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RESEARCH ARTICLE

Assessment of Coliform, *Escherichia coli*, and Enterococci in Raw and Treated Water in Negeri Sembilan, and Factors Associated with the Decontamination Procedure

Norfadzilah Azmee [a,c], Noor Azira Abdul-Mutalib[b,d*]

[a] Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; [b] Department of Food Service and Management, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; [c] Water Quality Unit, Syarikat Air Negeri Sembilan, Sg. Linggi Water Treatment Plant, Jalan Kuala Sawah, 71200, Rantau, Negeri Sembilan, Malaysia; [d] Laboratory of Food Safety and Food Integrity, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

***For correspondence:**
n_azira@upm.edu.my

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Abstract World Health Organization estimated that half of the world's population will encounter water-stressed living areas by the year 2025 due to climate change, population growth, and urbanization which already pose challenges for water supply systems. In Malaysia, water operators for each state are responsible to treat water and supplying it all over the state. This study focused on Negeri Sembilan state which has 21 water treatment plants that are responsible for supplying treated water to consumers. The objectives of this study were to assess microbial contaminants in raw and treated water using a rapid detection method (IDEXX Colilert method for coliform and *E. coli* detection, and IDEXX Enterolert-DW for enterococci detection in drinking water) and to quantify the number of microorganism with most probable number (MPN) method. A total of 96 samples were collected from both raw (n=17) and treated water (n=79) in Negeri Sembilan. Results have shown that the raw water sample in Seremban recorded the highest number of coliform (7258.8 MPN/100 ml), *E. coli* (5198.7 MPN/100 ml), and enterococci (997.5 MPN/100 ml). For treated water, all samples were recorded at <1 MPN/100 ml. This showed that the decontamination procedure especially chlorination done by the water treatment plant did reduce the number of microbes in drinking water. Further analysis showed that the level of ammonia did not influence the number of microorganisms. However, the presence of chlorine did reduce the number of microbes, whereas and high level of turbidity showed a high number of microbes. In conclusion, despite the high number of microbes in raw water samples, treatment using chlorine (0.2mg/L to 5.0mg/L) was effective in reducing their numbers (<1 MPN/100 ml) to provide clean water to the consumers.

Keywords: Coliform, Colilert, disinfection, drinking water, enterococci, urbanization.

Introduction

By the year 2020, the domestic and industrial sector is estimated to be the main water user in the country [1]. In Malaysia, water operators for each state are responsible to treat water and supply clean water to the consumers. This raw water is mostly derived from rivers and dams. In Negeri Sembilan, Syarikat Air Negeri Sembilan (SAINS) is responsible to supply treated water to all seven districts. In this state, four out of seven districts, comprising Seremban, Port Dickson, Rembau, and Tampin are classified as highly developed areas or rapidly increasing in the industrial sectors.

Generally, developed areas produce more pollutants and could affect aquatic resources, mostly related to discharges of water treatment plants, decontamination stations, hospitals, and industries. Furthermore, the correlation between pathogenic microbial concentrations and urban activities is well documented [2]. According to National Drinking Water Quality Surveillance (NDWQS), there are up to 40 different parameters in raw water and 131 different parameters in treated water to be tested, including taste, color, odor, microorganisms, chemical content, and pesticides.

World Health Organization (WHO) [3] has reported that contaminated water can transmit a lot of diseases such as diarrhea, cholera, dysentery, polio, and typhoid and it is estimated to cause 485,000 deaths each year. The cause of the outbreak comes from unsafe drinking water where the source of water was heavily contaminated with bacteria, parasites, or viruses. There are 368 million people who get their water from unprotected wells and springs, and 122 million people who get their water from lakes, ponds, rivers, and streams. Sharp geographic, sociocultural, and economic disparities persist, not only between rural and urban areas, but also within towns and cities, where people who live in low-income, informal, or illegal communities typically have less access to better drinking water sources than most other inhabitants [4]. Pathogenic bacteria were detected more frequently when the river water was associated with heavy rainfalls and with a sewage emergency discharge. This sudden elevated in pathogen concentrations may increase the risks of waterborne diseases. The evaluation of indicator organisms and pathogens at the raw water intake is a good method to reduce the risk of drinking water consumption [5]. This will ensure only clean water is supplied to the consumer.

Urbanization or the growth of the population, as well as the development of industries and new housing areas, may increase the level of organic matter in the river system. Therefore, the study of surface water pollution in the river is crucial. Based on the water quality assessment of Bertam River and its tributaries in Cameron Highlands, Malaysia by Eisakhani and Malakahmad [6], most of the causes of the decline in water quality are agricultural activities, feces that contain *E. coli,* and urban areas with poorly treated or untreated sewage poured in the river.

Disinfection treatment is critical for ensuring microbiological quality. Pathogenic bacteria in water can be eliminated with disinfectants, making the water safe for consumption. The use of multiple barriers, from catchment to consumer, to avoid or reduce microbial contamination of drinking water sources is based on the use of multiple barriers to prevent or reduce microbial contamination to levels that are not harmful to health. Chlorine, in the form of gaseous chlorine, chloramines, and sodium hypochlorite, is the most extensively used disinfectant for inactivating waterborne microorganisms in water treatment [7]. Pre-chlorination is employed before coagulation, sedimentation, or filtration to minimize pathogen levels; prevent biofilm or algal development; and oxidize any inorganic compounds such as iron and manganese before the main treatment steps. For the same reasons, intermediate chlorination is utilized after coagulation-sedimentation but before quick sand filtering. The disinfection of finished water as it reaches the distribution system is always done using final or post-chlorination [8]. Mostly post-chlorination is adapted in the water treatment system in Negeri Sembilan and a few locations had secondary dosing points to supply more chlorine for a farther route.

The objectives of this study were to assess the microbial status in different stages of water supply starting from the catchment area to the consumer; to study the differences between the numbers of microorganisms detected in drinking water supply and to relate it with microbial density and turbidity, free chlorine, and ammonia; and finally to determine the reliability of preventive measure taken towards microbial status in the targeted area.

Materials and Methods

Sampling Area

The study location involved all districts in Negeri Sembilan which consist of 96 sampling points and 4 types of water which were raw water, treatment plant outlet water, service reservoir outlet water, and distribution system water. Raw water was classified as untreated water while treatment plant outlet water, service reservoir outlet water, and distribution system water were classified as treated water. Treatment plant outlet water is the water produced after being chemically treated by the water operator and stored in the clean water tank before being distributed to people. Service reservoir outlet water is the water stored in a large balancing tank mostly located near to resident area before been pumping out to the resident area tank. Service reservoir outlet water could be stagnant for a long time due to fewer occupant number in the area. Distribution system water is water running through the underground pipeline and supply to resident house and factory**.** A minimum of 100 ml samples were collected at the specially designed sampling box provided by Syarikat Air Negeri Sembilan using the grab sampling method. Microbiological and chemical samples were immediately transported to the Water Quality Unit Laboratory in Rantau, Negeri Sembilan for analysis.

Sampling procedure, preservation, and transportation

Sampling procedure for onsite testing and chemical testing

Water was flushed out for around two to three minutes to ensure a fresh sample was taken. Samples were taken by using high-density polyethylene (HDPE) 2 L bottle and then were separated into two bottles for ammonia testing (5 drops of sulphuric acid were added to 100 ml of water samples) and on-site testing (addition of acid as samples preservation under or at pH 2).

Microbiological sampling procedure

The pipe was sterilized by the flaming procedure and sterilized containers with a dechlorination agent (sodium thiosulphate) were used for sample collection. Collected samples were brought to the laboratory at a controlled temperature (below 6°C for chemical analysis and 2 to 8°C for microbiology analysis).

In-situ Analysis

Free chlorine analysis

For free chlorine analysis, HACH Method 10069 or DPD calorimetric method (high range) was used (Standard Method for the Examination of Water and Wastewater 23rd Edition, 2017). Chlorine in the form of hypochlorous acid and hypochlorite ions (free chlorine or free available chlorine) reacts quickly with the DPD (N,N-diethyl-p-phenylenediamine) indicator to produce a magenta color that was equivalent to the chlorine concentration in the sample. The measurement wavelength is 520 nm for colorimeters. For measurement, blank reading was taken first which is the sample itself. Then, a content of one DPD Free Chlorine Powder Pillow for 25 ml samples was put into the sample. The sample cell was inverted for about 20 seconds to dissolve the reagent and the appearance of pink color indicated the presence of chlorine in the sample. The sample was read using a colorimetr and the result was recorded.

Turbidity analysis

American Public Health Association (APHA) 2130 B Nephelometric Method was used for turbidity analysis. The intensity of light scattered by the sample under specific conditions was compared to the intensity of light scattered by a standard reference suspension under the same conditions in this approach. The turbidity increases as the intensity of dispersed light increases. The sample was directly read using a turbidimeter. The sample was shaken before being poured into the sample cell to ensure homogeneity.

Chemical analysis

Ammonia analysis

Nitrogen ammonia analysis was done by using HACH Method 8155, Salicylate Method. Monochloramine is formed when ammonia molecules react with chlorine and formed 5-aminosalicylate when interacts with salicylate. The oxidation process produced a blue-colored molecule and a yellow hue from the excess reagent masks the blue color, resulting in a green-colored solution. The wavelength of the spectrophotometer and the colorimeter used were 655 nm, and 610 nm, respectively. The procedure of analysis was started by preparing a blank sample together with a tested sample. A total of 10 ml of deionized water was pipetted into the sample cell and 10 ml of the tested sample was pipetted into another sample cell. A sachet of Ammonia Salicylate powder pillow was added to each sample cell and was shaken to dissolve the reagent. The solution was then left for 3 minutes for reaction time. After that, a sachet of Ammonia Cyanurate powder pillow was added to each sample cell. The sample was then shaken again and left for another 15 minutes to allow the reaction to occur in the solution. After 15 minutes, the optical density reading of the samples was taken.

Microbiological analysis

Most probable number (MPN) for total coliform and E. coli

For total coliform and *E. coli* analysis, this study used the Idexx Colilert (IDEXX, USA) method which is also known as method 9223 B. Enzyme Substrate Test in Standard Method for the Examination of Water and Wastewater 23rd Edition. Colilert detects total coliforms and *E. coli* in water at the same time. The sample turns yellow when total coliforms metabolize Colilert's nutrient-indicator, ortho-Nitrophenyl-ß-galactoside (ONPG) and the sample fluoresces when *E. coli* metabolizes Colilert's nutrient-indicator, 4-methylumbelliferyl-β-D-glucuronide (MUG). The total coliform and *E. coli* detection were performed for raw treated water.

For raw water, a standardized 1:3 dilution procedure was applied where 25 ml of sample is diluted up to 100 ml with distilled water. Then, separately, 100 ml of raw and treated water sample was poured into a sterile and clear vessel container. One pack of colilert snap pack reagent was then added to the sample. The vessel container was closed tightly and shaken to dissolve the powder. Fifty-one wells of quanti-tray (gives up to 200 MPN) and Quanti-Tray/2000 (gives up to 2419 MPN) were used for treated and raw water, respectively. All procedure was done inside the laminar flow and strictly followed the aseptic technique. The sample then was incubated at 35±0.5°C for 24 h. After 24 h, the presence or absence of total coliforms was observed. Color changes from clear to yellowish were an indication of positive results. The well then was counted and MPN for treated water was obtained by referring to 51-Well Quanti-Tray MPN Table (https://www.idexx.com/files/qt51mpntable.pdf). Whereas, for raw water, Quanti-Tray/2000 MPN Table (https://www.idexx.com/files/qt97mpntable.pdf) was referred to. As this reagent detects total coliform and *E. coli* simultaneously, the *E. coli* result was also interpreted by referring to the tables. The presence of *E. coli* was determined by observing the sample under ultraviolet light (356 nm) and the presence of *E. coli* was indicated positive when blue fluorescence was emitted. As the raw water sample was diluted, the MPN obtained was multiplied by 3 to get the exact value of MPN of total coliform and *E. coli.*

Most probable number (MPN) for enterococci analysis

For enterococci analysis, the IDEXX Enterolert method was used which is also known as method 9230 D. Fluorogenic Substrate *Enterococcus* Test in Standard Method for the Examination of Water and Wastewater 23rd Edition. Instead of esculin, 4-methylumbelliferyl ß -D-glucoside (4-MUG) (a fluorogenic substrate) was applied in the enterococci protocol. When excited by long-wavelength (365 to 366nm) UV light, the ß -D-glucosidase enzyme hydrolyzes the substrate, resulting in a fluorescent signal. Non-enterococcus bacteria that make ß -D-glucosidase were inhibited, including certain *Serratia*, *Klebsiella*, and *Aerococcus* species. The method for raw and treated water was slightly different in terms of the type of quanti-tray used.

For the determination of the most probable number (MPN) of enterococci, the above procedure was repeated. However, IDEXX Enterolert was used instead of IDEXX Colilert, and the incubation was set to 41±0.5°C for 24 h.

Data analysis

Data collected were plotted using a bar graph representing the MPN of coliforms, *E. coli,* and enterococci. The value of free chlorine, turbidity, and ammonia was compared with the number of microorganisms.

Results and Discussion

Assessment of coliform, E. coli, and enterococci

In this study, a total of 96 samples were collected including both raw and treated types of water. Assessment of coliform, *E. coli,* and enterococci was done by rapid and simple detection method where IDEXX Colilert reagent was used for coliform and *E. coli,* whereas IDEXX Enterolert reagent was used for enterococci. Quanti-tray/2000 and Quanti-tray with 51 wells were used to estimate the MPN of those bacteria in raw and treated water, respectively. Figures 1, 2 and 3 show the number of coliform, *E. coli,* and enterococci by the districts where the samples were collected. The figures showed that all 17 raw water samples were contaminated with coliform, *E. coli,* and enterococci.

The highest numbers of coliform were obtained from the Intake Sungai Linggi (River) and Intake Ngoi-ngoi (Dam) samples which recorded 7258.8 MPN/100 ml. For *E. coli* analysis, the highest numbers were recorded in Intake Sungai Linggi (River) sample (5198.7 MPN/100 ml) while for enterococci the highest numbers recorded were Intake Ngoi-ngoi (Dam) sample (997.5 MPN/100 ml). Both of the samples from Intake Sungai Linggi (River) and Intake Ngoi-ngoi (Dam) were located in the Seremban area. The density of coliform and *E. coli* are different between September and October due to factors mostly related to weather conditions. Heavy rainfall in October will lead to more runoff from river banks and increasing discharge volume from farms and sewage. This will cause an increasing number of coliform and *E. coli* in raw water. This finding was proved in a study at Semenyih River, where the *E. coli* population ranged from 346±100.3 CFU/100 ml to 160500±11492.5 CFU/100 ml. The overall coliform population, on the other hand, ranged from 1293±649.4 CFU/100 ml to 219250±62245.3 CFU/100 ml.

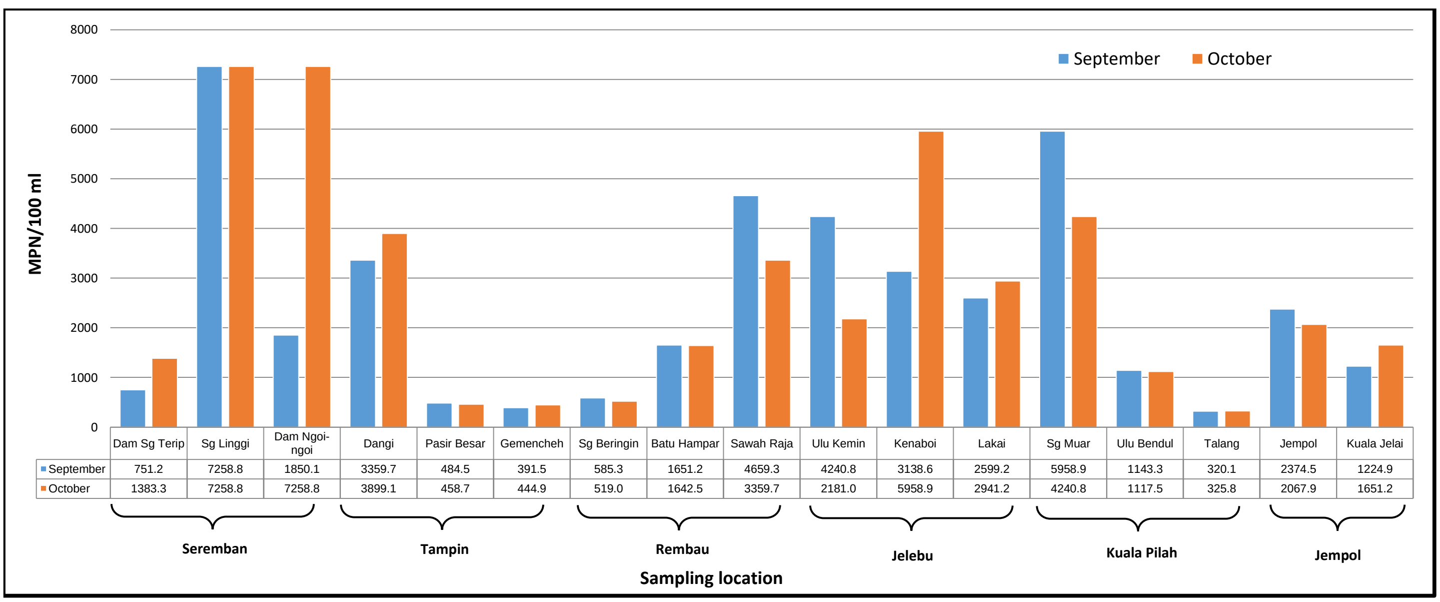

Figure 1. The density of coliform in raw water in Negeri Sembilan.

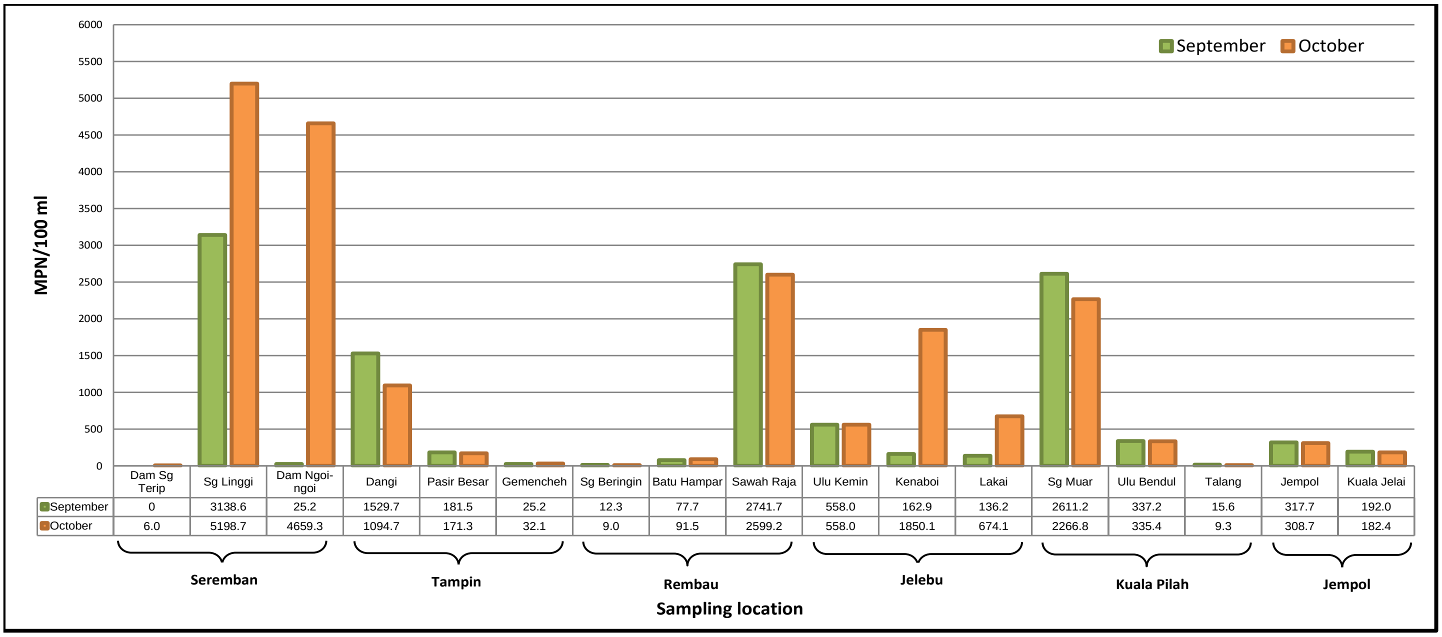

Figure 2. The density of *E. coli* in raw water in Negeri Sembilan.

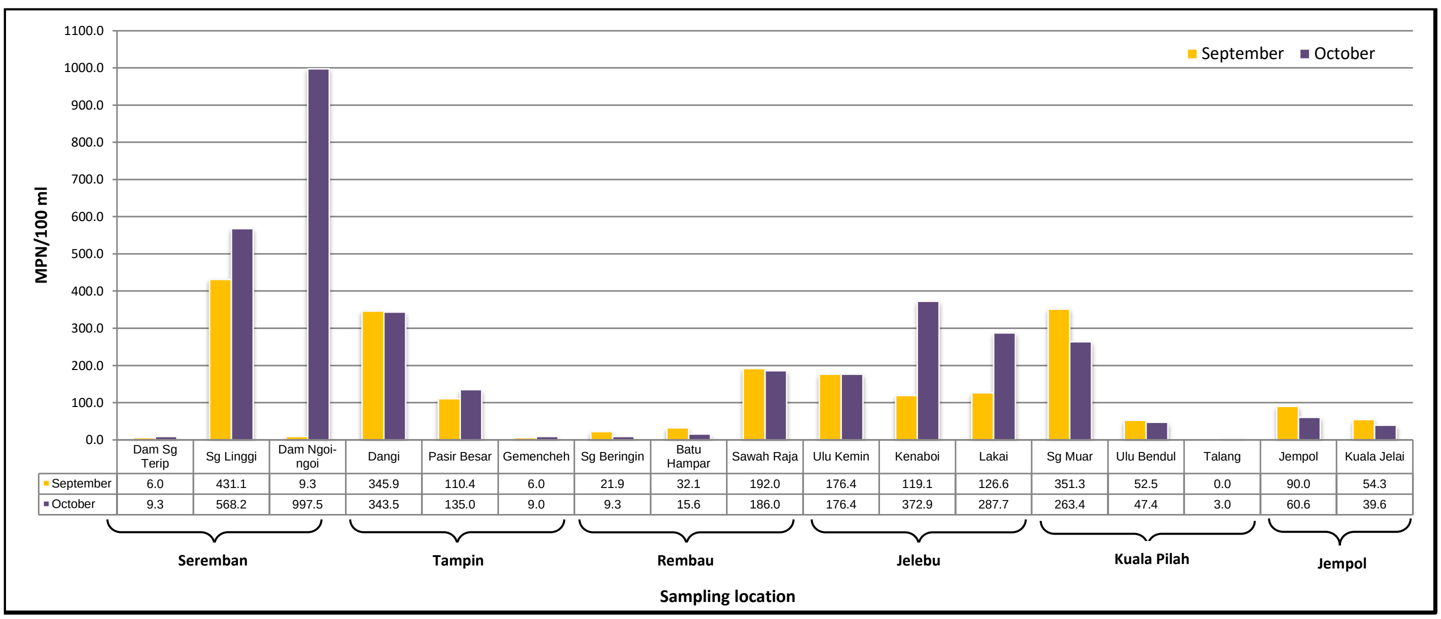

Figure 3. The density of enterococci in raw water in Negeri Sembilan.

The presence of coliform bacteria in surface water signifies contamination from humans, farm animals, and other point sources [9]. Even though the method of assessment is different, the results had shown the same trend. The primary goal is always to supply consumers with water that is microbiologically safe to drink. This is accomplished by employing proper disinfection procedures to destroy or remove bacteria, viruses, and cysts. As a result, disinfection process control is deemed crucial [10]. The decontamination process commonly used by water treatment plants is chlorination, which gives an excellent result in eliminating microbes in the water supply system. In an article from Pure Water Products, LLC [11] chlorine gas, sodium hypochlorite, and calcium hypochlorite are the three most prevalent chlorinecontaining compounds used in water treatment. The type of chlorine used is frequently determined by cost, storage, and pH requirements that must be met. Additionally, this study proves that the chlorination process applied for water treatment is able to reduce the number of MPN of the microbes to >100 (Table 1).

Table 1. MPN reading for treated water samples in the developed and rural area.

	Location			**Coliform, *E. coli, Enterococcus* MPN/100 ml**
Developed area	**Seremban**	TPO Sungai Terip	Kg. Rantau	<1
		TPO Sungai Linggi	Taman Tuanku Jaafar	
		TPO Ngoi-ngoi	Seremban 2	
		SRO Paroi/Senawang	Kolej Nilai	
		SRO Mantin	Kg. Tarun	
		SRO Nilai/Pajam		
	Port Dickson	SRO Si Rusa 2	Pekan Bukit Pelanduk	<1
		SRO Meter House	Telok Kemang NST	
		SRO Kg. Linggi	Klinik Kesihatan PD	
		Taman Desa PD	Hospital PD	
		Kem Sunggala	Taman Politeknik	
		Bandar Baru Springhill	Pengkalan Kempas	
	Tampin	TPO Dangi	SRO Gemencheh	<1
		TPO Pasir Besar	Pekan Gemencheh	
		TPO Gemencheh	Felda Jelai 4	
		SRO Dangi	Kem Gemas	
		SRO Pasir Besar	Hospital Baru Tampin	
	Rembau	TPO Pedas Lama	SRO Kendong	<1
		TPO Pedas Baru	Pekan Pedas	
		TPO Sawah Raja	Chembong Industries	
		SRO Bukit Kunyit	Kg. Acheh	
		SRO Rembau	Rumah Rakyat Kota	
Rural area	**Jelebu**	TPO Ulu Kemin	SRO Lakai	<1
		TPO Kenaboi	Peradong	
		TPO Lakai	Felda Pasoh 1	
		SRO Ulu Kemin	Kg. Chennah	
		SRO Kenaboi		
	Kuala Pilah	TPO Kuala Pilah Outlet	Market	<1
		TPO Ulu Bendul	UITM Beting	
		TPO Talang	Kg. Ibol	
		TPO Kepis	Kg. Melekai	
		SRO Kuala Pilah Barat	Senaling	
		SRO Bukit Tinggi	Bt. 3 Kg. Kepis	
		SRO Tudung Saji	Sek. Keb. Tengkek	
		SRO Bemban		
	Jempol	TPO Jempol	Tangki Pulapah	<1
		TPO Jelai	Felda Sg. Lui	
		SRO Taiso A	Taman ACBE	
		SRO Taiso B	Sek. Keb. St. Aidan	
		SRO Bukit Rokan Barat	Kg. Baru Rompin	
		Ladang Gaddes	Felda Palong 14	

The reading for treated water collected on 9 September and 5 October 2021 was <1 MPN/100 ml

SRO Service Reservoir Outlet
PD Port Dickson
TPO Treatment Plant Outlet
SRO Service Reservoir Outlet
Kg. Kampung

A study of microbiological and physicochemical water quality assessments of river water in an industrial region of the Northwest Coast of Borneo found that some sampling points recorded the least mean bacterial counts due to the location being farther from humans as well as animal activities [12]. Additionally, an increase in human activity will affect minerals and nutrient content in the water which can increase diseases [13].

The main purpose of river water quality monitoring consists in directly pointing to the sources of water contamination by using rapid bacterial indicators focusing on fecal bacteria such as *E. coli* and enterococc*i* [14]. The concentrations of *E. coli*, coliform bacteria, and enterococci, as well as turbidity, increased after rainfall according to the study made by Kistemann *et al*. [15]. Another previous study by Ferguson *et al*. [16] mentioned that water quality in the water was significantly affected by rainfall and sewage overflows following wet weather events which concentration of fecal coliforms and fecal streptococci in the water were both significantly increased with rainfall and sewage overflow. Microbial contamination detection of *E. coli* and coliform bacteria in river water shows the range of human health risks due to potentially pathogenic microorganisms [17].

Microbial load in developed areas drinking water system

The data on the microbial load clearly showed differences between developed areas and rural areas. In this study, four out of seven districts in Negeri Sembilan, which are Seremban, Port Dickson, Rembau and Tampin are categorized as developed areas. This classification is based on the length of the pipelines, the number of accounts, the population per area, and the total of water that is supplied to the area. Seremban and Port Dickson had vast populations while Rembau and Tampin were developing greatly. In a news article from Bernama [18], the government of Negeri Sembilan is optimistic that the state has the potential to expand due to an increase in various investments year after year. Since this state is about 70 km from Kuala Lumpur and is involved in mega-projects like Malaysia Vision Valley 2.0 (MVV 2.0), the East Coast Rail Link (ECRL), and the High-Speed Rail (HSR), it is likely to become major economic development drivers. In the MVV 2.0 area, the industrial and manufacturing sectors are concentrated in two key districts: Seremban and Port Dickson. The remaining four districts, Jempol, Kuala Pilah, Tampin, and Rembau, would be designated as agropolis and tourist sites.

The increasing population and industry had bad impacts on the drinking water source. Research regarding water pollution in Malaysia by Afroz & Rahman [19], states that several fluctuations in the annual amount of pollution in Malaysian rivers can be attributed to development in terms of industrialization, urbanization, and population increase. Pollutants deposited on the surface of the ground are washed away by rain in urban areas. Wastewater from residential, commercial, and industrial zones emits a foul stench, especially when the trash is present, and its quality degrades rainfall systems and contaminated rivers. Although some pollution comes from natural causes, the majority of pollution is created by human activity. A study by Leong *et al*. [20] at Bintulu, Sarawak also showed a higher bacterial density in the developed areas. Study sites were located near schools and homes that are exposed to diverse human activities and get direct waste drainage from housing, the water samples collected from a few locations had higher mean bacterial counts. Furthermore, Bintulu is an industrial town that generates wastewater runoff from oil, gas, and plantation crops like palm oil. The total availability of water is lowered because the expense of treating contaminated water is too high, and polluted water is not always suitable for consumption.

Camara *et al*. [21], discovered that human activities such as increasing urbanization, intensive agriculture, and deforestation in Malaysia have escalated land use and affected water quality across the country. The study showed that 87% of the reviewed studies revealed urban land use as a major source of water pollution, followed by agricultural land use and forest land use (82% and 77% in review studies respectively). However, it revealed that agricultural and forest-related activities had a greater impact on water quality due to their significant positive correlation with physical and chemical water quality indicators, whereas urban development activities had a greater impact on water quality due to changes in hydrological processes such as runoff and erosion. The investigation was done upstream of the water source to confirm related activities that lead to the increasing pollution of the water source. Activities such as sand mining, landslide, and farming have been captured as examples of increasing contaminants in the water source. An investigation was done accordingly in this study and found that a few activities happened upstream that may affect the level of microbiological numbers in the raw water. An investigation was done with the help of Syarikat Air Negeri Sembilan via their sanitary survey activity. Some of the activities that contributed to the high number of microbes in the developed area were sand mining activity (Sungai Linggi upstream), deforestation (Kenaboi and Lakai water treatment plant upstream), leveling land for agricultural activity (Kenaboi and Lakai water treatment plant upstream), and animal husbandry activity (Pedas Lama water treatment plant upstream).

Relationship between microbiological, physical, and chemical analysis in the most developed area

Although this study recorded a low MPN value (<1MPN/100 ml) of all coliforms*, E. coli,* and enterococci in treated water, there still has a chance for the microbes tested present in the treated water. Treatment of raw water reduces the microbial burden, although many distribution systems later see an increase in bacterial numbers as they get further away from the treatment point. Regrowth is the word for this increase, and it is recognized as a severe concern in many water distribution systems. Table 2 shows the number of coliform*, E. coli,* and enterococci in connection with the level of free chlorine, turbidity, and ammonia in the drinking water system in Seremban and Port Dickson collected in September and October 2021. Free chlorine, turbidity, and ammonia were selected as physical and chemical analyses that were possible relation to the microbial load in the water. From the data, free chlorine is the most related to microbial load in the water. Data showed a high number of coliforms*, E. coli,* and enterococci when there are 0.00 mg/L chlorine doses in the water and an absence of coliform*, E. coli* and enterococci when chlorine was dosed in the water.

Research conducted by Rasheed *et al*. [22] proved that *E. coli* can be inactivated by chlorine, at a dosage of 1.0 mg/L at 25°C where the maximum inactivation recorded was 7 log removals. When using a 1.0 mg/L chlorine dose, 45 minutes of exposure time was sufficient for maximum inactivation of *E. coli*; however, when using 0.5 and 0.25 mg/L chlorine doses, a longer exposure time was needed for complete disinfection. Tap water and well water, on the other hand, required more contact time.

For turbidity analysis, Intake *Sungai Linggi* recorded the highest number of coliforms*, E. coli,* and enterococci which were 7258.8 MPN/100 ml, 3138.6 MPN/100 ml, and 431.1 MPN/100 ml, respectively, and recorded the highest turbidity level. However, further analysis showed that there was no relationship between the ammonia level and the number of coliforms*, E. coli,* and enterococci in water. For the ammonia analysis, the highest reading was at SRO Meter House which is 0.70 mg/L but coliforms*, E. coli,* and enterococci were not detected in the water.

Previous research found that microbial count was inversely related to chlorine residual, whereas turbidity had a direct relationship. Microbial counts decrease as the chlorine concentration increases, and vice versa [23]. In another research conducted by Karikari and Ampofo [24], turbidity was found to have a positive connection with coliform and fecal coliform, indicating that an increase in turbidity would increase coliform count. Turbidity is the presence of suspended particles in water that can be induced by a variety of organic and inorganic components which can impair the microbiological quality of drinking water. Ammonia can be found in the source water used to make drinking water, or it can be added to treated water with chlorine to make chloramines, which are used as disinfectants. However, ammonia in drinking water is undesirable because nitrification can result in dangerous quantities of nitrite, as well as a change in water taste and odor, as well as an increase in heterotrophic bacteria, including opportunistic pathogens. From this study, there was no evidence of coliform*, E. coli,* and enterococci were related to the ammonia level. Despite that, the composition of the ammonia-oxidizing bacteria populations in the saturated sandy soil surrounding the extraction well is likely reflected in the ammonia-oxidizing bacteria population in raw and treated water at the water treatment plant [25].

All the previous studies strongly stated that there are a high number of pathogenic bacteria in raw water sources compared to treated water and activities upstream of the catchment area do affect the number of bacteria in the raw water.

Table 2. The level of free chlorine, turbidity, ammonia in connection to the number of coliform, *E. coli* and enterococci collected in Seremban dan Port Dickson.

Location		Free chlorine (mg/L)		Turbidity (NTU)		Ammonia (mg/L)		Coliform (MPN/100 ml)		*E. coli* (MPN/100 ml)		Enterococci (MPN/100 ml)	
		*S	O	S	O	S	O	S	O	S	O	S	O
Seremban	Intake Sungai Terip (Dam)	0.0	0.0	3.50	3.51	0.15	0.42	751.2	1383.0	<1	6.0	6.0	9.3
	Intake Sungai Linggi (River)	0.0	0.0	71.00	92.10	0.38	0.19	7259	7259	3139	5199	431.1	568.2
	Intake Ngoi-ngoi (Dam)	0.0	0.0	17.80	95.80	0.30	0.30	1850	7259	25.2	4659	9.3	997.5
	TPO Sungai Terip	3.3	3.2	2.10	1.51	0.01	0.02	<1	<1	<1	<1	<1	<1
	TPO Sungai Linggi	2.2	2.3	3.13	1.26	0.09	0.04	<1	<1	<1	<1	<1	<1
	TPO Ngoi-ngoi	3.7	2.2	3.18	1.41	0.05	0.10	<1	<1	<1	<1	<1	<1
	SRO Paroi/Senawang	2.8	2.6	1.46	1.95	0.11	0.02	<1	<1	<1	<1	<1	<1
	SRO Mantin	2.0	2.2	2.11	1.38	0.13	0.02	<1	<1	<1	<1	<1	<1
	SRO Nilai/Pajam	3.0	2.1	2.94	2.71	0.02	0.01	<1	<1	<1	<1	<1	<1
	Kg. Rantau	1.2	1.0	3.90	2.71	0.03	0.03	<1	<1	<1	<1	<1	<1
	Taman Tuanku Jaafar	2.0	2.1	2.16	1.88	0.04	0.85	<1	<1	<1	<1	<1	<1
	Seremban 2	2.0	2.7	1.36	2.13	0.03	0.02	<1	<1	<1	<1	<1	<1
	Kolej Nilai	3.0	2.0	1.01	2.16	0.04	0.01	<1	<1	<1	<1	<1	<1
	Kg. Tarun	2.5	1.9	1.31	2.12	0.22	0.02	<1	<1	<1	<1	<1	<1
Port Dickson	SRO Si Rusa 2	2.4	2.0	4.76	1.61	0.13	0.01	<1	<1	<1	<1	<1	<1
	SRO Meter House	2.2	1.9	4.32	1.59	0.70	0.01	<1	<1	<1	<1	<1	<1
	SRO Kg. Linggi	3.5	1.9	0.78	1.66	0.13	0.05	<1	<1	<1	<1	<1	<1
	Taman Desa PD	0.8	0.9	1.70	0.91	0.03	0.02	<1	<1	<1	<1	<1	<1
	Kem Sunggala	1.6	2.0	3.90	2.12	0.05	0.04	<1	<1	<1	<1	<1	<1
	Bandar Baru Springhill	1.0	1.5	3.66	4.79	0.07	0.09	<1	<1	<1	<1	<1	<1
	Pekan Bukit Pelanduk	0.9	1.4	1.80	3.71	0.01	0.01	<1	<1	<1	<1	<1	<1
	Telok Kemang NST	1.0	1.7	1.42	1.80	0.07	0.06	<1	<1	<1	<1	<1	<1
	Klinik Kesihatan PD	0.7	1.6	1.92	1.68	0.01	0.02	<1	<1	<1	<1	<1	<1
	Hospital PD	0.9	1.4	1.31	1.62	0.06	0.08	<1	<1	<1	<1	<1	<1
	Taman Politeknik	0.9	1.3	1.57	1.37	0.12	0.08	<1	<1	<1	<1	<1	<1
	Pengkalan Kempas	2.3	2.1	0.82	1.82	0.05	0.06	<1	<1	<1	<1	<1	<1

* S = September 2021; O = October 2021

Conclusion

In conclusion, it was shown that there was a high microbial number in the high development area in Negeri Sembilan and the chlorination process reduced the microbial loads in the treated water. The study also proves that the number of coliforms, *E. coli*, and enterococci in drinking water supply isolated from a developed area was much higher than in rural areas. The study also showed that there was a relationship between the number of microorganisms and the concentration of chlorine and turbidity in water. Preventive measures like chlorination at the end of the water treatment process did decrease the number of microbes in the targeted area for a cleaner water supply. This study is deemed essential as it will increase awareness and knowledge among consumers on safe and clean water thus, reducing water-borne disease outbreaks in Malaysia.

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