreading microbial pollutants via terrestrial run-off and other mechanisms (Sidhu et al., 2012; Heany et al., 2014). While there is agreement that the presence of *E. coli* in high densities suggests recent faecal pollution of human or animal origin, moderate to low densities of *E. coli* are more problematic for ascertaining occurrence of pollution, where substantial effort and resources are required to identify the causes (Whitman et al., 2006; Nafsin et al., 2022).

Faecal coliforms present in high abundance in waters not only impact the environmental quality, but also affect the animal and human health. These pathogens may be accidentally ingested via water during recreational activities such as swimming, or via food contaminated by contact. Some *E. coli* strains (extraintestinal pathogenic *E. coli*, ExPEC) are also known to emerge to cause infections at nonintestinal sites such as urinary tract, blood stream and prostate (Manges et al., 2019). Hence, exposure through small cuts or mucous membranes during recreational activities may result in infections.

The environment is the most important determinant of microbiota of fish and fish products (Yukgehnaish et al., 2020). The presence of *E. coli* in Merchang Lagoon and Raban Lake bring negative repercussion on the cage aquaculture activities in the waters, because it may mean possible presence of *E. coli* in the fish cultured. Recovery of *E. coli* from digestive tract and muscle of freshwater fish *Jenynsia multidentata* and *Bryconamericus iheringi* has been demonstrated in bioassays at a critical bacterial load of 10^2 and 10^3 CFU/mL for digestive tract and muscle respectively (Guzman et al., 2004). On the other hand, we also previously isolated *Plesiomonas shigelloides* from the muscle and intestines of tilapia cultured in Kenyir Lake (Nadirah et al., 2012). Although the lagoon and lake water is not the source of drinking water, the presence of *E. coli* or other possible faecal pathogens in the ecosystem may still pose a public health hazard, and impact on the livelihood of the resident communities.

Similar viewpoints have also been previously described in the contexts of marine recreation and estuarine cage aquaculture in Malaysia (Jalal et al., 2010; Jalal et al., 2012; Dewi et al., 2022). Remediation of faeces-polluted waters should be explored from biological, chemical, ecological and engineering perspectives (Anawar and Chowdhury, 2020). Bioactive compound from blue swimming crab *Portunus pelagicus* and probiotics (lactobacilli, bifidobacteria, enterococci) have been demonstrated to be effective against *E. coli.* Lytic bacteriophages should also be investigated for potential use as biocontrol agent against *E.coli.* Antibacterial properties of native plants such of mangroves may be explored for possibility of phytoremediaton (Laith et al., 2017; Fijan et al., 2018; Nurhafizah et al., 2017; Pacifico et al., 2019; Shamsuddin et al., 2013).

4. Conclusion

Our present study indicates faecal pollutions in Merchang Lagoon and Raban Lake, and the results suggest possible negative repercussions on public health and aquaculture activities in the waterbodies. The findings implicate the need for identifying the pollution source, as well as regular and systematic monitoring of microbial water quality of the ecosystems by the respective competent authorities. It also calls for greater public awareness on waterborne microbial hazard in recreational and economic activities, and realisation about the importances of proper sewage management, long-term environmental preservation and conservation. Further studies are recommended to look into the following issues and concerns: (1) possible *E. coli* naturalisation in the waterbodies; (2) the strains involved and their pathogenicity levels; (3) the resulting impacts of persistence phenomenon towards biodiversity, ecosystem services, human health and safety, local economies and livelihood sustainability; (4) decontamination and remediation strategies against faecal polluted waters.

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