Correlation between virus reduction and trihalomethanes risk after chlorine disinfection

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ความสัมพันธ์ระหว่างการลดลงของไวรัสและความเสี่ยงของไตรฮาโลมีเทนหลังกระบวนการฆ่าเชื้อด้วยคลอรีน

น.ส.วราภรณ์ ขันทอง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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วราภรณ์ ขันทอง : ความสัมพันธ์ระหว่างการลดลงของไวรัสและความเสี่ยงของไตรฮาโลมีเทนหลังกระบวนการฆ่าเชื้อด้วยคลอรีน. (Correlation between virus reduction and trihalomethanes risk after chlorine disinfection) อ.ที่ปรึกษาหลัก : อ. ดร. จตุวัฒน์ แสงสานนท์, อ.ที่ปรึกษาร่วม : ผศ. ดร.วรพจน์ กนกกันฑพงษ์

ถึงแม้ว่าการฆ่าเชื้อในน้ำเสียเป็นสิ่งสำคัญในการช่วยลดอันตรายจากเชื้อโรคในน้ำ แต่ก็มีความเสี่ยงที่อาจเกิดขึ้นซึ่งเป็นผลพลอยได้จากการฆ่าเชื้อโรค การสร้างสมดุลระหว่างการลดปริมาณจุลินทรีย์ และการจัดการการเกิดผลพลอยได้ที่มาจากการฆ่าเชื้อถือเป็นสิ่งสำคัญในการรักษาความเสี่ยงทั้งด้านจุลินทรีย์ และสารเคมีให้อยู่ในระดับที่ยอมรับได้ ในการประเมินความเสี่ยงของการศึกษานี้อยู่บนพื้นฐานของปริมาณไตรฮาโลมีเทนผ่านการกลืนกิน การสูดดม และการสัมผัสทางผิวหนัง [JS1] การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบความสัมพันธ์ระหว่างการลดลงของไวรัสและการเกิดขึ้นของไตรฮาโลมีเทน และเพื่อประเมินระดับความเสี่ยงของมะเร็งหลังการใช้คลอรีนในการฆ่าเชื้อ และการใช้กระบวนการบำบัดเพื่อลดความเสี่ยงของมะเร็งหลังการใช้คลอรีนของน้ำเสียที่ปล่อยออกมาจากโรงบำบัดน้ำเสียโดยใช้ระบบตะกอนเร่งแบบไซคลิกในโรงบำบัดน้ำเสียช่องนนทรีและฟีดแบบขั้นตะกอนเร่งชนิดตะกอนเร่งและการกรองขนาดเล็กพิเศษในโรงบำบัดน้ำเสียบางซื่อ จากการศึกษานี้ช่วงปริมาณคลอรีนที่ 4.8 ถึง 14 มิลลิกรัมต่อลิตร เพียงพอที่จะยับยั้งการทำงานของแบคทีริโอฟาจ 1–6 ล็อคริดักชั่นในโรงบำบัดน้ำเสียช่องนนทรี ในขณะที่โรงบำบัดน้ำเสียบางซื่อ ใช้ช่วงความเข้มข้นของคลอรีน 1.5 ถึง 5.0 มิลลิกรัมต่อลิตร ซึ่งเพียงพอตามแนวทางการลดไวรัสตามที่องค์การอนามัยโลกกำหนด ผลลัพธ์บ่งชี้ว่าปริมาณของคลอรีนมีผลกระทบอย่างมีนัยสำคัญต่อการยับยั้งการทำงานของแบคทีริโอฟาจ และระดับของการลดลงของล็อครีดักชั่นของไวรัสจากกระบวนการของคลอรีน ระดับความเข้มข้นของคลอรีนมีผลต่อระดับคลอรีนอิสระตกค้างหลังจากคลอรีนตั้งต้นมีค่าอยู่ในช่วง 0.147 ถึง 2.613 มิลลิกรัมต่อลิตร ตามความเข้มข้นของขีดจำกัดคลอรีนตกค้างในการนำน้ำกลับมาใช้ใหม่ด้านการเกษตรกรรมขององค์การอนามัยโลก นอกจากนี้ ความเข้มข้นของคลอรีนดูเหมือนจะมีอิทธิพลต่อการก่อตัวของไตรฮาโลมีเทน ความเข้มข้นของ ไตรคลอโรมีเทน, โบรโมไดคลอโรมีเทน, ไดโบรโมคลอโรมีเทน และไตรโบรโมมีเทน ที่

สาขาวิชา	พิษวิทยาอุตสาหกรรมและการประเมินความเสี่ยง	ลายมือชื่อนิสิต ....
ปีการศึกษา	2566	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม ....

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While wastewater disinfection is essential to mitigate the threats from waterborne pathogens, there arises a potential risk from disinfection byproducts. Striking a balance between achieving microbial reduction and managing the occurrence of disinfection byproducts is crucial for maintaining both microbial and chemical risks at acceptable levels. The cancer risk assessment was based on THMs through of oral ingestions, inhalation absorptions, and dermal absorptions exposure. This study aims to investigate the relationship between virus reduction and THMs formation, and to assess their respective cancer risk levels post-chlorination, and determined treatment process was used to decreased cancer risk post-chlorination. Effluent wastewater was collected from wastewater treatment plant employing cyclic activated sludge systems (CASS) in Chongnonsi wastewater treatment plant (CN-WWTP) and activated sludge type step feed and ultra-microfiltration in Bangsue wastewater treatment plant (BS-WWTP). From this study, the chlorine dose range of 4.8 to 14 mg/L is enough to inactivate 1–6 log of bacteriophage in CN-WWTP, while BS-WWTP used chlorine concentration range 1.5 to 5.0 mg/L, sufficient to according to the guidelines for virus reduction required by the World Health Organization (WHO). Results indicate that the dose of chlorine has a significant impact on the inactivation of bacteriophages and the level of log reduction. From chlorination process the chlorine concentration brought up the

Field of Study:	Industrial Toxicology and Risk Assessment	Student's Signature ....
Academic Year:	2023	Advisor's Signature ....
		Co-advisor's Signature ....

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CHAPTER 1
INTRODUCTION

1.1 Statement of problem

Water consumption has increased at a rate of roughly 1% year since 1980, and it is predicted that there will be a 20% to 30% increase in demand by 2050. Population growth and social and economic development were the main factors. Additionally, more than two billion people in some nations have insufficient water, and four billion people lack water for at least one month of the year. Similarly, three out of ten people do not have access to enough clean water, and six out of ten do not have access to appropriate sanitation(UNESCO et al., 2019).

Due to urbanization, socioeconomic factors, and intensive agriculture, the population is growing, and water demand is increasing (**Carey & Migliaccio, 2009; UNEP, 2016**). This has an impact on each area's wastewater treatment plant (WWTP) infrastructure and wastewater volume. Approximately 80% of the world's untreated wastewater is discharged directly into rivers (UNESCO, 2017). The river receiving the most wastewater has the highest nutrient, organic carbon, and pathogen content. As a result, important aspects of aquatic environments like eutrophication, the hydrologic characteristics of the receiving waters are affected, decreasing dissolved oxygen (DO) levels, increasing fish mortality, and phytoplankton blooms that are toxic(**Carey & Migliaccio, 2009**).

Water recycling or reuse is a water management approach that reduces water pollutant loads and aquatic environmental contamination (Carey & Migliaccio, 2009). Reusing water is part of the water circulation system, and opportunities for sufficient, and secure water supply all contribute to sustainable water use(UNEP, 2016). Reusing water can be utilized for a variety of purposes, including agriculture, irrigation, domestic use, public parks, gardening, and indirect reuse (Alexandrou et al., 2018). The technique or type of treatment used determines the application of water reuse in terms of water quality. The three categories of treatment are as follows: primary treatment (grit chambers, sedimentation, screening), secondary treatment (activated sludge), and advanced tertiary treatment (reverse osmosis, filtration) (Carey & Migliaccio, 2009). Reusing water relieved strain on rivers, supplied renewable, divided nutrients for agriculture, and disposed of sewage before releasing it into rivers (Carey & Migliaccio, 2009; WATER REUSE, 2018).

A combination of bacteria, protozoa, helminths, and viruses make up the pathogens in wastewater discharge. There are numerous pathogens such as salmonella up to 105, enteroviruses up to 106, and adenovirus up to 106 (USEPA et al., 2012). However, some Asian nations use untreated wastewater for agricultural irrigation and aquaculture, which is associated with a risk to human health (Liao, Chen, Xu, et al., 2021). 90% of the 1.8 million deaths caused by diarrheal illnesses each year in underdeveloped nations are children under the age of five. According to

World Health Organization performance guidelines, the following conditions must be treated and reduced: 2–4 log reduction for bacteria, 3–5 log reduction for viruses and 2–4 log reduction for protozoa (WHO, 2019). There are several different forms of disinfectants, including ozone, chlorine, chlorine dioxide, and chloramines (USEPA et al., 2001). Particularly chlorine is widely used in many countries due to its low cost and superior performance to other disinfectants in the removal of pathogens.

The effects of the disinfectant to occur disinfection by-products (DBPs) may potentially have long-term effects (WHO, 2022). Numerous studies have demonstrated that DBPs can cause long-term dermal, inhalation, and ingestion exposure (Chowdhury et al., 2009). IARC Classified some agents DBPs is carcinogenic to humans. Cancer risk and chronic effects are two possible health risk effects of DBPs. Trihalomethane (THMs) damage DNA and cause DNA strand breaks in primary human lung epithelial cells (Landi et al., 2003). Chloroform is thought to be carcinogenic and may increase the risk of bladder cancer in humans (Costet et al., 2011; Richardson et al., 2007). DBPs are produced as a result, of a disinfectant's interaction with factors as follows: dose, reaction time, pH, temperature (**Cortés & Marcos, 2018; Huang et al., 2016**), total organic carbon (TOC), dissolved organic carbon (DOC) (Alexandrou et al., 2018), amino acids (Yang et al., 2012), and total nitrogen (TN) (Watson et al., 2012).

While microbial risk can be mitigated, the potential hazard posed by disinfection byproducts, particularly in terms of chronic effect and cancer risk, cannot be overlooked. Balancing the risks between microbial reduction and the formation of disinfectant byproducts (DBPs) is essential for safe water reuse. This study aims to explore the relationship between virus reduction and the formation of trihalomethanes including the relationship between virus reduction and cancer risk levels from trihalomethanes. Furthermore, the method that wastewater was treating an important consideration. Data from this study had used as a guide to take treatment methods between biological treatment process and biological-ultrafiltration treatment processes, control levels of bacteriophage virus indicators to 3-5 log reduction an according to the guidelines required by the World Health Organization (WHO), and risk assess of trihalomethanes formation on the regulatory limit of $1x10^{-6}$ of the USEPA (USEPA, 1999, 2005), which is a common threshold used in risk assessments.

1.2 Research hypotheses

1.2.1 The effluent wastewater after the biological treatment process has a higher precursor concentration compared to the biological ultrafiltration method, which can lead to the formation of THMs

1.2.2 Biological treatment combined with ultrafiltration reduces precursors more effectively than the biological treatment process alone. Therefore, the risk of

THMs from biological treatment plus ultrafiltration is lower than that from the biological treatment process.

1.2.3 The relationship between virus reduction and THMs risk is inverse, and a 5-log reduction in virus concentration indicates an unacceptable level of THMs risk.

1.3 Research objectives

1.3.1 To determine the chlorine concentration required for aroud 3 to5 log reduction of the virus in effluent of biological treatment process and biological treatment combined with ultrafiltration.

1.3.2 To determine the formation of THMs following chlorine disinfection after biological treatment process and biological treatment combined with ultrafiltration.

1.3.3 To determine risk of THMs at difference virus log reduction target.

1.4 Scope of the study

1.4.1 Focusses on effluent wastewater from two wastewater treatment plant (WWTP) in Bangkok, Thailand as follows, Chongnonsi wastewater treatment plant (CN-WWTP) and Bangsue wastewater treatment plant (BS-WWTP).

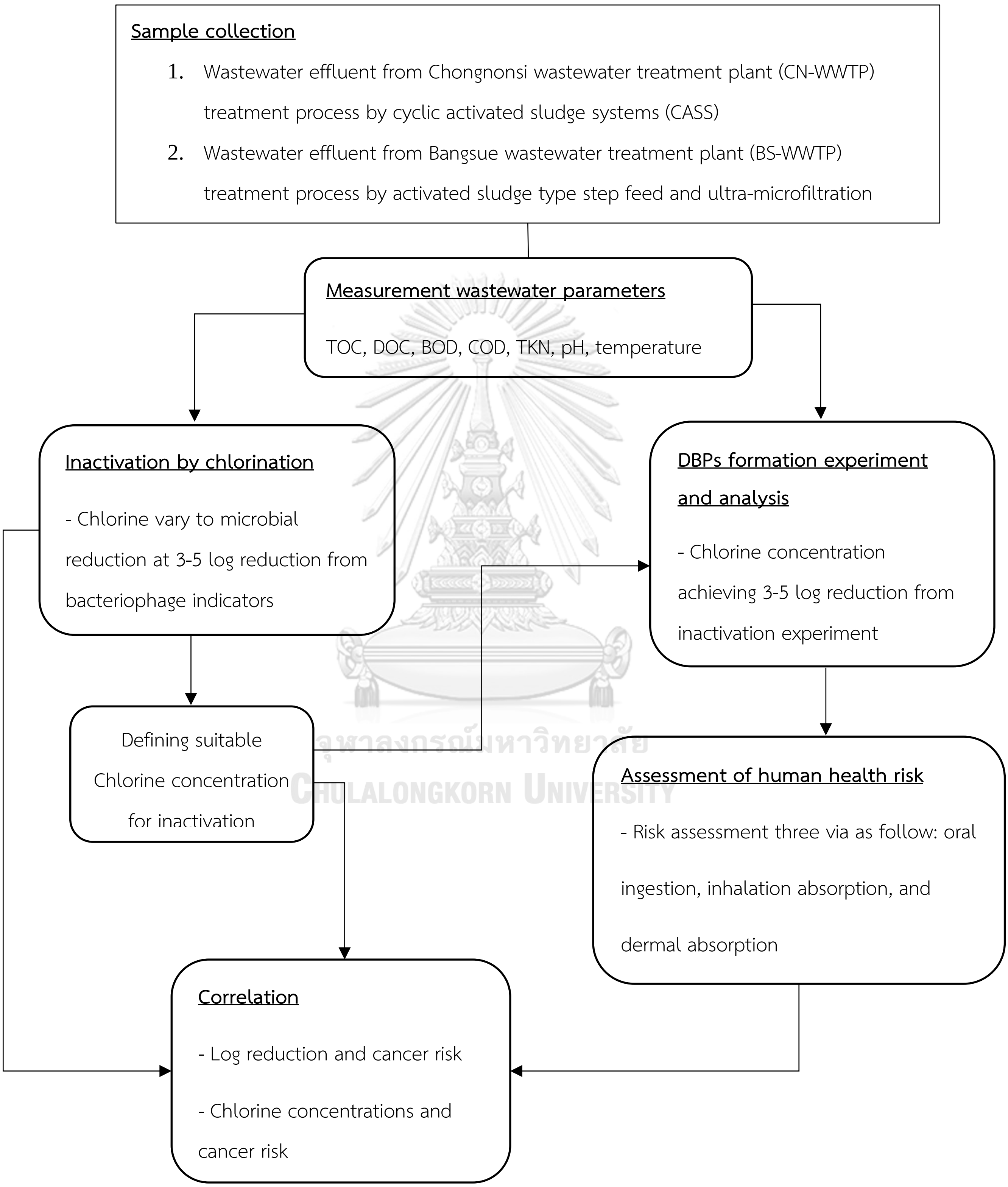
1.4.2 The water quality parameter was measured TOC, DOC, BOD, COD, TKN, pH, and temperature.

1.4.3 Research monitoring occur THMs from chlorine treatment in either log reduction of virus on bacteriophage indicator.

1.4.4 The log reduction of virus study at 3-5 log reduction by WHO regulations criteria for household water treatment.

1.4.5 The risk assessment focus on cancer risk from THMs on recommendation of WHO guidelines for drinking water quality.

1.5 Experimental setup



CHAPTER 2
LITERATURE REVIEW

2.1 Situations of water demand

Since 1980, there has been an increase in water demand that has grown at a pace of about 1% year, with estimates for the need in 2050 ranging from 20% to 30%. Population growth and social and economic development were the main factors (UNESCO et al., 2019). Furthermore, the agriculture sector has the highest usage when compared to other sectors as shown in Fig.1. Due to climate change, 685 million people in more than 570 cities may face freshwater shortages of at least 10% by 2050. Additionally, more than two billion people in some nations have insufficient water, and four billion people lack water for at least one month of the year (UNESCO et al., 2019). Similarly, three out of ten people do not have access to enough clean water, and six out of ten do not have access to appropriate sanitation (UNESCO et al., 2019). Africa has the most limited access to water resources globally, while Asia Pacific is next (The World Bank, 2020). The rate of urbanization and population increase in Asia-Pacific, and Africa is 2.4% (Liao, Chen, Wu, et al., 2021). Groundwater use is unsustainable in 29 of the 48 nations as of 2016. As a result, of the scarcity of water, the effects of climate change, and natural disasters. Between 1996 and 2015, various nations, including India with 17.5 million people and the People's Republic of China with 16.5 million people, had droughts that caused people to lose access to water, agricultural production to decline, local food shortages, and wildfires. Additionally, droughts have increased by 29% since 2000 (UNESCO et al., 2019).

Urbanization and socioeconomic development conditions. As a result, the population is growing, and water use is increasing (Carey & Migliaccio, 2009). This has an impact on each area's wastewater treatment plant (WWTP) infrastructure and wastewater volume. The river receiving the most wastewater has the highest nutrient, organic carbon, and pathogen content. As a result, receiver waters' hydrologic characteristics, quality of water, and important aspect of aquatic systems (B. E. Haggard, 2001; Carey & Migliaccio, 2009; Martins et al., 2004). Since 1990, water pollution has been a significant issue with rivers in Asia, Africa, and Latin America. First off, one third of all rivers are estimated to be contaminated by pathogens. There are issues with contaminants in drinking water and health risks from contact. Second, about one in seven of all rivers are affected by organic contamination (UNEP, 2016). Which raises concerns about aquatic habitats such as eutrophication, declining dissolved oxygen (DO) levels, increasing fish mortality, and developing phytoplankton toxicity (Burkholder' & Parsons2, 1992; Carey & Migliaccio, 2009; S. R. Carpenter, 1998)). Another reason is that one in ten rivers are affected by saline pollution, which raises questions regarding their usage for industrial and other purposes besides irrigation. Due to increased wastewater input into rivers, the aforementioned issue exists. Because of factors such as population growth, social and economic advancement, extensive agricultural production, and inadequate or no wastewater treatment (UNEP, 2016). Approximately 80% of the world's untreated wastewater is discharged directly into rivers (UNESCO, 2017).

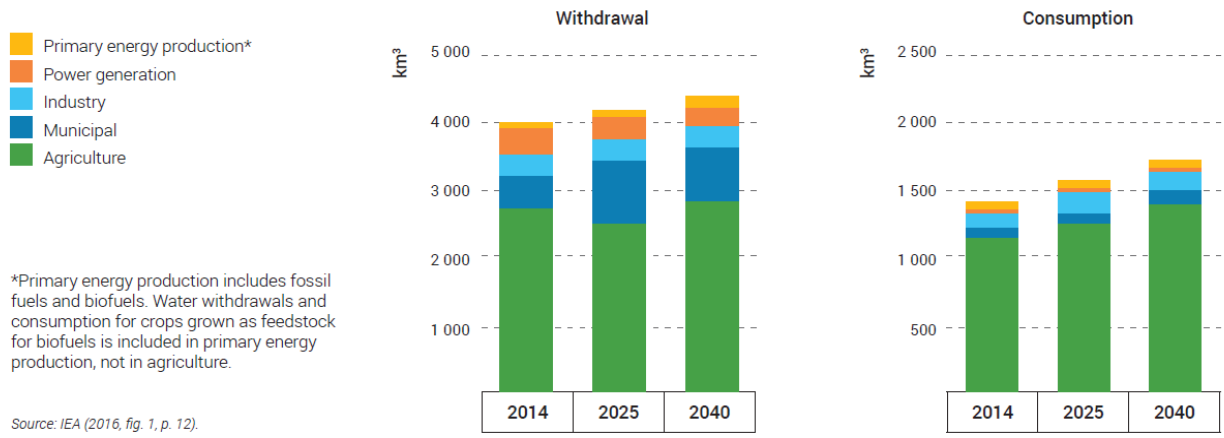
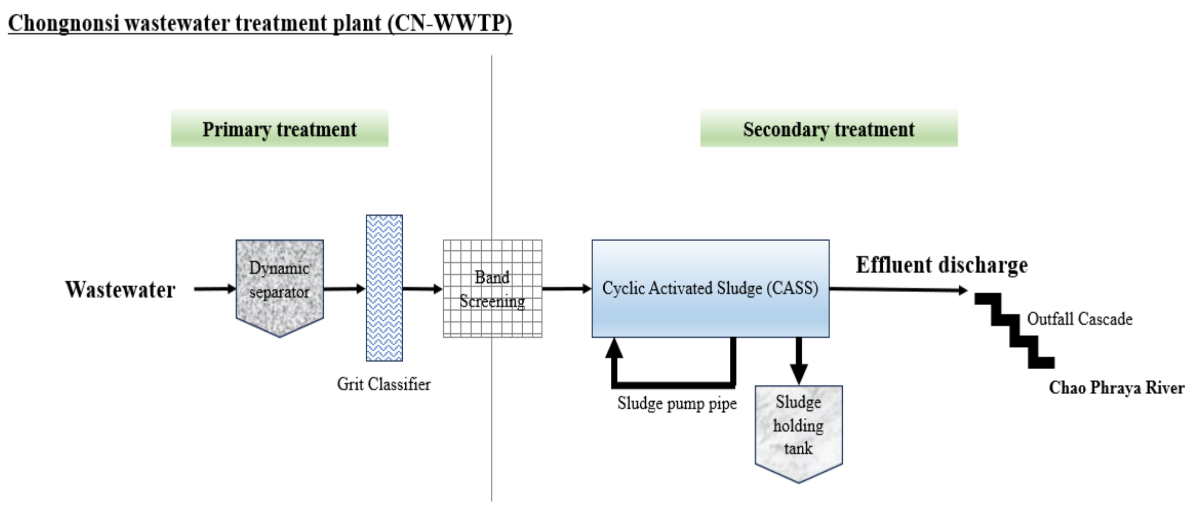


Figure 1 Global water demand by sector to 2040 (UNESCO et al., 2019)

2.2 Stages of wastewater treatment plant

The technique or type of treatment used determines the application of water reuse in terms of water quality. The three categories of treatment are as follows: primary treatment (grit chambers, sedimentation, screening, and flotation), secondary treatment (removal of nutrients, activated sludge), and advanced tertiary treatment (chemical and biological nutrient removal, filtration) (Carey & Migliaccio, 2009). Specifically, this study utilized Chongnonsi Wastewater Treatment Plant (CN-WWTP) and Bangsue Wastewater Treatment Plant (BS-WWTP). CN-WWTP employs primary treatment with screening and grit removal, followed by secondary treatment with activated sludge and nutrient removal. Furthermore, BS-WWTP, similar to CN-WWTP, utilizes advanced tertiary treatment with ultra-microfiltration to enhance nutrient removal as shown in Fig. 2. The United States uses the most recycled water in the world. In 2027, increase volumes by 37% (**Carey & Migliaccio, 2009; WATER REUSE, 2018**) China has the largest reuse water in the Asia-Pacific, with a daily capacity of 200 million cubic meters (Qu et al., 2019) Singapore's NEWater project, which offers water recycling, 30 percent of water demand (Lefebvre, 2018) Reusing water relieved strain on rivers, supplied renewable, divided nutrients for agriculture, and disposed of sewage before releasing it into rivers (**Carey & Migliaccio, 2009; WATER REUSE, 2018**).



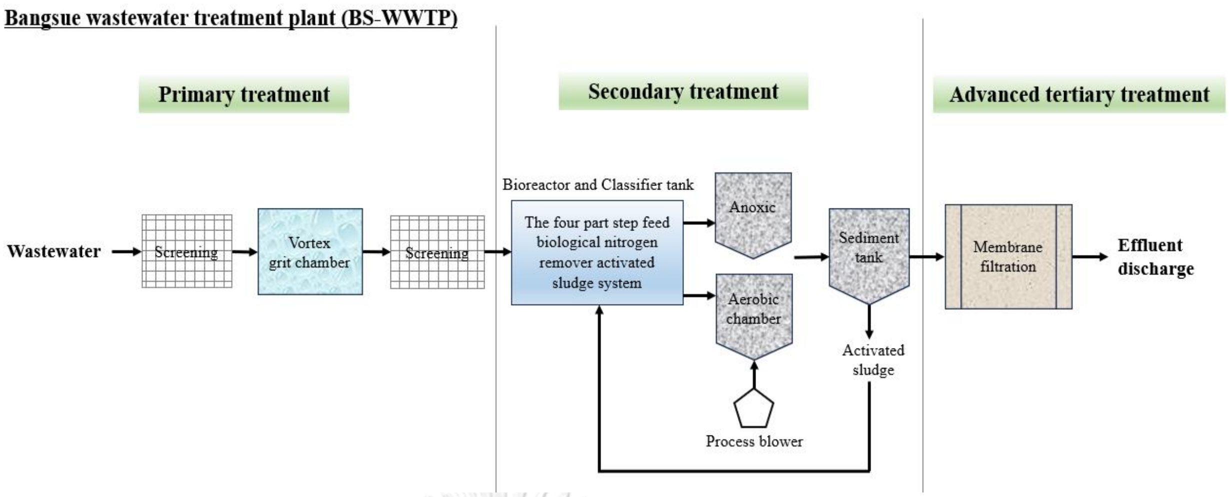


Figure 2 Flow diagram of the stages of treatment plant in CN-WWTP and BS-WWTP

2.3 Risk from wastewater reuse

Worldwide, there is concern about a pathogen in the drinking water that may be causing a diarrhea epidemic. The majority of poor and undeveloped nations have unclean utilities and drinking water, which can lead to acute sickness in people. There are many different types of waterborne pathogens that can cause infectious diseases, such as Campylobacter up to 10^4 per liter, Salmonella and Vibro cholera up to 10^5 per liter in raw wastewater (USEPA et al., 2012). Giardia is one type of protozoa up to 10^5 per liter include Cryptosporidium up to 10^4 per liter in raw wastewater (USEPA et al., 2012). One kind of helminth lives in untreated wastewater, such as Ascaris and Ancylostoma up to 10^3 per liter (USEPA et al., 2012). One kind of helminth lives in untreated wastewater, such as Enteroviruses and Adenovirus up to 10^6 per liter Caliciviruses up to 10^9 per liter and Rotavirus up to 10^5 per liter (USEPA et al., 2012). There is a crisis over the availability of clean water in sufficient quantities for drinking and for other uses due to the world's growing population. Most countries are starting to repurpose wastewater as a new option for various uses such agricultural, home (flushing, watering, and washing), parks, gardening, indirect reuse, and supplementing non-drinkable water (Alexandrou et al., 2018). Several nations, including California, Florida, Hawaii, Virginia, and Washington, clean their wastewater before using it, but many others throughout the world use untreated, raw wastewater. That is an issue for human health in many cases, such as the case of infection-related diarrhea in Mexico and the increased danger to children under the age of five (Blumenthal' et al., 2001). Diarrheagenic Escherichia coli infections among preschoolers in Hanoi (Hien et al., 2007). Farmers in Malamulele, South Africa, and children's hookworm and Giardia lamblia illnesses are caused by wastewater reuse in vegetable irrigation (Gumbo et al., 2010). In addition to studies on the negative health effects of used, untreated raw wastewater. For instance, during irrigation dermatitis and fungal growth in farmers aquatic plant culture, farmworkers and children are exposed to fecal coliform bacteria (Anh et al., 2009; Blumenthal et al., 2000).

2.4 Log reduction of virus

In order to reduce the risk and potential effects of pathogens on human health, drinking water and household water are currently disinfected. Most often, disinfection uses chemicals like chlorine, chlorine dioxide, chloramines, ozone, and potassium permanganate to remove microorganisms (USEPA et al., 2001). Particularly chlorine is utilized extensively in many nations due to its inexpensive cost and superior efficacy to other disinfectants in the elimination of germs. World Health Organization regulations criteria for household water treatment as follows 2 – 4 log reduction required for bacteria, 3 – 5 log reduction required for viruses and 2 – 4 log reduction required for protozoa as shown in Table 1 (WHO, 2019). Contrarily, there are no standards for wastewater reuse that would protect humans from the risks posed by microbial contamination. While Virginia required E. coli, Enterococci, and fecal coliform as indicators, Arizona, Florida, and Hawaii required fecal coliform as a bacterial indicator. Additionally, Florida required Giardia and Cryptosporidium for the quality of wastewater reuse (USEPA et al., 2012). Thailand uses a variety of wastewater treatment technologies, including contact stabilization activated sludge, vertical loop reactors, activated sludge with nutrient removal, and cyclic activated sludge systems, however there are no regulations addressing pathogen risk or disinfectant treatment. Despite the fact that chlorine has a number of advantages over other agents for protecting people from pathogens, particularly when wastewater is reused, numerous studies have shown that the formation of disinfection by-products (DBPs) is also worrying for their toxicity and potential negative effects on human health (G. F. Craun et al., 1994).

Table 1 Performance criteria for reduction require treatment technologies (WHO, 2019)

<table>
<tr><td>Performance classification</td><td>Bacteria log reduction</td><td>Viruses log reduction</td><td>Protozoa Log reduction</td><td>Interpretation (with correct and consistent use)</td></tr>
<tr><td>★★★</td><td>≥ 4</td><td>≥ 5</td><td>≥ 4</td><td rowspan="2">Comprehensive protection</td></tr>
<tr><td>★★</td><td>≥ 2</td><td>≥ 3</td><td>≥ 2</td></tr>
<tr><td>★</td><td colspan="3">Meet at least 2-star criteria for two class of pathogens</td><td>Target protection</td></tr>
<tr><td>-</td><td colspan="3">Fail to meet criteria for 1-star</td><td>Little or no protection</td></tr>
</table>

2.5 Mechanism of chlorination

Chlorine widely used to inactivate microbial. Chlorine is a yellow-green gas at room temperature and utterly reactive element include strong oxidising agent. When chlorine add in water were formed (Onyutha & Kwio-Tamale, 2022)

$$Cl_2 + H_2O \rightarrow HOCl + HCl$$

$$HOCl \leftrightarrow H^+ + OCl^-$$

2.6 Formation of DPBs

DBPs are produced by organic and inorganic materials (humic acids, Fulvic acids), halogenate organic, free bromine, iodine, and nitrogen in raw water with disinfectant as shown in Table 2 (Alexandrou et al., 2018; USEPA et al., 2001). However, raw wastewater, which includes medicines, pesticides, parabens, and other contaminants, produces DBPs more frequently than raw water or surface water (C. Postigo et al., 2015). The DBPs are divided into two categories: nitrogenous disinfection by-products (N-DBPs) and carbonaceous disinfection by-products (C-DBPs) as shown in Table 3 (**Shah & Mitch, 2012**). The amount of chlorine used, reaction duration, pH (THMs like a basic pH and HAAs like a cidic pH), temperature, and the presence of ions in the water are all factors that can affect the creation of byproducts (**Cortés & Marcos, 2018; Huang et al., 2016**). Additionally, C-DBPs depend on dissolved organic carbon (DOC) and total organic carbon (TOC) (Alexandrou et al., 2018). While N-DBPs are dependent on amines, amino acids (Yang et al., 2012) and, total nitrogen (TN) (Watson et al., 2012).

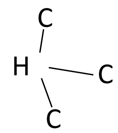
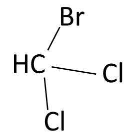
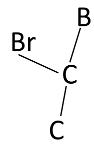
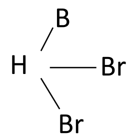
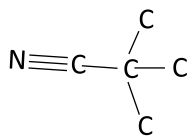
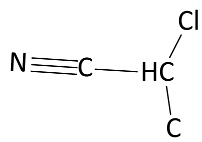
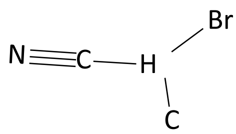
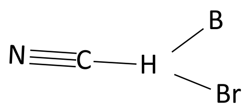
The C-DBPs group is composed of trihalomethanes (THMs) (chloroform, bromodichloromethane, dibromochloromethane, and bromoform) and haloacetic acids (HAAs) (bromoacetic acid, dibromoacetic acid, chloroacetic acid, dichloroacetic acid, trichloroacetic acid) (Richardson et al., 2007). The N-DBPs category also includes haloacetonitriles (dichloroacetonitrile, bromochloroacetonitrile, dibromoacetonitrile, trichloroacetonitrile), haloacetamides and halonitromethanes.

Surface water contains THMs in concentrations ranging from 0.05 to 380 µg/l and HANs in concentrations between 0.5 and 219 µg/l. However, medications, organic chemicals, hormones, insecticides, textile colors, fuels (**Cortés & Marcos, 2018**), personal care product and industrial chemical are combined in wastewater (USEPA et al., 2012) lead to potential risks. As a result, during the formation process, there may be a risk to human health from DBPs.

Table 2 The natural organic matter and implication of DBPs formation (Bond et al., 2012; Korshin & Benjamin, 2000)

Chemical group	C-DBPs	N-DBPs
Humic acids	Primary source	Possibly importance
Carbohydrates	Important or probably	Unimportant
Amino acids	Minor or Important	Significant
Proteins	Important or may be significant	Undetermined
Carboxylic acid	Important	Probably minor

Table 3 Classified chlorination DBPs formations (Hrudey, 2009; Zhang et al., 2012).

Class	Name	Subclass	CAS number	Cancer Characterization	Structure
C-DBPs	Trihalomethanes (THMs)	Chloroform (Trichloromethane)	67-66-3	2B	
		Bromodichloromethane	75-27-4	2B	
		Dibromochloromethane	124-48-1	C [a]	
		Bromoform (Tribromomethane)	75-25-2	C [a]	
N-DBPs	Haloacetonitriles (HANs)	Trichloroacetonitrile	545-06-2	3	
		Dichloroacetonitrile	3018-12-0	3	
		Bromochloroacetonitrile	83463-62-1	3	
		Dibromoacetonitrile	3252-43-5	2B	

2B: Possibly carcinogenic to humans C: possible human carcinogen

[a] : Classified by USEPA Group

2.7 Toxicity and risk of DBPs

Due to several studies and organizations showing chronic exposure from ingestion, inhalation, and skin contact, disinfection byproducts (DBPs) must be concerned about their negative health effects on people (Chowdhury et al., 2009). Since1970, numerous studies have focused on disinfection byproducts (DBPs) found in drinking water, and some may have discovered new DBPs that increase toxicity through causing cytotoxicity and genotoxicity (Shen et al., 2010).

World Health Organization recommended amount of DBPs in water containing trihalomethane (THMs) < 1.0 μg/l (sum of the ratio of the concentration of each) and the following guidelines for a subgroup of THMs; chloroform (CHCl3) 300 μg/l, bromodichloromethane ($CHBrCl_2$) 60 μg/l, dibromochloromethane ($CHBr_2Cl$) 100 μg/l and bromoform (CHBr3) 100 μg/l. In addition to the haloacetonitrile (HANs) rules restriction, the following subgroup; dichloroacetonitrile (DCAN) 20 μg/l and dibromoacetonitrile (DBAN) 70 μg/l (WHO, 2022).

In a study, it was discovered that THMs harm the DNA of primary human lung epithelial cells. In another investigation, it was discovered that human lymphoblastic leukemia cells CCRF-CEM caused DNA strand breaks (Landi et al., 2003). Additionally, HepG2 cells DNA damage was observed to be increased by all THMs group (Zhang et al., 2012). In particular, the IARC classified chloroform and bromodichloromethane as group 2B (possibly carcinogenic to humans), while dibromochloromethane and bromoform are categorized as group 3. (Not classifiable as to its carcinogenicity to humans) (IARC Monographs, 2018).

The chloroform is a volatile material that may be released into the air during showers and other household activities, making inhalation (60–70%) a substantial route of exposure (WHO, 2022). According to Taiwan's exposure model for cancer risk, inhaling chloroform carries a higher risk of developing cancer than the recommended risk value for Class A carcinogens (1.00×10^{-06}) (Wang et al., 2007a). In addition, both animals and people can absorb a large amount of chloroform via their skin while bathing. Other organs like the nervous system, liver, kidneys, lungs, fat, and blood are being exposed to the chloroform by diffusion (WHO, 2004). As a result, bladder cancer in humans (Costet et al., 2011), kidney tumors in rats, and liver tumors in mice are all promoted by the substance's carcinogenicity (Richardson et al., 2007).

Haloacetonitrile (HANs) is more cytotoxic and genotoxic than C-DBPs. The toxicity is governed, in particular, by the restrictions for dichloroacetonitrile (DCAN) and dibromoacetonitrile (DBAN) (Dong et al., 2018). In contrast, there is no toxicological standard for bromochloroacetonitrile (BCAN) and trichloroacetonitrile (TCAN). Although the IARC has not categorized the HANs group as being carcinogenic to humans, numerous studies have shown that dichloroacetonitrile and bromochloroacetonitrile can be mutagenic in bacterial assays. In a different investigation, three HANs (DBAN, DCAN, and TCAN) were discovered to be responsible for DNA strand breakage in human lymphoblastic cells (DANIEL, 1986; E. L. C. Lin et al., 1986). Another study discovered that three HANs (DBAN, DCAN, and TCAN) greatly increased DNA damage (Zhang et al., 2012). Additionally, the HANs group demonstrated in vitro induced chromatid exchange, DNA breakage, and adducts in mammalian cells (WHO, 2022).

DCAN can cause development toxicity in zebrafish embryos, which can result in a considerable reduction in hatchability, an increase in malformation, and mortality when the concentration exceeds 100 μg/l It can also accumulate in adult zebrafish (T. Lin et al., 2016). Similarly, the results of the rat demonstrate that the body and spleen weights were lower, the liver and kidney weight ratios were higher, and there was damage to the liver and kidneys (Dong et al., 2018). Additionally, the DCAN may cause oxidative stress-mediated apoptosis in LO2 cells and activate apoptotic signals via p53 (Luo et al., 2017). Additionally, fetal mouse brain damage from maternal DCAN exposure may include neurodegeneration, oxidative stress, and an imbalance in apoptosis (Esmat et al., 2012). Furthermore, the persistent presence of DCAN in drinking water causes genotoxicity, which damages mammalian DNA (Chowdhury et al., 2009; Richardson et al., 2007).

CHAPTER 3
MATERIALS AND METHODS

3.1 Study sites, Sample collection and analysis

Domestic wastewater sample select two sites are difference method: Chongnonsi (CN) and Bangsue (BS) wastewater treatment plant (WWTP) from the Drainage and Sewerage Department in Bangkok, Thailand. The CN-WWTP were used treatment process by cyclic activated sludge systems (CASS) have area 32,000 m^2, size treatment 200,000 m^3/day. The BS-WWTP were used treatment by activated sludge type step feed and ultra-microfiltration have area 33,120 m^2, size treatment 120,000 m^3/day as shown in Fig. 3. The selected parameters for analysis included total organic carbon (TOC) and dissolved organic carbon (DOC) using a TOC analyzer, chemical oxygen demand (COD) using a COD analyzer, biological oxygen demand (BOD) using a BOD analyzer, total kjeldahl nitrogen (TKN) using a kjeldahl nitrogen analyzer, pH and temperature were recorded using a pH meter.

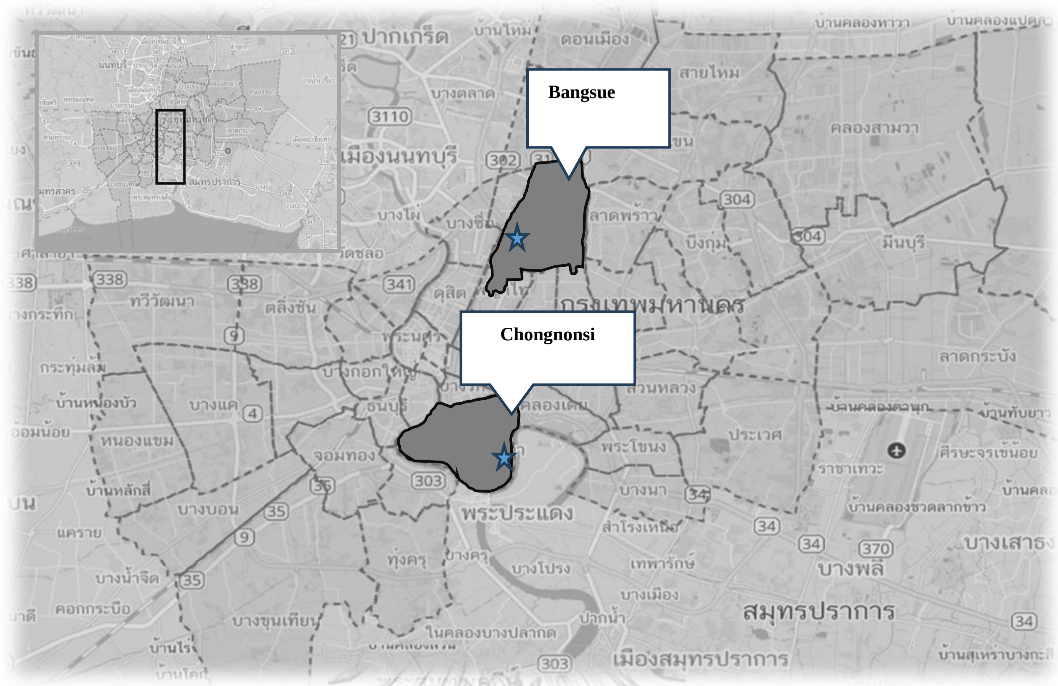


Figure 3 Two effluent wastewater treatment plant select sample in Bangkok, Thailand.

3.2 Virus reduction and bacteriophage assays

The *E.coli* host strain from Escherichia coli strain C-3000 frozen stock. Dissolve the stock 1 ml in Luria-Bertani Broth (LB Broth) 9 ml, incubate at 37 °C, shaker at 130 to 150 rpm, overnight (18 to 24 hours). Add glycerol 50% (glycerol 95%, 63 g and deionized water to the volume of 100 ml) 10 ml, and separate the solution 1 ml in a tube and keep at -20 °C. The *E.coli* C-3000 tube 1 ml from stock freezer add in LB Broth 9 ml and shake in a shaking incubator at 37 °C, shaker at 130 to 150 rpm, 5 to 6 hours.

The bacteriophage MS2 assays used *E.coli* C-3000 frozen stock in a tube of 1 ml and added 9 ml of LB Broth incubate at 37 °C, shaker at 130 to 150 rpm, 5 to 6 hours. Add MS2 1 ml to a tube containing 10 ml of *E.coli* C-3000 and LB Broth, incubate overnight (18 to 24 hours). Filter 0.45 µm. to separate *E.coli* C-3000 from MS2 and put 1 ml in either tube. The agar layer is prepared by using 10 g of LB Broth and 5 g of Bacto agar to fusion, adding calcium chloride 0.4 g (880 µl) and deionized water in 500 ml, and putting it on an autoclave. Add 1 ml of the MS2 sample to the agar layers on each plate, and add 1 ml of the agar solution to each plate. The plates were incubated at 37 °C overnight (18 to 24 hours). The enumerated MS2 coliphages by plaque-forming units PFU/ml. The MS2 must achieve a concentration of $1x10^{11}$ PFU/ml.

3.3 Inactivation by chlorination

The chlorine stock solution used for disinfectant was prepared from sodium hypochlorite solution (10%). The contact time was fixed at 0-second, 30-second, 2-minute, 5-minute, 15-minute, and 30-minute. The initial chlorination concentration was varied to get the microbial inactivation. The free chlorine residual was measured by diethyl-p-phenylene diamine (DPD) method using the HACH DR900. The required chlorine dose achieving microbial inactivation around 3 to 5 log inactivation was used for observing the DBPs formation and associated risk.

3.4 Disinfection by-products (DBPs) analysis

The solution standards of chloroform (TCM), bromodichloromethane (BDCM), and dibromochloromethane (DBCM) standard (purity>99%) were purchased from (Sigma Aldrich, Dorset, UK) bromoform (TBM) standard (purity 99%) was obtained from (Wako, Japan). Methyl tert-butyl ether (MTBE) (99.9%) and sodium sulfate were used for the extraction of DBPs. Sodium hypochlorite (10%) is used as a disinfectant. Sodium thiosulfate was used for quenching DBPs, and other chemicals were of analytical grade.

THMs in the sample were extracted by sodium sulfate 5 g. in tea color glass bottle, adding MTBE 2.5 ml., shaking 2 minutes, and were analyzed by gas chromatography (GC) (7890B, Agilent) with an electron capture detector (ECD) equipped with a fused silica capillary column was using Agilent DB-624 (30 m x 250 µm x 1.4 µm film thickness). Using helium carrier gas at a flow rate of 1 mL/min, the injection was carried out in split mode with 1 mL and a split ratio of 20:1 at 200 °C. The GC oven ran for 15.5 minutes, ramping up to 150 °C for 5 minutes at a rate of 10 degrees Celsius per minute. The initial temperature was 50 degrees. 290 °C was the constant temperature of the detector. This make-up gas was nitrogen at 60 mL/min.

3.5 Assessment of human health risk

The risk assessment is based on oral ingestion, inhalation, and dermal absorptions. The THMs exposure by oral ingestion were assumes contaminated in food or drinking water. For inhalation and dermal absorptions were contaminated during agriculture, industry, household, or other activities. The equations for the calculation of cancer risk exposure are shown below:

$$I\ (oral\ ingestion) = \frac{C_{Orl}\ x\ IR\ x\ FI\ x\ EF\ x\ ED\ x\ CF}{BW\ x\ AT} \quad (1)$$

$$I\ (inhalation\ absorption) = \frac{C_{Inh}\ x\ VR\ x\ AE\ x\ ET\ x\ EF\ x\ ED\ x\ CF}{BW\ x\ AT} \quad (2)$$

$$I\ (dermal\ absorption) = \frac{C_{Der}\ x\ SA\ x\ F\ x\ PC\ x\ ET\ x\ EF\ x\ ED\ x\ CF}{BW\ x\ AT} \quad (3)$$

where: parameters of value for exposure assessment as shown in Table 4.

The inhalation absorption was used two-resistance theory proposed by (Little et al., 1992) the equation was calculating the THMs concentration in shower room estimated by:

$$C_{air} = \frac{(Y_{s(t)} + Y_{s(i)})}{2} \quad (4)$$

$Y_{s(t)}$ is the initial THM concentration in the shower room (assumed as 0 mg/L)

$Y_{s(i)}$ is the THM concentration in the shower room at time t (min)

$$Y_{s(t)} = [1 - \exp(-bt)]\ (\frac{a}{b}) \tag{5}$$

$$b = \left\{\left(\frac{Q_L}{H}\right)[1 - \exp(-N)] + \ Q_G \right\}/V_s \tag{6}$$

$$a = \{Q_L\ x\ C_w\ [1 - \exp(-N)]\}/V_s \tag{7}$$

$$N = (K_{OL}A)/Q_L \tag{8}$$

N is a dimensionless coefficient that was calculated from K_{OL}

where: parameters of inhalation absorption for exposure assessment as shown in Table 4.

$$Cancer\ Risk\ (I) = CDI\ x\ SF \tag{9}$$

where: CDI is THMs exposure (mg/(kg x day); SF is THMs cancer slope factor ((kg x day)/mg)

$$Total\ Risk = (I_{orl}\ x\ SF_{orl}) + (I_{Inh}\ x\ SF_{Inh}) + (I_{Der}\ x\ SF_{Der}) \quad (10)$$

The parameters used for exposure assessment that a person would get cancer as a result of being exposed to THMs were shown in Table 4.

Table 4 Parameter and value for exposure assessment.

Parameter	Unit	Emblem	Value	Reference
Concentration of THMs	µg/L	C	In this study	This study
Exposure duration	year	ED	30	Legay et al., (2011); Mishaqa et al., (2022); USEPA, (1989)
Exposure frequency	day/year	EF	365	L. J.-H. Lee et al., (2002); Wang et al., (2007)
Body weight	kg.	BW	70	Mishaqa et al., (2022); Radwan et al., (2020, 2021)
Averaging Time	day	AT	70 x 365	L. J.-H. Lee et al., 2002; Wang et al., (2007)
Conversion factor	L/cm^3	CF	0.001	
Exposure time	Min/day	ET	35	Pardakhti et al., (2011)
Oral ingestion				
Ingestion rate	L/day	IR	2.0 (average in adult)	Radwan et al., 2020, 2021; US-EPA, (1989)
Oral bioavailability	-	FI	0.1 (for adult)	USEPA, (1989)
Inhalation				
THM concentration in air	mg/L	C	Little's model	Little et al., (1992)
Water Flow Rate	L/min	Q_L	5	Little et al., (1992); Pardakhti et al., (2011)

Air Flow Rate	L/min	Q_G	50	Little et al., (1992); Pardakhti et al., (2011)
Water temperature (T)	℃	T	40	S. C. Lee et al., (2004)
Bathroom volume	M^3	Vs	6	Genisoglu et al., (2019); Kujlu et al., (2020)
Ventilation rate	m^3/h	VR	0.83	Pardakhti et al., (2011)
Dimensionless Henry's	unit less	H	TCM: 0.25	S. C. Lee et al., (2004)
Absorption efficiency	Percent	AE	50%	S. C. Lee et al., (2004); Pardakhti et al., (2011)
Duration	min	t	11	Little et al., (1992)
law const 40 ℃			BDCM: 0.124	Little et al., (1992)
			DBCM: 0.0526	Pardakhti et al., (2011)
			TBM: 0.0501	
Over all mass transfer	L/min	$K_{OL}A$	TCM: 7.4	Little et al., (1992); Pardakhti et al., (2011)
coefficient			BDCM: 5.9	
			DBCM: 4.6	
			TBM: 3.7	
Dermal				
Skin surface area	m^2	SA	1.8	USEPA, (1989)
Fraction of skin in contact	percent	F	90%	S. C. Lee et al., (2004)
Permeability coefficient	cm/h	PC	TCM: 0.00683	Mishaqa et al., (2022)

BDCM: 0.00402

DBCM: 0.00289

TBM: 0.0026

The data for health risk assessment by oral ingestion, inhalation absorptions, and dermal absorptions was calculated by parameter relevant and cancer slope factor from Mishaqa et al., (2022); Pardakhti et al., (2011) were shown in Table 4 and Table 5. The equation for calculating cancer risk of THMs cancer risk was shown below

Table 5 Health risk assessment of toxicological data (Mishaqa et al., 2022; Pardakhti et al., 2011).

Name	Cancer Groups	Cancer Slop factor (SF) [mg/kg/day]$^{-1}$	
		Oral / Dermal	Inhalation
TCM	B1	3.1×10^{-2}	8.05×10^{-5}
BDCM	B2	6.2×10^{-2}	1.30×10^{-1}
DBCM	C	8.4×10^{-2}	9.50×10^{-2}
TBM	B2	7.9×10^{-3}	3.85×10^{-3}

B1: probable human carcinogen with limited human data

B2: probable human carcinogen with sufficient animal data

C: possible human carcinogen

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Characteristics of wastewater sample

The sample from CN-WWTP and BS-WWTP were collected difference three time in the year of 2023. Until analysis, all effluent wastewater samples were maintained at 4 °C. The parameters were analyzed before chlorination and before the analysis of disinfection byproducts (DBPs). The quality parameter of two effluent wastewater treatment plant were shown in Table 6.

Table 6 Characteristics of effluent wastewater from CN-WWTP and BS-WWTP.

Station	Date	TOC (mg/L)	DOC (mg/L)	COD (mg/L)	BOD (mg/L)	TKN (mg/L)	pH	Temperature (°C)
CN-WWTP	July 13, 2023	19.31	17.24	42.00	9.50	2.13	6.00	24.80
	August 10, 2023	24.62	18.49	52.80	14.00	3.05	6.20	23.55
	September 22, 2023	21.85	19.35	31.50	5.00	4.72	5.80	21.90
BS-WWTP	May 25, 2023	23.82	17.95	20.80	2.80	2.83	6.00	24.80
	June 6, 2023	19.69	17.72	20.80	3.00	1.63	6.00	24.80
	September 12, 2023	21.66	19.80	47.50	11.00	4.14	6.00	24.80

Table 7 shows the results of the characteristics of wastewater samples in CN-WWTP and BS-WWTP. The median and standard deviation (SD) values for TOC, DOC, COD, BOD, and TKN between CN-WWTP and BS-WWTP were not significantly different. The results show that the combination of biological treatment and ultrafiltration did not reduce precursors to a level lower than achieved by the biological treatment process alone.

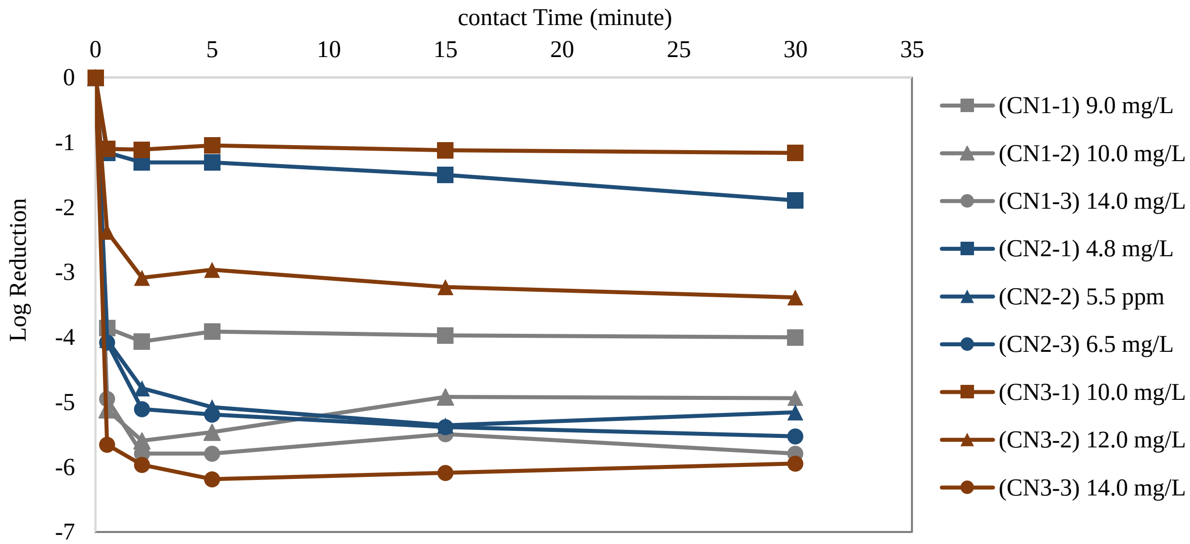
Table 7 The median, standard deviation, and p-value from characteristics of effluent wastewater in CN-WWTP and BS-WWTP.

Station	TOC			DOC			COD			BOD			TKN		
	Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value
CN-WWTP	23.23	2.65	0.27	18.92	1.06	0.91	42.15	10.65	0.68	9.5	4.50	0.71	3.88	1.31	0.57
BS-WWTP	20.67	2.06		18.76	1.14		34.15	15.42		7	4.68		2.88	1.26	

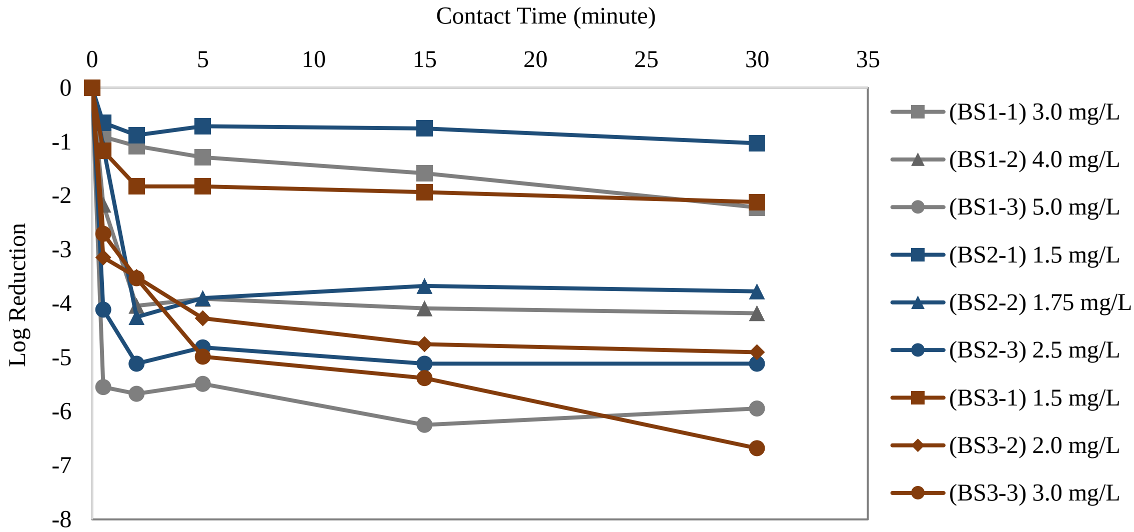
Confidence interval 95%

4.2 Virus inactivation and Chlorine residual level

Effluent wastewater samples from wastewater treatment plants with cyclic activated sludge systems (CASS-WWTP) in CN-WWTP, and activated sludge type step feed and ultra-microfiltration in BS-WWTP. The two effluent wastewaters were treated with varying chlorine concentrations to inactivate bacteriophage MS2, achieving 1-6 log reductions. Fig 4 illustrated the inactivation of bacteriophage MS2 by chlorine in wastewater. The results indicated that the initial chlorine dose around 4.8 to 14 mg/L in CN-WWTP, while BS-WWTP were used around 1.5 to 5.0 mg/L. Moreover, it was found that the initial chlorine concentration plays a crucial role, with increased concentrations leading to higher levels of log reduction. A notable observation from the study is the strong tailing effect seen in the chlorine inactivation of MS2, suggesting that higher initial chlorine concentrations are necessary for more effective microbial inactivation. These findings underscore the significant impact of chlorine concentration on log reduction, corroborating the research conducted by Kingsley et al., (2017).



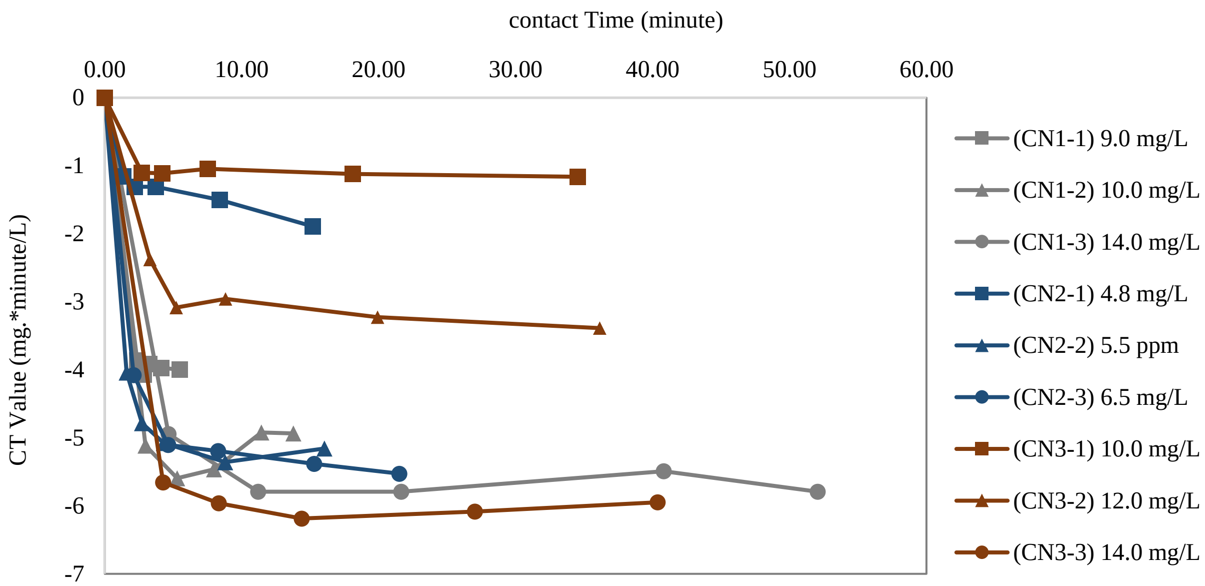
Series CN1 on July 13, 2023; Series CN2 on August 10, 2023; Series CN3 on September 22, 2023



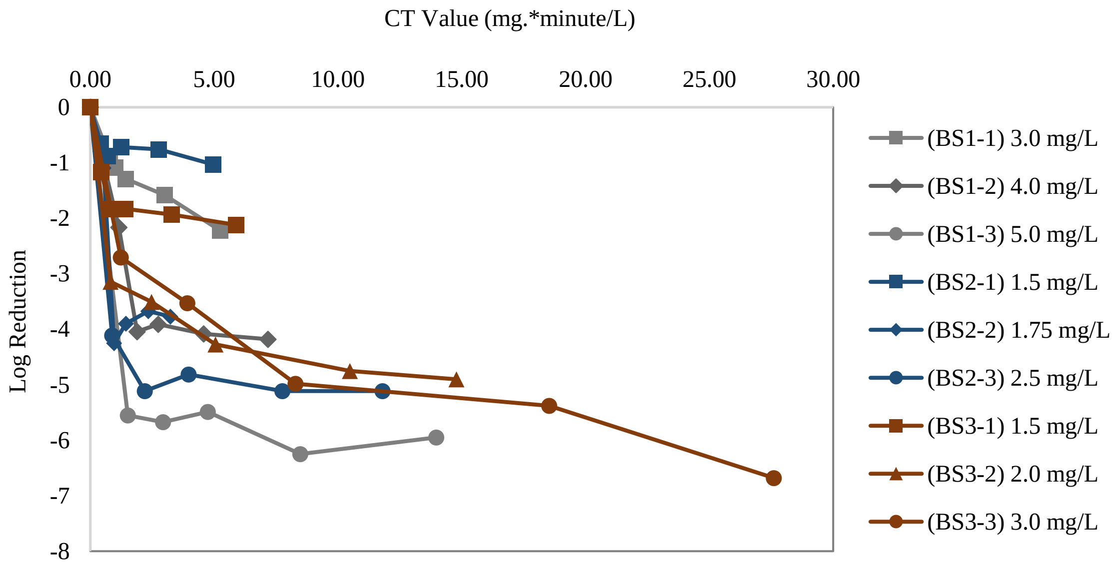
Series BS1 on May 25, 2023; Series BS2 on June 6, 2023; Series BS3 on September 12, 2023

Figure 4 Inactivation of bacteriophage MS2 by chlorination in effluent wastewater at 0-30 minute from BS-WWTP and CN-WWTP The figure presents data from three different dates.

The CT concept (chlorine concentration-time) has been widely utilized to estimate chlorination performance. Fig 5 illustrated the relationship between CT values and MS2 inactivation, varying the initial chlorine concentration. It was observed that MS2 inactivation occurred rapidly in the initial phrase, achieving 1 to 6 log reduction at varying initial chlorine concentration of 4.8 to 14 mg/L in CN-WWTP, while BS-WWTP were used chlorine concentration of 1.5 to 5.0 mg/L. The result demonstrated in CN3 was used chlorine concentration 10.0 to 14.0 mg/L inactivated virus 1-6 log reduction, while used 10.0 mg/L of chlorine at 1 log reduction the CT value 2.73-34.52 mg.*minute/L, used 12.0 mg/L of chlorine at 2-3 log reduction the CT value 3.31-36.15 mg.*minute/L, and used 14.0 mg/L of chlorine at 5-6 log reduction the CT value 4.25-40.37 mg.*minute/L, in the same way with whole chlorine concentration used included in BS-WWTP. Subsequently, the CT value increased reduction of MS2 became relatively constant. These results suggest that contact time had a lesser impact on inactivation compared to chlorine concentration, as evidenced in the CT value data. At the initial phrase of inactivation, there was a rapid increase in log reduction, primarily due to the high dose of chlorine reacting with the virus, followed by a gradual decrease in the initial chlorine reaction rate. These findings were consistent with previous studies of Kanna, (2016); Rashed et al., (2023). Notably, the influence of chlorine concentration on achieving the targeted log reduction level was significantly more pronounced than that of contact time. Therefore, relying solely on the CT (concentration-time) value might not provide a comprehensive estimation of the log reduction. This highlights the need for a more nuanced approach in evaluating the effectiveness of chlorine disinfection in wastewater treatment.



Series CN1 on July 13, 2023; Series CN2 on August 10, 2023; Series CN3 on September 22, 2023



Series BS1 on May 25, 2023; Series BS2 on June 6, 2023; Series BS3 on September 12, 2023

Figure 5 Correlation between log reduction of bacteriophage and CT Value in effluent wastewater at 0-30 minute from BS-WWTP and CN-WWTP The figure presents data from three different dates.

The initial of chlorine was considerable for chlorination process, because used determined chlorine concentration to achieve log reduction target. The water characteristic one of factor was rendered to chlorine concentration initial such as ammonia, TOC, DOC, TKN, nitrite, and pH. Table 6 was presented the characteristics of samples from CN-WWTP and BS-WWTP, parameter was measured TOC, DOC, BOD, COD, TKN, pH, and temperature. The result of water quality parameter was appeared TOC and DOC value was lower in CN1 of 19.31 mg/L and 17.24 mg/L, but used chlorine concentration to 3-5 log reduction higher of 9.0 to 14.0 mg/L. The COD was higher in CN2 of 52.8 mg/L, but used chlorine concentration resembled BS1 to 1-6 virus log reduction with 4.8 to 6.5 mg/L in CN2 and 3.0 to 5.0 mg/L in BS1, which BS1 the COD amount lower of 20.8 mg/L. Furthermore, the BOD value higher in BS3 of 11.0 mg/L used chlorine concentration 1.5 to 3.0 mg/L to achieve 1-5 log reduction which was near lower concentration. These finding were determined the organic matter or water quality value requisite to chorine dose and initial chlorine to achieve log reduction level target. However, the water quality value was not indicated chlorine concentration to used.

The chlorine residuals were referred to the concentration of chlorine that remains in water after the initial chlorination process. Chlorine residuals were critical for maintained disinfection, prevented bacterial regrowth, and guarantee that water arrived consumers remains safe to drink. USEPA was guideline minimum limit on chlorine residual for agriculture reused is 0.05 mg/L in sensitive crop and 1.0 mg/L present no problem to plants (USEPA et al., 2012). (WHO, 2014, 2017) Fig. 6 represent the concentration of free chlorine residuals were remained after initial chlorine in wastewater effluent. The free chlorine residuals average was inactivated bacteriophage achieve 1 to 6 log reduction on 0-30 minute found CN-WWTP was measured range 0.180 to 2.613 mg/L, while BS-WWTP was estimated concentration range 0.147 to 1.211 mg/L. The result in this study was appropriate chlorine concentration of 4.8 to 14 mg/L in CN-WWTP and used chlorine concentration of 1.5 to 5.0 mg/L in BS-WWTP. The free chlorine residual remained after chlorine initial enough to engendered of free residuals level in recommendation of the USEPA.

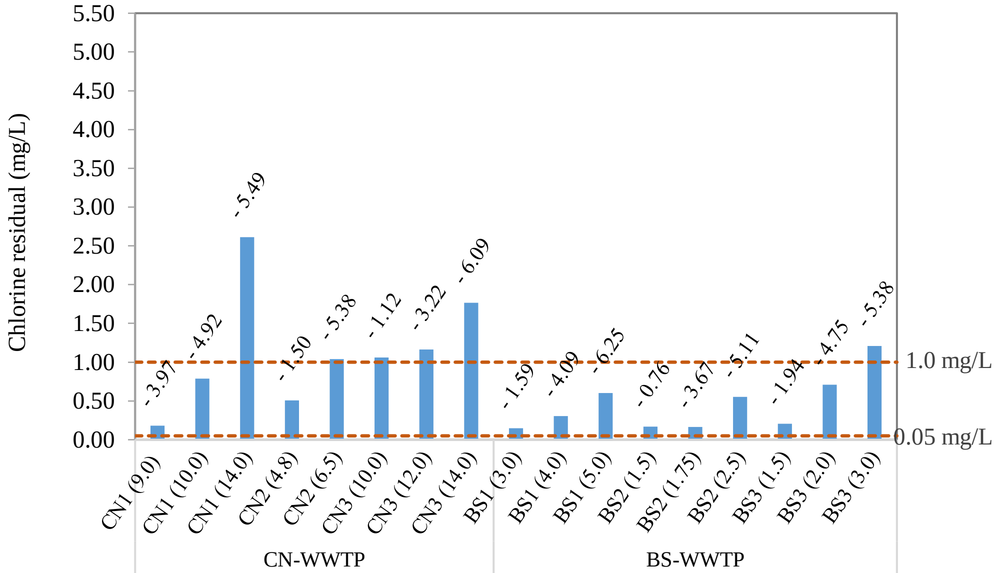


Figure 6 Free chlorine residuals average at 0.5-30 minute at 1-6 log reduction from reacted of chlorine in effluent wastewater from CN-WWTP and BS-WWTP. The figure was shown concentration of free chlorine residuals compare with guidelines of residual chlorine in water reuse of the USEPA.

4.3 THMs formation

Fig.7 showed the relationship between varying the initial chlorine concentration and THMs formation at 15 minutes contact time. Study results in CN1 were used chlorine concentration 9.0 to 14.0 mg/L, the THMs volume increased as follows: TCM of 11.301 to 14.448 µg/L, BDCM of 5.056 to 8.375 µg/L, DBCM of 2.882 to 4.137 µg/L, and TBM of 0.441 to 1.074 µg/L. From the result was imparted of CN-WWTP the volume of THMs increased accordingly with chlorine concentration, in the same way with the result in BS-WWTP. The results demonstrated that an increase in initial chlorine concentration generally correlates with a rise in THMs concentrations across the same sampling date.

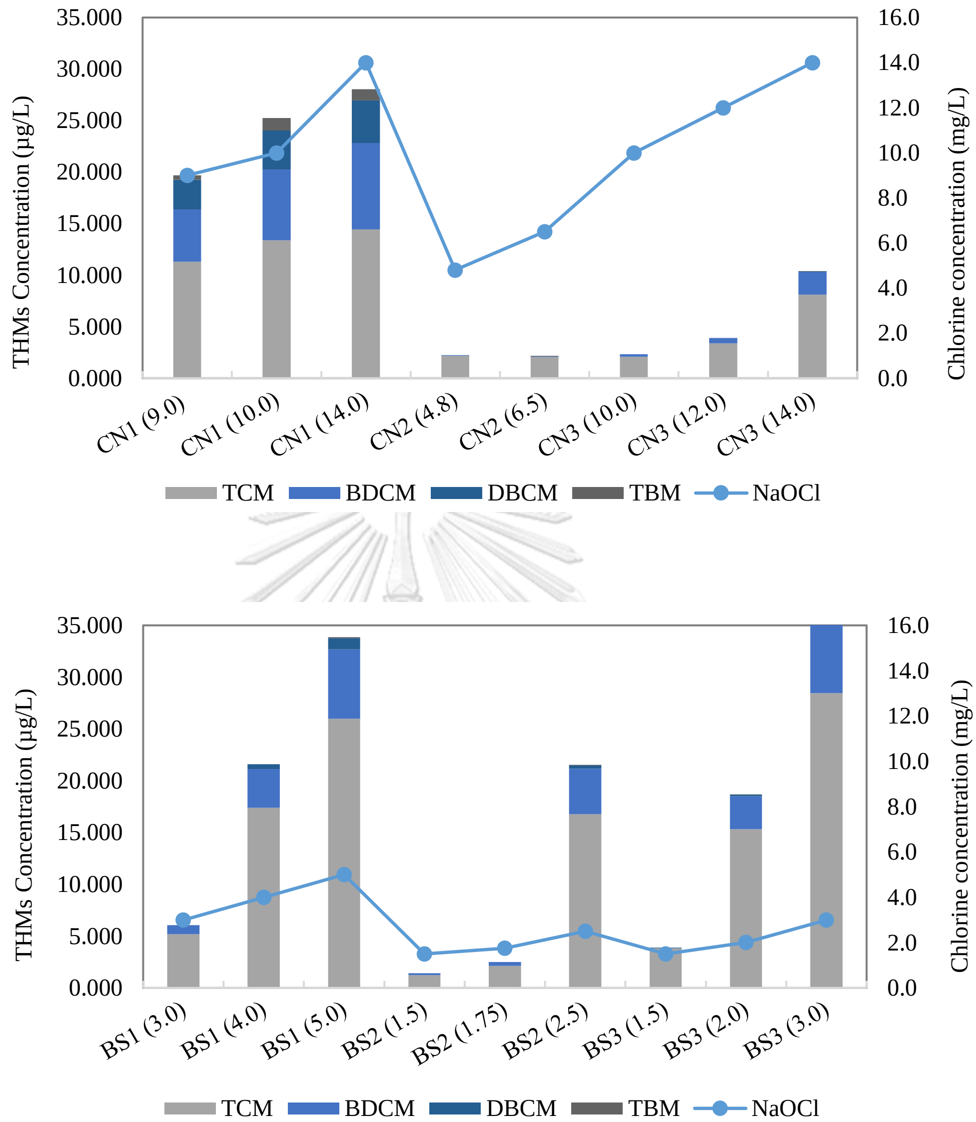


Figure 7 The relationship between concentration of chlorine was used to inactivation bacteriophage virus indicators 1-6 log reduction and THMs occurred in effluent wastewater from CN-WWTP and BS-WWTP

The correlation between chlorine concentration and THMs formation in CN-WWTP and BS-WWTP is significant, particularly in BS-WWTP for TCM and BDCM concentrations. This is attributed to the higher precursor levels in BS-WWTP compared to CN-WWTP. Thus, it indicates that the precursor in BS-WWTP is a determining factor, rather than the chlorine concentration. The results in CN-WWTP showed non-significance, as CN2 demonstrated no correlation, which in turn affected the overall lack of significance in CN-WWTP as shown in Table 8. The results show that biological treatment with ultrafiltration in BS-WWTP is correlated with the occurrence of TCM and BDCM. This correlation is due to the process method's ability to decrease chlorine demand substances, such as ammonia, amines, iron, manganese, and sulfides, leading to precursors that are determining factors in chlorination.

Table 8 A Spearman's correlation coefficient from concentration of chlorine and THMs formation in CN-WWTP and BS-WWTP.

Station	TCM		BDCM		DBCM		TBM	
	rs	p-value	rs	p-value	rs	p-value	rs	p-value
CN-WWTP	0.527	0.180	0.581	0.131	0.269	0.520	0.193	0.647
BS-WWTP	0.772	0.015*	0.785	0.012*	0.645	0.061	0.506	0.165

Confidence interval 95%

The correlation between chlorine concentration and THMs formation in CN-WWTP and BS-WWTP is significant, particularly in BS-WWTP for TCM and BDCM concentrations. This is attributed to the higher precursor levels in BS-WWTP compared to CN-WWTP. Thus, it indicates that the precursor in BS-WWTP is a determining factor, rather than the chlorine concentration. The results in CN-WWTP showed non-significance, as CN2 demonstrated no correlation, which in turn affected the overall lack of significance in CN-WWTP as shown in Table 8. The results show that biological treatment with ultrafiltration in BS-WWTP is correlated with the occurrence of TCM and BDCM. This correlation is due to the process method's ability to decrease chlorine demand substances, such as ammonia, amines, iron, manganese, and sulfides, leading to precursors that are determining factors in chlorination.

Table 9 A Spearman's correlation coefficient from concentration of chlorine and THMs formation in CN-WWTP and BS-WWTP.

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	rs	p-value	rs	p-value	rs	p-value	rs	p-value
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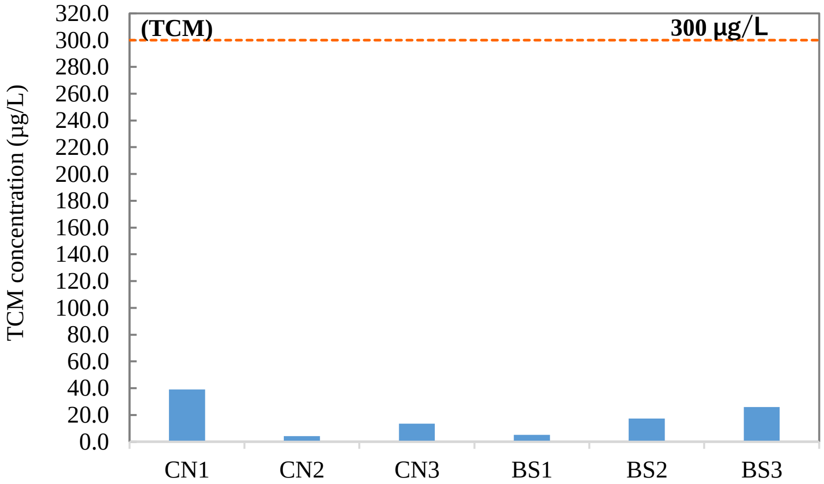
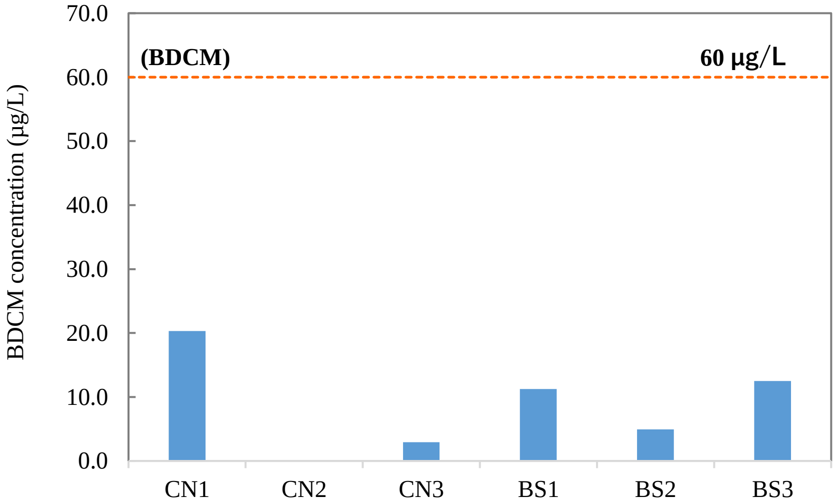
BS-WWTP	0.772	0.015*	0.785	0.012*	0.645	0.061	0.506	0.165

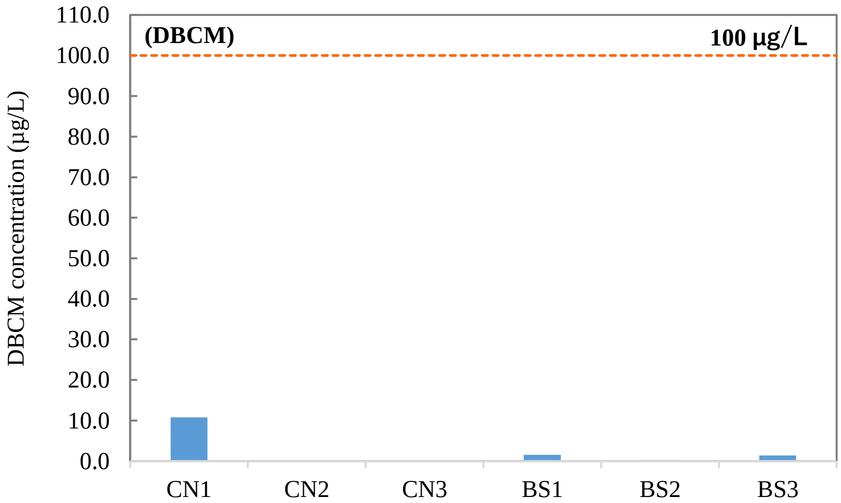
Confidence interval 95%

Interestingly, despite a lower initial chlorine concentration in BS-WWTP sample 1.5 to 5.0 mg/L compared to the CN-WWTP sample 4.8 to 14.0 mg/L, the formation of total THMs in BS-WWTP was higher. Fig. 7 indicated the maximum concentrations of total THMs were demonstrated in BS3 with 38.998 μg/L, followed by BS1 with total THMs of 33.874 μg/L, and the third for CN1 with total THMs of 28.034 μg/L. The BS3 and BS1 was used same methods with activated sludge system and ultra-microfiltration, while CN1 was used cyclic activated sludge systems only. From the results different by sampling date affected to used chlorine dose and THMs formation. Furthermore, treatment process method, presented the various of water quality value. Because of this were influenced to chlorine dose to achieve log reduction target an affected to formation of THMs. This study inferred to organic matter level is a part of were specified the initial chlorine concentrations, same the previous study of Niu et al., (2015).

Disinfection of effluent wastewater were occurred THMs formation from reacted of organic matter and chlorine. The result in Fig. 8 shown maximum of THMs concentration were looked in TCM in BS1 of 48.560 μg/L, the BDCM, DBCM, and TBM in CN1 of 20.311 μg/L, 10.795 μg/L, and 2.719 μg/L, respectively. World Health Organization was guidelines for drinking-water quality, which are TCM regulations of 300 μg/L, BDCM regulations of 60 μg/L, DBCM regulations of 100 μg/L and, TBM regulations of 100 μg/L (WHO, 2022). The concentration of THMs from the reaction of chlorination for inactivation in every dose of chlorine does not exceed the guidelines for drinking-water quality.


(TCM)
300 µg/L
TCM concentration (µg/L)
320.0
300.0
280.0
260.0
240.0
220.0
200.0
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
CN1
CN2
CN3
BS1
BS2
BS3


(BDCM)
60 µg/L
BDCM concentration (µg/L)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
CN1
CN2
CN3
BS1
BS2
BS3


(DBCM)
100 µg/L
DBCM concentration (µg/L)
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
CN1
CN2
CN3
BS1
BS2
BS3

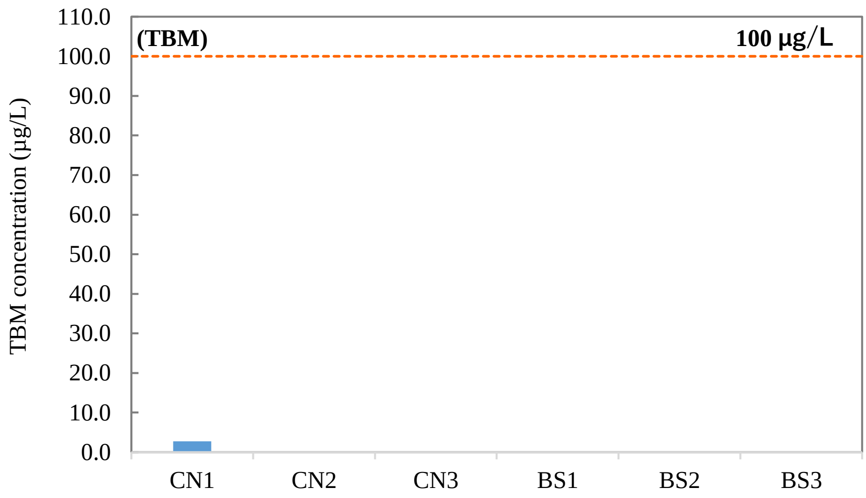


Figure 8 Concentration of THMs (µg/L) in each concentration of chlorine used for inactivation virus 1-6 log reduction compared with guidelines for drinking-water quality (WHO, 2022), *TCM: Chloroform, BDCM: Bromodichloromethane, DBCM: Dibromochloromethane, TBM: Bromoform.*

The percentage maximum of THMs value were presented TCM concentration higher than BDCM, DBCM, and TBM every chlorine initial dose as follow: in CN2 and BS3 with 98.273% and 98.737%, followed by BDCM higher in CN1 with 29.875%, DBCM higher in CN 1 with 14.955%, and TBM higher in CN 1 with 4.772% as shown in Fig. 9. The TCM remarkable was raised to measure in effluent wastewater, after the chlorination process. TCM was the predominant compound among all THMs group, with higher concentrations at every chlorine dose and across all bacteriophage log reductions followed by BDCM, DBCM and TBM. This result was agreed with the findings of Amjad et al., (2013); Pardakhti et al., (2011); Uyak, (2006).

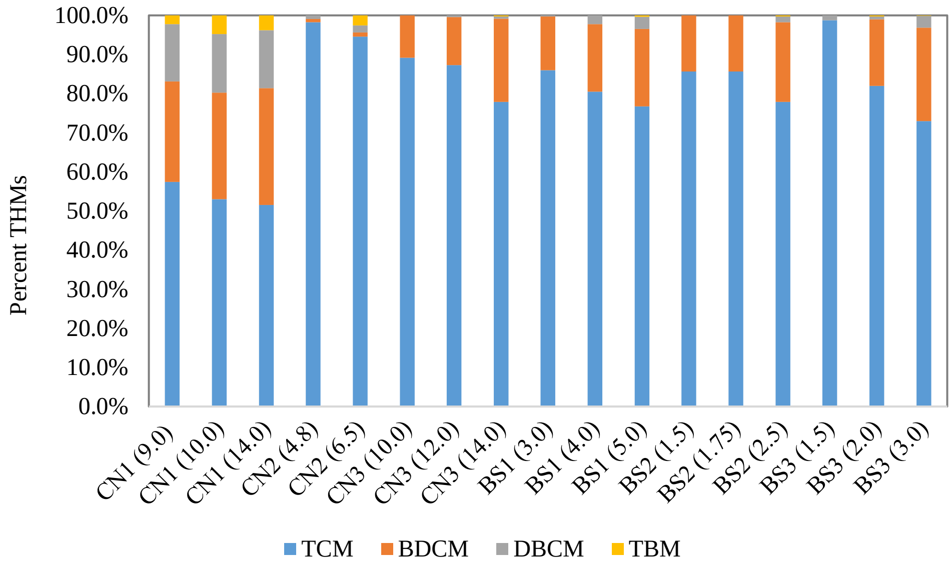


Figure 9 The percentage of THMs formation 15 minutes contact period were achieving 1-6 log reduction of bacteriophage MS2 in effluent wastewater CN-WWTP and BS-WWTP.

4.4 Relationship between inactivation and THMs formation

Chlorination disinfection targeting a 1-6 log reduction of bacteriophage virus indicators shows varying concentrations of THMs. The results show an increase in log reduction level to increase THMs amounts from chlorination disinfection. The concentration of chlorine is also significant for inactivation bacteriophage virus indicators and THMs occurrence in the same method treatment as shown in Fig. 10. The chlorine concentration has an effect on THMs present, same the previous study of Furst et al., (2018). Therefore, the concentration of THMs increased with the rising log reduction levels of bacteriophage virus indicators, potentially elevating the carcinogenic risk to humans even as the risk from microorganisms decreased.

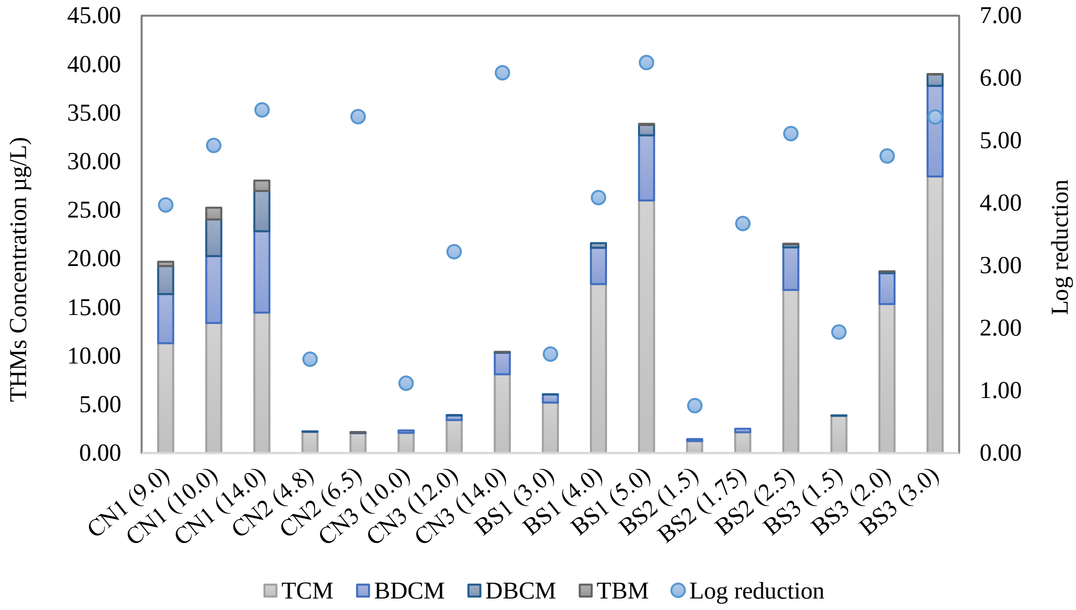


Figure 10 Correlation between period 1-6 log reduction of bacteriophage and concentration of THMs each concentration of chlorine in effluent wastewater from CN-WWTP and BS-WWTP.

The correlation between log reduction and THMs formation in CN-WWTP and BS-WWTP showed that an increase in log reduction led to an increase in THMs occurrence. Specifically, BS-WWTP demonstrated a significant increase in TCM, BDCM, DBCM, and TBM formations according to log reduction levels. The CN-WWTP showed no significant correlation between log reduction and THMs formation. This lack of significance was observed because CN2 demonstrated an increase in log reduction, while THMs formation remained relatively constant, affecting the overall non-significant correlation in CN-WWTP as shown in Table 9. The combination of biological treatment and ultrafiltration in BS-WWTP can decrease chlorine demand substances, leading to lower chlorine concentration to achieve a 1-6 log reduction of the virus. Consequently, chlorine concentration affects the ability of log reduction and THMs occurrence.

Table 10 A Spearman's correlation coefficient from log reduction and THMs formation in CN-WWTP and BS-WWTP.

Station	TCM		BDCM		DBCM		TBM	
	rs	p-value	rs	p-value	rs	p-value	rs	p-value
CN-WWTP	0.461	0.251	0.468	0.242	0.571	0.140	0.654	0.078
BS-WWTP	0.872	0.002*	0.829	0.006*	0.837	0.005*	0.875	0.002*

Confidence interval 95%

4.5 Risk assessment form log reduction level

The results in Table 10 indicated a direct relationship between cancer risk level and log reductions. Crucially, it was observed that higher log reduction levels correspond to increase the risk from THM concentrations. According to our findings, the cancer risk with total of oral ingestion, inhalation absorption, and dermal absorption were seen in BS1 at 6.25 log reduction with TCM occurred 25.989 mg/L demonstrated unacceptable risk with 1.02×10^{-6}, which compare 1.59 and 4.09 log reduction with 5.189 mg/L and 17.382 mg/L of TCM concentrations presented 2.03×10^{-7} and 6.81×10^{-7}, respectively. Including, the result in BS3 were present unacceptable risk at 5.38 log reduction with 28.455 mg/L of TCM concentration appeared cancer risk 1.12×10^{-6}, which contrast at 1.94 and 4.75 log reduction with 3.828 mg/L and 15.329 mg/L shown cancer risk 1.50×10^{-7} and 6.01×10^{-7}, respectively. These finding were founded cancer risk level in BS1 and BS3 shown unacceptable risk is negligible, because log reduction level form in experiment at 1-6 log reduction presented higher than guideline at 3-5 log reduction of the WHO. The summary from result shown THMs concentration increase was leaded to cancer risk increased, this observation was agreement with the established cancer risk levels reported in previous study of Kumari et al., (2015); Mishaqa et al., (2022) whereas, the finding was disagreeing the previous study of Wang et al., (2007).

Table 11 Total cancer risk level (oral ingestions, inhalation absorptions, and dermal absorptions) compare with level of log reduction.

Site	Log reduction	Cancer Risk level			
		TCM risk	BDCM risk	DBCM risk	TBM risk
CN1	3.97	4.43×10^{-7}	4.13×10^{-7}	3.06×10^{-7}	4.35×10^{-9}
	4.92	5.24×10^{-7}	5.62×10^{-7}	4.01×10^{-7}	1.19×10^{-8}
	5.49	5.66×10^{-7}	6.84×10^{-7}	4.40×10^{-7}	1.06×10^{-8}
CN2	1.50	8.55×10^{-8}	1.39×10^{-9}	2.26×10^{-9}	-
	5.38	8.02×10^{-8}	1.87×10^{-9}	4.12×10^{-9}	5.54×10^{-10}
CN3	1.12	8.15×10^{-8}	2.05×10^{-8}	-	-
	3.23	1.33×10^{-7}	3.88×10^{-8}	1.86×10^{-9}	-
	6.09	3.18×10^{-7}	1.81×10^{-7}	6.52×10^{-9}	2.77×10^{-10}
BS1	1.59	2.03×10^{-7}	6.76×10^{-8}	1.78×10^{-9}	-
	4.09	6.81×10^{-7}	3.05×10^{-7}	5.04×10^{-8}	-
	6.25	1.02×10^{-6}*	5.47×10^{-7}	1.13×10^{-7}	1.15×10^{-9}
BS2	0.76	4.81×10^{-8}	1.68×10^{-8}	-	-
	3.67	8.38×10^{-8}	2.93×10^{-8}	-	-
	5.11	6.58×10^{-7}	3.57×10^{-7}	3.36×10^{-8}	6.21×10^{-10}
BS3	1.94	1.50×10^{-7}	-	5.20×10^{-9}	-
	4.75	6.01×10^{-7}	2.60×10^{-7}	1.56×10^{-8}	4.49×10^{-10}
	5.38	1.12×10^{-6}*	7.61×10^{-7}	1.24×10^{-7}	5.22×10^{-10}

The total chemical score of THMs was exposure in one sampling date were through by oral ingestions, inhalation absorptions, and dermal absorptions from effluent wastewater in CN-WWTP and BS-WWTP were analysed. The result was found oral ingestion in two effluent wastewater treatment plant (CN-WWTP, BS-WWTP) exceeded the USEPA limit (1 x 10^{-6}) (USEPA, 1999, 2005)with cancer risk level in CN1 of 1.11 to 1.62 x 10^{-6}, and BS1 of 1.61 x 10^{-6}, BS2 of 1.00 x 10^{-6}, and BS3 1.91 x 10^{-6}. The cancer risk was through inhalation absorption and dermal absorption demonstrated acceptable risk of USEPA in two effluent wastewater treatment plant as shown in Fig. 11.

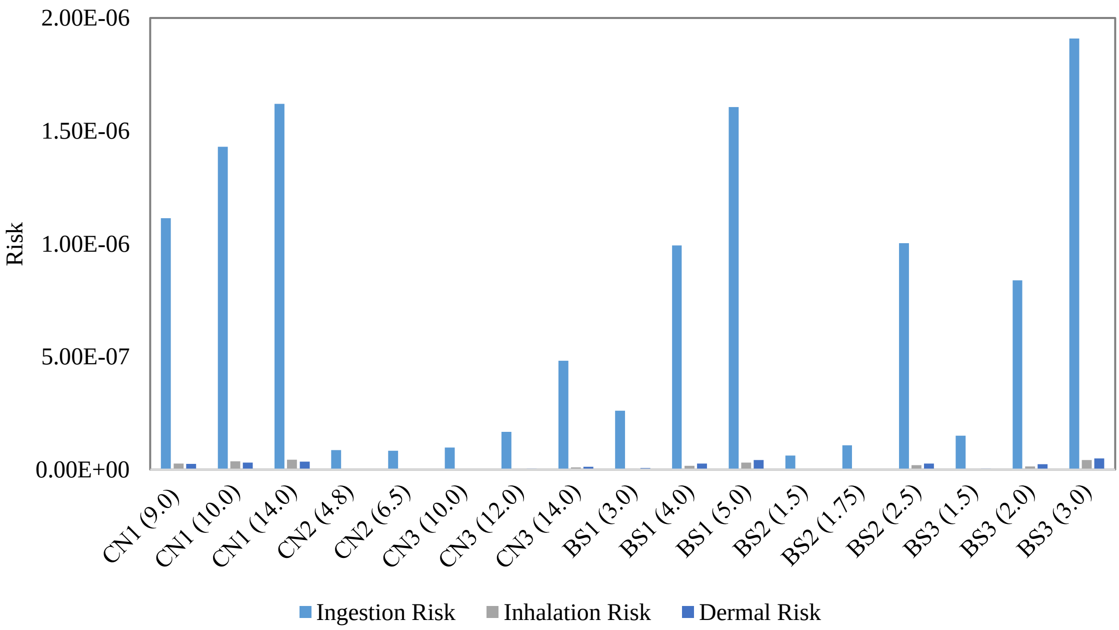


Figure 11 The cancer risk level from through of cancer risk from THMs volume were achieving 1-6 log reduction of bacteriophage MS2 from effluent wastewater in CN-WWTP and BS-WWTP.

The result in Fig.12 shown TCM cancer risk the prominent point in BS3 96.65% demonstrated the cancer risk level unacceptable of the USEPA. The observed of TCM relevant increasing cancer risk level according to the previous study of Kumari et al., (2015).

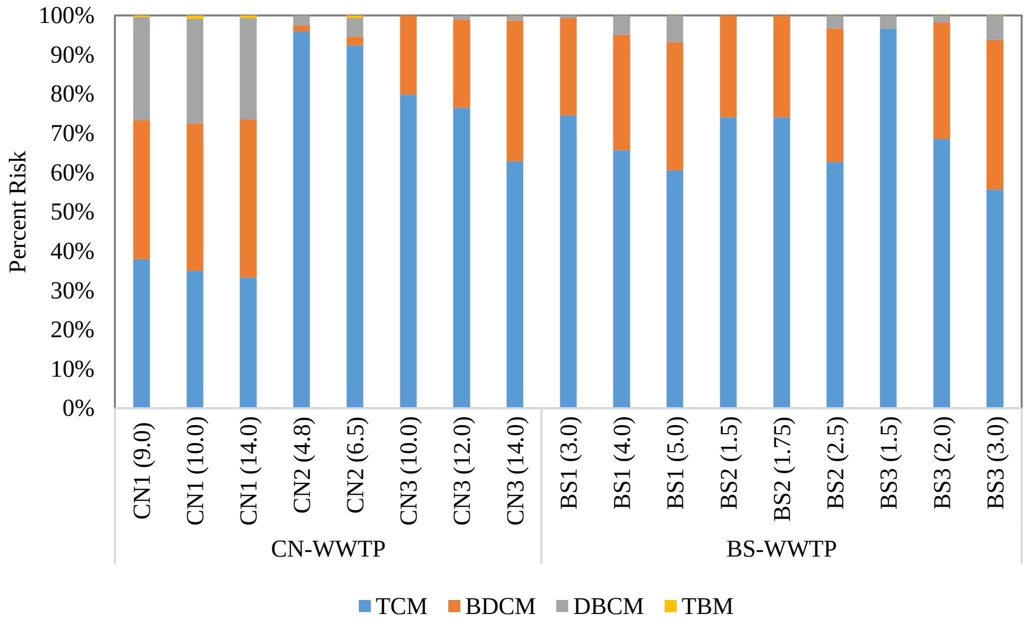
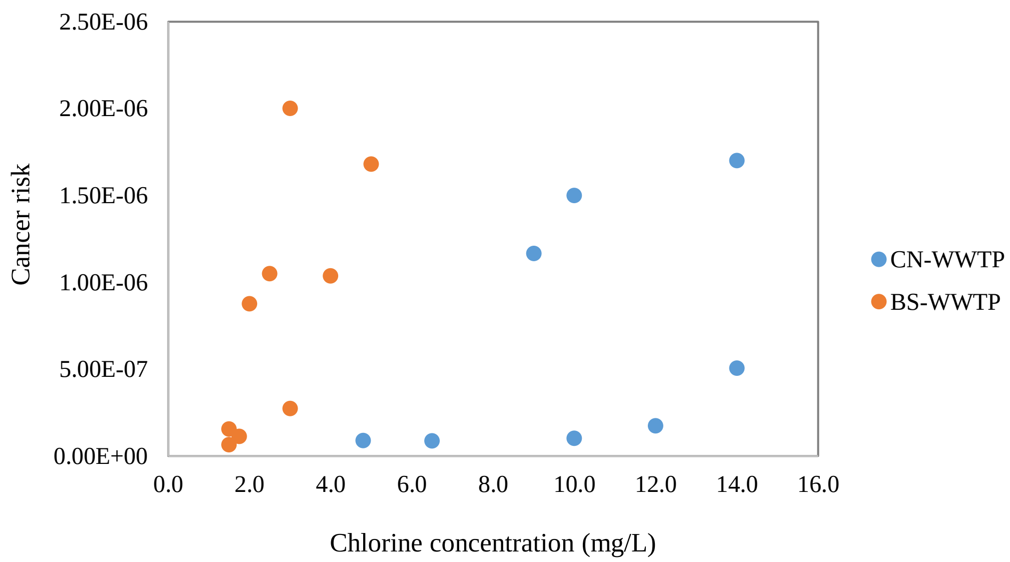


Figure 12 The percentage total cancer risk level of TCM, BDCM, DBCM, and TBM for activated virus 1-6 log reduction at 15 minutes of effluent wastewater in CN-WWTP and BS-WWTP.

The CN-WWTP were used treatment process by cyclic activated sludge systems (CASS), and BS-WWTP were used treatment by activated sludge type step feed and ultra-microfiltration. The difference between two treatment plant were used ultra- microfiltration method in BS-WWTP, because of this were referred to variant of organic matter, chlorine concentration to achieve 1-6 log reduction, THMs concentration, and cancer risk level. Fig. 13 illustrated in BS the total chemical score cancer risk level higher than CN with 2.00×10^{-6} and in CN shown 1.70×10^{-6}. The finding was determined ultra- microfiltration method needless was used to decreased cancer risk from disinfection process by chlorine disinfectant to 1-6 log reduction.

Fig. 13 illustrates the correlation, where an increase in chlorine concentration corresponds to an increase in cancer risk. Similarly, an increase in log reduction is associated with an increased cancer risk. This relationship is attributed to higher chlorine concentrations being associated with increased log reduction and THMs accumulation.



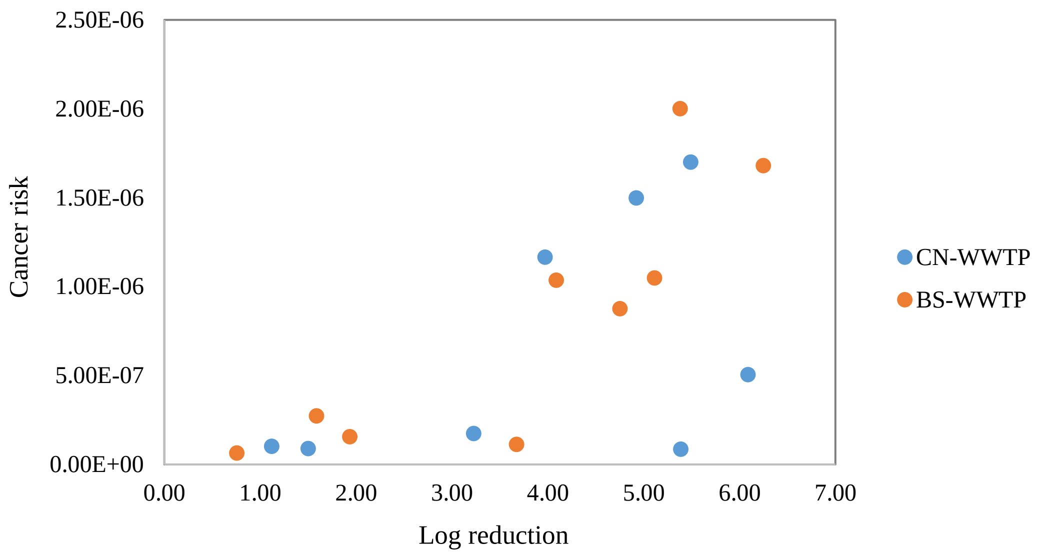


Figure 13 Compare the total cancer risk level with chlorine concentrations, and log reduction in CN-WWTP and BS-WWTP for inactivated virus 1-6 log reduction at 15 minutes.

The correlation results between cancer risk and log reduction, and cancer risk and chlorine concentration in CN-WWTP and BS-WWTP revealed a positive relationship. Specifically, in BS-WWTP, the correlation between cancer risk and log reduction showed a strong and significant association. Additionally, there was a significant positive relationship between cancer risk and chlorine concentration. In CN-WWTP, a positive relationship was observed but was not deemed significant. This lack of significance in CN-WWTP is attributed to CN2 not showing correlation between cancer risk and log reduction, and the correlation between cancer risk and chlorine concentration in CN-WWTP was not significant, as indicated in Table 11. The results show that biological treatment with ultrafiltration in BS-WWTP could decrease chlorine demand substances more than the biological treatment process alone in CN-WWTP. Therefore, the chlorine concentration in BS-WWTP leads to the log reduction of the virus and THMs occurrence, affecting the correlation with cancer risk levels.

Table 12 A Spearman's correlation coefficient from cancer risk with log reduction, and cancer risk with chlorine concentration in CN-WWTP and BS-WWTP.

Station	Cancer risk & log reduction		Cancer risk & chlorine concentration	
	rs	p-value	rs	p-value
CN-WWTP	0.447	0.267	0.564	0.146
BS-WWTP	0.901	0.001*	0.781	0.013*

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The chlorine concentration was crucial more than contact time and CT value in chlorination process to achieve at 1-6 log reduction of bacteriophage in secondary treated wastewater.

From this study, the disinfection targets at 1-6 log reduction of bacteriophage MS2 virus indicators were agreed to the guidelines drinking-water quality of the WHO, in the same way free residual chlorine concentrations accorded to recommendation water agriculture reuse of the WHO. The organic matter content from this study no had indicated the formation level of THMs in secondary treated wastewater.

TCM concentrations were consistently higher follow by BDCM, DBCM, and TBM concentrations across different chlorine concentrations and levels of log reduction. The dominance of TCM higher may be influenced by the specific conditions of the chlorination process and the composition of organic matter in the treated water.

The THMs concentration was increased with log reduction level increased. However, the amount of THMs occurred in two effluent wastewaters agreeable to the guideline of the WHO. The cancer risk level of THMs formation were assessed by through oral ingestions, inhalation absorptions, and dermal absorptions exposure. Cancer risk of TCM in 5-6 log reduction shown unacceptable limit of the USEPA also the cancer risk through oral ingestions were demonstrated exceed guideline of the USEPA. In contrast, cancer risk from BDCM, DBCM, and TBM were acceptable guideline of the USEPA also the inhalation absorptions, and dermal absorptions risk according to limit of the USEPA. Compare cancer risk from treatment wastewater process were founded unnecessary was used ultra-microfiltration in treatment process into decreased risk to acceptable level for achieve 1-6 log reduction of the WHO.

5.2 Recommendations

5.2.1 The next experiment should involve using another wastewater treatment process to compare THMs formation and explore optional methods for removing precursors, which are factors in THMs occurrence.

5.2.2 Study the types of precursors that indicate the level of disinfection byproducts (DBPs) formation in the chlorination process, such as hydrophobic and hydrophilic precursors.

5.2.3 The level of log reduction can change based on the goal of reusing wastewater, and the microbial indicators used can be adjusted accordingly.

5.2.4 The next study will involve experimenting with other DBPs, such as N-DBPs and HAAs.

5.2.5 Data from this study can serve as a guide to consider treatment methods, levels of microbiological control, and DBP risk when determining applications of water reuse for sustainable water management.

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