# โครงการ
# การเรียนการสอนเพื่อเสริมประสบการณ์

**ชื่อโครงการ** การตรวจตราและดูประสิทธิภาพในการบำบัดจากโรงบำบัดน้ำเสียของไวรัสเอนเทอโรในสิ่งแวดล้อม

Detection of enteroviruses and their removal efficiency in the wastewater treatment plants

**ชื่อนิสิต** นายกฤตนิพิชญ์ เพ็ชรอนันต์ **เลขประจำตัว** 6033303323

**ภาควิชา** วิทยาศาสตร์สิ่งแวดล้อม

**ปีการศึกษา** 2563

**คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย**

# Detection of enteroviruses and their removal efficiency in the wastewater treatment plants

Kritnipit Phetanan

A Senior Project Submitted in Partial Fulfillment of the Requirements
for the Bachelor’s Degree of Science Program in Environmental Science
Department of Environmental Science, Faculty of Science,
Chulalongkorn University
Academic Year 2020

# SENIOR PROJECT

| | | |
|---|---|---|
| **Project Title** | **Detection of enteroviruses and their removal efficiency in the wastewater treatment plants** | |
| **Student Name** | Mr. Kritnipit Phetanan | Student ID 603 33033 23 |
| **Department** | Environmental Science | |
| **Academic Year** | 2020 | |

**Faculty of Science, Chulalongkorn University**

**Project Title** **Detection of enteroviruses and their removal efficiency in the wastewater treatment plants**

**Student Name** Mr. Kritnipit Phetanan Student ID 603 3303323
**Project Advisor** Jatuwat Sangsanont, Ph.D.
**Department** Environmental Science
**Academic Year** 2020

---

Accepted by the Department of Environmental Science, Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Bachelor's Degree

.............................................................................. Head of Department of Environmental Science
(Assistant Professor Pasicha Chaikaew, Ph.D.)

**Project Committee**

................................................................. Chairman
(Assistant Professor Vorapot Kanokkantapong, Ph.D.)

................................................................. Committee
(Associate Professor Pantana Tor-ngern, Ph.D.)

JATUWAT SANGSANONT
................................................................. Project advisor
(Jatuwat Sangsanont, Ph.D.)

| | | | |
|---|---|---|---|
| **หัวข้อ** | **การตรวจตราและดูประสิทธิภาพในการบำบัดจากโรงบำบัดน้ำเสียของไวรัสเอนเทอโรในสิ่งแวดล้อม** | | |
| **โดย** | **นาย กฤตนิพิชญ์ เพ็ชรอนันต์** | **รหัสประจำตัวนิสิต** | **603 33033 23** |
| **อาจารย์ที่ปรึกษา** | **อาจารย์ ดร. จตุวัฒน์ แสงสานนท์** | | |
| **ภาควิชา** | **วิทยาศาสตร์สิ่งแวดล้อม** | | |
| **ปีการศึกษา** | **2563** | | |

---

## บทคัดย่อ

ไวรัสเอนเทอโรเป็นไวรัสอาร์เอนเอที่ก่อให้เกิดโรคทางน้ำเสียในมนุษย์ และส่งผลทำให้เกิดโรคหลากหลายในมนุษย์ ไม่ว่าจะเป็นโรคที่เกี่ยวกับระบบทางเดินอาหารหรือโรคมือเท้าปากก็ล้วนมีสถิติการติดเชื้อไวรัสเอนเทอโรนี้มากในประเทศไทย เนื่องจากไวรัสเอนเทอโรเป็นไวรัสที่มีความคงทนในสิ่งแวดล้อมเป็นอย่างมาก จึงเป็นเรื่องปกติที่จะพบเจอได้ในสิ่งแวดล้อมทุกด้านโดยเฉพาะสิ่งแวดล้อมด้านน้ำ โรคมือเท้าปากเป็นหนึ่งในโรคที่เกิดจากการติดเชื้อของไวรัสเอนเทอโร โดยส่วนมากมักจะเกิดการติดเชื้อในเด็กทารกจนถึงเด็กเล็กเนื่องจากเด็กในวัยนี้ยังไม่มีภูมิคุ้มกันที่มากพอ การจัดการน้ำเสียไม่ว่าจะเป็นการตรวจตราไวรัสในสิ่งแวดล้อมอยู่เป็นประจำเพื่อทำการหาปริมาณความเข้มข้นของไวรัสที่เราสนใจหรือการควบคุมน้ำเสียโดยการสร้างระบบควบคุมคุณภาพมาตรฐานน้ำเสียเป็นสิ่งสำคัญ ซึ่งนำไปสู่การสืบหาว่าปริมาณของผู้ป่วยที่เป็นโรคมือเท้าปากจากการติดเชื้อไวรัสเอนเทอโรสามารถแทนได้จากปริมาณความเข้มข้นของไวรัสเอนเทอโรในน้ำเสียหรือไม่ และยังหาความสามารถในการบำบัดน้ำเสียของโรงบำบัดน้ำเสีย ในงานวิจัยชิ้นนี้ ได้ทำการเก็บตัวอย่างน้ำเสียจากโรงบำบัดน้ำเสียในกรุงเทพมหานครทั้ง 3 โรง ได้แก่ โรงบำบัดน้ำเสียดินแดง โรงบำบัดน้ำเสียหนองแขมและโรงบำบัดน้ำเสียช่องนนทรีด้วยวิธีการจ้วงตักทั้งสามโรงบำบัด ได้ทำการศึกษาเกี่ยวกับสหสัมพันธ์ของปริมาณความเข้มข้นของไวรัสเอนเทอโรในน้ำเสียกับปริมาณผู้ป่วยที่ติดเชื้อไวรัสเอนเทอโรและเกิดโรคมือเท้าปากว่าทั้งสองมีความสัมพันธ์กันมากน้อยเท่าใด พบว่าปริมาณความเข้มข้นของไวรัสเอนเทอโรไม่ได้มีความสัมพันธ์อย่างมีนัยสำคัญกับปริมาณผู้ป่วยที่ติดเชื้อไวรัสเอนเทอโรและเกิดโรคมือเท้าปาก รวมถึงได้ทำการศึกษาหาประสิทธิภาพในการบำบัดไวรัสในน้ำเสียของโรงบำบัดน้ำเสียทั้ง 3 โรงบำบัดที่ได้ทำการศึกษาขึ้นมา พบว่าโรงบำบัดน้ำเสียดินแดงมีประสิทธิภาพในการบำบัดไวรัสในน้ำเสียอย่างน้อยเท่ากับ 1-log reduction ซึ่งทั้งสามโรงบำบัดน้ำเสียนี้ไม่ได้มีการเพิ่มกระบวนการฆ่าเชื้อโรคที่ใช้สำหรับการฆ่าเชื้อการปนเปื้อนทางชีวภาพมาโดยเฉพาะ

คำสำคัญ: ไวรัสเอนเทอโร, การตรวจตราทางสิ่งแวดล้อม, การตรวจตราทางคลินิค, Wastewater-based epidemiology (WBE), โรคมือเท้าปาก, โรงบำบัดน้ำเสีย, ประสิทธิภาพในการบำบัด

| | | |
|---|---|---|
| **Project Title** | **Detection of enteroviruses and their removal efficiency in the wastewater treatment plants** | |
| **Student Name** | Kritnipit Phetanan | Student ID 603 33033 23 |
| **Project Advisor** | Jatuwat Sangsanont, Ph.D. | |
| **Department** | Environmental Science | |
| **Academic Year** | 2020 | |

---

# ABSTRACT

Enteroviruses are small and non-enveloped RNA viruses and are also known as viruses that cause waterborne diseases such as gastrointestinal disease or hand-foot-mouth disease in humans from mild symptoms to severe syndromes in Thailand. Since enteroviruses are highly persistent and stabilize the environment, they're normally found in every media in an environment especially in sewage. Hand-foot-mouth disease is one of the diseases caused by the infection of enterovirus. Mostly infect in infants to child because they have a low amount of immunity. Wastewater management is crucial in the case of protecting and avoiding infection by monitoring the interested virus routinely. Improved and sustainable infrastructure for water quality and facilities is also an important requirement for the reduction of human health risks and environmental protection. Therefore, to investigate that can the infected hand-foot-mouth cases be substituted by the prevalence of enterovirus in sewage, also, looking for the effectiveness of wastewater treatment plants. In this study, wastewater samples are collected by grab sampling from three wastewater treatment plants: Dindaeng wastewater treatment plant, Nong Khaem wastewater treatment plant, and Chong Nonsi wastewater treatment plant. Studying the correlation between the prevalence of enterovirus in wastewater and the infected hand-foot-mouth disease cases. The results were determined that there is no correlation between the prevalence of enterovirus in wastewater and the infected hand-foot-mouth disease cases. Also, studying the three wastewater treatment plants removal efficiency. The results found that the Dindaeng wastewater treatment plant has at least 1-log reduction removal efficiency. The reason is these three wastewater treatment plants have not added a disinfection process that can treat the biological contaminations.

Keywords: Enterovirus, Environmental Surveillance, Clinical Surveillance, Wastewater-based epidemiology (WBE), Hand-foot-mouth disease, Wastewater treatment plant, The virus removal efficiency

# ACKNOWLEDGEMENTS

Firstly, I would like to thank my advisor, Jatuwat Sangsanont, Ph.D. for always supporting my senior project to succeed. He always steered me in the right direction with his kindness, motivation, inspiration, and helped me all the time of my research. Without his guidance, this project would not have been successfully conducted.

I would also like to acknowledge my research chairman, Assistant Professor Vorapot Kanokkantapong, Ph.D., and my project committee, Associate Professor Pantana Tor-ngern, Ph.D. for their valuable comments, recommendations, and suggestions.

My thank also goes to Nasamon Wanlapakorn, Ph.D., clinical instructor and researcher of Center of Excellence in Clinical Virology Department of Pediatrics, Faculty of Medicine, Chulalongkorn University for useful information in this research project.

I would also like to thank my co-working research project, Miss Phitchaya Kongsuwan, who always by my side during this research with her passionate participation for her help, advice, and teamwork.

Finally, I must express my great appreciation to my family for supporting me throughout my years of study. I am so grateful to receive good encouragement. This accomplishment would not have been possible without them. Thank you.

Kritnipit Phetanan

# CONTENTS

## LIST OF TABLES AND FIGURES

**Page**

**Tables**

**Figures**

# CHAPTER I
# INTRODUCTION

## 1.1 Introduction

Enteroviruses are small and non-enveloped RNA viruses in the family of *Picornaviridae* and are also known as the viruses that cause waterborne diseases in humans from mild symptoms to severe syndromes (Pallansch et al., 2013). By ingesting, touching, or inhaling, people are easily infected with the pathogens. Naturally, Enteroviruses are highly persistent and stabilize the environment. Scientists found that enteroviruses can be up to $10^{11}$ viral particles per gram-feces from an infected individual (Bosch, 1998) which can be discharged into the environment via the sewage system. Although they are found after being treated in the wastewater treatment plants, they still carried out the infectivity (Cioffi et al., 2020). Enteroviruses are etiological agents for many cases of nonbacterial gastroenteritis, respiratory infection, conjunctivitis, and hepatitis, causing high morbidity and mortality in immunocompromised and in immunocompetent individuals worldwide (Kapikian, 2001; Lenaerts et al., 2008; Okoh et al., 2010). Hand-foot-mouth disease is one of the diseases that the infectivity comes from enterovirus 71 (Y. Li et al., 2018; Yang et al., 2017). Affecting most infants and children below 5 years old then showing the cases with exanthema, fever, and other mild clinical manifestations, and recovering within several days. The approximate child mortality rate was 7.5 per 1,000 live birth for Bangkok which was caused by unsatisfied basic needs including household sanitation, school attendance, and especially the sewage system (Disease, n.d., 2012). Hand-foot-mouth disease plays an important role in the case of these unsatisfied basic needs. Also, there is no such antiviral drug that cures the hand-foot-mouth disease directly (Owino et al., 2019). Therefore, protecting and avoiding being infected by enteroviruses especially enterovirus 71 causing the hand-foot-mouth disease need to be well-prepared.

Wastewater management is crucial in the case of protecting and avoiding infection hand-foot-mouth disease. Nowadays, many anthropogenic activities such as water irrigation, water usage in industries have increased the risk of infected viral pathogens via wastewater. Improved and sustainable infrastructure for water quality and facilities is an important requirement for the reduction of human health risks and environmental protection (Montwedi et al., 2021). Since water contamination with pathogens has become a global concern because the viruses are highly spread. There are two main ways for better management: wastewater monitoring, and wastewater control. A variety of studies on monitoring viruses in wastewater have focused on the prevalence of human enteric viruses in wastewater and wastewater environments (Farkas, Hillary, et al., 2020). Wastewater monitoring is needed because wastewater-based epidemiology (WBE) is a useful method for analyzing infected people by using prevalence data and clinical data. On the other hand, wastewater treatment plants have functioned for controlling purposes. They are made for keeping all quality parameters under normal conditions (Borowa et al., 2006). Therefore, wastewater management such as wastewater monitoring, and wastewater control is needed.

The detection of enteroviruses has been reported in rivers and sediments, in wastewater treatment plants (WWTPs) (Cioffi et al., 2020). To detect enteroviruses from sewage, environmental surveillance has been suggested to be a strategy for monitoring enteroviruses routinely. Besides, with high sensitivity, environmental surveillance has been conducted in many parts of the world to study the molecular epidemiology of non-polio enteroviruses (NPEVs) and other enteric viruses (Pang et al., 2019; Wang et al., 2014). Also, environmental surveillance can be done for the reason of informing asymptomatic infected people data (Ahmed et al., 2021). Still, there are questions about the substitution between the prevalence of enteroviruses in wastewater treatment plants and the number of infected cases of hand-foot-mouth disease. Moreover, the relationship between viral load and clinical disease severity has been rarely studied in hand-foot-mouth disease cases to date (Song et al., 2020). The integrated knowledge by using the clinical data from hospitals and environmental surveillance data called wastewater-based epidemiology (WBE) has been used for calculating the asymptomatic infected cases.

To prevent pandemics and to avoid the spreading of enteroviruses via water, wastewater treatment plants are one of the important tools for treating the viruses before spreading through the environment. These help a lot in lower the risk of infection to enteroviruses. The virus removal reduction for wastewater treatment plants depending on the construction for the basic needs of the communities or the discharge point through the environment. For instance, crop irrigation needs at least 6-$\log_{10}$ of the virus, whereas water potable needs at least 12-$\log_{10}$ of the virus (Gerba et al., 2017). Generally, the activated sludge treatment process is treated by biological agents via three phases: anaerobic, anoxic, and aerobic (Borowa et al., 2006). According to the study, water quality standards in different countries are strict due to the purpose of water reclamation (Manikandan et al., 2021). However, wastewater treatment plants in Thailand were not constructed for virus removal purposes. No disinfection treatment process had been constructed and still, the performance of different secondary treatments (nutrient removal/ cyclic system/ vertical loop reactor) of enteroviruses removal efficiency is questionable. The most effective treatment will be recommended for future guidelines or future modifications for wastewater treatment plants.

As mentioned above, wastewater management is crucial for better water quality and sanitation. The goals of this study are to determine the correlation between the prevalence of enterovirus from the wastewater treatment plant and hand-foot-mouth disease, also to investigate the removal efficiency of viral gene loads in wastewater treatment plants in Bangkok.

## 1.2 Objective

1. To determine the correlation between the prevalence of enterovirus from the wastewater treatment plant and the number of patients with hand-foot-mouth disease.

2. To investigate the removal efficiency of viral gene loads in wastewater treatment plants in Bangkok.

## 1.3 Benefits

The study creates a better understanding of the correlation between the prevalence of enteroviruses found in wastewater and the confirmed clinical cases of hand-foot-mouth disease which can lead to the preparation and prediction for new strategies to monitor and control the spread of waterborne diseases. Also, the knowledge of the virus removal efficiency can be used to apply the wastewater treatment plant type for future guidelines.

# CHAPTER II
# THEORETICAL BACKGROUNDS AND LITERATURE REVIEW

## 2.1 **Enterovirus Background**

Enterovirus belongs to the single-stranded RNA family of viruses called *Picornaviridae* and is identified to have more than 67 human enterovirus serotypes (Chang, 2008). An outbreak of enterovirus causes global awareness in the clinical circle and proved fatal in many children. With these many serotypes, enterovirus 71 is one of the major causes of many diseases like hand-foot-mouth disease which is most frequently affecting children under 5 years old. Enterovirus is easily infected to new birth children who have not been exposed to any viruses and most of the time are infected by direct oral from the secretion that is contaminated in the environment. Also, stool samples show the prevalence of enterovirus from infected people (Solomon et al., 2010).

No signal determined whether EV71 will show the symptoms of asymptomatic, hand-foot-mouth disease, or neurological disorder (Solomon et al., 2010). From January 2020 to June 2020, 6202 cases are confirmed to be infected by enterovirus and cause Hand-foot-mouth disease in Thailand. Although EV71 has become a notifiable disease, concurrent virological surveillance is still necessary.

## 2.2 **Hand-foot-mouth disease**

Hand-foot-mouth disease has first confirmed in 1969 (Schmidt et al., 1974). Based on the surveillance database from China, EV71 is the major type of enterovirus that causes hand-foot-mouth disease among people in China. Of all infected hand-foot-mouth diseases, EV71 was responsible for 70% of all severe cases and 90% of close-to-death cases (Liu et al., 2015). Hand-foot-mouth disease shows many symptoms from mild led to fatal. Children with mild symptoms will catch a fever of more than 39° C, showing rashes among the hand, foot, mouth area, and also ulcer inside the mouth, throat, and tongue. Many countries in the Asia Pacific show high fatality. For instance, in Taiwan, there were 129,106 reported cases of hand-foot-mouth disease and 405 severe cases with 34 deaths due to EV71 in 1998 (Chang, 2008). Also, in Cambodia, there were around 69% fatalities out of 78 infections between April and July 2012 (Sabanathan et al., 2014).

## 2.3 **Environmental Surveillance**

Due to the presence of not only fecal and suspended solids in the sample but also chemicals induced by domestic usage, urban and rural runoffs, industrial activities, etc., that create a complex state of the sample from which the viral genetic material must be isolated (Haramoto et al., 2018). Wastewater shows the great potential of the emitted pathogens data. The use of environmental surveillance for the detection of the virus has been developed for raw sewage. Because it is nearly impossible for testing the infected pathogens for each individual, it is essential to have the central for the origin or place where the disease is begun. Environmental surveillance is a tool used to monitor the extent and duration of the spread of the virus in specific populations. It can give a measure of contaminants and also warn us of possible threats emerging in that particular confinement (Pärnänen et al., 2019). Wastewater analysis is important for monitoring viral pandemics and its efficiency depends on many factors like location, sanitation, meteorological condition, and sampling methods (Ivanova et al., 2019). Environmental surveillance is successfully used for aiding strategies for many viruses such as the Aichi virus and poliovirus (X. W. Wang et al., 2005) and is essential for detecting asymptomatic infections.

## 2.4 **Enterovirus concentration method**

Many methods have been developed for several virus concentrations in water and wastewater matrices (Haramoto et al., 2018). Because of a variety of viruses, a viral concentration method is needed to be performed for a specific type of virus. Since several concentration methods are suitable for a variety of viruses. That can lead to the different concentration methods mean the different recovery efficiency of the virus due to the concentration process. Virus concentration is particularly important because the concentration of enterovirus in wastewater is expected to be low in the beginning. Several methods have been conducted for virus concentration. For instance, virus concentration by centrifugal ultrafiltration. The sewage was filtered through a membrane of polyethersulfone to remove bacterial cells and debris. The viral bacterial was concentrated through filtered water by a centrifugal device. In addition, virus concentration by adsorption onto a negatively charged membrane is adjusted the pH by HCL and filtered through negatively charged nitrocellulose membrane via glass funnel (Carrillo-Reyes et al., 2021). To provide an effective early warning system or to inform decisions on an easing of restrictions safely, the methods must be sensitive enough to detect a very small number/low concentration of viruses in a wastewater sample.

## 2.5 **Detection by molecular biology**

Among the PCR techniques, many different types of PCR are used for several purposes. For example, classical PCR, real-time quantitative PCR (qPCR), and reverse transcriptase quantitative PCR (RT-qPCR) use to detect the genetic with sensitivity and specificity (Fehr et al., 2015). Also, need a standard curve for a specific quantification. Because there is a variety of enterovirus in the environment, new methods are useful for multiple detections. qPCR is a suitable method for targeting genes of pathogens that have been widely used in environmental health and environmental surveillance research (Farkas, Mannion, et al., 2020). qPCR is a technique that uses antisense DNA probes and primers (small complementary pieces of DNA), to amplify viral RNA; however, since RNA itself cannot be directly amplified, it must first be converted into a DNA form (Bustin & Nolan, 2020; Hamza & Bibby, 2019; Jahne et al., 2020). Besides, different viruses need different primers and probes. qPCR is the combination of reverse transcription and quantify the RNA targets. However, the limitation of using qPCR is the number of copies per volume can not be high as ideal for environmental samples (Ishii et al., 2014).

## 2.6 **Microbial Indicator**

The presence of microbial indicators in the sewage can be used to evaluate the safety of the water. *E. coli* is commonly used as faecal indicators to estimate the water quality of waterborne pathogens. Since the use of *E. coli* is believed to be a good agent for studying waterborne pathogen behaviors. E. coli is proposed to mimic the environmental conditions and fulfill the information. However, some viruses have different removal efficiency in wastewater treatment plants due to their processes and environmental degradation processes. Bacteriophages are proposed as an effective candidate for estimate the water quality standard (Wu et al., 2011). They are low risk to humans and more like enterovirus because of structure, size, morphology, environmental resistance, and their resistance from wastewater treatment plants. The removal efficiency of human enterovirus can be followed the patterns of bacteriophages.

## 2.7 **Wastewater-based epidemiology**

Wastewater-based epidemiology (WBE) is a new approach based on the chemical analysis of biomarkers in raw wastewater and has been used as an environmental tool for providing real-time information or prewarning on an incidence of disease to get qualitative and quantitative information within a given wastewater catchment. Most of the time, WBE is used for the early detection of drug consumption on the drug market. The consumption rates for some illicit drugs show significant differences between years, seasons, regions, special events, and weekdays/weekends. Right now, WBE is approached more than using it as a drug consumption assessment. WBE plays an important role in the detection of virus quality and quantity for assessing the infected information. WBE gives information about between substances which is the prevalence of enterovirus in this case and predicts the information of the diseases based on the biomarkers which are exposed to the wastewater by human lifestyles. Although WBE is varied in use as a tool, the use of WBE as the epidemiology of some pathogens that cause diseases such as cryptosporidiosis, giardiasis, and microsporidiosis is poorly understood in developing countries despite their wide occurrence. WBE analyzes the distribution of these pathogens in wastewater to deduce the extent of their transmission in urban areas and the likely sources of infection in humans (N. Li et al., 2012). Furthermore, providing an early warning and effective epidemic supervision is the main prospect of WBE. A variety of studies showed the predictable infected people including asymptomatic infected people such as COVID-19 disease cases(Ahmed et al., 2020; Westhaus et al., 2021).

## 2.8 **Wastewater treatment system**

Normally, the wastewater treatment system includes 3 main processes; primary, secondary, and tertiary steps which are the steps of filtration of the solid disposal, separate organic and inorganic solids, remove suspend or dissolved solids, and biological or chemical approaches for improving water quality respectively. Among these stages, the aeration stage which is found in activated sludge wastewater treatment plant type is normally found in Thailand wastewater system. 8 wastewater treatment plants mainly treat the sewage around

Bangkok such as Sri Phaya, Rattanakosin, Chong Nonsi, Nong Khaem, Tung Kru, Dindaeng, Chatuchak, and Bangsue. All of them are activated sludge system wastewater treatment system that has some different treated function. The sewerage system, which is Dindaeng wastewater treatment plant, Chong Nonsi wastewater treatment plant, and Nong Khaem wastewater treatment plant has an area coverage of 37, 28.5, and 44 kilometers with the total efficiency of 350,000 $m^3$/day, 200,000 $m^3$/day, and 157,000 $m^3$/day respectively. These three WWTPs have the same components of primary treatment, which is for the removal of floating suspended solids, biological treatment (activated sludge) which is for the removal of organic matters, and some different treatment: nutrients removal (Dindaeng), cyclic system (Chong Nonsi), and vertical loop reactor (Nong Khaem).

For the Dindaeng wastewater treatment plant, there is a conventional activated sludge commonly include an aeration tank, which is used for biological degradation, and a secondary clarifier (sedimentation tank), where the sludge is separated from the treated wastewater. BOD, TSS, nitrogen, and phosphorus can be reduced by this wastewater treatment plant.

For the Nong Khaem wastewater treatment plant, the wastewater in an oxidation ditch circulates in the horizontal loop but the wastewater in a vertical loop reactor circulates in a vertical loop around a horizontal baffle. There is an upper and bottom zone, separated by a horizontal baffle. Oxygen is added by bubble diffusers. The sludge from this treatment is separated from the treated wastewater. Nitrogen and phosphorus can be removed from this wastewater treatment plant.

For Chong Nonsi wastewater treatment plant, it can be performed by all the functions of a conventional activated sludge plant such as biological removal of pollutants, solids/liquid separation, and treated effluent removal by using a single tank in an alternating stage of operation. Thus, the sludge from this process is silted in the same tank of wastewater.

Apart from the wastewater treatment plant in Thailand, there are several types of wastewater treatment plant systems that can deal with virus removal. The wastewater pond system is very common worldwide. Several studies showed that 1-log reduction of virus can be removed from 14.5 – 20.9 days (Verbyla & Mihelcic, 2015). However, the efficiency is depended on the characteristics of each pond, including chemical composition and optical properties.  UV inactivation and chlorine treatment are reported to be around 1.2 – 1.8 log reduction. Moreover, they can be obtained more by reducing the *E. coli* and coliphage up to 2.5 – 3 log reduction (Simhon et al., 2019). Ozone treatment is highly effective for removing the virus and has been used as a disinfectant for the disinfection of sewage effluents used for irrigation of crops, and discharge to surface water, due to the issue of high chlorine demand and problematic disinfectant by-products. Algal systems for virus removal are the use of algae for treating the virus in the sewage. The result of the treatment is obtained to be the same as the activated sludge process with 1 – 3 log reduction values depending on the virus types (Delanka-Pedige et al., 2020).

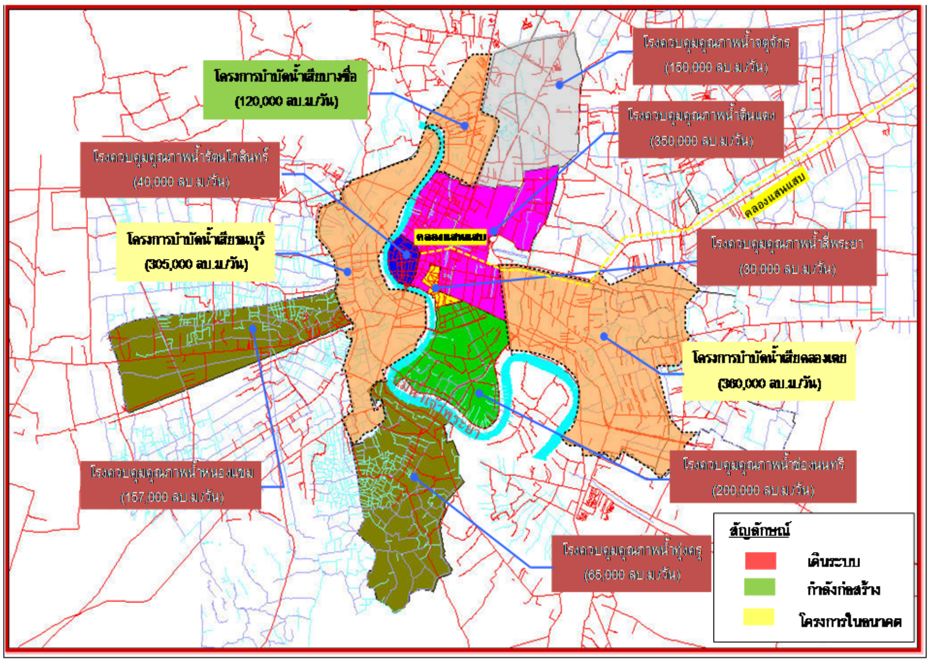

**Fig. 2.1** Wastewater treatment plants collected areas

Source: https://web.facebook.com/PCD.go.th/photos/a.174461189303943/834985949918127/?type=1&theater

## 2.9 **The virus removal efficiency of the wastewater treatment plant**

The removal efficiency is calculated from the prevalence of virus before treated by wastewater treatment plant deducted by the prevalence of virus after treated by the wastewater treatment plant. The efficiency is easily determined by the viral contaminants in the effluent of the wastewater treatment plant. Eliminating these contaminants has proven to be challenging, and researchers have proposed various alternatives to traditional treatment methods (Venugopal et al., 2020). A few suggestions for upgrading the purification process are pretreatment by nanofiber filtration, electrocoagulation, photocatalysis, and the use of specific plant species such as *Eucalyptus camaldulensis* and *Arundo donax*. Other ways of treatment include the use of membrane bioreactors that have anoxic and oxic zones, wetlands, stabilization ponds, or aeration. Log reduction value (LRV) is measured by the effectiveness of each wastewater treatment plant. For instance, 1-log reduction means 90% removal of target pathogens, 2-log reduction means 99% removal of target pathogens (Sheet, 2014). Normally, the log reduction value is assigned for the effective management of human sanitation. Many pathogens that caused waterborne disease in wastewater are highly variable. Making it impossible to measure the pathogens individually. Therefore, suitable reference pathogens are selected from each type of pathogen and detected to confirm the effectiveness of the treatment process. The WHO established the guidelines for safety in managing wastewater (OMS, 2020). The study showed that the virus removal reduction factor that wastewater plants need to provide depending on the reclaimed water use. For instance, crop irrigation needs at least a 6-log reduction. However, a 12-log reduction is needed for potable water (Lesimple et al., 2020). From the previous study, the activated sludge process showed the efficiency of a 1-log reduction in reducing the viral pathogens (Frigon et al., 2013).

# CHAPTER III
# MATERIALS AND METHODS

## 3.1 The overall processes

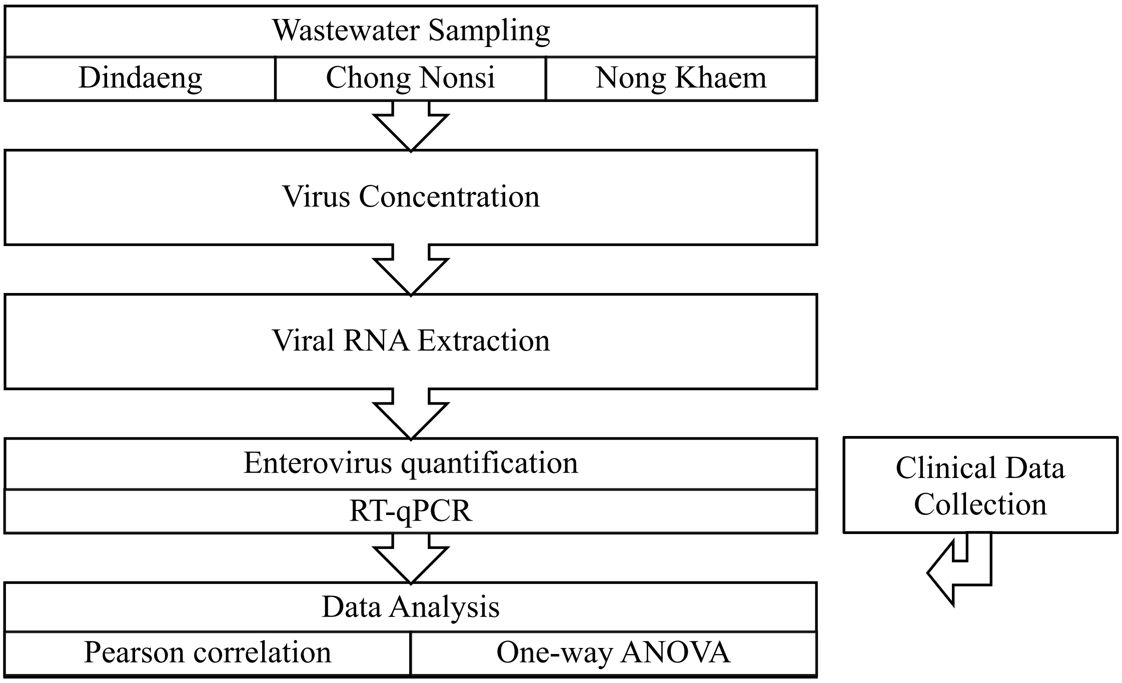

**Fig. 3.1** Overall processes

## 3.2 Research materials

### 3.2.1 Laboratory Instruments and Equipment

1) Denver ultra-basic pH meter
2) Forceps
3) Lab Spoon
4) Precisa 2200 CSCS Balances, Scales, and Weighing
5) Hirayama HVA-85 Autoclave
6) Sanden Intercool Freezer
7) Argo Refrigerator
8) GAST RAA-V110-ED Pumps
9) PCR Tube
10) Vortex-Genie 2
11) 1.5 mL Microcentrifuge Tube
12) QuantStudio™ 6 Flex Real-Time PCR System
13) QIAamp® Viral RNA Mini Kit
14) Bottle
15) Beaker
16) Carboy Bottle

17) Wash Bottle
18) Filter Flask
19) Filter
20) Funnel
21) Pipette Tips
22) Test Tube
23) Pipets
24) Cylinder
25) Aluminum Foil
26) Micropipette
27) MSE Falcon 6/300 centrifuge
28) Amicon ® Ultra-15

3.2.2 Chemical reagents

1) $MgCl_2$
2) $H_2SO_4$
3) NaOH
4) 100 x Tris-EDTA buffer
5) Bacteriophage MS2 stock solution
6) Probe
7) Enterovirus primers
8) RNA extraction kit
9) Ethanol (96–100%)

## 3.3 Wastewater sampling

Influent and effluent wastewater samples were collected at three wastewater treatment plants (WWTPs): Dindaeng wastewater treatment plant, Chong Nonsi wastewater treatment plant, and Nong Khaem wastewater treatment plant. About 1 liter of wastewater samples was collected by grab sampling twice a month from December 2019 to May 2020 and stored in a container (<8 °C) until further analysis.

## 3.4 Virus concentration

Before concentrating the water sample, virus spiked is needed to be done for checking the recovery of enteroviruses. A bacteriophage sample called MS2 was added to the water samples to obtain a concentration of $10^3$ PFU/mL. Then, the viruses were concentrated by adding 5.3 g of $MgCl_2$ into sewage specimens previously reported by Katayama et al., 2002 to obtain a concentration of 25 mM sample solution. The samples were filtered through an electronegative membrane filter. Afterward, the filtered samples were rinsed 200 mL of 0.5 mM $H_2SO_4$ (pH 3.0) which helps to remove the magnesium bounded. The captured viruses were eluted by 10 mL of 1 mM NaOH (pH 10.8) and recovered in a 100 μL 100x Tris-EDTA buffer tube which is also added 50 μL of 100 mM $H_2SO_4$ (pH 1.0) to neutralization. Finally, 10 mL of samples were stored at -80 °C freezer. Then, secondary concentration is processed by the laboratory equipment named MSE Falcon 6/300 centrifuge (United Kingdom) for further concentrated viruses. By setting up the speed of 6000 rpm with 20 °C 15 minutes, resulted in a higher concentration of enteroviruses.

### 3.5 Viral RNA extraction and quantification assay

Before detecting enteroviruses, the second concentrated water samples which are loaded in Amicon ® Ultra-15 were extracted by using QIAamp® Viral RNA Mini Kit. The extracted RNA viruses were followed by Handbook (March 2018). Quantitative polymerase chain reactions (qPCRs) were carried out on QuantStudio™ 6 Flex Real-Time PCR System testing for the enteroviruses. qPCR was performed as reported by (Fumian et al., 2010), using primers and probes as described by (Zeng et al., 2008). To prevent contaminations, quality control is needed by using different rooms (reagent preparation, sample preparation, and amplicon detection). The qPCR is performed on a total volume of 10 µL/reaction along with 2 µL of the samples with a prepared master mix by using various reagents. 4X TaqMan Fast Virus is prepared for 2 µL per reaction. 10 M Forward Primer and 10 M Reverse Primer are prepared for 0.4 µL per reaction as shown in **Table 3.1**. 10 M Probe is prepared for 0.3 µL per reaction as shown in **Table 3.1**. Finally, DEPC $H_2O$ is prepared for 4.4 µL per reaction in a total of 8 µL of master mix per reaction. There are four steps of qPCR condition preparations. Firstly, the reverse transcription step is set up at 50 °C for 5 minutes. Next, RT- inactivation step is set up at 95°C for 10 minutes. Then, the denaturation step is set up at 95°C for 15 seconds. Lastly, the annealing step is set up at 60°C for 1 minute in a total of 45 cycles. The positive control was from the sequenced sample with enteroviruses, identified in a specific sequencing run. The negative control was the sterile water without DNA and RNA. The assay efficiency ($10^{(-1/slope)}$) was calculated by the slope of the standard curve which shows on the software by plotting the log copy number compared with the cycle threshold value (Ct).

**Table 3.1** Primer/Probe name sequences for enterovirus detection by qPCR

| Primer/Probe name | Sequence (5’ → 3’) |
|---|---|
| EV-5’UTR-qF (Forward primer) | TCCTCCGGCCCCTGAATG |
| EV-5’UTR-qR (Reverse primer) | ATTGTCACCATAAGCAGCCA |
| EV-5’UTR-probe | FAM-GCAGCGGAA/ZEN/ CCGACTACTTT-Iowa Black FQ |

### 3.6 Data collection on clinical surveillance

The reported number of Hand-foot-mouth diseases were obtained from the Center of Excellence in Clinical Virology at the Faculty of Medicine, Chulalongkorn Hospital, N-Health, and Thonburi Hospital database. The clinical data were collected anonymously and securely from December 2019 to May 2020 with the analyzed numbers, patients’ gender, and ages. The number of patients was calculated by using the ratio of the confirmed cases caused by enterovirus over the total cases.

### 3.7 Statistical analysis

The correlation between the confirmed cases by clinical surveillance and the determined enteroviruses in wastewater samples by qPCR by Pearson correlation test using SPSS 20.0 software. The comparison between these two variables' relationships was plotted in a year range from December 2019 to May 2020. It is evaluated the coefficients from -1 to 1 week with a significance level of 5%.

### 3.8 Calculation of wastewater treatment plants efficiency

The LRV equation was achieved from (Prado et al., 2019) and calculated by using the following equation:

$$Log\ reduction\ value(LRV) = \log_{10} influent\ concentration - \log_{10} effluent\ concentration$$

$$\%\text{Removal Efficiency} = \frac{\text{Effluent concentration} - \text{Influent concentration}}{\text{Influent concentration}}\, x\ 100$$

The results are analyzed by the one-way ANOVA for the significant differences in wastewater treatment plant efficiency.

### 3.9 The limit of detection

The lowest amount of analysis which can be detected with more than a stated percentage of confidence but not necessarily quantified as an exact value (Kralik & Ricchi, 2017; OIE, 2010).

# CHAPTER IV
# RESULTS AND DISCUSSION

In this study, the results were determined from 36 wastewater samples from December 2019 to May 2020 with 3 wastewater treatment plants: Dindaeng, Nong Khaem, and Chong Nonsi. Finding the enterovirus concentration by using the qPCR method. 6 samples were examined for each of the influent and effluent of the wastewater treatment plants.

## 4.1 The prevalence of enterovirus concentration in wastewater samples

Among the 36 samples were tested for enterovirus, 10 out of 36 were identified as positive and can be calculated through enterovirus concentration (MPN/mL). By finding the standard curve for a known enterovirus concentration, the slope of the standard for 5'UTR assay was calculated the Ct values back for a real enterovirus concentration in the unit of MPN/mL as shown in **Table 4.1** and then summarized as shown in **Fig.4.1**. For each wastewater treatment plant, Dindaeng was shown the positive enterovirus concentration only in December 2019. The log concentrations were 0.3224 MPN/mL in the influent and 0.0536 MPN/mL in the effluent. For Nong Khaem, the data was shown positive in January 2020, February 2020, and May 2020. The log concentrations were 0.0110 MPN/mL in influent, 0.1673 MPN/mL in influent, and 0.0175 MPN/mL in influent respectively. For Chong Nonsi, the data was shown a positive from December 2019 to March 2020. The log concentrations were 0.0593 MPN/mL in the effluent of December 2019, 0.4700 MPN/mL in the influent and 0.2384 MPN/mL in the effluent of January 2020, 0.0950 MPN/mL in the effluent of February, and 0.4185 MPN/mL in the influent of March 2020. The others that were identified as negative because the Ct values of qPCR are greater than 40 were converted into the detection limit concentration and written as N.D. In this study, we found the limit of detection is equal to 0.0208 MPN/mL. From **Fig.4.1**, the bar chart showed the common highest peak of Chong Nonsi treatment plant in the influent which was in January 2020 and March 2020. Dindaeng treatment plant was the third peak which was found in the influent. In April and May, none of the enterovirus concentrations can be found by this study. Thus, the detection of limit value is assumed for their concentration. These concentrations of enterovirus were confirmed by using one-way ANOVA for influent and effluent. They were found to be non-significantly ($p = 0.390$, $0.142$) respectively.

The results of these microbial indicators were used to plot the correlation graphs between the enterovirus concentration as shown in **Fig.4.2** and **Fig.4.3**. The previous study (Chaihard, 2020) that investigated the prevalence of microbial indicators such as *E. coli* and bacteriophages during the same period showed the average concentration of *E. coli* and bacteriophages in the unit of CFU/mL and PFU/mL respectively. From **Fig.4.2**, the data were used to plot from January 2020 to March 2020 in the total number of three months. The highest average log concentration for enterovirus was showed in the influent of Chong Nonsi in January 2020 whereas the highest average log concentration for *E. coli* was showed in the influent of Nong Khaem in January 2020. The graph showed a small negative correlation between the prevalence of average *E. coli* and average enterovirus since the correlation coefficient was -0.2960. The correlation was found to be non-significantly (p = 0.569). From **Fig.4.3**, the data were used to plot from January 2020 to March 2020 in the total number of three months. The highest average log concentration for enterovirus was showed in the influent of Chong Nonsi in January 2020 whereas the highest average log concentration for bacteriophage was also showed in the influent of Nong Khaem in January 2020. The graph showed a medium negative correlation between the prevalence of average bacteriophage and average enterovirus since the correlation coefficient was -0.4044. The correlation was found to be non-significantly (p = 0.426). These two graphs interpreted that none of the average log concentration of microbial indicators can be used to determine the prevalence of enterovirus. Since the correlation coefficient showed a low interpretation amount. Also **Fig.4.2** and **Fig.4.3**, the R-Squared that were 0.0876 and 0.1636 respectively ensured the prevalence of microbial indicators cannot be substituted as the prevalence of enterovirus.

To date, water quality monitoring depends on fecal indicators such as *E. coli*. Many countries' water standard qualities have accepted *E. coli* as an indicator that showed much associated waterborne illness (Rock & Rivera, 2014). With the clearer image, the higher the *E. coli*, the higher risk of getting sick from waterborne diseases. However, there are studied that found out the limitation of accepting only *E. coli* as a biological water quality standard (Field & Samadpour, 2007; Wu et al., 2011). In addition, more microbial indicators such as coliphage have suggested solving the mentioned limitation (Field & Samadpour, 2007; Harwood et al., 2014). In case of the data are more sufficient, there could be a significant correlation between the pathogens and microbial indicators like coliphage. The previous studies showed that it is crucial to continuously identify the amount of enterovirus because there need to be many matched conditions for the substitution of microbial indicators and pathogens (Wu et al., 2011). Since the prevalence of enterovirus cannot calculate by finding only the number of microbial indicators, monitoring enterovirus always seems to be helpful for human health sanitation and epidemiological study. For instance, there are many enterovirus strains found in different periods and different strains of enterovirus caused various diseases (Bisseux et al., 2020).

**Table 4.1** The average enterovirus concentration (MPN/mL) of each month in each wastewater treatment plant.

| **Month/Year** | **Wastewater Type** | **Enterovirus Concentration (MPN/mL)** | | |
|---|---|---|---|---|
| | | **Dindaeng** | **Nong Khaem** | **Chong Nonsi** |
| 12 / 2019 | Influent | 0.3224 | N.D. | N.D. |
| | Effluent | 0.0536 | N.D. | 0.0593 |
| 1 / 2020 | Influent | N.D. | N.D. | 0.4700 |
| | Effluent | N.D. | N.D. | 0.2384 |
| 2 / 2020 | Influent | N.D. | 0.1673 | N.D. |
| | Effluent | N.D. | N.D. | 0.0950 |
| 3 / 2020 | Influent | N.D. | N.D. | 0.4185 |
| | Effluent | N.D. | N.D. | N.D. |
| 4 / 2020 | Influent | N.D. | N.D. | N.D. |
| | Effluent | N.D. | N.D. | N.D. |
| 5 / 2020 | Influent | N.D. | N.D. | N.D. |
| | Effluent | N.D. | N.D. | N.D. |

Note: N.D. indicates the result below the detection limit

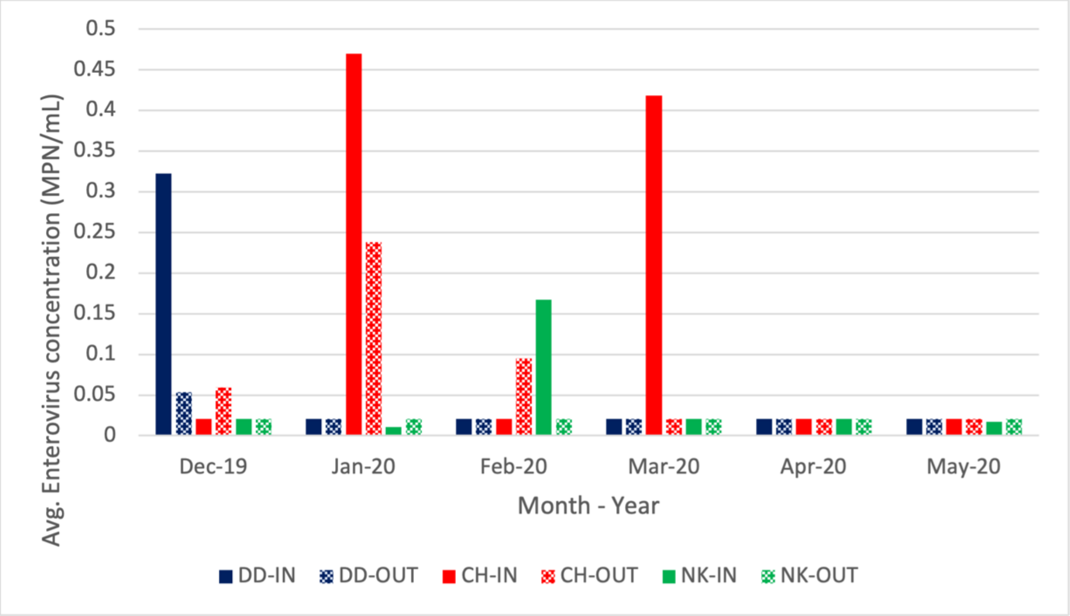

**Fig. 4.1** Bar Chart showed the average enterovirus concentration (MPN/mL) of each month in influent and effluent from each wastewater treatment plant.

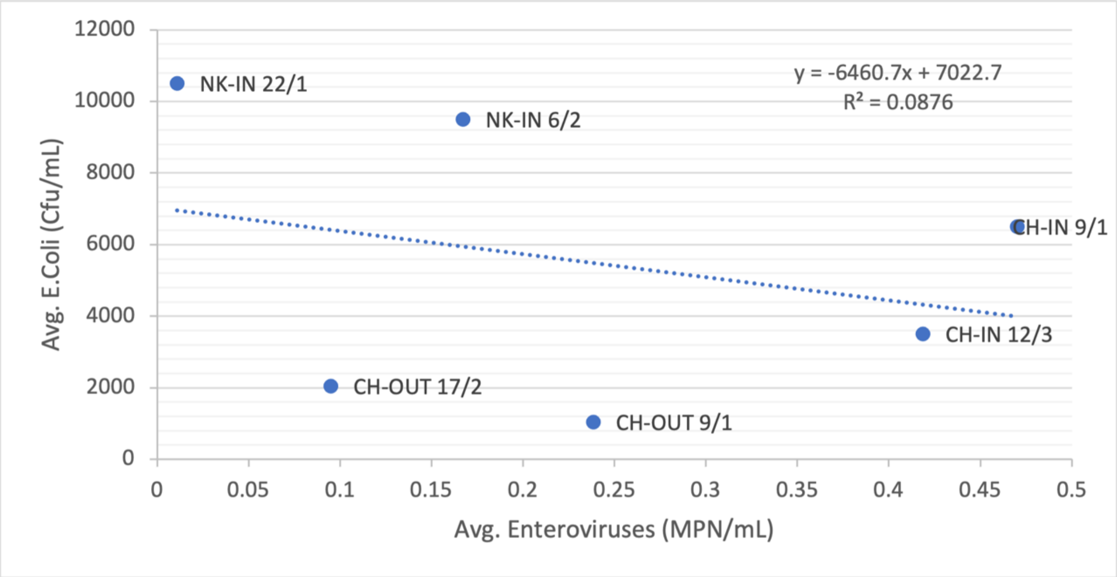

**Fig. 4.2** The correlation graph between the average *E. coli* concentration and the average enterovirus concentration.

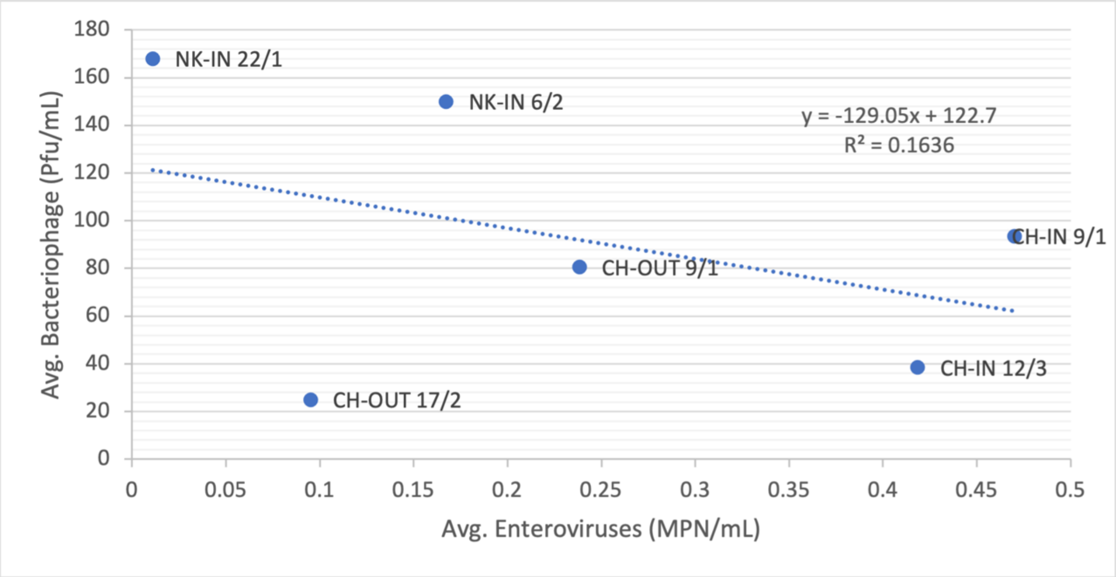

**Fig. 4.3** The correlation graph between the average bacteriophage concentration and the average enterovirus concentration.

### 4.2 The comparison between the prevalence of enterovirus and the clinical data

The clinical data collected anonymously from three hospitals: The Center of Excellence in Clinical Virology at the Faculty of Medicine, Chulalongkorn Hospital, N-Health, and Thonburi Hospital database were used to compare with the average log concentration of enterovirus. The infected cases were qPCR as a positive of panEV with the clinical diagnosis of hand-foot-mouth disease (HFMD). From **Table 4.2**, the highest average log concentration of enterovirus was 0.171 which was in January 2020. The number of infected cases that were found to be hand-foot-mouth diseases was equal to 2. On the other hand, the lowest average log concentration of enterovirus was 0.021 which was the detection of limit and was found in

April and May 2020. The number of infected cases that were found to be hand-foot-mouth diseases was equal to 0. This was related to the prevalence of enterovirus. The average log concentration of enterovirus was used to plot the correlation graph between the enterovirus concentration and clinical cases as shown in **Fig.4.4**. The data were plotted with the combination of bar chart and line graph by using the number of cases and enterovirus concentration respectively in 6 months. The graph showed a positive correlation between the prevalence of enterovirus and the number of cases since the correlation coefficient was 0.6807. The correlation was found to be non-significantly ($p = 0.137$).

There were studies in Japan and Taiwan that showed most hand-foot-mouth diseases from June to August which is the summer season in Japan (Gonzalez et al., 2019; Messacar et al., 2020), and April to July which is the pre-summer season in Taiwan respectively (England, 1999). The result corresponded to the previous study that occurred in Japan. There were low numbers of infection cases from the clinical data from December to May. Yet, the other months were not collected since the study was finding the prevalence of enterovirus in wastewater from December 2019 to May 2020. However, the prevalence of enterovirus especially enterovirus A-71 in the sewage is mostly seemed to be found in January, and July which also corresponded to the study (Bisseux et al., 2020; Tao et al., 2020). Thus, although there was a positive correlation between the prevalence of enterovirus and infected people with hand-foot-mouth disease, the enterovirus concentrations that we found in the sewage were not detected to the same timelines as the data of enterovirus A-71 infected cases in hospital. Still, there were some different conditions like temperature and humidity which can be caused some curious issues. Besides, the detection of enterovirus in the sewage might come from the infection of other enterovirus-caused diseases except for hand-foot-mouth disease. Moreover, the low number of samples might cause some statistical errors. The reason is wastewater-based epidemiology (WBE) that needed the various prevalence of enterovirus with the correlate infection cases to show the real-time infected people information.

**Table 4.2** The number of infected cases per month and average log concentration of enterovirus.

| Month / Year | Number of Cases | Concentration (MPN/mL) |
|---|---|---|
| December / 2019 | 1 | 0.121 |
| January / 2020 | 2 | 0.171 |
| February / 2020 | 1 | 0.070 |
| March / 2020 | 0 | 0.153 |
| April / 2020 | 0 | N.D. |
| May / 2020 | 0 | N.D. |

Note: N.D. indicates the result below the detection limit

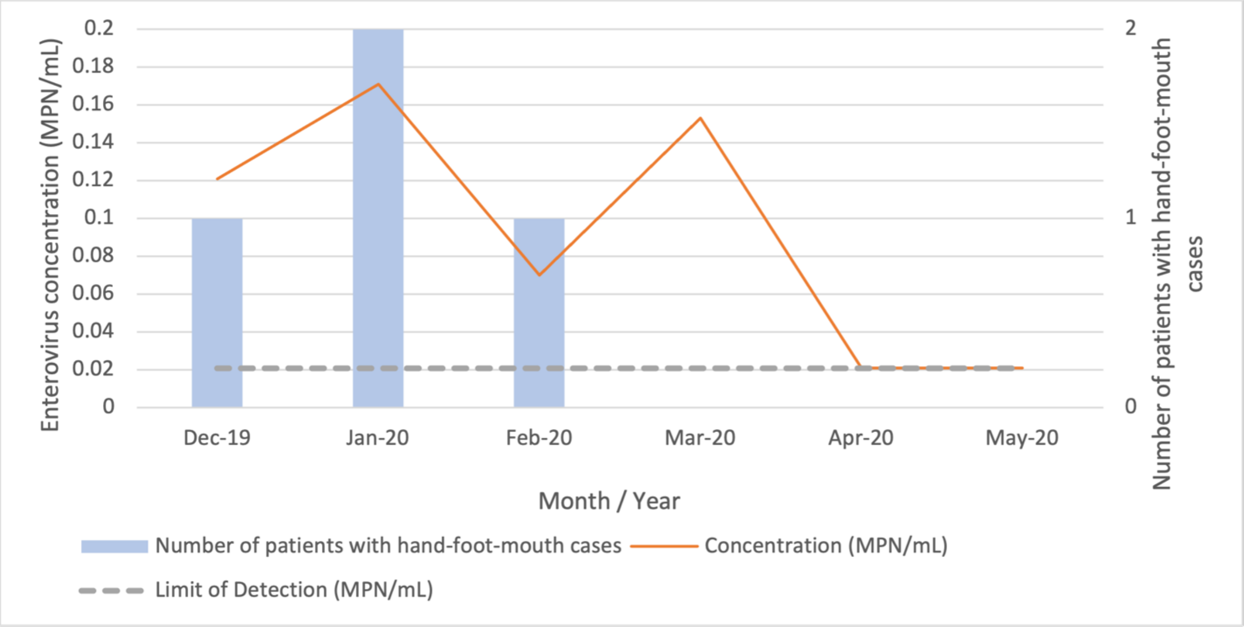

**Fig. 4.4** The correlation graph between the average enterovirus concentration and the infected cases.

### 4.3 The removal efficiency of enterovirus in wastewater treatment plants

The log reduction value and removal efficiency percentage were summarized as shown in **Fig.4.5** and **Fig.4.6**. From three wastewater treatment plants, the log reduction value was calculated by taking the logarithm of the enterovirus concentration in the effluent and influent water of a treatment process. Nong Khaem had the highest log reduction value for more than 0.9045. In addition, the arrow that showed on the bar charts for the Nong Khaem treatment plant is meant for at least of the value. Since the result of the Nong Khaem treatment plant was coming from the limit of detection, therefore, it is inaccurate to find the exact log reduction value. Dindaeng had the second-highest log reduction value for 0.7791. Chong Nonsi had the lowest log reduction value of the three wastewater treatment plants for 0.2948.

The removal efficiency percentages were seemed to be the same trend as the log reduction value. From three wastewater treatment plants, the removal efficiency percentage was calculated by the difference between effluent and influent water of a treatment process, divided by the influent, and multiplied by a hundred. Nong Khaem had the highest removal efficiency for more than 87.54 percentage. In addition, the arrow that showed on the bar charts for the Nong Khaem treatment plant is meant for at least of the value. Since the result of the Nong Khaem treatment plant was coming from the limit of detection, therefore, it is inaccurate to find the exact removal efficiency percentage. Dindaeng had the second-highest removal efficiency for 83.37. Chong Nonsi had the lowest removal efficiency of three wastewater treatment plants for 49.28.

The results of these microbial indicators were used to plot the correlation graphs between the enterovirus log reduction value and removal efficiency percentage as shown in **Fig.4.7**, **Fig.4.8**, **Fig.4.9**, and **Fig.4.10** respectively. The previous study (Chaihard, 1377) that investigated the prevalence of microbial indicators such as *E. coli* and bacteriophages during the same period showed the log reduction value and removal efficiency percentage of *E. coli* and bacteriophages respectively. From **Fig.4.7**, the data were used to plot from January 2020 to March 2020 in the total number of three months. The highest log reduction value for enterovirus was shown in March 2020 whereas the highest log reduction value for *E. coli* was shown in February 2020. The graph showed a medium negative correlation between the log reduction value of *E. coli* and enterovirus since the correlation coefficient was -0.3891. The correlation was found to be non-significantly (p = 0.746). From **Fig.4.8**, the highest log reduction value for enterovirus was shown in March 2020 whereas the highest log reduction value for bacteriophage was shown in February 2020. The graph showed a medium positive correlation between the log reduction value of bacteriophage and enterovirus since the correlation coefficient was 0.3241. The correlation was found to be non-significantly (p = 0.790). From **Fig.4.9**, the highest removal efficiency percentage for enterovirus was shown in March 2020 whereas the highest removal efficiency percentage for *E. coli* was shown in February 2020. The graph showed a medium negative correlation between the removal efficiency percentage of *E. coli* and enterovirus since the correlation coefficient was -0.3111. The correlation was found to be non-significantly (p = 0.799). From **Fig.4.10**, the highest removal efficiency percentage for enterovirus was shown in March 2020 whereas the highest removal efficiency percentage for bacteriophage was shown in February 2020. The graph showed a large positive correlation between the removal efficiency percentage of bacteriophages and enterovirus since the correlation coefficient was 0.8392. However, the correlation was found to be non-significantly (p = 0.366).

The trend of the log reduction value and the removal efficiency of three wastewater treatment plants were related to each other. Although the Nong Khaem wastewater treatment plant seemed to have the highest removal efficiency, yet the data lacked information because the removal efficiency of the Nong Khaem wastewater treatment plant was calculated by using the limit of detection concentration. Based on the data from the Department of Drainage and Sewerage System in Bangkok (DDS), the Dindaeng wastewater treatment plant is a biological activated sludge process with nutrients removal type, while Nong Khaem wastewater treatment plant is a vertical loop Reactor (VLR), and Chong Nonsi wastewater treatment plant is a cyclic activated sludge system (CASS) (JICA, 2011). The Dindaeng wastewater treatment plant has a higher removal efficiency due to the separated aeration tank from the sedimentation tank that means there is a low chance of getting contaminated or mixing the sewage with sludge. In contrast, the Chong Nonsi wastewater treatment plant has no separation tank process. The mixing between sewage and sludge from unseparated tanks can cause microbial contamination. Overall, due to the result, these three wastewater treatment plants can reduce about 1-log reduction which is typical for activated sludge in wastewater treatment plants without disinfection process (Frigon et al., 2013). Right now, there was a usage of the treated secondary effluent wastewater for watering urban trees and irrigation. However, Thailand has not set up any water reclamation standards. The reason is that the cost of processing tap water is lower than the cost of making water reuse in Thailand. Moreover, to be safe irrigating reclaimed water, there is a study showed at least 5.5-log virus removal reduction needed (Arden et al., 2020). It is suggested that microbial control and surveillance are needed for reclaimed water.

The knowledge of microbial concentration and community structure over advanced treatment processes in a full-scale water reclamation plant (Cui et al., 2020).

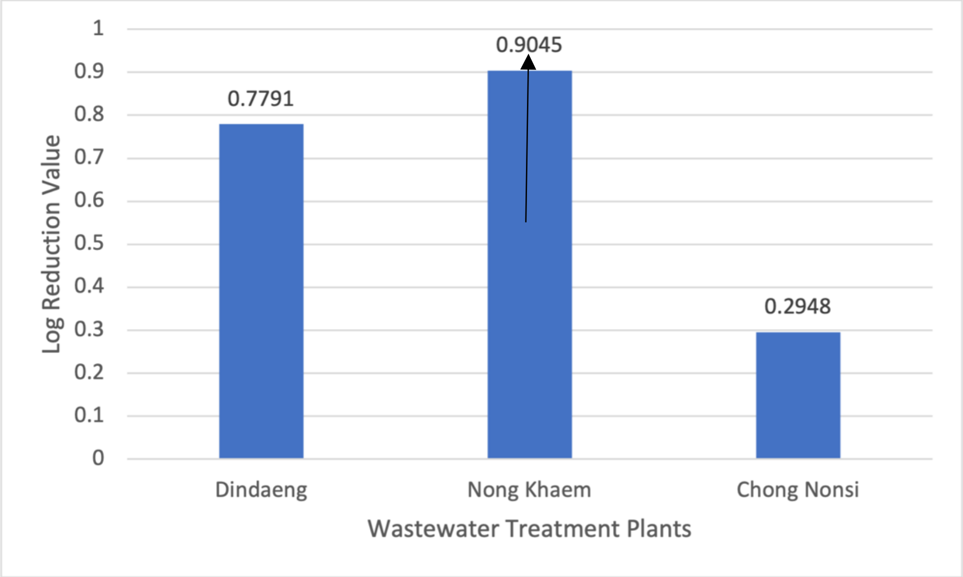

**Fig. 4.5** The log reduction value for each wastewater treatment plant.

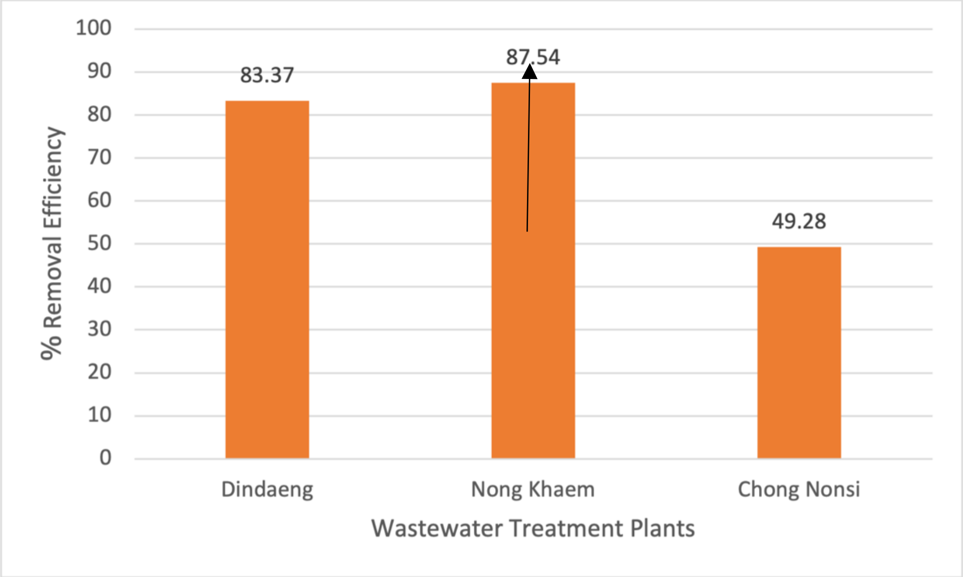

**Fig. 4.6** The removal efficiency percentage for each wastewater treatment plant.

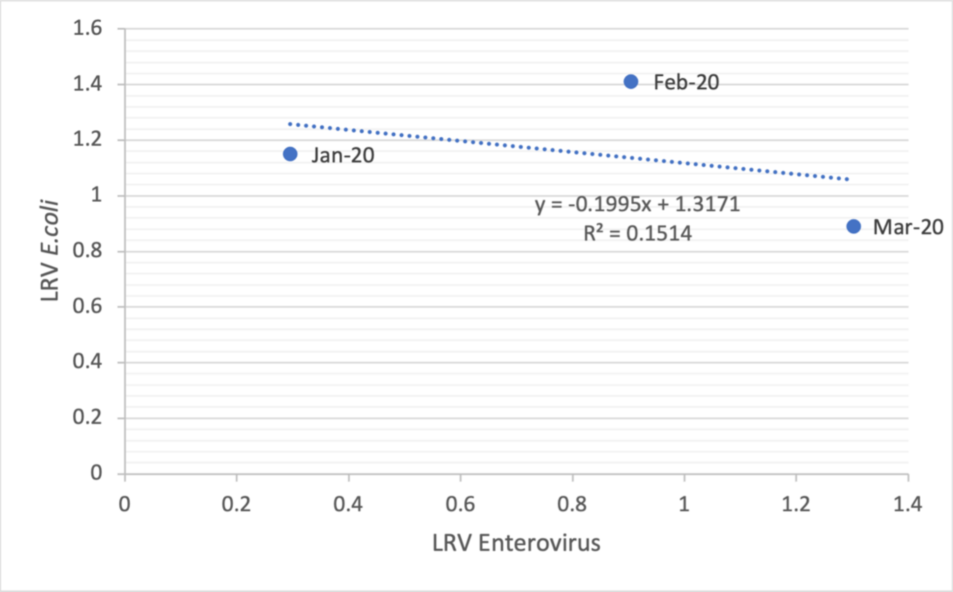

**Fig. 4.7** The correlation graph between the log reduction value of enterovirus and the log reduction value of *E. coli.*

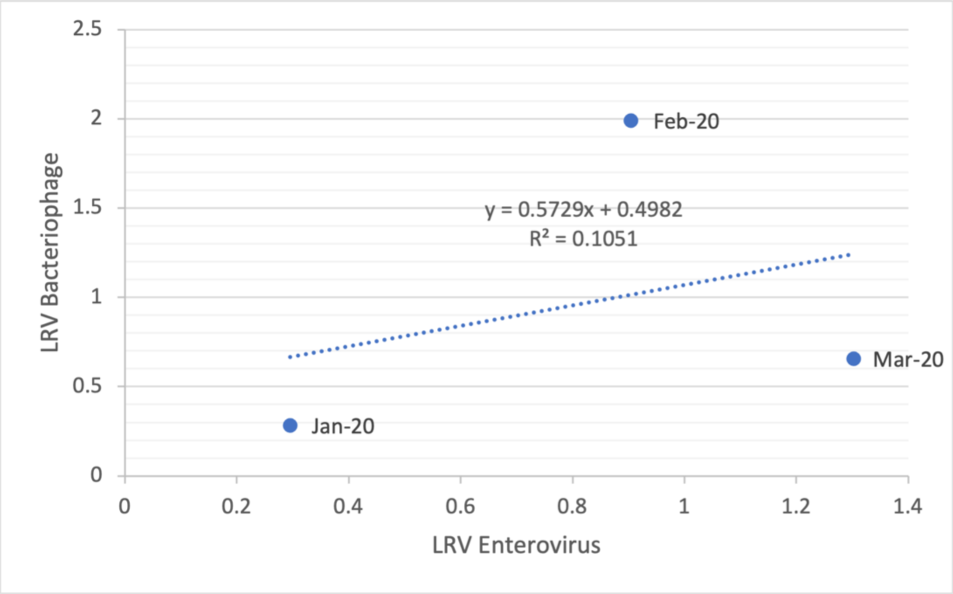

**Fig. 4.8** The correlation graph between the log reduction value of enterovirus and the log reduction value of bacteriophage.

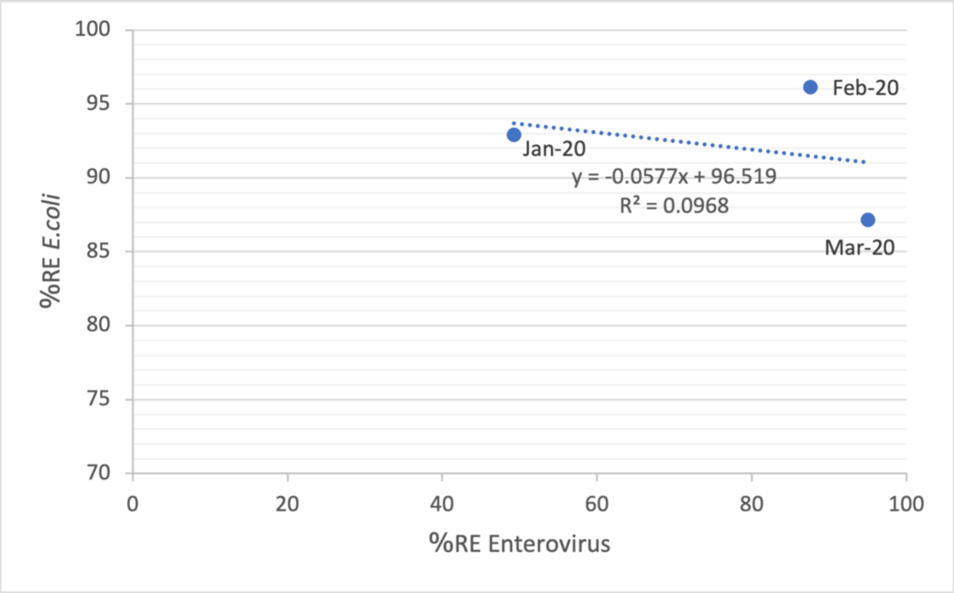

**Fig. 4.9** The correlation graph between the removal efficiency percentage of enterovirus and the removal efficiency percentage of *E. coli.*

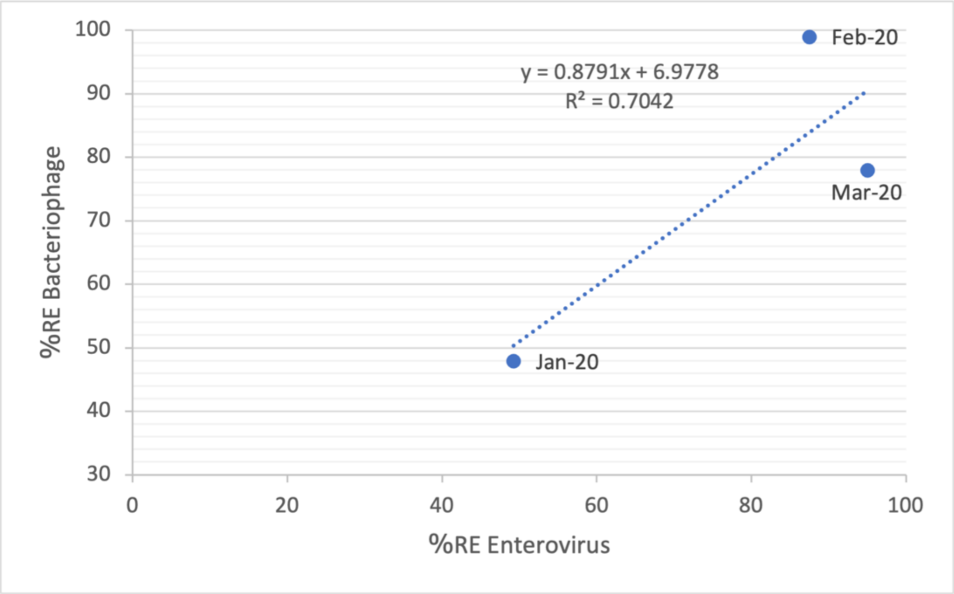

**Fig. 4.10** The correlation graph between the removal efficiency percentage of enterovirus and the removal efficiency percentage of bacteriophage.

# CHAPTER V
# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

From the overall results, the detected enterovirus concentration seemed to be no difference between each wastewater treatment plant. Moreover, enterovirus cannot be surrogated by microbial indicators such as *E. coli* and bacteriophage. Still, wastewater monitoring for checking water quality such as chemical and biological contamination should be done routinely because the detection of microbial indicators in the sewage cannot use for the detection of enterovirus in the sewage. The enterovirus concentrations and the hand-foot-mouth disease cases are not correlated to each other. The number and timeline of samples might be too low that the data are not enough for the expected result. Besides, the detection of enterovirus in the sewage might come from the infection of other enterovirus-caused diseases except for hand-foot-mouth disease.

From the three wastewater treatment plants, Dindaeng and Nong Khaem treatment plants are seemed to have similar removal efficiency. In addition, from the result, wastewater treatment plants should design for disinfection purposes of virus because enterovirus removal efficiency cannot be surrogated by microbial indicators removal efficiencies such as *E. coli* and bacteriophage. Wastewater management is a crucial thing that cannot be ignored in Thailand. Wastewater treatment plants are effective to reduce contaminated sewage for at least 1-log reduction. However, the removal efficiency is not enough for treating most viruses including enterovirus. Treated sewage in Thailand is not suitable for wastewater reuse and irrigation. Finally, the combination of using environmental surveillance data and clinical data called wastewater-based epidemiology (WBE) is a new approach for finding real-time infected people and helps to find asymptomatic infected people. Although this study showed non-correlated data between enterovirus concentration and hand-foot-mouth disease cases, more information and virus surveillance in the environment is needed.

## 5.2 Recommendation

To accomplish a better result in the future, in-depth environmental surveillance is needed with more samples and information of coverage area of wastewater treatment plants. Furthermore, an established standard value for virus contamination is needed for better water quality. If the water reclamation system has played an important role in the future, the installation of an advanced treatment process such as the disinfection process is required.

# REFERENCE

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J. W., Choi, P. M., Kitajima, M., Simpson, S. L., Li, J., Tscharke, B., Verhagen, R., Smith, W. J. M., Zaugg, J., Dierens, L., Hugenholtz, P., Thomas, K. v., & Mueller, J. F. (2020). First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. *Science of the Total Environment*, *728*. https://doi.org/10.1016/j.scitotenv.2020.138764

Ahmed, W., Tscharke, B., Bertsch, P. M., Bibby, K., Bivins, A., Choi, P., Clarke, L., Dwyer, J., Edson, J., Nguyen, T. M. H., O'Brien, J. W., Simpson, S. L., Sherman, P., Thomas, K. V., Verhagen, R., Zaugg, J., & Mueller, J. F. (2021). SARS-CoV-2 RNA monitoring in wastewater as a potential early warning system for COVID-19 transmission in the community: A temporal case study. *Science of the Total Environment*, *761*, 144216. https://doi.org/10.1016/j.scitotenv.2020.144216

Arden, S., Morelli, B., Schoen, M., Cashman, S., Jahne, M., Ma, X. C., & Garland, J. (2020). Human health, economic and environmental assessment of onsite non-potablewater reuse systems for a large, mixed-use urban building. *Sustainability (Switzerland)*, *12*(13). https://doi.org/10.3390/su12135459

Asami, T., Katayama, H., Torrey, J. R., Visvanathan, C., & Furumai, H. (2016). Evaluation of virus removal efficiency of coagulation-sedimentation and rapid sand filtration processes in a drinking water treatment plant in Bangkok, Thailand. *Water Research*. https://doi.org/10.1016/j.watres.2016.05.012

Bisseux, M., Didier, D., Audrey, M., Christine, A., Hélène, P. L., Jean-Luc, B., & Cécile, H. (2020). Monitoring of enterovirus diversity in wastewater by ultra-deep sequencing: An effective complementary tool for clinical enterovirus surveillance. *Water Research*, *169*. https://doi.org/10.1016/j.watres.2019.115246

Borowa, A., Brdys, M. A., & Mazur, K. (2006). Modelling of wastewater treatment plant for monitoring and control purposes by state - Space Wavelet Networks. *IFAC Proceedings Volumes (IFAC-PapersOnline)*, *1*(PART 1), 250–255. https://doi.org/10.3182/20060830-2-sf-4903.00044

Bosch, A. (1998). Human enteric viruses in the water environment: A minireview. *International Microbiology*. https://doi.org/10.2436/im.v1i3.39

Bustin, S. A., & Nolan, T. (2020). RT-QPCR testing of SARS-COV-2: A primer. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms21083004

Chaihard, T. (1377). *Thanaporn*. 68–70.

Chang, L. Y. (2008). Enterovirus 71 in Taiwan. In *Pediatrics and Neonatology*. https://doi.org/10.1016/S1875-9572(08)60023-6

Cioffi, B., Monini, M., Salamone, M., Pellicanò, R., Di Bartolo, I., Guida, M., La Rosa, G., & Fusco, G. (2020). Environmental surveillance of human enteric viruses in wastewaters, groundwater, surface water and sediments of Campania Region. *Regional Studies in Marine Science*. https://doi.org/10.1016/j.rsma.2020.101368

Cui, Q., Liu, H., Yang, H. W., Lu, Y., Chen, Z., & Hu, H. Y. (2020). Bacterial removal performance and community changes during advanced treatment process: A case study at a full-scale water reclamation plant. *Science of the Total Environment*, *705*, 135811. https://doi.org/10.1016/j.scitotenv.2019.135811

Disease, M. (n.d.). *ก | โรคมือเท้าปาก.*

England, T. N. (1999). *Journal Medicine ©*. *341*, 929–935.

Farkas, K., Hillary, L. S., Malham, S. K., McDonald, J. E., & Jones, D. L. (2020). Wastewater and public health: the potential of wastewater surveillance for monitoring

COVID-19. In *Current Opinion in Environmental Science and Health*. https://doi.org/10.1016/j.coesh.2020.06.001

Farkas, K., Mannion, F., Hillary, L. S., Malham, S. K., & Walker, D. I. (2020). Emerging technologies for the rapid detection of enteric viruses in the aquatic environment. In *Current Opinion in Environmental Science and Health*. https://doi.org/10.1016/j.coesh.2020.01.007

Fehr, A. R., & Perlman, S. (2015). Coronaviruses: An overview of their replication and pathogenesis. In *Coronaviruses: Methods and Protocols*. https://doi.org/10.1007/978-1-4939-2438-7_1

Field, K. G., & Samadpour, M. (2007). Fecal source tracking, the indicator paradigm, and managing water quality. *Water Research*, *41*(16), 3517–3538. https://doi.org/10.1016/j.watres.2007.06.056

Frigon, D., Biswal, B. K., Mazza, A., Masson, L., & Gehr, R. (2013). Biological and physicochemical wastewater treatment processes reduce the prevalence of virulent Escherichia coli. *Applied and Environmental Microbiology*, *79*(3), 835–844. https://doi.org/10.1128/AEM.02789-12

Fumian, T. M., Leite, J. P. G., Castello, A. A., Gaggero, A., Caillou, M. S. L. de, & Miagostovich, M. P. (2010). Detection of rotavirus A in sewage samples using multiplex qPCR and an evaluation of the ultracentrifugation and adsorption-elution methods for virus concentration. *Journal of Virological Methods*. https://doi.org/10.1016/j.jviromet.2010.08.017

Gerba, C. P., Betancourt, W. Q., & Kitajima, M. (2017). How much reduction of virus is needed for recycled water: A continuous changing need for assessment? In *Water Research*. https://doi.org/10.1016/j.watres.2016.11.020

Gonzalez, G., Carr, M. J., Kobayashi, M., Hanaoka, N., & Fujimoto, T. (2019). Enterovirus-associated hand-foot and mouth disease and neurological complications in japan and the rest of the world. *International Journal of Molecular Sciences*, *20*(20), 1–16. https://doi.org/10.3390/ijms20205201

Hamza, I. A., & Bibby, K. (2019). Critical issues in application of molecular methods to environmental virology. In *Journal of Virological Methods*. https://doi.org/10.1016/j.jviromet.2019.01.008

Haramoto, E., Kitajima, M., Hata, A., Torrey, J. R., Masago, Y., Sano, D., & Katayama, H. (2018). A review on recent progress in the detection methods and prevalence of human enteric viruses in water. In *Water Research*. https://doi.org/10.1016/j.watres.2018.02.004

Harwood, V. J., Staley, C., Badgley, B. D., Borges, K., & Korajkic, A. (2014). Microbial source tracking markers for detection of fecal contamination in environmental waters: Relationships between pathogens and human health outcomes. *FEMS Microbiology Reviews*, *38*(1), 1–40. https://doi.org/10.1111/1574-6976.12031

Ishii, S., Kitamura, G., Segawa, T., Kobayashi, A., Miura, T., Sano, D., & Okabe, S. (2014). Microfluidic quantitative PCR for simultaneous quantification of multiple viruses in environmental water samples. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.02578-14

Ivanova, O. E., Yarmolskaya, M. S., Eremeeva, T. P., Babkina, G. M., Baykova, O. Y., Akhmadishina, L. V., Krasota, A. Y., Kozlovskaya, L. I., & Lukashev, A. N. (2019). Environmental surveillance for poliovirus and other enteroviruses: Long-term experience in moscow, russian federation, 2004-2017. *Viruses*. https://doi.org/10.3390/v11050424

Jahne, M. A., Brinkman, N. E., Keely, S. P., Zimmerman, B. D., Wheaton, E. A., & Garland, J. L. (2020). Droplet digital PCR quantification of norovirus and adenovirus in decentralized wastewater and graywater collections: Implications for onsite reuse. *Water Research*. https://doi.org/10.1016/j.watres.2019.115213

JICA. (2011). *Wastewater treatment plant in Bangkok*.

Kapikian, A. Z. (2001). A rotavirus vaccine for prevention of severe diarrhoea of infants and young children: Development, utilization and withdrawal. *Novartis Foundation Symposium*. https://doi.org/10.1002/0470846534.ch10

Katayama, H., Shimasaki, A., & Ohgaki, S. (2002). Development of a virus concentration method and its application to detection of enterovirus and Norwalk virus from coastal seawater. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.68.3.1033-1039.2002

Kato, R., Asami, T., Utagawa, E., Furumai, H., & Katayama, H. (2018). Pepper mild mottle virus as a process indicator at drinking water treatment plants employing coagulation-sedimentation, rapid sand filtration, ozonation, and biological activated carbon treatments in Japan. *Water Research*, *132*, 61–70. https://doi.org/10.1016/j.watres.2017.12.068

Kralik, P., & Ricchi, M. (2017). A basic guide to real time PCR in microbial diagnostics: Definitions, parameters, and everything. *Frontiers in Microbiology*, *8*(FEB), 1–9. https://doi.org/10.3389/fmicb.2017.00108

Lenaerts, L., De Clercq, E., & Naesens, L. (2008). Clinical features and treatment of adenovirus infections. In *Reviews in Medical Virology*. https://doi.org/10.1002/rmv.589

Lesimple, A., Jasim, S. Y., Johnson, D. J., & Hilal, N. (2020). The role of wastewater treatment plants as tools for SARS-CoV-2 early detection and removal. *Journal of Water Process Engineering*, *38*(July), 101544. https://doi.org/10.1016/j.jwpe.2020.101544

Li, N., Xiao, L., Wang, L., Zhao, S., Zhao, X., Duan, L., Guo, M., Liu, L., & Feng, Y. (2012). Molecular Surveillance of Cryptosporidium spp., Giardia duodenalis, and Enterocytozoon bieneusi by Genotyping and Subtyping Parasites in Wastewater. *PLoS Neglected Tropical Diseases*. https://doi.org/10.1371/journal.pntd.0001809

Li, Y., Chang, Z., Wu, P., Liao, Q., Liu, F., Zheng, Y., Luo, L., Zhou, Y., Chen, Q., Yu, S., Guo, C., Chen, Z., Long, L., Zhao, S., Yang, B., Yu, H., & Cowling, B. J. (2018). Emerging enteroviruses causing hand, foot and mouth disease, China, 2010–2016. *Emerging Infectious Diseases*. https://doi.org/10.3201/eid2410.171953

Liu, S. L., Pan, H., Liu, P., Amer, S., Chan, T. C., Zhan, J., Huo, X., Liu, Y., Teng, Z., Wang, L., & Zhuang, H. (2015). Comparative epidemiology and virology of fatal and nonfatal cases of hand, foot and mouth disease in mainland China from 2008 to 2014. In *Reviews in Medical Virology*. https://doi.org/10.1002/rmv.1827

Manikandan, G., Karunakaran, G., Akshaya, T., & Senbagam, T. (2021). Materials Today : Proceedings Reuse of sewage water in Ramanathapuram for construction purpose. *Materials Today: Proceedings*, *xxxx*, 3–7. https://doi.org/10.1016/j.matpr.2021.03.395

Messacar, K., Spence-Davizon, E., Osborne, C., Press, C., Schreiner, T. L., Martin, J., Messer, R., Maloney, J., Burakoff, A., Barnes, M., Rogers, S., Lopez, A. S., Routh, J., Gerber, S. I., Oberste, M. S., Nix, W. A., Abzug, M. J., Tyler, K. L., Herlihy, R., & Dominguez, S. R. (2020). Clinical characteristics of enterovirus A71 neurological disease during an outbreak in children in Colorado, USA, in 2018: an observational cohort study. *The Lancet Infectious Diseases*, *20*(2), 230–239. https://doi.org/10.1016/S1473-3099(19)30632-2

Montwedi, M., Munyaradzi, M., Pinoy, L., Dutta, A., Ikumi, D. S., Motoasca, E., & Van der Bruggen, B. (2021). Resource recovery from and management of wastewater in rural South Africa: Possibilities and practices. *Journal of Water Process Engineering*, *40*(May 2020), 101978. https://doi.org/10.1016/j.jwpe.2021.101978

OIE. (2010). Principles and methods of validation of diagnostic assays for infectious diseases. *OIE Terrestrial Manual*, *May 2009*, 1–18.

Okoh, A. I., Sibanda, T., & Gusha, S. S. (2010). Inadequately treated wastewater as a source of human enteric viruses in the environment. In *International Journal of Environmental Research and Public Health*. https://doi.org/10.3390/ijerph7062620

OMS. (2020). Water , sanitation , hygiene and waste management for the COVID-19 virus. *World Health Organisation*, *March*, 1–9.

Owino, C. O., & Chu, J. J. H. (2019). Recent advances on the role of host factors during non-poliovirus enteroviral infections. *Journal of Biomedical Science*. https://doi.org/10.1186/s12929-019-0540-y

Pallansch, M. A., Oberste, M. S., & Lindsay Whitton, J. (2013). Enteroviruses: Polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In *Fields Virology: Sixth Edition*. https://doi.org/10.1002/0470857285.ch6

Pang, X., Qiu, Y., Gao, T., Zurawell, R., Neumann, N. F., Craik, S., & Lee, B. E. (2019). Prevalence, levels and seasonal variations of human enteric viruses in six major rivers in Alberta, Canada. *Water Research*, *153*, 349–356. https://doi.org/10.1016/j.watres.2019.01.034

Pärnänen, K. M. M., Narciso-Da-Rocha, C., Kneis, D., Berendonk, T. U., Cacace, D., Do, T. T., Elpers, C., Fatta-Kassinos, D., Henriques, I., Jaeger, T., Karkman, A., Martinez, J. L., Michael, S. G., Michael-Kordatou, I., O'Sullivan, K., Rodriguez-Mozaz, S., Schwartz, T., Sheng, H., Sørum, H., … Manaia, C. M. (2019). Antibiotic resistance in European wastewater treatment plants mirrors the pattern of clinical antibiotic resistance prevalence. *Science Advances*. https://doi.org/10.1126/sciadv.aau9124

Prado, T., de Castro Bruni, A., Barbosa, M. R. F., Garcia, S. C., de Jesus Melo, A. M., & Sato, M. I. Z. (2019). Performance of wastewater reclamation systems in enteric virus removal. *Science of the Total Environment*, *678*, 33–42. https://doi.org/10.1016/j.scitotenv.2019.04.435

Rock, C., & Rivera, B. (2014). *Supplementary Material ESM3 : w q w q w q*. *March*, 90.

Sabanathan, S., Van Tan, L., Thwaites, L., Wills, B., Qui, P. T., & Van Doorn, H. R. (2014). Enterovirus 71 related severe hand, foot and mouth disease outbreaks in South-East Asia: Current situation and ongoing challenges. In *Journal of Epidemiology and Community Health*. https://doi.org/10.1136/jech-2014-203836

Schmidt, N. J., Lennette, E. H., & Ho, H. H. (1974). An apparently new enterovirus isolated from patients with disease of the central nervous system. *Journal of Infectious Diseases*. https://doi.org/10.1093/infdis/129.3.304

Sheet, F. (2014). Log removal values in wastewater treatment. *Australia, Water Research*, 1–2.

Solomon, T., Lewthwaite, P., Perera, D., Cardosa, M. J., McMinn, P., & Ooi, M. H. (2010). Virology, epidemiology, pathogenesis, and control of enterovirus 71. In *The Lancet Infectious Diseases*. https://doi.org/10.1016/S1473-3099(10)70194-8

Song, C., Li, Y., Zhou, Y., Liang, L., Turtle, L., Wang, F., Wu, P., Qiu, Q., Yang, J., Wang, K., Cui, P., Cheng, Y., Zhang, T., Guo, C., Zeng, M., Long, L., Peiris, M., Zhou, C., Cowling, B. J., & Yu, H. (2020). Enterovirus genomic load and disease severity among children hospitalised with hand, foot and mouth disease. *EBioMedicine*, *62*. https://doi.org/10.1016/j.ebiom.2020.103078

Tao, Z., Chen, P., Cui, N., Lin, X., Ji, F., Liu, Y., Xiong, P., Zhang, L., Xu, Q., Song, Y., & Xu, A. (2020). Detection of enteroviruses in urban sewage by next generation sequencing and its application in environmental surveillance. *Science of the Total Environment*, *728*. https://doi.org/10.1016/j.scitotenv.2020.138818

Venugopal, A., Ganesan, H., Sudalaimuthu Raja, S. S., Govindasamy, V., Arunachalam, M., Narayanasamy, A., Sivaprakash, P., Rahman, P. K. S. M., Gopalakrishnan, A. V., Siama, Z., & Vellingiri, B. (2020). Novel wastewater surveillance strategy for early detection of coronavirus disease 2019 hotspots. In *Current Opinion in Environmental Science and Health*. https://doi.org/10.1016/j.coesh.2020.05.003

Wang, H., Tao, Z., Li, Y., Lin, X., Yoshida, H., Song, L., Zhang, Y., Wang, S., Cui, N., Xu, W., Song, Y., & Xu, A. (2014). Environmental surveillance of human enteroviruses in Shandong Province, China, 2008 to 2012: Serotypes, temporal fluctuation, and molecular epidemiology. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.00851-14

Wang, X. W., Li, J., Guo, T., Zhen, B., Kong, Q., Yi, B., Li, Z., Song, N., Jin, M., Xiao, W., Zhu, X., Gu, C., Yin, J., Wei, W., Yao, W., Liu, C., Li, J., Ou, G., Wang, M., … Li, J. (2005). Concentration and detection of SARS coronavirus in sewage from Xiao Tang Shan hospital and the 309th Hospital of the Chinese People's Liberation Army. *Water Science and Technology*. https://doi.org/10.2166/wst.2005.0266

Westhaus, S., Weber, F. A., Schiwy, S., Linnemann, V., Brinkmann, M., Widera, M., Greve, C., Janke, A., Hollert, H., Wintgens, T., & Ciesek, S. (2021). Detection of SARS-CoV-2 in raw and treated wastewater in Germany – Suitability for COVID-19 surveillance and potential transmission risks. *Science of the Total Environment*, *751*. https://doi.org/10.1016/j.scitotenv.2020.141750

Wu, J., Long, S. C., Das, D., & Dorner, S. M. (2011). Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. *Journal of Water and Health*, *9*(2), 265–278. https://doi.org/10.2166/wh.2011.117

Yang, B., Liu, F., Liao, Q., Wu, P., Chang, Z., Huang, J., Long, L., Luo, L., Li, Y., Leung, G. M., Cowling, B. J., & Yu, H. (2017). Epidemiology of hand, foot and mouth disease in china, 2008 to 2015 prior to the introduction of EV-A71 vaccine. *Eurosurveillance*. https://doi.org/10.2807/1560-7917.ES.2017.22.50.16-00824

Zeng, S. Q., Halkosalo, A., Salminen, M., Szakal, E. D., Puustinen, L., & Vesikari, T. (2008). One-step quantitative RT-PCR for the detection of rotavirus in acute gastroenteritis. *Journal of Virological Methods*. https://doi.org/10.1016/j.jviromet.2008.08.004

## BIOGRAPHY

| | |
|---|---|
| Name: | Kritnipit Phetanan |
| Date of birth: | June 12, 1998 |
| Place of birth: | Bangkok |
| E-mail: | France.krit@gmail.com |
| Phone number: | +66944869769 |
| Graduation: | Bachelor of Environmental Science,<br>Chulalongkorn University Bangkok, Thailand |