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Health Risks Assessment related with E. coli, Salmonella Species and Helminth Eggs (Ascaris Lumbricoides) in the Water Courses in Thailand [in fecal sludge]: a case study of Klong Luang District, Pathum Thani Province, and Mandalay Region


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Abstract

Annual infection risk and single exposure related with *E. coli, Salmonella* species, and Helminth eggs (*Ascaris lumbricoides*) for canal water, irrigation water, tap water, flooding water and fecal sludge materials of Khlong Luang District, Pathum Thani province by using risk assessment for quantitative microbial (QMRA) were assessed. The exposure assessment was constructed using households interview surveys and literature reviews during their activities such as swimming, fishing, farming, flooding and sanitation workers. Results showed that a high contamination of water by a mean of 10^{3} CFU per100 ml for *E. coli* (47%), 10^{5} MPN per 100 ml for *Salmonella* species (69%) in canal water, 10^{2} CFU per 100 ml for *E. coli* (30%), 10^{4} MPN per100 ml for *Salmonella* species (60%) in irrigation water, 10^{3} CFU per 100 ml for *E. coli* (47%), 10^{5} MPN per 100 ml for *Salmonella* species (69%) in flooding water, 10^{5} CFU per 100 ml for *E. coli* (69%), 10^{4} MPN per 100 ml for *Salmonella* species (60%) in fecal sludge matter**.** In this study, Helminth eggs (*A. lumbricoides*) were insignificant all water samples and justifying that *E. coli* 8% were *E. coli* O 157: H7. In Khlong Nueng, annual risks of pathogenic infection from accidental ingestion of water for swimming and fishing with *E. coli* and *Salmonella* species for canal water, flooding water, and sanitation workers have exceeded the acceptable level of 36/1000 population per event and also annual risks, whereas irrigation water has a lower annual risk. Higher concentrations of pathogenic microorganisms might cause gastrointestinal track, diarrhea, headache, skin infection, eye infection in those areas due to poorly managed sanitation and unintentionally flooding. The study recommends requirement for treatment plants for wastewater, awareness raising in the communities in peri-urban settings with the management of local government.

Key words: canal, irrigation, tap, flooding, fecal sludge matter, QMRA

ထိုင်းနိုင်ငံ၏ ရေစီးလမ်းကြောင်းနှင့် လူ၏ အညစ်အကြေးတွင် ပါရှိသည့် ပိုး (၃) မျိုး ဖြစ်သည့် *E.coli*, Salmonella Species and Helminth Eggs (*Ascaris Lumbricoides*) တို့နှင့် ပတ်သက်၍ ကျန်းမာရေးဆိုင်ရာ ဆန်းစစ်ခြင်း

တင်နွဲ့ရီ၊ ဦးစီးအရာရှိ
ပတ်ဝန်းကျင်ထိန်းသိမ်းရေးဦးစီးဌာန

စာတမ်းအကျဉ်း

ထိုင်းနိုင်ငံ၊ Pathum Thani province, Khlong Luang ခရိုင်ရှိ ကန်ရေ၊ စိုက်ပျိုးရေ၊ သုံးရေ၊ ရေကြီးရေလျှံမှုနှင့် လူ့အညစ်အကြေးများမှ*E. coli, Salmonella* species, and Helminth eggs (*Ascaris lumbricoides*) ပိုး(၃)မျိုးကိုစမ်းသပ်၍ ပိုးပါဝင်မှုများမှ တစ်ကြိမ်ထိတွေ့ခြင်းဖြင့် မည်သို့ဖြစ်စေသည်၊ နှစ်စဉ်ထိတွေ့ခြင်းဖြင့် မည်သို့ဖြစ်စေသည်ကိုလေ့လာမှုပြုသော စာတမ်းဖြစ်ပါသည်။ Khlong Neung နှင့် Khlong Song ကန်များမှ Khlong Neung ကန်ထဲတွင်ရေကူးခြင်း၊ ငါးဖမ်းခြင်း၊ Khlong Song ကန်ရေအား မီတာ(၁၅၀၀) အကွာတွင်သွယ်တန်း၍ စိုက်ပျိုးရေးလုပ်ငန်း ဆောင်ရွက်ခြင်း၊ မိုးသည်းထန်စွာရွာသွန်းမှုမှ ရေကြီးရေလျှံဖြစ်ပေါ်ခြင်း၊ မိလ္လာနောက်လိုက် လုပ်ငန်းဆောင်ရွက်ခြင်းများမှ ကျန်းမာရေးဆိုင်ရာ မည်ကဲ့သို့ဖြစ်စေသည်ကို ကန်ဘေးတစ်လျှောက်ရှိ လူနေအိမ်များနှင့် မိလ္လာနောက်လိုက်များအား အင်တာဗျူးကောက်ယူခြင်းနှင့် ထုတ်ဝေပြီးဖြစ်သော စာတမ်းများကိုကိုးကား၍ မည်ကဲ့သို့ ထိတွေ့မှုဖြစ်စေသည်ကို လေ့လာမှုပြုလုပ်ခဲ့ပါသည်။ Khlong Neung ကန်၏ အထက်ပိုင်းတွင် စက်မှုဇုန်တည်ရှိပြီး ကန်အလယ်၌ သား၊ ငါး၊ ဟင်းသီး ဟင်းရွက်ရောင်းသော ဈေးတည်ရှိကာ ၎င်းတို့၏ စွန့်ပစ်ရေများအား တိုက်ရိုက်သော်လည်းကောင်း၊ သွယ်ဝိုက်၍ သော်လည်းကောင်း စွန့်ပစ်လျက် ရှိပါ သည်။ ကန်ရေထဲတွင် 10^{3} CFU per100 ml for *E. coli* (47 %) နှင့် 10^{5} MPN per 100 ml for *Salmonella* species (69 %) ပါဝင်ကြောင်း၊ စိုက်ပျိုးရေထဲတွင် 10^{2} CFU per 100 ml for *E. coli* (30%) နှင့် 10^{4} MPN per100 ml for *Salmonella* species (60%) ပါဝင်ကြောင်း၊ ရေကြီးရေလျှံမှုတွင် 10^{3} CFU per 100 ml for *E. coli* (47%) နှင့် 10^{5} MPN per 100 ml for *Salmonella* species (69%) ပါဝင်ကြောင်း၊ လူ့အညစ် အကြေးထဲတွင် 10^{5} CFU per 100 ml for *E. coli* (69%)နှင့် 10^{4} MPN per 100 ml for *Salmonella* species (60%)ပါဝင်ပါသည်။ Helminth eggs (*Ascaris lumbricoides*) သည် မည်သည့် ရေနမူနာတွင်မျှ ပါဝင်မှုမရှိပါ။ E.coli၏ 8%အားရယူ၍ရောဂါဖြစ်စေသည့် E.coli O157:H7 အားတွက်ချက်ခဲ့ပါသည်။ Khlong Neung ကန်ထဲတွင် ရေကူးခြင်း၊ ငါးဖမ်းခြင်း၊ မိုးသည်းထန်စွာ ရွာသွန်းမှုမှ ရေကြီး ရေလျှံ ဖြစ်ပေါ်ခြင်း၊ မိလ္လာနောက်လိုက်လုပ်ငန်းဆောင်ရွက်ခြင်းများမှ တစ်ကြိမ် ထိတွေ့ခြင်းဖြင့်၊ နှစ်စဉ်ထိတွေ့ခြင်းဖြင့် အန္တရာယ်ဖြစ်စေနိုင်သည်မှာ သတ်မှတ်ထားသည့် လူဦးရေ ၁၀၀၀ တွင် ၃၆ ယောက်ထိ ခွင့်ပြုထားသော်လည်း ၃၆ ယောက်ကျော်အထိ ကျော်လွန်နေပြီး စိုက်ပျိုးရေတွင် သတ်မှတ်ထားသည်ထက် အနည်းငယ် ကျော်လွန်

နေခြင်း၊ ခန့်မှန်းမထားသော ရောဂါ ဖြစ်စေသည့် ပိုးဝင် ရောက်မှုမှ ဝမ်းလျော ဝမ်းပျက် ဖြစ်ပွားခြင်း၊ ဆီးသွားလမ်းကြောင်းဆိုင်ရာ ရောဂါဖြစ်ပွားခြင်း၊ ခေါင်းကိုက်ခြင်း၊ အရေပြားယားယံခြင်း၊ မျက်စိနာခြင်း စသည်တို့ ဖြစ်ပွား စေနိုင်သဖြင့် စွန့်ပစ်ရေဆိုး သန့်စင်မှု၊ ၎င်းနှင့် ပတ်သက်သည့် ဟောပြောပွဲများ ဆောင်ရွက်ခြင်း စသည်တို့ဆောင်ရွက်ခြင်းမှ လူ့အညစ်အကြေးမှတစ်ဆင့် ပိုးကူးစက်မှု လျော့နည်းစေနိုင်ကြောင်း တင်ပြအပ်ပါသည်။

Health Risks Assessment related with E. coli, Salmonella Species and Helminth Eggs (Ascaris Lumbricoides) in the Water Courses in Thailand [in fecal sludge]: a case study of Klong Luang District, Pathum Thani Province, and Mandalay Region

1. Introduction

Water scarcity issues are caused by a variety of factors including population growth, changing climate, urban development, agricultural area expansion, and other industrial activities in many countries around the world. Moreover, human excreta like fecal sludge have been primarily released directly into urban or peri-urban environments, where it can have adverse effects such as diarrhea, cholera, and helminthiasis (Lalander et al., 2013). In addition, wastewater from households, industries, and markets is discharged both directly and indirectly into canal water systems, most of which is untreated, creating an environmental and health risk to water bodies in the nearby areas due to the chemical pollution and microbial contamination (Anceno et al., 2007a; Diallo et al., 2008; Surinkul & Koottatep, 2009; Yajima & Koottatep 2010). Without treatment or without proper treated wastewater include the chemicals of the toxic, pathogenic microorganisms, and higher concentrations quantities of nutrients for organic and inorganic, which might damage bathers of recreational water through aerosol inhalation, accidentally water ingestion, and infection of wound (Abdelzaher et al., 2010, Abessa et al., 2005; Braga et al., 2000; Westrell et al., 2004; World Health Organization [WHO], 1998).

Excreta are full of nutrients like nitrogen and phosphorus, as well as diseases including bacteria, viruses, protozoa, and helminths. Inhalation, accidental ingestion, and dermal contacts (swimming, washing, etc.) are examples of direct exposure, as are indirect exposures such as ingestion of contaminated water, food (such as contaminated drinking water consumption), crops of wastewater- fed and the fish, and person-to-person. Bacteria, viruses, protozoa, and helminths are the four types of microorganisms. Bacteria and viruses live in the environment for a short time, depending on temperature, moisture content, and other factors. Protozoa and helminths spend more time in the environment than other organisms. Depending on temperature, moisture, nutrients, and other factors, various infections may stay alive in the surrounding environment for a longer or shorter time periods. Furthermore, increasing rainfall and more frequent floods can cause more damage to on-site and decentralized sanitation systems, resulting in fecal sludge overflow, which can cause to high levels of fecal pathogen disease, such as diarrheal and parasitic infection.

Fecal microbial contamination levels, such as *Escherichia coli, Salmonella* species, and Helminth eggs, can be discovered in the environment where humans live. Young children have more possibilities to interact with the environment through touching and mouthing than adults and the elderly. Furthermore, when various factors have been involved, such as fishing, swimming, sanitation workers, and farmers in dirty water, diseases can be easily transmitted to the host, such as by food and water ingestion with contamination as well through person to person and animal contact.

Some urban dwellers, particularly children, may be exposed to high concentrations of fecal microbial pollution and associated enteric infections that might have detrimental effect on public health in urban environments with a higher population density and inadequate sanitation services. As a result, a risk assessment for quantitative microbes (QMRA) was employed to determine the amount of microorganism's risk presented by pathogens of the waterborne across a number of exposure pathways. The objective of this research is to learn more about the health concerns connected with *E. coli, Salmonella* species, and Helminth Eggs (*A. lumbricoides*) in water, Thailand.

2. Objectives

The objectives of Thailand my research are as follows:

a. To estimate fecal indicator organisms (*E. coli*), actual pathogenic organisms (*Salmonella* species), and Helminth Eggs (*A. lumbricoides*) concentrations in selected areas.

b. To investigate different FS practices in the selected areas.

c. To use fecal indicator organisms (*E. coli*), actual pathogenic organisms (*Salmonella* species), and Helminth Eggs (*A. lumbricoides*) to analyze the effects of human health risk employing QMRA.

d. To propose the most effective health-risk mitigation strategies for FS practices in developing countries' peri-urban areas.

e. To access the current situations of the stakeholders about sanitation practices in the selected areas

The objectives of Mandalay my research are as follows:

a. To investigate different FS practices in sanitation workers.

b. To use fecal indicator organisms (*E. coli*), actual pathogenic organisms (*Salmonella* species), and Helminth Eggs (*A. lumbricoides*) to analyze the effects of human health risk employing QMRA.

c. To propose the most effective health-risk mitigation strategies for FS practices in sanitation workers in Mandalay city and developing countries' peri-urban areas.

3. Materials and Methods

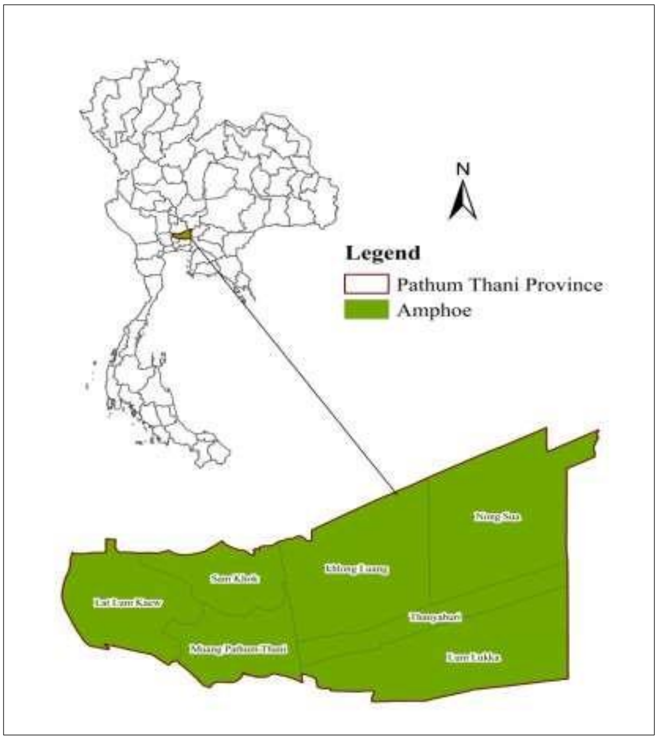
3.1 The current situations of Khlong Neung and Khlong Song Sub-districts of Khlong Luang district, Pathum Thani Province

3.1.1 Study Area

Khlong Nueng and Khlong Song sub-districts of Khlong Luang district, Pathum Thani province are selected for my study area. The study area where is residential areas, industrial (Industrial Estate), large-scale food marketplaces (Talad Thai) and institutional (academic and medical).

Thailand country has different kinds of canal systems which are man-made. Bangkok, in particularly, has 1,165 canals covering 2,280 kilometers, more than twice the length of the city's road network (1,014) km. 45.6 million m^3 of wastewater is stored in this canal system. 490 sub-canals in Bangkok transport surface wastewater for 2.7 million m^3 (Diallo et al., 2008). Khlong Luang is a district northeast of Pathum Thani province. It located 45 kilometers away from north of Bangkok's city center. The Khlong Luang district municipality is a community of peri-urban that comprises 20 villages of Khlong Nueng 99,075 people and 15 villages of Khlong song (43, 038) people, as well as farmers in Khlong Song (111) (DoA, 2021). In Klong Luang district, households, industries, and markets wastewater is released into the canal systems, most of which is untreated, resulting in the chemical pollution and the microbial contamination of the water bodies in the surrounding areas, creating environmental and health risks (Anceno et al., 2007b; Diallo et al., 2008; Surinkul & Koottatep, 2009; Yajima &

Koottatep 2010). Study area of Khlong Luang district, Pathum Thani province in Thailand was shown in Figure 3.1.



1_Figure 3.1 Study area of Khlong Luang district, Pathum Thani province in Thailand

The number of people in Khlong Luang district and the number of people Khlong Nueng and Khlong Song sub- districts were described in Table 3.1 and Table 3.2.

1_Table 3.1: The Number of People in Khlong Luang District

No.	Sub-district	Total number of villages	Population (people)	Total Farmer (Households)
1.	Khlong Nueng	20	99, 075	96
2	Khlong Song	15	43, 038	111
3	Khlong Sam	16	90, 438	296
4	Khlong Shi	16	20, 383	519
5	Khlong Hha	16	15, 486	549
6	Khlong Hok	14	13, 272	638
7	Khlong Chet	9	7, 599	549
Total		106	289, 291	2,758

Source: Department of Agriculture, Khlong Luang District (2021)

2_Table 3.2: The Number of People in Khlong Nueng and Khlong Song Sub- Districts

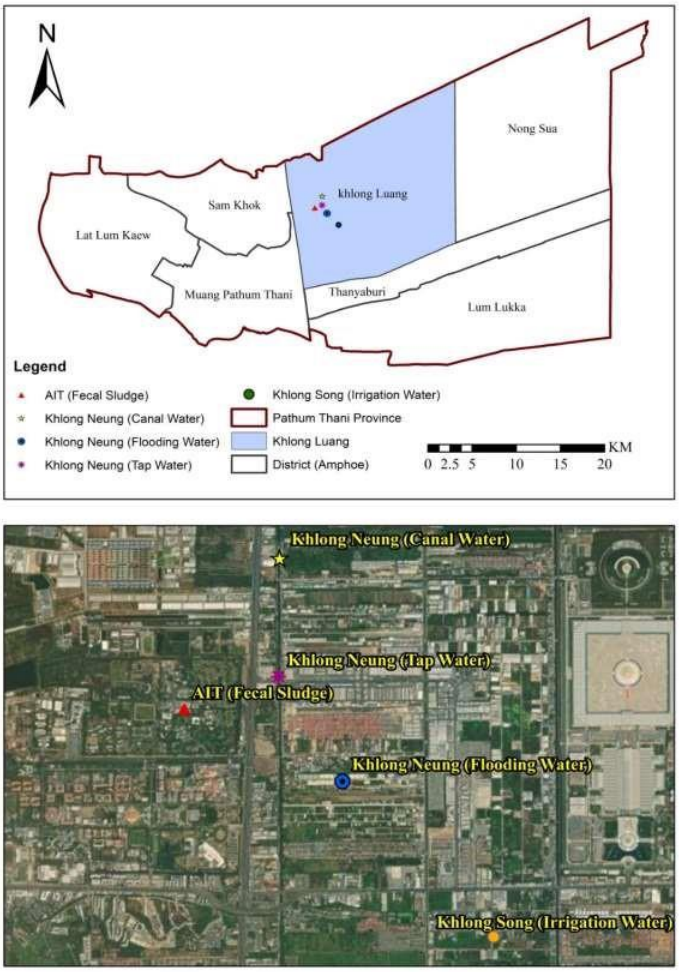
No.	Sub-district	Households	Male	Female	Total
1	Khlong Nueng	45, 500	25, 653	31, 149	56, 802
2	Khlong Song	11, 165	9, 160	10, 062	19, 222

Source: Data from Tha Khlong Municipality, September (2021)

3.1.2 Sampling Sites

Canal water, irrigation water, tap water, flooding water and FS matter were used in this study. Canal water samples were taken at Talad Thai from the Khlong Nueng canal, with a focus on coming from the market in the canal. The water coming from the market, which includes places where fish, pork, and cattle are sold, is directly discharged into the canal. Near Talad Thai market, tap water was collected from a household shop along the canal. For those locations, tap water is mostly supplied by Nava Nakorn Industrial Estate in Pathum Thani. Bathing, utensil washing, and laundry cleaning are all done with tap water. Irrigation water for the Khong Song people comes from the Khlong Song canal, which is around 1500 meters (1.5 kilometers) away from the irrigation areas.

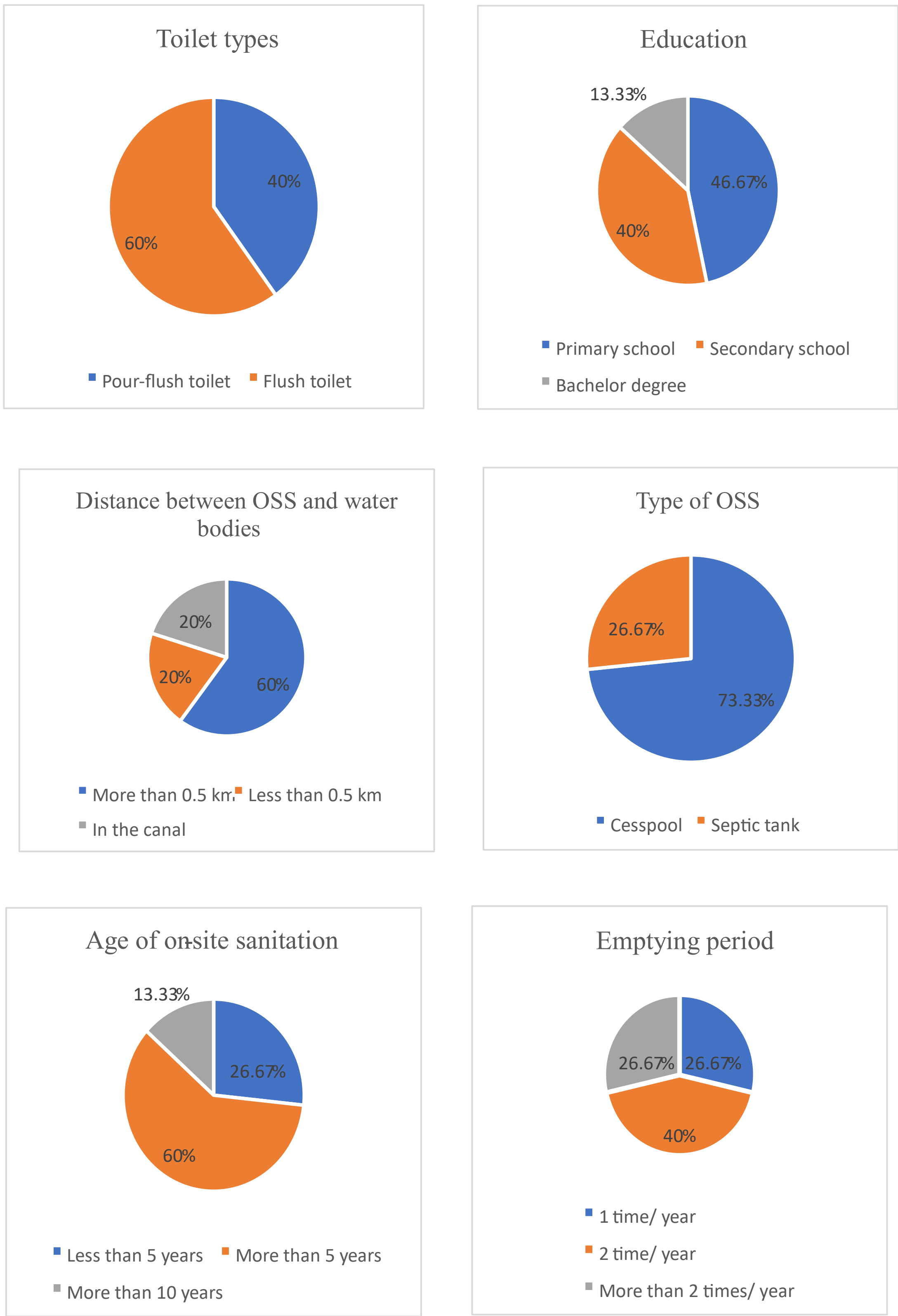
According to field observations by researcher, some farmers conserve water by digging the ground. Agricultural seasoning has exploited and used the stored water to generate agricultural products. Near the Talad Thai market, floodwaters have been collected. There was no flooding during the time when the flooding water was collected. However, when there was a significant rainfall, samples were taken. Fecal sludge materials (liquid), particularly in large buckets, have collected on the AIT campus, originating from academic buildings of AIT (Asian Institute of Technology). Sampling locations of the study area, Khlong Luang District, Pathum Thani province in Thailand was described in Figure 3.2.



2_Figure 3.2 Sampling locations of the study area, Khlong Luang district, Pathum Thani province in Thailand

3.1.3 Interview Survey for the Stakeholders

The data from household's interview survey for the current conditions of the stakeholders about the sanitation awareness are shown as follows in Figure 3.3.



3_Figure 3.3: The data from household's interview survey about sanitation awareness

3.1.4 The Collection of the Sample

The sample collection is shown as follows in Table 3.3.

3_Table 3.3: sample collection

Sample	Number of sampling sites	Parameters	Frequency
Canal water (Talad Thai market)	1	*E. coli*, *Salmonella* species Helminth Eggs	Twice a month
Irrigation water	1	*E. coli*, *Salmonella* species Helminth Eggs	Twice a month
Tap water	1	*E. coli*, *Salmonella* species Helminth Eggs	Once a month
Flooding water	1	*E. coli*, *Salmonella* species Helminth Eggs	One time
Fecal sludge	1	*E. coli*, *Salmonella* species Helminth Eggs	One time

3.1.5 Laboratory Analysis

APHA et al. (2017) was used to analyze the spread plate method (CFU) for *Escherichia coli* (*E. coli*), the standard five tube method of Most Probable Number (MPN) for *Salmonella* spp., and method for Zinc Sulphate for Helminth eggs (*Ascaris Lumbricoides*). MPN/100 ml of wastewater, CFU/100 ml of wastewater, and number of eggs per 1000 ml of wastewater were given as the results. Laboratory analysis for each pathogen was shown in Table 3.4.

4_Table 3.4: Laboratory Analysis Details for each Pathogen

No	Parameter	Unit	Method	Frequency	Reference
Water Quality Parameters					
1.	*E. coli*	Numbers	Spread Plate method	Twice a month	APHA (2017)
2.	*Salmonella* species	Numbers	Most Probable Number	Twice a month	
3.	Helminth Eggs (*Ascaris Lumbricoides*)	Numbers	Zinc Sulphate method	Twice a month	

3.1.5.1 Spread plate method for E. coli

The Examination of Waste and Wastewater Standard Methods adopted spread plate approach (APHA, 2017). CFU/100 ml is dependent on a plate counting colonies. The following procedure was used to analyze *E. coli* and *E. coli* concentrations on EMB agar was shown in Figure 3.4:

1. First, the researcher used an auto pipette with sterilize tips to pipette 0.1ml amount, 0.01 ml amount, and 0.001 ml amount of sample onto an Eosin Methylene Blue (EMB) agar surface plate with a dilution of 0.9 ml.
2. It distributes sample around medium surface by rotating plate manually using sterilized glass spreaders on EMB agar surface plate.
3. Finally, before inverting, the material must completely absorb into the EMB agar medium.
4. Finally, incubate the EMB agar plate at 37° C for 24 hr.
5. Count the colonies number characteristic on each plate and multiply by 100 ml to get the CFU/100 ml.

   CFU/ 100 ml = colonies counted × 100/sample plated actual volume, ml **Eq-3.1**

Where:

CFU = colony forming unit

4_Figure 3.4: The concentrations of E. coli on EMB agar

3.1.5.2 Most Probable Number (MPN) for Salmonella species analysis

Salmonella species were identified using the method of Most Probable Number (MPN), a fermentation with multiple tube technique, standard methods for water and wastewater analysis which were adapted (APHA, 2017). MPN/100 ml is based on the number of organisms with the probability of statistical. The following procedure was used to analyze *Salmonella* species (excluding S. para typhi and S. typhi species) and concentrations of *Salmonella* spp., on SS agar was shown in Figure 3.5:

1. 1 ml diluted sample was inoculated into 10 ml Enterobacteriaceae Enrichment (EE) broth; and 24 hr incubation with 37° C.
2. Next, broth for EE culture-medium (1 mL) had been moved broth for Soya peptone with Rappaport-Vassiliadis (RVS) (10 mL) and at 37° C for 24 hr incubation.
3. Thirdly, one loop culture from step 2 RVS was transferred to a *Salmonella* Shigella (SS) plate agar and incubated for 24 hours with 37° C.
4. Finally, positive findings are obtained when the color of the colony on SS agar in multiple tube fermentation is black.

The following equation was employed to calculate the number of Most Probable (MPN) of species:

$$MPN/\ 100ml = (Table\ MPN\ /\ 100\ ml) \times 10/\ V \qquad \textbf{Eq -3.2}$$

Where:

V = sample portion volume at the selected dilution which are the lowest

5_Figure 3.5: The concentrations of Salmonella species on SS agar

3.1.5.3 Zinc sulphate method for Helminth Eggs (Ascaris Lumbricoides) analysis

The method for zinc sulphate, which was adapted from water and wastewater examination standard methods, had been employed to analyze helminth eggs (*Ascaris Lumbricoides*) (APHA, 2017). The following approach was used to analyze Helminth eggs (*Ascaris Lumbricoides*).

1. Depending on the parameters of the sample, a 1 L sample was employed using filter paper. The sediments collected on the filter paper were collected and washed off in a beaker with distilled water (DI).
2. Second, pour the beaker of water into many 50 mL centrifuge tubes, centrifuge at 3000 rpm with 3 minutes, then discard the fluid which were supernatant.
3. Finally, the combination for the deposits in an appropriate tube; in a 50 ml centrifuge tube, this should be no more than 5 ml.
4. Next, re-suspend the deposit in a few ml of $ZnSO_4$ and mix thoroughly with a vortex. Continue to add more $ZnSO_4$ and mix until the tube is nearly filled.
5. The fifth one, centrifuge the tubes for 3 minutes at 3000 rpm. Then, transfer 10 mL of the supernatant to four new tubes with 50 m. To fill tubes with DI water to lower the specific gravity (SG) for $ZnSO_4$ and thus the eggs are not damaged and can deposit after centrifugation.
6. Remove and discard the fluid of the supernatant after centrifuging the tubes at 3000 rpm for 3 minutes. After that, combine the deposits in one tube and recover all of the eggs from the other tubes with a few ml of DI water. Then vortex and centrifuge for 3 minutes at 3000 rpm to obtain one deposit.

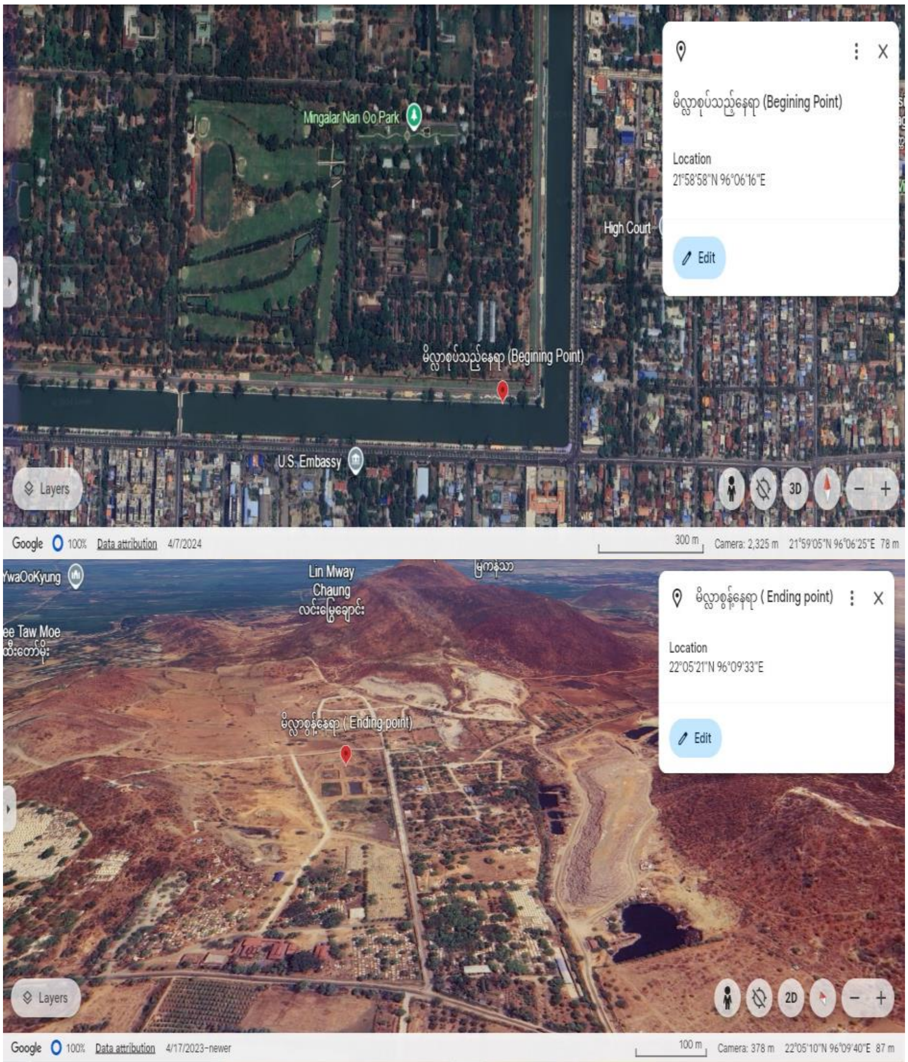
7. Finally, the deposit is transferred to three microscope slides in triplicate, with 1-2 drops of iodine solution or 0.85 % NaCL added to each slide, each one covered with a coverslip, and viewed under a microscope to count each type of helminth ova.

The number of eggs can be computed by the usage of the following equation:

Number of egg/ L= No. of egg in one slide × 1000/ 0.25 **Eq -3.3**

3.1.6 Study Area of Mandalay Region

The study area of collection of sanitation is situated in the Mandalay palace campus with 500 gallons vehicle, Aung Myae Thar San Township, Mandalay. The final disposal site of sanitation is currently located near Aye Yeik Nyein cemetery, and was shown in Figure 3.6.



6_Figure 3.6: Study area of Aung Myae Thar San Township, Mandalay

Data collection

Data was collected to the study area on 23 December 2024. Sanitation workers were interviewed surveys on 23 and on 24 December 2024, and 3 January 2025.

3.1.7 General information of Mandalay

The area of Mandalay is 121.55 square kilometer, has 6 townships (96 squares) that includes Aung Myae Thar San Township, Chan Aye Thar San Township, Mahar Aung Myae

Township, Chan Mya Thar Si Township, Pyikyitakon Township and Amarapura Township. The total population of Mandalay is about 1.7 million and pour-flush toilet with 94% of a city (sources from on March 2023 သန်းခေါင်စာရင်း).

i) Environmental Conditions of Concern

The location is situated in the upper part of Kyarnikan village, Aye Yeik Nyein, near the monetary cemetery, besides Kyarnikan solid waste final disposal site, and between hills. The grasses of the final disposal site in fecal sludge are eaten by cows and birds. If the funeral people are going to the cemetery. Final disposal site of solid waste besides cemetery was shown in Figure 3.7.

7_Figure 3.7: Final disposal site of solid waste besides cemetery

ii) FS Collection and Disposal in Mandalay

Sanitary vehicles from MCDC have serviced 26 vehicles for the whole city that involves 1000 gallons (8 vehicles), 800 gallons (12 vehicles), and 500 gallons (6 vehicles). Aung Myae Thar San township possesses 4 vehicles (big 3 vehicles and small 1 vehicle) about 18 ခေါက် / week., Chan Aye Thar San township possesses 5 vehicles (big 4 vehicles and small 1 vehicle) about 12 ခေါက်/week, Mahar Aung Myae township possesses 4 vehicles (big 3 vehicles and small 1 vehicle) about 15 ခေါက်/week, Chan Mya Thar Si township possesses 4 vehicles (big 3 vehicles and small 1 vehicle) about 15 ခေါက်/week, Pyi Kyi Ta Kon township possesses 5 vehicles (big 4 vehicles and small 1 vehicle) about 15 ခေါက်/ week and Amarapura township possesses 4 vehicles (big 3 vehicles and small 1 vehicle) about 12 ခေါက်/week. The final disposal site of fecal sludge location is situated 11 km north of Mandalay city at the Aye Yeik Nyein cemetery.

iii) Oxidation pond

The oxidation pond has 3 ponds. There is facultative pond (200′× 100′× 6′), aerobic pond (100′× 100′× 5′), and finishing pond (50′× 50′× 4′) with stones. It was built in the 2015-

2016 budget year. Waste pond (မြေသားကန်) was built (200′× 100′× 12.6′) in 2021 (information from water and sanitation department, MCDC, 2023 report). The oxidation pond was shown in Figure 3.8, 3.9 and 3.10.

8_Figure 3.8: Final Disposal Site of fecal sludge matter (Waste Pond) (မြေသားကန်) No.4

9_Figure 3.9: Final Disposal Site of fecal sludge matter (Aerobic Pond) No.2

10_Figure 3.10: Final Disposal Site of fecal sludge matter (Facultative Pond) No.1

Sewage Treatment Plant (50000 Gal/ Day)

Project location is situated on near Gandama village, 4.78 acres owned by MCDC, Patheingyi township, Mandalay Region. Sewage Treatment per day is about 50,000 gallons. Construction period is from 22-8-2023 to 25-2-2024. It includes sewage treatment plant (50,000 gal/ day), constructing in sludge drying bed, constructing in control room, constructing in waste pond by soil, constructing in Transformer, constructing in fencing chain link, constructing in gate door, constructing in road with small stones in a project. In sewage treatment system, there are physical treatment and biological treatment. In sewage treatment system, it includes raw materials from sewage truck, grit chamber, pre-sedimentation tank, equalization tank, aeration tank, sedimentation tank: treated water tank and digester tank; pressurized sand filter and sludge drying bed, natural pond. Sewage Treatment Plant (50000 Gal/ Day) was shown in Figure 3.11.

11_Figure 3.11: Sewage Treatment Plant (50000 Gal/ Day)

iv) Sanitation workers

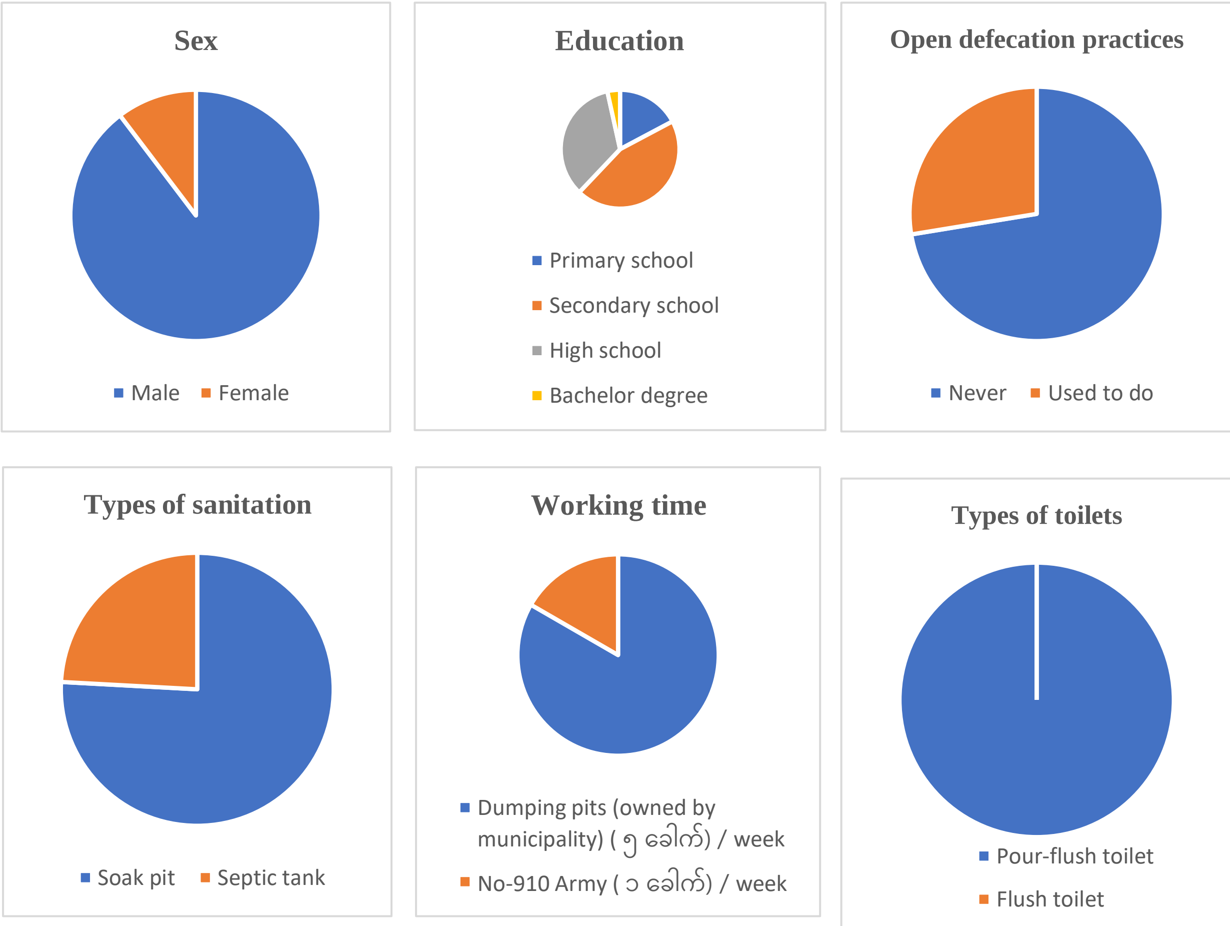
Sanitation workers have worked 6 days per week. 5 days per week per township sanitary were discharged in Aye Yeik Nyein Cemetery and 1 days per week per township sanitary were discharged in 910 Army. According to sanitation workers interview survey, fecal sludge matter can be removed from approximately 39,000 gallons/ day of a city. Approximately 288 days for a year for sanitation workers for daily exposure is assumed, accidentally ingested dose rate of 1 to 5ml each day. Cesspools and soakage pits were used by 75 %, while septic tanks were used by 25 %. Dumping pits owned by municipality from collection of fecal sludge were about 85% (information from water and sanitation department, MCDC) based on the interview surveys.

Moreover, according to an interview survey of sanitation workers, relevant staff dispose of fecal sludge on land, in the upper part of Kyarnikan village, Aye Yeik Nyein, near monetary, cemetery, besides Kyarnikan solid waste final disposal site, and between hills. All sanitation workers wear mostly masks, and gloves and boots on sometimes, and fecal sludge matter is disposed on ground without retaining wall. Sanitation workers in site was shown in Figure 3.12.

12_Figure 3.12: Fecal sludge matter collection of study area

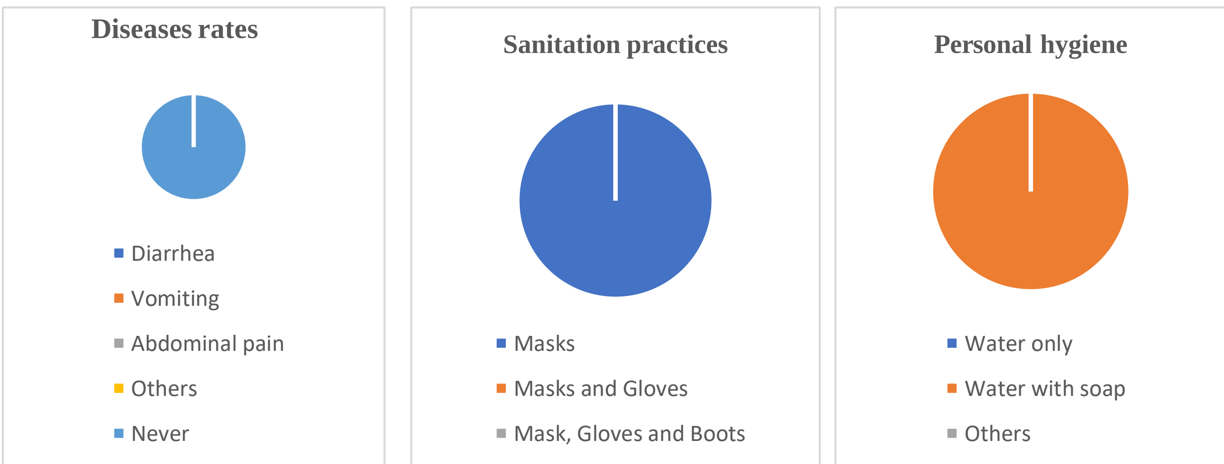
v) Sanitation workers behavior practices

The observer conducted interviews with 29 sanitation workers out of 38 people for 6 townships for the whole city of Mandalay. This was shown in Figure 3.13.



13_Figure 3.13: Sanitation workers behavior practices

According to sanitation workers interview surveys, pour-flush toilet was used 100%, water with soap with hand after finishing toilet with 100%, open defecation behavior practices



with used to do 27%, and after working from fecal sludge collection truck tank, never used to do for disease rate was 100% for sanitation workers of a city.

3.2 A Quantitative Risk Assessment on Microbes-QMRA

A Quantitative Microbial Risk Assessment-QMRA has been the assessment of pathogenic microbes' diseases quantitatively.

A Quantitative Risk Assessment for microorganisms, which had been composed of 4 stages:

1) Identification of the Hazard
2) Assessment on Exposure
3) Assessment on Dose-response
4) Risk assessment to determine health risks associated with infectious feces contamination of various environmental media.

3.2.1 Identification of the hazard

The goal for hazard identification has been to find harmful microorganisms or microbial toxins in food and water. Clinical and surveillance data can be used to identify hazard information (WHO, 2003). The first stage of QMRA is hazard identification, which involves determining which fecal pathogenic microbes are likely to cause acute or chronic health impacts in humans. In this study, fecal pathogenic organisms such as *E. coli*, *Salmonella* species, and Helminth Eggs (*A. lumbricoides*) were used. The hazard identification for local communities was shown in Table 3.5.

5_Table 3.5: The Hazard Identification for Local Communities and sanitation workers

Data collection	**Source**	**Usage**
Local community: Population, The amount of households Area, Type of On-site sanitation	Local government offices, private and government sector, internet website	Canal water, flooding water, Access of public health
Agricultural field data collection	Department of Agriculture	Irrigation water, Access of public health
Geological characteristics of local community, Soil structure formation, Groundwater table depth, microorganism transmission pathways	Literatures	Potential transmission pathways, Access of public health
Water supply source,	Local government offices	Assess of public health
Historical information from waterborne disease	Department of Public Health	Assess of public health
Fecal sludge collection: quantities, frequency	Interview survey	Access of public health

3.2.2 Assessment on Exposure

The objectives of assessment for expose are to calculate concentrations and pathogens doses that are relatively to cause illnesses in humans. Using the production-to-consumption approach, it is also possible to estimate the occurrence and pathogen level in a particular part of consumption for food over time, or in a specified water volume, with associated uncertainty. While a mean value can be used, an estimation of the distribution of exposures is needed for greater accuracy. This usually includes determining annually consumption frequencies for food and water, as well as weights and volumes for a population which were given or sub-populations, and using data to identify population exposure to pathogenic microorganisms through a specific commodity for food or water (WHO, 2003).

In this study, exposure assessment was used to calculate the microbial ingested dose through environmental transmission pathways such as consumption of contaminated water, such as canal water, tap water, FS tank, irrigation water, and floods water. This process determines the amounts of microbial concentrations in water, such as *E. coli, Salmonella* species, and Helminth eggs (*A. lumbricoides*), as well as the ingestion dose of water/contact material per exposure. Water volume ingestion dose assumptions were used and shown in Table 3.6.

6_Table 3.6: Water Volume Ingestion Dose Assumptions

Scenarios	**Condition**	**Ingestion dose of water volume**	**Frequency of the year**	**Source**
1	Flooding	10-30 ml	6 time/year	From literatures
2	Working	1-5 ml	144 time/ year	From this study
			672 time/ year	From Mandalay study
3	Farming	10-50 ml	112 time/ year	From this study
4	Swimming	20-50 ml	6 time/ year	From literatures
5	Fishing	10 ml	6 time/ year	From this study

3.2.3 The assessment of dose-response

The model for dose- response Beta-Poisson have been found to be capable of describing such dose-to-dose variability of the Poisson distribution, and can be generalized as a function of two parameters: a median dose infection (N_{50}) and a parameter for a slope ($\dot{\alpha}$) of a curve for dose-response that is specific for describing the organism the distribution of probability (Beta) N_{50}. (Gerba et al., 2002, Hunter et al., 2003) has been required dosage or pathogen expose (i.e., the organism's number on average with consumed) to cause infected. (Ryu et al., 2005) by:

Beta-Poisson dose-response model $P_{inf} = 1 - [1 + d/N_{50}\,(2^{1/\dot{\alpha}} - 1)]^{-\dot{\alpha}}$ **Eq-3.4**

The simply beta-Poisson dose-response model for QMRA, for rotavirus, Campylobacter spp., pathogenic *E. coli*- *E. coli* O157: H7, pathogenic *Salmonella* spp. and *A. lumbricoides* had been used (Teunis & Havelaar, 2000; McBride et al., 2013; Haas et al., 2014) is characterized by:

$$P_{inf} = 1 - [1 + (d/\beta)]^{-\dot{\alpha}}$$

With a median infectious dose defined as

$$N50 = \beta\,(2\ 1/\dot{\alpha} - 1)$$

Where, P_{inf} = daily infection risk probability

d = daily exposure pathogenic dosage

N50 = mediumly infection dosage

ἀ = parameter for slope which are characterized relationships for dose-response indicated as pathogenic affected constant

For pathogenic, *E. coli* O157: H7, recently shown for dose-response by Teunis et al., (2008) coming from data outbreak: $\alpha = 0.373$, $\beta = 39.71$ which implies $N_{50} = 7.336$ (Teunis et al., 2008) or for pathogenic *E. coli* spp. $\alpha = 0.373$, $N_{50} = 214.94$ (Teunis et al., 2008), and for *Salmonella* spp. $\alpha = 0.3126$, $N_{50} = 23{,}600$ (Haas et al., 1999) and for Helminth eggs (*Ascaris lumbricoides* $\alpha = 0.0104$; $N_{50} = 859$ (Mara & Sleigh, 2010).

3.2.4 The characterization of the risk

The risk assessment (i.e., an estimation of the chance and several of detrimental health consequences that might occur in particularly population, with related uncertainties) is obtained by combining the three previous phases (WHO, 2003). Computing the annual infection risk probability is part of the risk characterization process (P). It is tied to a person's multiple exposures (Sakaji & Funamizu, 1998) and was computed as:

Annual risk of infection $P = 1\text{-}(1\text{-}P\ inf)\ n$ **Eq-3.5**

Where, P = estimated annually infected risks probability from n exposure yearly because of Per event pathogenic dosage (d)

N = the days number yearly that individual has been exposed to pathogenic dosage (d) agents or totally exposure number events that occur

Probable Risk of Infection for bacteria such as *E. coli* and *Salmonella* species, in canal water, irrigation water, flooding water and sanitation workers for single exposure and annual risk

4. Results

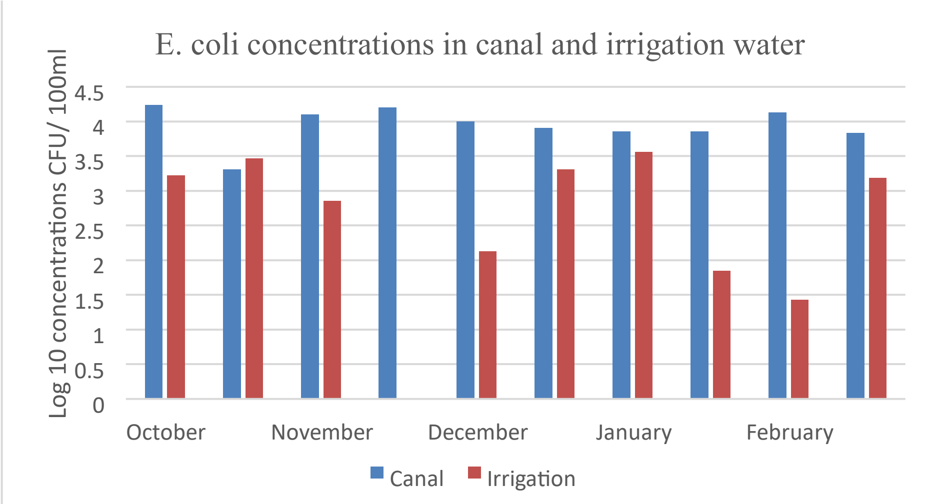
4.1. Microbial pathogenic concentrations

4.1.1. Microbial pathogenic concentrations in canal and irrigation water of Thailand research

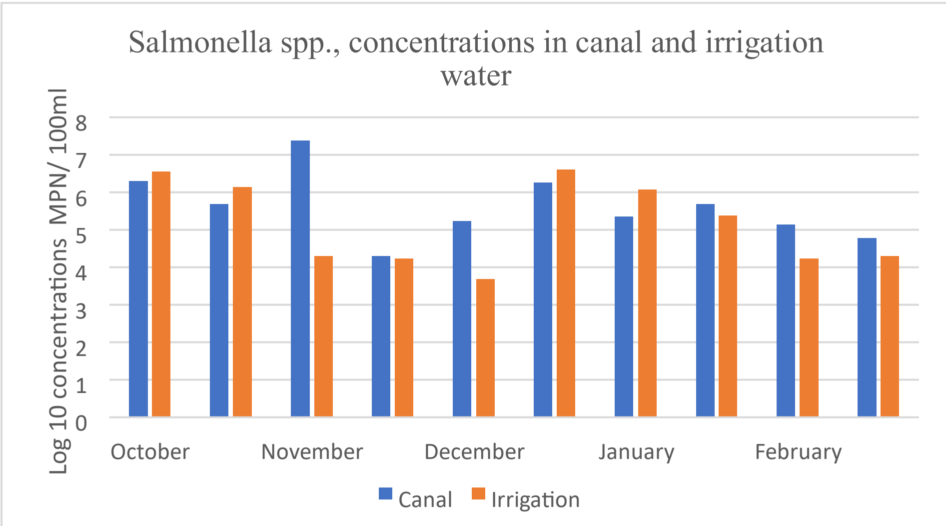
Summary for bacteria *E. coli* concentrations and Salmonella species concentrations in canal and irrigation water are as follows in Figure 4.1 and 4.2.

The concentrated *E. coli* in canal water were higher than in irrigation water. Because most peri-urban households stayed upstream of the Khlong Nueng canal, built toilets in the canal, and disposed of fecal sludge and grey water such as bathing, laundry, and washing utensils directly discharged into the canal, and kept domestic dogs in those regions.

The amounts of *E. coli* in irrigation water were lower than those found in canal water. Because some peri-urban homes stayed upstream of the Khlong Song canal and built toilets in the canal, discharging of fecal sludge and grey water directly into the canal for bathing, laundering, and washing utensils, and keeping domestic dogs in those areas. Some households, however, are located downstream of Khlong Song, the irrigation water sample collection canal. No correlation was found between canal water and irrigation water with each pathogen concentration (p value> 0.05). Correlation was found between canal water and irrigation water with sampling months (p value < 0.05). It is statically significant.



14_Figure 4.1: Summary for bacteria E. coli concentrations in canal and irrigation water



15_Figure 4.2: Summary concentrations for Salmonella spp., in canal water and irrigation water

Salmonella spp. concentrations in canal water were slightly higher than in irrigation water. *Salmonella* spp., on the other hand, can live in extreme temperatures and precipitation. During November's rains, water from the Khlong Nueng and Khlong Song canals was moved downstream by water flow from upstream places. Moreover, due to most households in the peri- urban settings stayed in the upstream of the Khlong Nueng canal and built toilets in the canal and disposed fecal sludge directly into the canal and also grey water such as bathing, laundry cleaning, washing utensils and keeping domestic dogs in those areas. Talad Thai market also sells meat such as chicken, beef, and poultry, discharged directly into canal water. For the Khlong Nueng canal, animal meat can carry *Salmonella* spp., and for Khlong Song canal, farmers may utilize animal feces as fertilizer in agricultural fields. As a result, both canal water and irrigation water have increased *Salmonella* spp. concentrations.

4.1.2. Microbial pathogenic concentrations in flooding water

The concentrated *E. coli* and *Salmonella* species, had been calculated by using described in the previous chapter equation (1) and (2). The log 10 concentrations number of pathogenic microorganisms for bacteria *E. coli* CFU per 100 ml and *Salmonella* species MPN per 100 ml in flooding water were shown in Table 4.1.

7_Table 4.1: Summarized for Concentrated E. coli and Salmonella Species in Flooding Water

Flooding **water**	Sampling **point**	Avg. Temp **°C**	Avg. Humi **(%)**	Log10 **concentration/ 100ml**	Weird **%**
E. coli CFU per 100 ml	Near Talad Thai market	27.9	80	3.690196	47
Salmonella species MPN per 100 ml	Near Talad Thai market			5.968483	69

The quantities of bacteria *E. coli* and *Salmonella* species in flooding water were found to be an average about 10^{3} = (3 log) and also 47%, and 10^{5} = (5 log) and also 69 % respectively.

The whole flooding water data set was transformed to logarithms, transformed percentage (%). *E. coli* and *Salmonella* species were found in floodwater. Flooding water was collected near Talad Thai market in October during heavy rains, and the water could not entirely drain. The average temperature and humidity in October are 27.9 °C and 80%, respectively.

Furthermore, when significant rainfall was predicted in those areas, the local authority removed various species of plants from waterways. As a result, flooding has become less frequent in such areas. The prevalence of *E. coli* and *Salmonella* species near the Talad Thai market suggests that it is linked to human feces and warm-blooded animals. Animal or human excrement injected into contaminated food, such as by a food-service worker in a commercial eatery, causes the majority of infections (*Salmonella*, 2021) (en.m.widepedia.org). *Salmonella* infections can also be spread by raw or undercooked meat, poultry, eggs, or egg products consumption (Gong et al., 2022).

4.1.3. Microbial pathogenic concentrations in Fecal Sludge.

The *E. coli* and *Salmonella* species concentrations were calculated by using the described in the previous chapter equation (1) and (2). The log 10 concentration number of pathogenic microorganisms for bacteria *E. coli* CFU per 100 ml, *Salmonella* species MPN per 100 ml in fecal sludge matter were described in Table 4.2.

8_Table 4.2: Summarized for E. coli and Salmonella Species in FS Matter

FS matter	**Sampling point**	**Avg. Temp °C**	**Avg. Humi (%)**	**Log10 concentration 100 ml**	**Weird %**
E. coli	AIT campus	27	72	5.651503	69
Salmonella species	AIT campus			4.681241	60

The quantities of *E. coli* and *Salmonella* species in fecal sludge materials were found to be an average about 10^{5} = (5 log) and also 69%, and 10^{4} = (4 log) and also 60 % respectively. The entire fecal sludge matter data set was converted to logarithms, and the transformed percentages were calculated (percent). The quantities of *E. coli* in fecal sludge are higher in two species. The whole fecal sludge matter data set was converted to logarithms and the percentages were modified (percent). In fecal sludge matter, *E. coli* and *Salmonella* species were detected. *E. coli* has larger quantities in fecal sludge than the other species. Healthy humans and animals have *E. coli* bacteria in their intestines. The majority strains for E. coli are not hazardous, and they are important for healthy human digestive system part (www.cdc.gov). *E. coli* and *Salmonella* species are common in human feces.

4.2. Probable Risk of Infection for *E. coli* and *Salmonella* species of Thailand research

4.2.1. Probable Risk of Infection for *E. coli* and *Salmonella* species in canal water for Thailand research

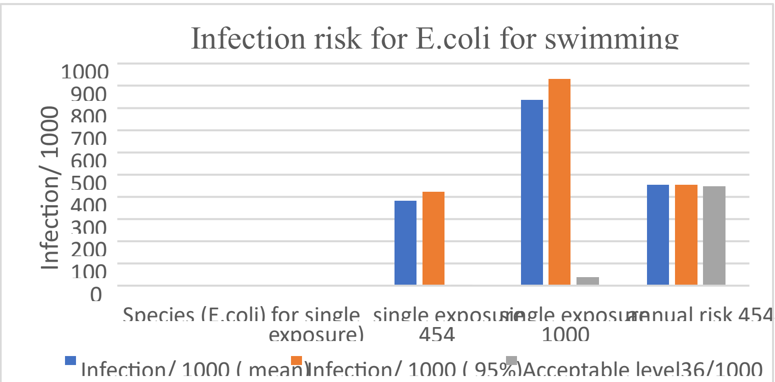
i) Scenario 1: swimming in canal water

People who live along the canal do not use canal water for each situation at this time, according to the researcher's observations and interviews. When the majority of the rainfall and flooding occurred, however, the researcher found that some residents living near and along the canal began fishing and swimming in it. The water ingestion doses for swimming (20-50 ml for 6 times per year) and fishing (10 ml for 6 times per year) were developed using the model with dose- response Beta Poisson described in previous chapter. Probability of infection for single exposure and annually risk infection can be calculated by using equation (3.4) and (3.5).

Table 4.3 and Figure 4.3 demonstrate the risk of *E. coli* infection from swimming in canal water:

9_Table 4.3: E. coli infection Risk when Swimming in Canal Water

Species	Probable risk of infection	Probability of infection (for single exposure)	single exposure, Khlong Nueng 454 people (2%)	single exposure for 1000 people	Annual risk of infection, (Khlong Nueng 454 people (2%))
E. coli	Infection/ 1000 (Mean)	0.8	380	836	454
	Infection/ 1000 (95%)	0.9	422	929	454
Acceptable level36/1000		0.04	16	36	98



16_Figure 4.3: Infection Risk for E. coli for Swimming in Canal Water

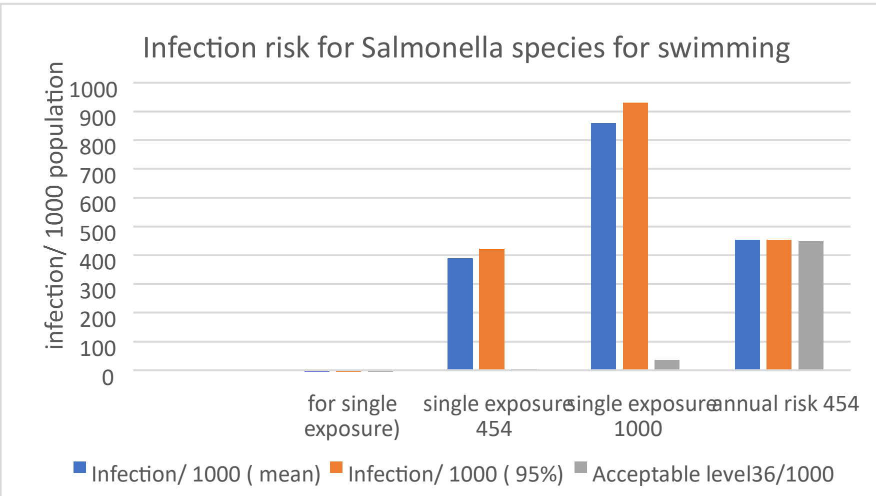
Khlong Nueng has a population of 56802. According to a 15-household interview survey, there are 22, 721 people, 56802 of whom are justified based on less than 0.5 km (20%) and inside the canal (20%) with distance between on-site sanitation and water bodies. In 15 households, 60 percent of the residents eat fish caught in the canal. As a result, for 22, 721 people, the probability of swimming and fishing is 2% (454 people).

Firstly, the probability for infection for *E. coli* after a single exposure to canal water is between 0.8 and 0.9, which is greater than the permitted level of 0.04. Second, a single exposure for Khlong Nueng 454 persons (2 percent) is around 380 and 422, which is greater than the permitted level of 16. Finally, a single exposure for 1000 people is 836 and 929 which is more than the permissible amount of 36. Finally, the annual infection risk for Khlong Nueng, approximately 454 persons, or 2% of the population are 454 and 454 people which is greater than the tolerable amount of 98.

Table 4.4 and Figure 4.4 demonstrate the risk of *Salmonella* species infection from swimming in canal water:

10_Table 4.4: Risk of Infection for Salmonella Spp., for Swimming in Canal Water

Species	**Probable risk of infection**	**Probability of infection (for single exposure)**	**single exposure, Khlong Nueng 454 people (2%)**	**single exposure for 1000 people**	**Annual risk of infection, (Khlong Nueng 454 people (2%))**
Salmonella spp.,	Infection/ 1000 (Mean)	0.9	389	858	454
	Infection/ 1000 (95%)	0.9	422	930	454
Acceptable level36/1000		0.04	16	36	98



17_Figure 4.4: Risk of infection for Salmonella species for swimming in canal water

Firstly, for *Salmonella* species, the probability for infection for a single exposure when swimming in canal water is between 0.9 and 0.9, which is greater than the permissible level of 0.04. Second, a single exposure for Khlong Nueng 454 individuals (2 percent) is approximately 389, 422 people, which is greater than the permitted amount of 16. Thirdly, a single exposure for 1000 people is between 858 and 930 persons, which is greater than the permissible level

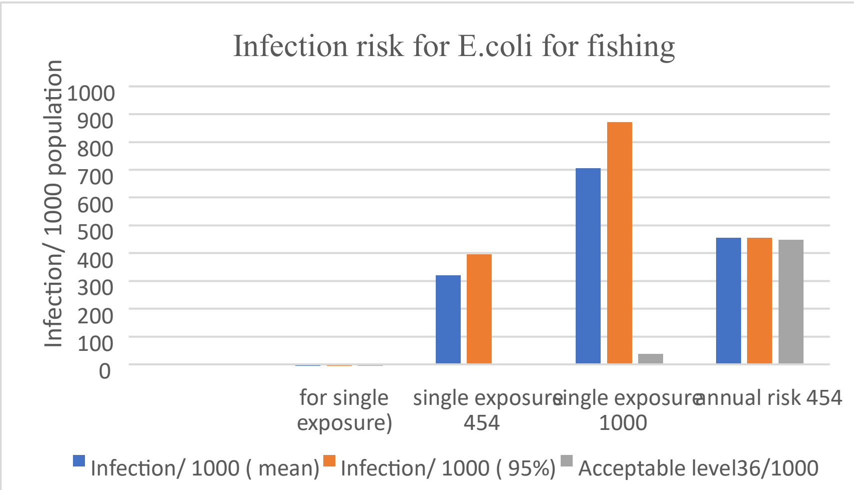
36. Finally, the yearly infection risk for Khlong Nueng 454 individuals, or 2% of the population, is between 454 and 454 people, which is greater than the permissible limit of 98.

ii) Scenario 2: fishing in canal water

Infection risk for *E. coli* for fishing in canal water was described in Table 4.5 and Figure 4.5:

11_Table 4.5: Risk for Infection for E. coli for Fishing in Canal Water

Species	Probable risk of infection	Probability for infection (for single exposure)	Single exposure (Khlong Nueng 454 people (2%))	Single exposure for 1000 people	Annual risk of infection (Khlong Nueng 454 people (2%))
E. coli	Infection/ 1000 (Mean)	0.7	320	705	454
	Infection/ 1000 (95%)	0.87	395	871	454
Acceptable level36/1000		0.04	16	36	98



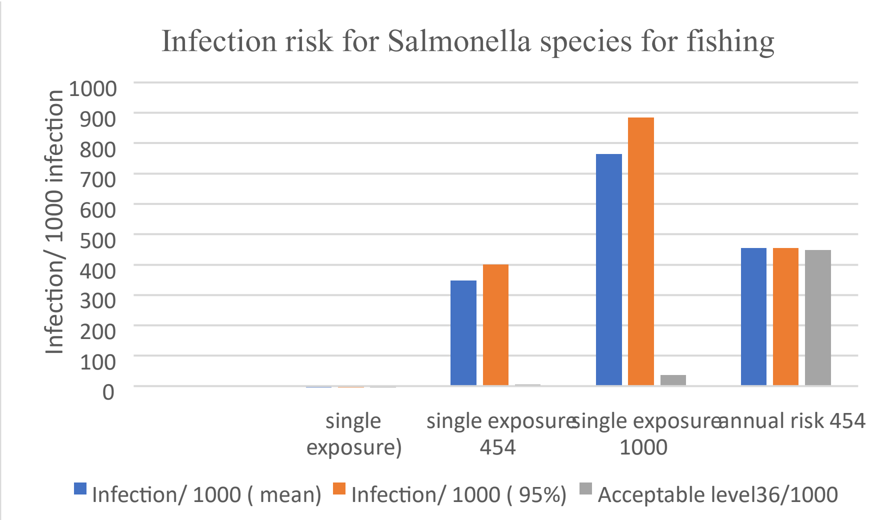
18_Figure 4.5: Infection risk for E. coli for fishing in canal water

Firstly, probability for infected for per event expose with canal water for *E. coli* when fishing is between 0.7 and 0.87, which is greater than the permitted level of 0.04. Second, a single exposure for Khlong Nueng 454 people (2 percent) is around 320 and 395 which is greater than the permissible level of 16. Third, a single *E. coli* exposure for 1000 people is between 705 and 871 people, which exceeds the permissible level 36. Finally, the annual infection risk in Khlong Nueng or 2% of the population, is between 454 and 454 people which is more than the permissible threshold of 98

Infection risk for *Salmonella* species for fishing in canal water was described in Table 4.6 and Figure 4.6:

12_Table 4.6: Risk for Infection for Salmonella Spp., for Fishing in Canal Water

Species	Probable risk of infection	Probability of infection (for single exposure)	Single exposure (Khlong Nueng 454 people (2%))	Single exposure for 1000 people	Annual risk of infection (Khlong Nueng 454 people (2%))
Salmonella spp.,	Infection/ 1000 (Mean)	0.8	347	765	454
	Infection/ 1000 (95%)	0.9	401	884	454
Acceptable level36/1000		0.04	16	36	98



19_Figure 4.6: Risk of infection for Salmonella species for fishing in canal water

Firstly, the probability for infection for a single exposure to canal water for *Salmonella* spp., when fishing is between 0.8 and 0.9, which is greater than the permitted level of 0.04. Second, for Khlong Nueng 454 people (2 percent), a single exposure is approximately 347 and 401 people, which is greater than the allowed limit 16. Third, a single exposure for 1000 people is between 765 and 884 people, which is greater than the tolerable limit 36. Finally, the annual infection risk for Khlong Nueng 454 people, or 2% of the population, is between 454 and 454 people, which is greater than the permissible limit of 98.

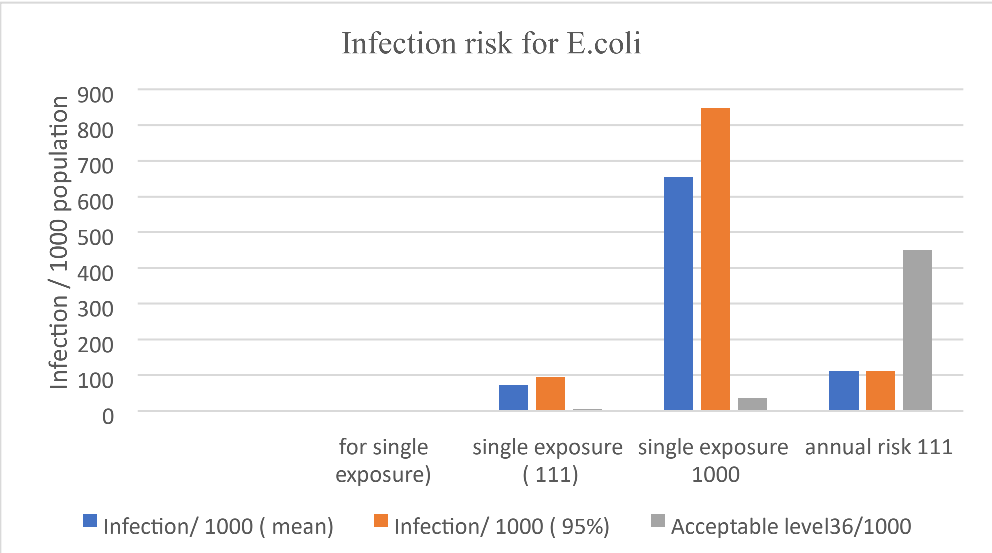
4.2.2. Probable Infection risk from *E. coli* and *Salmonella* species, for farming in irrigation water for Thailand research

i) Scenario 1: farming in irrigation water

According to the interview survey, the majority of farmers work for four months. Irrigation water is supplied by the Khlong Song canal, which is approximately 1500 meters (1.5 km) from the canal to the irrigating areas. In the previous chapter, the ingestion dose of water for farming (10-50 ml) was discussed. According to the interview survey, the majority of farmers work for four months 112 times per year. Khlong Song is populated to 111 farmers (DoA, 2021). Equation (3.4) was used to compute the probability for infection for per event using the Beta-Poisson model. Equation (3.5) was employed to compute the yearly infection risk. Infection risk for *E. coli* for farming in irrigation water was described in Table 4.7 and Figure 4.7:

13_Table 4.7: Risk of Infection E. coli for Farming in Irrigation Water

Species	**Probable risk of infection**	**Probability for infection (for single exposure)**	**Single exposure (Khlong Song 111)**	**single exposure for 1000 people**	**Annual risk of infection (Khlong Song 111)**
E. coli	Infection/ 1000 (Mean)	0.65	73	653	111
	Infection/ 1000 (95%)	0.85	94	847	111
Acceptable level36/1000		0.04	4	36	448



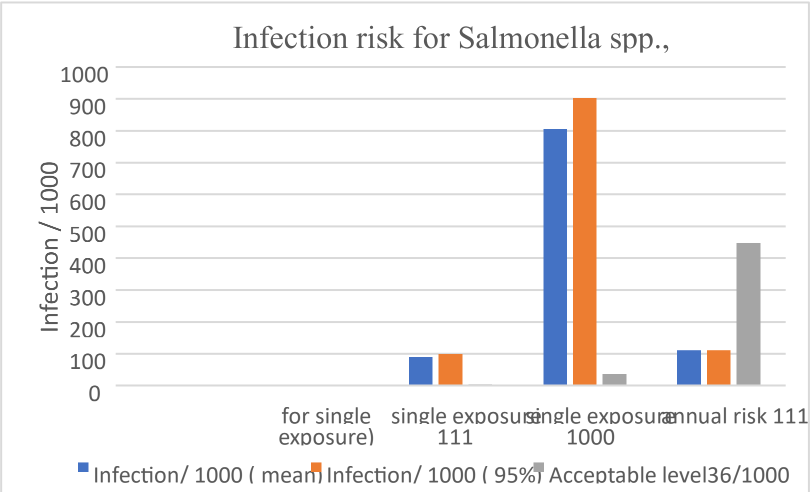
20_Figure 4.7: Infection risk for E. coli for farming in irrigation water

Firstly, the probability for infection for per event exposure in irrigation water for farming is between 0.65 and 0.85 for *E. coli*, which is greater than the acceptable level of 0.04. Second, for Khlong Song 111 people, a single exposure is 73, and 94, which is greater than the allowed level 4. Third, a single exposure for 1000 people is 653 and 847 people, respectively, exceeding the acceptable level 36. Finally, the annual infection risk for Khlong Song 111 people is between 111 and 111 people, which is lower than the allowed amount of 448.

Infection risk for *Salmonella* spp., for farming in irrigation water was described in Table 4.8 and Figure 4.8.

14_Table 4:8: Risk for Infection for Salmonella Spp., for Farming in Irrigation Water

Species	Probable risk of infection	Probability of infection (for single exposure)	Single exposure (Khlong Song 111)	single exposure for 1000 people	Annual risk of infection (Khlong Song 111)
Salmonella spp.,	Infection/ 1000 (Mean)	0.8	89	804	111
	Infection/ 1000 (95%)	0.9	100	903	111
Acceptable level36/1000		0.04	4	36	448



21_Figure 4.8: Risk of infection for Salmonella spp., in irrigation water

Firstly, Probability of infection for single exposure for farming in irrigation water is between 0.8 and 0.9 for Salmonella species is higher than the acceptable level 0.04. Secondly, a single exposure for Khlong Song 111 people is approximately 89 and 100 people for *Salmonella* species is higher than the acceptable level 4. Thirdly, a single exposure for 1000 people is between 804 and 903 people for *Salmonella* species is higher than the acceptable level 36. Finally, annual infection risk for Khlong Song 111 people, between 111 and 111 people for *Salmonella* species is below the acceptable level 448.

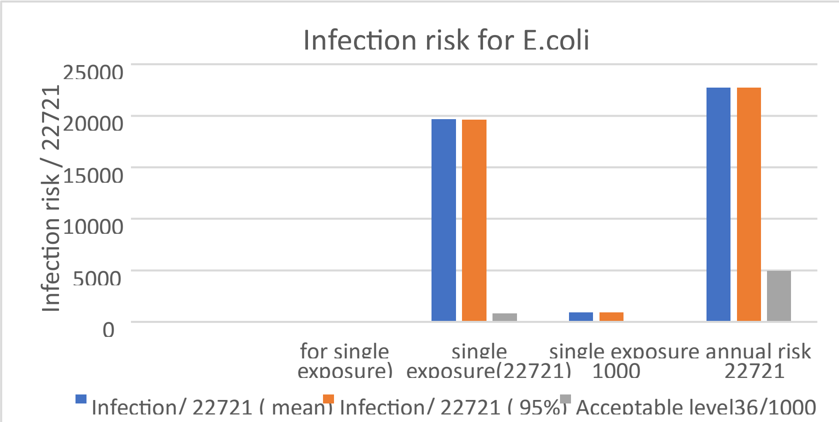
4.2.3. Probable Risk of Infection for *E. coli* and *Salmonella* species for flooding water for Thailand research

i) Scenario 1: Flooding water

Human excreta and warm-blooded animals are associated to *E. coli* and *Salmonella* spp., found near Talad Thai market. The ingestion dose of water for flooding was shown to be 10-30 ml six times per year in the previous chapter. Khlong Nueng has a population of 56802. Because the distance between on-site sanitation and water bodies is less than 0.5 km (20 percent) and in the canal, 22, 721 people, or 56,802 people, are justified (20 percent). As a result of the floodwater injection, it is estimated that 22,721 individuals in Pathum Thani province, which is primarily affected by flooding, will be affected. Equation (3.4) was used to compute the probability for infection for per event exposure using the model Beta-Poisson. Equation (3.5) was utilized to compute the yearly infection risk. Risk of infection for bacteria *E. coli* and *Salmonella* species, for flooding water had been described in Table 4.9 and Figure 4.9:

15_Table 4.9: Infection Risk E. coli for Flooding Water

Species	Probability risk of infection	Probability for infection (for single exposure)	Single exposure, (Khlong Nueng 22721 people)	Single exposure for 1000 people	Annual risk of infection (Khlong Nueng 22721)
E. coli	Infection/ 1000 (mean%)	0.86	19665	866	22721
	Infection/ 1000 (95%)	0.86	19605	863	22721
Acceptable level36/1000		0.036	818	36	4908



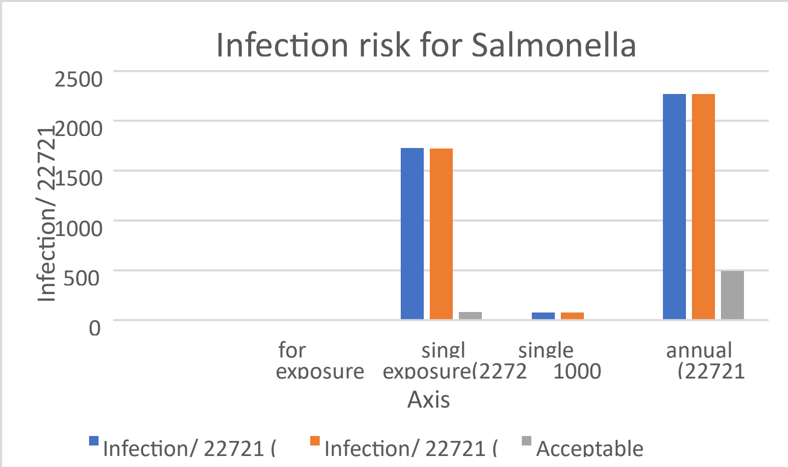
22_Figure 4.9: Risk of infection for E. coli for flooding water

Firstly, the probability of infection for flooded water is between 0.86 and 0.86 for *E. coli*, which is higher than the acceptable level of 0.04. Second, a single exposure in Khlong Nueng results in about 19665 and 19605 people for *E. coli*, respectively, which is greater than the acceptable level of 818. Third, a single *E. coli* exposure for 1000 people is between 866 and 863, which is greater than the acceptable level of 36. Finally, the annual infection risk in Khlong Nueng is around 22721 people, and the *E. coli* infection risk is about 22721 people, which is greater than the acceptable threshold of 4908.

Risk of infection for *Salmonella* spp., for flooding water was described in Table 4.10 and Figure 4.10.

16_Table 4.10: Risk for Infection for Salmonella Species for Flooding Water

Species	Probability risk of infection	Probability of infection (for single exposure)	Single exposure, (Khlong Nueng 22721 people)	Single exposure for 1000 people	Annual risk of infection (Khlong Nueng 22721)
Salmonella species	Infection/ 1000 (mean%)	0.8	17297	761	22717
	Infection/ 1000(95%)	0.8	17211	758	22716
Acceptable level36/1000		0.036	818	36	4908



23_Figure 4.10: Risk of infection for Salmonella spp., for flooding water

Firstly, for *Salmonella* spp., the probability for infection for per event exposure to flooded water is between 0.8 and 0.8, which is greater than the acceptable level of 0.04. Second, a single exposure to *Salmonella* species in Khlong Nueng, 22721 people, is about 17297 and 17211 people, which is greater than the acceptable threshold of 818. Third, for *Salmonella* spp., a single exposure for 1000 people is between 761 and 758 people, which is greater than the acceptable level 36. Finally, the annual infection risk for *Salmonella* species in Khlong Nueng, 22721 individuals, are between 22717 and 22716 people, which is higher than the acceptable level of 4908.

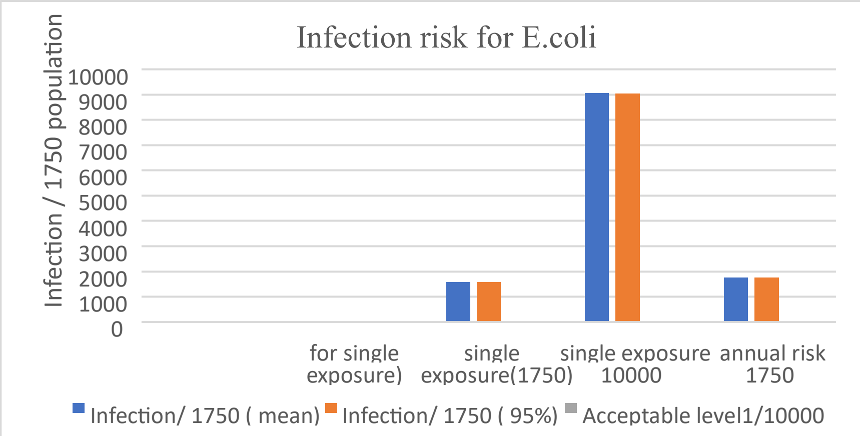
4.2.4. Probable Infection risks from *E. coli* and *Salmonella* species for sanitation workers for Thailand research

i) Scenario 1: Sanitation workers

The ingestion dose of water for sanitation workers along the canal and near Talad Thai market was 1-5 ml, and fecal sludge collectors collected 3 times per week for a total of 144 times per year, according to the published material in the previous chapter and interview survey. Six people are working for 156 households for a year, according to interview surveys. As a result, sanitation workers are estimated to number 1750 for 45500 households for Khlong Nueng. Equation (3.4) was used to compute the infection for probability for a single exposure using model for Beta-Poisson. Equation (3.5) had been employed to calculate yearly infection risk. Infection risk for bacteria *E. coli* for sanitation workers was described in Table 4.11 and Figure 4.11:

17_Table 4.11: Risk for Infection for E. coli for Sanitation Workers

Species	Probable risk of infection	Probability for infection (for single exposure)	Single exposure (Khlong Nueng 1750)	Single exposure for 10000 people	Annual risk of infection (Khlong Nueng 1750)
E. coli	Infection/ 10000 (mean%)	0.91	1585	9057	1750
	Infection/ 10000 (95%)	0.90	1582	9040	1750
Acceptable level1/10000		0.0001	0.18	1	25



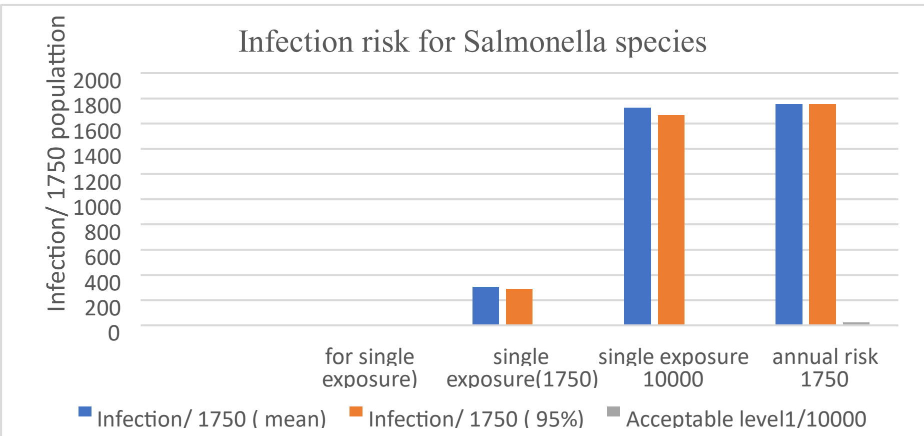
24_Figure 4.11: Risk for infection for E. coli for sanitation workers

Firstly, the probability for infection for sanitation workers after per event exposure to *E. coli* is between 0.91 and 0.90, which is greater than the acceptable level of 0.0001. Second, a single exposure for Khlong Nueng is approximately 1585 people, and a single exposure for *E. coli* is approximately 1582 people, both of which are greater than the acceptable level of 0.18. Third, a single *E. coli* exposure for 10,000 people is approximately 9057 and 9040, which is greater than the acceptable level 1. Finally, the annual infection risk for Khlong Nueng, which has a population of 1750 people, is between 1750 and 1750 individuals, which is greater than the acceptable level of 25 for *E. coli*.

Risk for infection for *Salmonella* spp., for sanitation workers was described in Table 4: 12 and Figure 4.12:

18_Table 4.12: Risk Infection for Salmonella Spp., for Sanitation Workers

Species	**Probable risk of infection**	**Probability of infection (for single exposure)**	**Single exposure (Khlong Nueng 1750)**	**Single exposure for 10000 people**	**Annual risk of infection (Khlong Nueng 1750)**
Salmonella spp.,	Infection/ 10000 (Mean)	0.2	302	1725	1750
	Infection/ 10000 (95%)	0.1	291	1665	1750
Acceptable level1/10000		0.0001	0.18	1	25



25_ Figure 4.12: Risk for infection for Salmonella spp., for sanitation workers

To begin with, the probability for infection for sanitation workers after per event exposure is between 0.2 and 0.1 for *Salmonella* species, which is greater than the acceptable level of 0.0001. Second, a single exposure for Khlong Nueng, 1750 people, is around 302 and for *Salmonella* species, 291 people, is greater than the acceptable level of 0.18. Third, a single *Salmonella* spp. exposure for 10,000 people is between 1725 and 1665 people, which is greater than the acceptable level 1. Finally, the yearly infection risk for *Salmonella* spp. in Khlong Nueng (1750 people) is between 1750 and 1750 persons, which is greater than the acceptable level of 25.

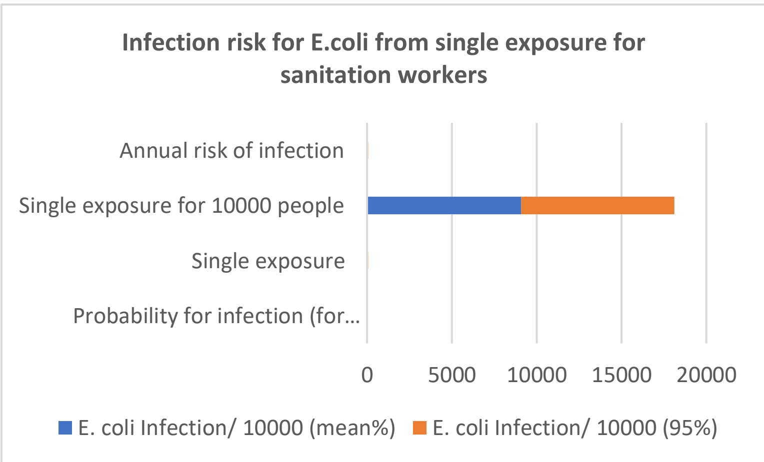
4.2.5. Probable Infection risks from *E. coli* and *Salmonella* species for sanitation workers for Mandalay Region

Scenario 1: Sanitation workers

The ingestion dose of water for sanitation workers for the whole city of Mandalay was 1-5 ml, and fecal sludge collectors collected approximately 14 times per week for a total of 672 times per year per township, according to the published material in the previous chapter and interview survey. As a result, sanitation workers are estimated to number 38 people for 1.7 million people for Mandalay city. Equation (3.4) was used to compute the infection for probability for a single exposure using model for Beta-Poisson. Equation (3.5) had been employed to calculate yearly infection risk. Infection risk for bacteria *E. coli* for sanitation workers was described in Table 4.13 and Figure 4.13:

19_Table 4.13: Risk for Infection for E. coli for Sanitation Workers

Species	Probable risk of infection	Probability for infection (for single exposure)	Single exposure (Mandalay city 38)	Single exposure for 10000 people	Annual risk of infection (Mandalay city38)
E. coli	Infection/ 10000 (mean%)	0.91	34	9100	38
	Infection/ 10000 (95%)	0.90	34	9000	38
Acceptable level1/10000		0.0001	0.0038	1	0.0038



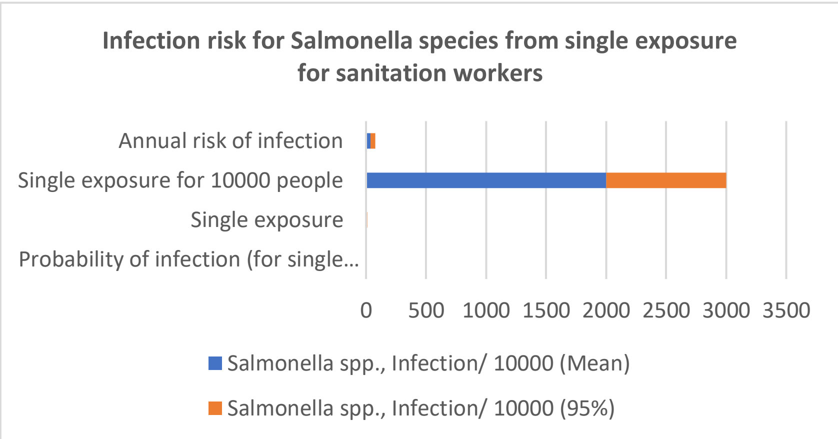
26_Figure 4.13: Risk for infection for E. coli for sanitation workers

Firstly, the probability for infection for sanitation workers after per event exposure to *E. coli* is between 0.91 and 0.90, which is greater than the acceptable level of 0.0001. Second, a single exposure for Mandalay city is approximately 34 people, and a single exposure for *E. coli* is approximately 34 people, both of which are greater than the acceptable level of 0.0038. Third, a single *E. coli* exposure for 10,000 people is approximately 9100 and 9000, which is greater than the acceptable level 1. Finally, the annual infection risk for Mandalay city, which has a population of 38 people for sanitation workers, is between 38 and 38 individuals, which is greater than the acceptable level of 0.0038 for *E. coli*.

Risk for infection for *Salmonella* spp., for sanitation workers was described in Table 4: 14 and Figure 4.14:

20_Table 4.14: Risk Infection for Salmonella Spp., for Sanitation Workers

Species	**Probable risk of infection**	**Probability of infection (for single exposure)**	**Single exposure (Mandalay city 38)**	**Single exposure for 10000 people**	**Annual risk of infection (Mandalay city 38)**
Salmonella spp.,	Infection/ 10000 (Mean)	0.2	7	2000	38
	Infection/ 10000 (95%)	0.1	3	1000	38
Acceptable level1/10000		0.0001	0.0038	1	0.0038



27_Figure 4.14: Risk for infection for Salmonella spp., for sanitation workers

To begin with, the probability for infection for sanitation workers after per event exposure is between 0.2 and 0.1 for *Salmonella* species, which is greater than the acceptable level of 0.0001. Second, a single exposure for Mandalay city, 38 people, is around 7 and for *Salmonella* species, 3 people, is greater than the acceptable level of 0.0038. Third, a single *Salmonella* spp. exposure for 10,000 people is between 2000 and 1000 people, which is greater than the acceptable level 1. Finally, the yearly infection risk for *Salmonella* spp. in Mandalay city (38 people) is between 38 and 38 people, which is greater than the acceptable level of 0.0038.

5. Discussion and Conclusion

5.1 Discussion for Thailand research

Four water samples were taken, as well as fecal sludge matter, which was analyzed for bacteria *E. coli* and *Salmonella* species; and parasitic worms' eggs from Helminth (*A. lumbricoides*). Helminth eggs (*A. lumbricoides*) play no role in this research. It was not taken into account while calculating the risk of infection. For (454 people or 2%), swimming and fishing in canal water; 1750 people for sanitation workers; and 22721 people flooding water for yearly risk infection, infection/1000 or infection/10000 (95 percent) for bacteria *E. coli* and *Salmonella* species, had been greater than permission level for *E. coli* and *Salmonella* spp. However, the acceptable limit (111 individuals) for yearly risk infection was lower than the infection/1000 (Mean) and infection/1000 (95 percent) for bacteria *E. coli* and *Salmonella* species in water which were irrigated.

The origin of feces discovered in canal water, irrigation water, flooding water, and fecal sludge materials is a fecal indicator organism (*E. coli*). While mostly *E. coli* is harmless, some, such as *E. coli* O 157: H7, are pathogenic bacteria that can cause severe diarrhea, fever, vomiting, abdominal cramps. *Salmonella* species (pathogenic bacteria) are the most commonly cause for foodborne disease. They have ability to resist severe environmental conditions. It can range from mild to severe, depending on the individual's behavior and the number of bacteria present.

Canal water sample collection is surrounded by hut infrastructure, such as an industrial estate, in the upstream, middle, and downstream, where migrant's people stay and established toilets in the canal and discharged both FS and grey water directly into the canal. The water from the market was collected in the canal that runs under the bridge. Beef, pork, chicken, fish, egg products, and other meats are sold at the meat market. *Salmonella* can be discovered in a range of items that have been contaminated, including meats, fruits and vegetables, and ready-to-eat foods (Magoulas, 2016).

Furthermore, irrigation water was transported from the Khlong Song canal. It is around 1.5 miles away (km). Farmers dug holes in the ground to store water in their agricultural fields. Animal excrement could be used as a natural fertilizer by the farmers. As a result, *E. coli* and *Salmonella* strains have been found in canal and irrigation water. The *E. coli* and *Salmonella* species concentrations, on the other hand, varied over the sample months according to temperature, relative humidity, and rainfall. In the upstream, the Khlong Song canal for irrigation water is surrounded by a few huts.

E. coli and *Salmonella* species were also found in flooded water. Flooding water was accumulated near the Talad Thai market and could not be absorbed by the ground during rainfall. *Salmonella* species could be transmitted mostly by contaminated food from Talad Thai market. Furthermore, some people used open defecation near a shelter behind the market late at night. The microorganisms can be re-suspended and survive under the shelter after the rain has fallen. *E. coli* and *Salmonella* species can survive in favorable environmental conditions and multiply, with no intermediate host and high infective dosage to the host. Furthermore, *E. coli* (fecal indicator organism), *Salmonella* species (actual pathogenic organisms), and Helminth eggs can be detected in fecal sludge and sewage sediment, which can be dangerous to sanitation workers.

As a result, households in peri-urban slum areas were more vulnerable to public health concerns. Furthermore, Thailand is currently dealing with fecal sludge management issues such as dumping fecal sludge in public areas or fields, a lack of FS treatment facilities that cover the entire region, and FS treatment plant hygiene (DoH, MoPH, 2021), and as a result, public health risks will increase as ground water levels rise and flooding areas expand.

Furthermore, Pathum Thani province is not only a low-lying place, but it also dumps fecal sludge in public areas or fields in Thailand, resulting in pathogenic microbial pollution for the inhabitants when flooding increases. Moreover, if future high rainfall and floods are caused by climate change, groundwater levels can be raised. As a result, microbial pathogenic contamination has the potential to spread.

5.2 Discussion for Mandalay research

Excreta are full of nutrients like nitrogen and phosphorus, as well as diseases including bacteria, viruses, protozoa, and helminths. Bacteria lives in the intestines of human beings and animals which are mostly warm- blooded animals.

According to *(Koottatep et al., 2021),* fecal sludge matter includes in urine mostly nutrients nitrogen-85%, phosphorous- 60%, Potassium- 54% as well as in feces mostly COD-47%, fecal coliforms- 10^7-10^9/ 100ml. Therefore, grasses, bushes and many other plants are currently cultivated in old final disposal site of fecal sludge matter for a city as well as eutrophication. The grasses of final disposal site in fecal sludge are also eaten by cows and birds. It will cause animal contact. Human infections can be transmitted by mammals and birds (waterfowl) in general (Fayers et al., 1997).

Total coliform, fecal coliform, and *salmonella* all occur concentrated in the upper layers of the sediment in water routes, according to Labelle et al. (1980), and can survive there until disturbed by disturbances like as dredging and rapid rainwater runoff. Goyal et al. (1978) also found that sediments act as reservoirs for pathogens, giving some shelter for them, and that the quantity of total coliforms present in sediment was connected to the concentrations of enteric viruses present in seawater. Therefore, pathogens can have between plants as shelter for them under favorable conditions.

According to *(Koottatep et al., 2021),* the periods for pathogen survival in FS matter, fecal coliform organism survival time average in wet FS matter at temperature with ambient in climatic temperate (10-15 ° C) is < 150 days, in climatic tropical (20-30 ° C) is < 50 days. Pathogens are inactivated as a result of a complex interaction of mechanisms with pH, temperature, ultraviolet radiation in the sunlight reaching the surface of the pond, and photo-oxidative reactions taking advantage of the high concentration of DO. Therefore, the sunlight can reach the surface of the pond if the grasses and bushes are cut off.

Septic tank systems are the mostly often reported groundwater pollution sources in the United States, due to untreated wastewater volume discharged in the groundwater (Hagedorn, 1984). Pit latrines pollution may become more difficult to control where groundwater levels rise (WHO, 2009). Where groundwater levels rise, pit latrines need to be aware of the depth of toilets, and also where groundwater levels rise, fecal sludge matter from pit latrines and septic tanks etc., need to be collected as quick.

To operate daily sewage treatment plant with skillful person for the whole city.

If the funeral people are going to the cemetery, it will cause person to person contact. Moreover, the site is surrounded by the hills. If heavy rain is become, the rainfall will be going through from high level to low level, the weak resistance of the lower part of the hills, it will affect as well as diseases like diarrhea. Therefore, if the ponds are constructed with stones, the diseases rate will be less frequent.

The road between cemetery and final disposal site of fecal sludge matter is currently made of dry soils with sand which can happen slip with vehicles in the heavy rainfall. It will cause person to person contact, animal to person contact and vehicles to person contact.

Furrow irrigation is cheap but more evaporation loss. Therefore, if appropriate precautions have been taken, wastewater can be used for irrigating agricultural land.

5.3. Conclusion and Recommendation

Conclusion for Thailand research

***With microbial pathogenic concentrations might cause public health at risks in study areas*:**

1. A concentrated with an average 10^3 CFU per 100 ml for *E. coli* (47%), 10^5 MPN per 100 ml for *Salmonella* spp. (69%) in canal water, 10^2 CFU/ 100 ml for *E. coli* (30%), 10^4 MPN/ 100 ml for *Salmonella* species (60%) in irrigation water, 10^3 CFU/ 100 ml for *E. coli* (47%), 10^5 MPN/ 100 ml for *Salmonella* species (69%) in flooding water, 10^5 CFU/ 100 ml for *E. coli* (69%), 10^4 MPN/ 100 ml for *Salmonella* species (60%) in fecal sludge matter in selected areas were detected.
2. In Khlong Nueng, annual risks of pathogenic infection from accidental ingestion of water for swimming and fishing with *E. coli* and *Salmonella* species for canal water, flooding water, and sanitation workers have exceeded the acceptable level of 36/1000 population per event and also annual risks, whereas irrigation water has a lower annual risk.
3. Sanitation workers collected FS removal from 36 residences per month (about 36, 000 L per month) using a collection truck. In the specified areas, FS was disposed of in the fields as the final disposal site.
4. The proper strategies for reducing public health risks are: The nature of harmful microorganisms such as bacteria allows them to survive and multiply in favorable environmental conditions such as temperature, relative humidity, and rainfall variations, as well as in the presence of shelter. The following are effective ways for reducing public health risks:

Recommendations for Mandalay research

- If sewage treatment plant (50,000 gal/ day) will be operated, it can reduce environmental and social (public health) impacts for the people of the whole city. If it is safe for sludge drying bed, it is appropriate measures for agricultural practices.
- Various types of plants such as grasses and bushes throughout the designated ponds in order to prevent temperature disturbances should be cut off in old final disposal site of fecal sludge matter of a city in summer weather.
- The site should be fenced, designated as restricted area and signed properly.
- Concrete road for final disposal site of fecal sludge matter should be built along Aye Yeik Nyein Cemetery to the final disposal site of fecal sludge matter of a city.
- Farmers should use PPE such as gloves, masks, and shoes to avoid accidental ingestion and inhalation. Improved irrigation practices, such as switching from flood irrigation with hosepipe to sprinkler or drip, are needed to limit exposure by minimizing the amount of wastewater reuse accidentally swallowed.

- Sanitation workers should wear (PPE) like masks, boots, and gloves to protect unintentional ingestion or contaminated water inhalation, as well as local government need to enhance public awareness of the public health consequences of the sanitation service chain.
- The site should be above seasonal flood level and not subjected to water logging during heavy rain, or due to a high-water table during the rainy season.
- The site should be more than 200m from the boundary of any residential property, or other sensitive receptors such as schools.
- The site should be a minimum distance of 200m from any downstream source of public or private water supply such as river or any water course, borehole, well or exposed aquifer.
- The land should be downstream of any public water supply abstraction point, or if upstream, it should be at least 5 km from the point.
- Regular tanker movements should be able to take place safely and not cause a nuisance to the public (especially in near the river areas).

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8. APPENDIX

Khlong Nueng households in peri-urban areas in Pathum Thani province, Thailand

Irrigation water from Khlong Song canal, in Pathum Thani province, Thailand

Sannitation workers in their fieldwork

Fecal Sludge truck in their fieldwork

Fecal sludge collection

Mandalay, Sanitation workers interview survey