Eur. Phys. J. Plus (2022) 137:689
https://doi.org/10.1140/epjp/s13360-022-02891-5

THE EUROPEAN PHYSICAL JOURNAL PLUS

Regular Article

Experimental study on total coliform violations in the complied NH_2CL, O_3, and UV treated municipal water supply system

V. Ramani Bai[1,2,a], **A. Chun Kit**[1], **G. Kangadharan**[3], **R. Gopinath**[4], **P. Varadarajan**[5], **A. J. Hao**[6]

[1] Faculty of Engineering, Technology and Built Environment, UCSI University Kuala Lumpur, Kuala Lumpur, Malaysia
[2] UCSI-Cheras Low Carbon Innovation Hub Research Consortium, Kuala Lumpur, Malaysia
[3] Alpha Cambridge International School, Tiruchirappalli, India
[4] Baba's, Selayang Industrial Park, Selangor, Malaysia
[5] Bharat Heavy Electricals Ltd, Tiruchirappalli, India
[6] University of Nottingham, Semenyih, Malaysia

Received: 15 February 2021 / Accepted: 30 May 2022

Abstract Water quality has become a severe concern on a global scale, owing mostly to the rapid increase of the nation's development. According to Malaysia's Natural Resources and Environment Ministry, poor water management is the primary cause of the country's water quality problems. Many river systems are polluted by home and industrial pollutants, according to the findings of research in Malaysia and comparable difficulties in a few other nations. Hence, the following are the research's goals: (1) To look into what is causing the infractions. (2) To undertake the inquiry, develop a thorough hypothesis. (3) To detect dangerous germs by sampling the most usually infected regions. (4) To develop a test for Total Coliform violations in chlorine-treated water at the water treatment plant and in water distribution systems. As a result, the most major barrier to ensuring the safe delivery of treated water to consumers and protecting human health from water-related diseases is the drinking water treatment process. As a result, practically all water treatment systems around the world, including those in the USA, use a chlorine-based procedure to disinfect the water system during treatment. According to studies, the ideal way of disinfecting treated water is both safe and beneficial. Any sort of pandemic or biologically caused disease has no societal implications. Many countries began to suffer in 2009 as a result of e-coli and total coliform contamination in their water systems, leading to ambiguity in disinfection methods. Some water from UNMC's coolers was within the guidelines, while some exceed them. Water coolers at Block E (614 m) and Block B (605 m), for example, measured 12 CFU/100 ml and 11 CFU/100 ml, respectively. Water coolers should be cleaned regularly to ensure that they perform correctly. Further, the microbial population was found to be higher at water storage tanks than that is at the water cooler. This demonstrates how a water cooler fulfils its purpose of filtering and trapping germs to provide clean drinking water.

1 Introduction

At the national, regional, and local levels, access to safe drinking water is becoming increasingly significant as a health and development concern. The biggest risk to public health from bacteria in the water is related to the use of contaminated drinking water, which involves ingestion of contaminated water, such as human or animal faeces.

Pathogenic bacteria, viruses, protozoa, and helminths can all be found in faeces. During water treatment, disinfection is an important step that acts as a barrier against numerous microorganisms. Coliform bacteria are aerobic and facultative anaerobic, gram-negative, non-spore-forming rods capable of fermenting lactose and producing acid and gas in less than 48 h at a temperature of 35 °C (32–37 °C) [1].

Coliform bacteria are bacteria found in the environment and the faeces of warm-blooded animals and humans. Coliform bacteria are linked to treatment efficacy, and they should be absent from well-treated plant effluents. Because coliform bacteria are not pathogens, they are unlikely to cause illness. The presence of coliform in drinking water, on the other hand, suggests the presence of disease-causing organisms (pathogens) in the water system. Normally, health symptoms associated with polluted drinking water range from no ill effects to diarrhoea (gastrointestinal distress). The presence of pathogens will lead to a decrease in water quality. The majority of diseases can contaminate drinking water.

Water is an essential resource to our life; therefore a satisfactory and good quality water supply must be available to all. Group of people is at the greatest risk of waterborne diseases, especially those living under unsanitary conditions. Generally speaking, it is complex, time-consuming, and expensive to test drinking water for all possible pathogens. However, it is easier and cheaper to assess

[a] e-mail: chinnavramani@gmail.com (corresponding author)

Published online: 14 June 2022

the presence of coliform bacteria. The water system operators need to take action such as finding out the source of contamination or pollutants and restoring safe drinking water if coliform bacteria are found in water.

1.1 About total coliform

The major aim is to determine the water quality in the University of Nottingham Malaysia Campus, through the identification of total coliform in the chlorine-treated water. The municipal water is being supplied from the state's water supplier, Syarikat Bekalan Air Selangor (SYABAS). The coliform in the water supply may show the ineffectiveness of the water treatment system as well as the contamination included in the water supply distribution system and as per quality reports [2]. The presence of coliform, presented in terms of concentration, is an indicator to see whether the water supply in our campus is faecal contaminated or not. Thus it is to check the degree of contamination.

1.2 Objectives and scope of the study are

- Investigation of the presence of total coliform in complied chlorine-treated water supply from SYABAS (water supply inlet) and its variations.
- Conduct laboratory works (microbiological approach) to differentiate coliform bacteria and non-coliform bacteria presented in water samples.
- Identify the possible sources of total coliform occurrence and causes of contamination.
- Lastly, compare the water quality status of the campus against the Malaysian drinking water quality standards.

2 Literature review

The identification of the violation of microorganisms such as e-coli, total coliform, and other unidentified microbes in the disinfected treated water was a big issue early this century. The disinfectant such as NH2CL, O3, and UV in treated municipal water supply systems are having traces of coliform. They were noticed at the storage, distribution network, and destinations. A detailed review of past research and selected publications were presented in the following section. The review was conducted under four different heads related to this problem. They are (i) Type of microbes in water supply, (ii) Cause of violation of microbes in water supplies, (iii) Hypothesis and methods to solve the problem of the coliform violation, and (iv) Successful methods and solutions arrived in the past research.

2.1 Type of microbes in water supply

The article presents a considerable number of solids and high levels of BOD5, COD, and nitrogenous compounds in tannery wastewater were found by this study. The effluent showed a high BOD5/ COD ratio explained by the presence of organic matter in the wastewater. Optimum pH and temperatures values exhibited in the unit operations suggest that the integrated biological system in the current study is suitable for the rapid growth of bacteria responsible for nitrification and denitrification [3].

Investigation on green synthesis of AgNPs using Muntingia calabura leaf extract as reducing and stabilizing agents was conducted. The AgNPs formation was monitored using a UV–Vis spectrophotometer. Characterizations of AgNP's size and shape were observed by TEM. The elemental analysis was analysed using XDS. The maximum surface Plasmon resonance for AgNPs was detected at 425–430 nm. This study revealed that the AgNPs were polydispersed and polycrystalline. The microbial inhibition test against Escherichia coli and Bacillus cereus showed that the Muntingia leaf mediated AgNPs had inhibited the growth of these bacteria, as indicated by the formation of the inhibition zone. The average inhibition zone for Escherichia coli was 10.3 ± 0.5 mm and for Bacillus cereus at 9.5 ± 0.6 mm. TEM results showed that the synthesised AgNPs have a spherical form with sizes ranging from 22 to 37 nm. Hence, the synthesised AgNPs can potentially be applied for water treatment and medicinal purposes [4].

2.2 Cause of violation of microbes in water supplies

The use of pesticides not only affects vectors and other pests but can also be detrimental to human health. Pesticides can leach when plants are irrigated with surface water and surface water. This water can be a source of drinking water in areas contaminated with pesticides. This past study aimed to determine the presence of pesticides in surface waters and the effectiveness of pesticide removal in existing drinking water treatment facilities (DWTPs) and potential risks to consumer health. This study was conducted in Tanjung Karang, Selangor, Malaysia. In conventional DWTP, the pesticide removal efficiency is 77% (imidacloprid), 86% (propiconazole and bupropion), 88% (tebuconazole), and 100% (pymetrozine, tricyclazole, chlorantranil), respectively. liprol, azoxystrobin, and trifloxystrobin). Conventional DWTP could not completely remove the four pesticides. Therefore, in the future, it is necessary to consider an advanced treatment system to protect the health of society [5]. Environmental laws are expected to be expanded to include a variety of municipally derived ECs. However, there is currently a lack of knowledge about their destiny during

wastewater treatment and in the environment. The stated removals of ECs by WwTWs are questionable due to the limitations of previously employed sampling methodologies. As a result, the removal performance of various WwTW process types under varying operational situations must be re-evaluated using appropriate sample techniques [6]. The availability and distribution of these freshwater resources have been linked to social well-being, economic development, and political development. The negative health effects of river water supply, dam construction, irrigation development, and flood mitigation have increased the incidence of malaria, Japanese encephalitis, schistosomiasis, lymphatic filariasis, and other diseases in many parts of the world. As a result, the most important part of maintaining good water quality is conducting a study on the quality of river water. River water quality parameters such as pH, Dissolved Oxygen (DO), biochemical oxygen demand, suspended solids, chlorides, phosphates, nitrates, and sodium are critical for the survival of the river ecosystem's living beings [7].

2.3 Hypothesis and methods to solve the problem of the coliform violation

This article studied the nitrate in groundwater supplies using fuzzy logic as it is risking health. The input parameters were mapped to the outputs derived to show the level of health risk and human cancer risk. It is uncertain about the size of the population subjected to nitrate and the cost incurred for reducing the risk. Two input parameters were used to obtain three output results. Fuzzy logic, in this work, provides a realistic and appropriate assessment but it can only be used for decision making [8].

This research involved watershed assessment and described the use of Fuzzy logic to the Riparian Restoration Ranking (R3) System on some montane watersheds in Arizona. A Composite Fuzzy Score was obtained from 14 input parameters. This system can assess the watershed environment at different scales, without the need to identify a reference datum. However, it is a classification tool only. Biological wastewater treatment is the focus of this research using Fuzzy logic. The Takagi–Sugeno inference system was used to estimate the concentration of the substrate. This method of estimation is not expensive and complicated as compared to online measurements but if a change that deeply modified the process occurred in the reactor a static error appears between measured and calculated values, and it becomes necessary to recalibrate the observers [9]. Mamdani's method of fuzzy can adjust its rule based on local conditions, Able in assessing vulnerability in the case of data scarcity [10].

Because of improper classification of waste, they are hazardous, and more money is spent on their disposal every year. By following this proper classification system and its disposal method, we can economically spend money on hospital waste disposal [11].

The developed climate change coastal resource information system is a simple, yet flexible database online. The database structure allowed for variation in the level of detail provided for each variable and country. In addition to the ability to view, query, and report monitoring data, the DMIS also allows users to display the data spatially using a GIS including Water quality analysis online [12].

A sampling on 12 physical and chemical parameters measured at 15 stations during 2013–2014 in Haraz River, northern Iran was presented. The results of the measurements were analysed using multivariate statistical analysis methods including cluster analysis (CA), principal component analysis (PCA), factor analysis (FA), and discriminant analysis (DA). According to the CA, PCA, and FA, the stations were divided into three groups high pollution, medium pollution, and low pollution. The research findings confirm the applicability of multivariate statistical techniques in the interpretation of large data sets, water quality assessment, and source apportionment of different pollution sources [13].

2.4 Successful methods and solutions arrived in the past research

The analysis of water quality was conducted using the Mamdani inference system. A fuzzy water quality index was obtained from the twenty-seven (27) input parameters. These are about twenty-seven water quality parameters are analysed in this study. While it is a powerful deduction tool it can only be used for decision making [14]. This work focused on wastewater treatment and employed Fuzzy C mean (FCM) clustering is a process monitoring tool in an activated sludge wastewater treatment facility. FCM makes it simple to compare different classes, or a class and an object because the membership total equals unity. Although it is imperfect in FCM in terms of membership value contour lines and stretches unevenly [15].

The research work described the use of fuzzy control and nonlinear estimation in wastewater treatment plants. The Mamdani inference was used to determine unmeasured variables. The 2 inputs used were e (error signal) and d_e (derivative of e) and a single output was obtained. It is an inexpensive method, but the main advantage lies in the efficiency of the system to reduce the impact of unmeasured disturbances. However, it does require the measurement of all the state variables [16].

A pilot-scale study in the wastewater treatment plant was carried out. The purpose was recovering the stable operation when there is a disturbance caused. The paper investigates the state of the art of a pilot-scale wastewater plant, its changing trend and so to send the best commands to the final control. First, the five input parameters give a trend using the Takagi–Sugeno-Kang method. These 2 outputs are used as inputs in a Mamdani Fuzzy logic system to obtain the best pump commands. In another Takagi–Sugeno-Kang system, the five inputs are used. The overall system is compact, simple, powerful, and competent to be used in low-cost computing platforms [17]. The researchers looked at the microbial ecology of DWDS, which have typically relied on growing organisms from bulk water samples. Microarrays, metabolomics, and metaproteomics are three approaches that can fill this gap; however, their applicability in DWDS has yet to be investigated. Future studies should combine a variety of tools to explore and link microbial diversity and activity to better understand the relationship between microbes and system function. Environmental metagenomics

combined with other approaches such as metatranscriptomics, metaproteomics, and metabolomics in a system biology approach might allow us to gain a better knowledge of DWDS [18].

The water quality index using the Mamdani Fuzzy inference system was studied. Six input parameters are used, namely BOD, COD, DO, pH, NH3, SS, and a fuzzy water quality index is obtained. This method is a robust determination, deduction, assessment, and extension of the tool for biological parameters [19].

An online coastal database system including a water quality database was developed. Since the demand for seacoast construction and exploitation of resources are ever-growing globally, firm policies are to be developed. A fine decision-making tool is needed to have control over the exploitation of coasts in terms of land and water to perfect the management of these resources [20].

The study created a dataset that is arbitrarily used for training, validation, and testing processes for each cyclic update in Levenberg–Marquardt backpropagation for the numerical treatment of the dynamics of the COVID-19 model. The effectiveness and reliable performance of the design LMANNs are endorsed based on assessments of achieved accuracy in terms of mean squared error-based merit functions, error histograms, and regression studies [21].

This article builds upon the previous developments of the aggregative risk analysis approach. Each basic risk item in a hierarchical framework is expressed by a triangular fuzzy number, which is derived from the composition of the likelihood of a failure event and the associated failure consequence. An analytic hierarchy process is used to estimate the weights required for grouping non-commensurate risk sources. The evidential reasoning is proposed to incorporate newly-arrived data for the updating of existing risk estimates. The exponential ordered weighted averaging operators are used for defuzzification to incorporate attitudinal dimensions for risk management. It is envisaged that the proposed approach could serve as a basis to benchmark acceptable risks in water distribution networks. Faeces can be the source of pathogenic bacteria, viruses, protozoa, and helminths. Disinfection is an important process and an effective barrier to many pathogens during the water treatment [22]. The infection is spread via vectors from humans to humans or from animals to humans. Vector-host infections are estimated to account for 17% of all infectious diseases, resulting in around one million fatalities per year. The majority of these infectious disease outbreaks are recorded in tropical and sub-tropical countries, as well as communities where sanitation is an issue and safe drinking water is scarce [23, 24]

2.5 Summary of literature review

The existing treatment plants could not completely remove the microbes, pesticides, and other hazardous substances that come with raw water. Complex treating and cleaning methods must be considered to protect public health in the future. Particularly, the behaviour of microbes in current treated water has not been sufficiently studied. Consequently, the deletion performance of different types of treatment processes in different operating situations should be reconsidered and revaluated using correct sampling methods. Hence an experimental study has been planned in the current research on the violation of microorganisms such as e-coli, total coliform, and other unidentified microbes in the disinfected treated water using NH2C, O3, and UV and in the municipal water supply system.

3 Fundamental of microbial analysis

The presence of coliform bacteria group in the municipal water supply is one of the principal indicators of the suitability of water, to see whether the water supply is faecal contaminated. Escherichia Coli (E. Coli) also acts as a criterion of the degree of pollution. Here, a microbiological approach is used to investigate the water quality in The University of Nottingham Malaysia Campus.

3.1 Microbial aspects of water

Securing the safety of the water supply is based on the application of multiple barriers, starting from the water catchment area to users, to prevent or reduce the contamination of water to an insignificant level. For instance, protection of water catchment, proper water treatment, and operation of the distribution system is necessary to maintain water quality.

The presence of a coliform group of bacteria in the municipal water supply is one of the principal indicators of the suitability of water, to see whether the water supply is faecal contaminated. There is possible that pathogens may exist in water sources if coliform bacteria are found. Drinking water is the only way in the case that pathogens are transmitted through the faecal-oral route. Contamination of hands, foods, utensils, and clothes may become the cause. Microbial drinking water safety is not only related to direct faecal contamination but also the organisms that grow in a water distribution network such as Legionella.

Waterborne pathogens may grow in water, for instance, Legionella. Other host-dependent waterborne pathogens are not able to grow in water but can persist, for example, Noroviruses and Cryptosporidium. Host-dependent waterborne pathogens will gradually lose viability and the ability to infect after leaving the host, but the rate of decay is always exponential, and they will become undetectable after a certain period. For the case of pathogens with low persistence, they will find new hosts rapidly and can be spread by person-to-person contact.

3.2 Characteristics of indicator organisms

Microbial indicators are micro-organisms that indicate the potential issues of microbiological water quality, but they are not pathogenic. There are some properties that an effective indicator organism should have when it is used to detect contamination of water. Firstly, it shows when faecal pathogens are present in water. Secondly, it can be detected after dilution. Thirdly, the organism itself should be relatively fast and easy to detect. Lastly, it should be sensitive as pathogens to disinfection and should survive longer.

3.2.1 Total coliforms

Total Coliform refers to a large group of gram-negative, rod-shaped bacteria that share some characteristics. Total coliform consists of thermotolerant coliform, bacteria of faecal origin, and some other bacteria that are isolated from environmental sources. Hence, the appearance of total coliforms may or may not indicate faecal contamination. The presence of total coliform may be due to the entry of soil or organic matter into the water. Total coliforms can be grown on medium or agar that consists of lactose, with a temperature of 35 °C.

3.2.2 Faecal coliforms (thermotolerant coliform)

Faecal coliform is commonly used to denote coliform organisms that ferment lactose to produce acid and gas and is suitable to grow at a temperature around 44 °C. However, some organisms with these properties may not be of faecal origin, and hence "thermotolerant coliform" is more commonly used. The existence of thermotolerant coliforms always indicates faecal contamination. Generally, most of the thermotolerant coliforms are the gut organism E. Coli. In the laboratory, thermotolerant coliforms can grow on media that consists of lactose. Do note that the absence of E. coli does not necessarily guarantee the absence of faecal contamination. The absence of evidence does not logically denote evidence of absence.

3.2.3 Faecal streptococci

The presence of faecal streptococci is also evidence of faecal contamination in water. This kind of bacteria tends to persist longer in the environment compared to thermotolerant coliforms or total coliforms. Faecal streptococci can grow in a medium which consists of sodium azide, at a temperature of 37 °C to 44 °C. It can be detected by the reduction of a dye or hydrolysis of aesculin.

3.2.4 Disinfection

Disinfectants that are widely used in water treatment are oxidizing agents (halogens, halogen compounds, ozone) and physical agents (ultraviolent and radiation). Currently, chlorine is used as a primary disinfectant in potable water treatment. The use of chemical disinfectants during treatment normally comes with the formation of by-products, but the health risk from the by-products is extremely small compared to the risk associated with waterborne diseases.

3.3 Water treatment

Besides the protection of water sources, water treatment processes are crucial to removing contamination of the drinking water system. The treatment process consists of several steps such as pre-and post-treatment. The coagulation, flocculation, sedimentation, and filtration stages are designed to remove particles in the water source, including microorganisms. These stages should be optimised to achieve reliable performance. In between, chemical coagulation is the most critical part to determine the removal efficiency of coagulation, flocculation, and clarification processes.

For the disinfection stage, the adequate concentration of disinfectant is the key element to achieving the necessary level of microbial risk reduction. The most commonly used technique is chlorination. Besides, ozonation, ultraviolet irradiation, and chloramination are also very effective to kill bacteria and also reasonably effective to inactivate viruses and protozoa. After the treatment is done, storage of water before supply to customers can improve disinfection by increasing disinfectant contact times.

3.4 Water distribution system

A water distribution system is established to transport treated water from the water treatment plant to the consumer. It should be designed with insufficient capacity so that it can meet the demand of consumers. Besides, the secondary purpose of a water distribution system is to provide water for the fire-fighting purpose. Hydraulic analysis has to be done, which involves the calculation of flow rate and head loss in the pipe and the pressure at critical points. A typical water distribution system should consist of pipes, nodes, and loops. Moreover, ground storage reservoirs also need to be considered in detail.

Pipelines are the most important element in the water distribution system, which provides access for water to reach every potential user. Normally, there is a potential opportunity for microbial or chemical contamination to exist due to the nature of the distribution system, such as long-distance pipelines, storage tanks, and interconnections with industrial users. To control the water quality, water must be microbial safe before entering the distribution system.

3.5 Public health

Outbreaks of waterborne diseases may bring serious effects to large numbers of people. Hence, the control of such outbreaks becomes a priority to make sure good water quality. Some of the pathogens in contaminated water may lead to severe diseases. For example, typhoid, infectious hepatitis, cholera, and disease caused by E. Coli. and Shigella spp. Therefore, it is important to provide a clean and safe water supply to everyone to ensure public health.

3.6 Stressed organism

A stressed organism is an injured bacteria. The indicator bacteria including total coliforms, faecal coliforms, and faecal streptococci may become stressed or injured in waters. Bacteria's structural and metabolic properties have to be taken care of to make sure that the entire sample collecting process did not harm the organism. A stressed organism will be incapable of growth and colony formation normally under standard conditions because of structural or metabolic damage.

This situation will lead to an inaccurate finding and outcome for microbiological parameters of water quality. As the total coliform is calculated in terms of the number of colonies counted during the experiment, their colonization is crucial. The bacteria may be injured due to extreme temperature, solar radiation, and PH value. This also will lead collectively to significant underestimations of the number of indicator bacteria in water.

4 Experimental analysis of coliform bacteria in the water supply system

The purpose of the experiment was to determine the presence of coliform bacteria in the municipal water supply in the University of Nottingham Malaysia Campus. Coliform bacteria are microorganisms and are having a very small particle size. Therefore, it is not visible to the naked eye. Certain procedures need to be followed. Furthermore, certain procedures will be conducted in the microbiological laboratory to differentiate coliform bacteria and non-coliform bacteria. The presence of coliform bacteria, particularly Faecal Coliform or Escherichia Coli (E. Coli), renders the water potentially unsatisfactory and unsafe.

The entire process of investigation of water quality was divided into six stages and was presented in the following sections. It included preparation of equipment (Sect. 4.1), preparation of agar (Sect. 4.2), sample collection stage (Sect. 4.3), preservation and storage (Sect. 4.4), testing of samples (Sect. 4.5), and getting results by calculations (Sect. 4.6).

4.1 Preparation of the experiment

It is necessary to check the availability of equipment and apparatus that are required throughout the entire laboratory process. The specifications of the equipment were studied, for instance, the temperature of the incubator and refrigerator was maintained at a suitable value.

4.1.1 Washing and sterilization

Washing and sterilization of equipment was an important stage in laboratory work. The glassware or plasticware was cleaned and washed thoroughly with distilled water. For glassware, it was sterilized by dry heat for at least 60 min at a temperature of 170 °C. The bottles used to collect samples were non-reactive borosilicate glass or plastic bottles and were rinsed with distilled water before using them.

4.1.2 Handling of equipment

The hands were washed properly with soap and wear gloves before carrying the equipment. Hands were sprayed with alcohol or ethanol. The worktable was cleaned using alcohol or ethanol to kill the bacteria. During the experiment, safety equipment was worn such as a face mask and goggles. Furthermore, all the equipment were carried carefully, especially glassware such as flasks and beakers to make sure that they did not have chipped edges or etched inner surfaces.

4.2 Preparation of agar

Eosin-Methylene Blue (EMB) agar was the medium used to detect the growth of coliform colonies. The bacteria which do not ferment lactose will appear to be colourless colonies. Other details on EMB Agar are described in Sect. 4.7. One portion of EMB

agar is around 37.4 g. This portion of EMB agar was added to 1000 ml of distilled water. The mixture was placed in glass bottles (Scott Bottles) which were autoclavable, stirred until the agar was completely dissolved and left for 15 min at a temperature of 121 °C. The lid of the glass bottle was loosened before putting into Autoclave. After the autoclaving process was done, a time was given for the temperature to cool down to 60 °C.

Pouring agar must be done in a laminar fume chamber, which was cleaned with ethanol. It was dispersed by swirling the medium before pouring into sterilized Petri dishes. Pouring should be done when agar was still hot and in liquid form with high workability.

Agar was poured into many Petri dishes with a diameter of 90 mm until the agar half fills Petri dishes (see Fig. 1). Whenever the air bubble was found, Petri dishes were swirled gently to get rid of bubbles and make the agar a homogeneous one. When the agar is completely cold, the lid was placed and inserted into a plastic bag. Next, sealed the plastic bag and stored them in the refrigerator at an appropriate temperature.

4.3 Sample collection

About 25 locations were selected as sample collection points. They were located at Block A, B, B1, C, D, E, F1, F2, F3, G, H, and Block N on the University campus, including the water supply inlet to campus, water tanks, the water outlets such as the washbasins and water coolers. The complete list of sample collection points are presented in Fig. 2.

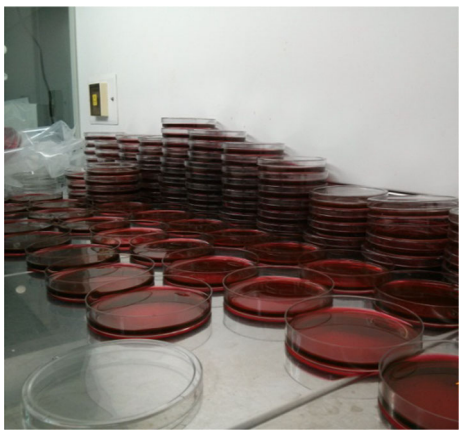


Fig. 1 EMB Agar poured into petri dishes, which done in laminar fume chamber

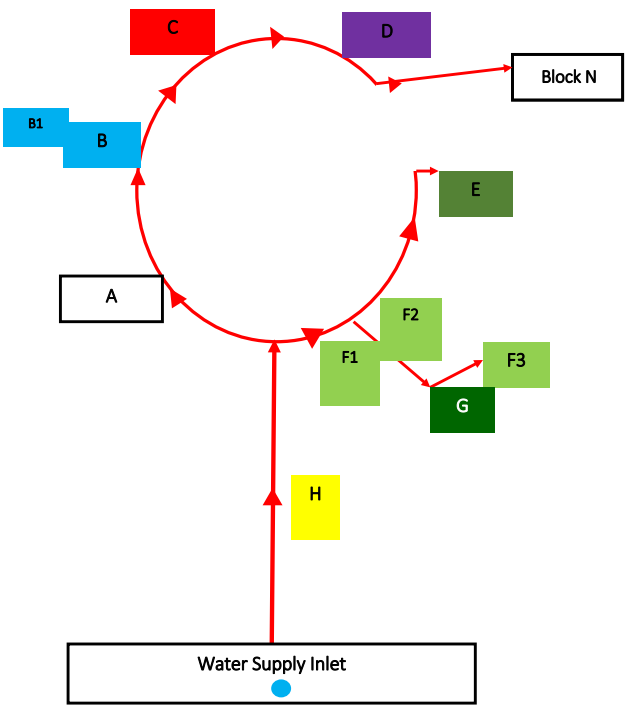


Fig. 2 Direction of water from source to all blocks—water distribution network of the campus

4.3.1 Sample collection points

Water samples were collected at these locations at two different times in a day viz., morning at 7 am and evening at 3 pm. The schedule and sequence of sample collection were prepared to adopt in the study. For the control sample or standard liquid, purified water or distilled water were used. The distance between the water supply inlet and the sample collection points was measured. The distance measured was an approximate value considering horizontal distance and elevation. It can provide a rough distance that water needs to travel to reach the outlet, and it is useful for the analysis part.

4.3.2 Volume of water samples

For each location, the sample bottle with a capacity of 500 ml was used. This was to collect a water sample with a volume of approximately 300–350 ml. 200 ml to produce two specimens with a sample size of 100 ml, and the rest of the water is for backup purposes in case there were any water spills during the experiment.

4.3.3 Order of collecting samples

By knowing the flow of water supply in the distribution system, the order of sample collection had been planned in detail. The collection work was done by following the flow of the water supply. Firstly, a water sample was collected at the water supply inlet, which was the starting point of the water supplied to campus.

Secondly, a sample was collected at the water tank of several buildings. When the water supply had reached Block H first, water samples were collected at Block H, starting from the water tank. It followed the water samples at the outlet such as the water at washbasins and water coolers. These steps were repeated for each building by following the sequence as in Fig. 2.

4.3.4 Sampling technique

When collecting a water sample from the water tank, the outlet valve was opened fully underneath the tank to let the water runoff for two to three minutes to ensure that the flow line was clear before collecting the sample. For a water tank without an outlet valve after opening the cover of tanks, a cup was used to take out some water.

4.3.5 Labelling of water samples

During the sample collection process, labelling work must be done for easy referencing. For each sample bottle, a labelling sticker was pasted to indicate the collection date, collection time, and sample code which could give locations. This would prevent confusion while laboratory work was carried out (see Fig. 3).

4.4 Preservation and storage of samples

Generally, the microbiological analysis must be started as soon as possible immediately after sample collection to avoid any unpredictable changes in the microbial violation. For the most accurate results, the sample bottles would be kept in a cooler box filled with ice after being collected. It is also advisable to carry cooler boxes if the transportation of samples from collection points to the laboratory takes longer and cannot be processed within one hour after collection. In the current research, it was well organised to analyse the sample on the day of the collection itself. In case of failure to follow this, samples were kept in the refrigerator overnight only. Since the samples for coliform analysis should not keep in the fridge for more than 48 h after collection.

Fig. 3 Labelling stickers pasted on sampling bottles

4.5 Testing of samples

The membrane filtration technique was applied in the laboratory. It is a process to filter water samples and trap bacteria on a membrane filter. This is commonly used in microbiology. The complete and detailed laboratory works and procedures included the preparation work, set up of filtration unit, operation of the filtration process, placing membrane filter with bacteria on Petri dishes filled with EMB agar, labelling of Petri dishes, incubation of Petri dishes, and counting procedure of many colonies. All these steps were repeated for every specimen, and it took time to complete the entire set of testing.

4.6 Coliforms count calculations

After the incubation, the Petri dishes were taken out and started counting the number of colonies on Petri dishes. Counting could be assisted by using a colony counter. There were different colours of colonies identified and were recorded; the number of colonies for each colour respectively.

4.6.1 Calculation of colony forming unit

After the counting process, the coliform density of each specimen (Colony Forming Unit, CFU/100 ml) was calculated using the formula shown below:

$$\frac{\text{coliforms}}{100\,\text{ml}} = \frac{\text{numberofcoloniescounted} \times 100}{\text{samplesize}}$$

If there was no coliform colony found, coliform colonies were counted as "<1 coliform/100 ml". For good quality of water, the occurrence of coliforms normally would be minimal. In this research, the sample size was set to be 100 ml, so the number of colonies counted on Petri dishes were directly being taken as coliform density (CFU/100 ml).

4.6.2 Average colony forming unit

For the case that the same bottle of water samples was tested twice, their respective coliform density was observed first, then followed by the calculation of average coliform density (CFU/100 ml). A geometric mean was used to obtain the average value (mean), which could be achieved by using the following method.

*Method: The N*th*Root Method:*

$$\text{Geometric Mean} = \sqrt[n]{x_1 x_2 x_3 \ldots\ldots x_n}$$

where $x_1, x_2, x_3 \ldots x_n$ are the coliform densities observed from the "n" number of specimens respectively.

For example, if a bottle of a water sample was tested twice, and the first value was 25 CFU/100 ml, the second value is 16 CFU/100 ml. By using the equation, the average coliform density was calculated as 20 CFU/100 ml.

4.7 E. Coli on EMB agar

This was additional laboratory work to confirm the colour of Escherichia Coli (E. Coli). They had used the Eosin-Methylene Blue (EMB) Agar in this research work. In this simple process, EMB agar was poured into Petri dishes.

Firstly, the inoculating loop was sterilized by burning it on Bunsen burner flame until it turned to orange colour (see Fig. 4) flame. Waited for approximately 20 s for the inoculating loop to cool down or put the loop in the solid agar. Used the inoculating loop to pick an E. Coli colony from the E. Coli culture plate. The next Streak Plate Technique was used to place the E. Coli on a fresh EMB agar plate.

The first streak was with bacteria picked, streaking like the pattern as shown in Fig. 5. After the first streak, the inoculating loop was sterilized again and proceeded to the second streak. The second streak was without picking any bacteria from the culture plate, started by touching the lines formed. This had been brought some bacteria when streaking. After finished, sterilize again the inoculating loop. The third streak will be the same as the second streak, without picking bacteria but just start by touching the line formed previously and draw a line with a zig-zag pattern as in Fig. 5.

Sealed with parafilm and labelled the petri dish, then put it in the incubator with a temperature of 35 °C for 24 h. The observation was made after incubation. The E. Coli presented as Dark Blue with Green Metallic Sheen colonies on the EMB agar plate (see Fig. 6).

4.8 Differentiation of coliform bacteria

In this stage, the bacteria colony presented on membrane filter on EMB agar which was done earlier will be transferred to Nutrient Agar using Streak Plate Technique. Nutrient Agar provides nutrients for most of the bacteria to grow. The purpose of the streak plate technique is to dilute the bacteria and end up with a single colony.

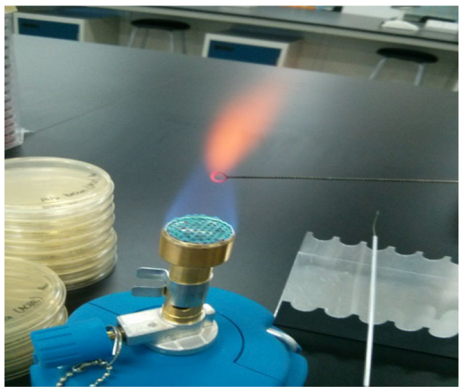


Fig. 4 Sterilization of inoculating loop using Bunsen burner

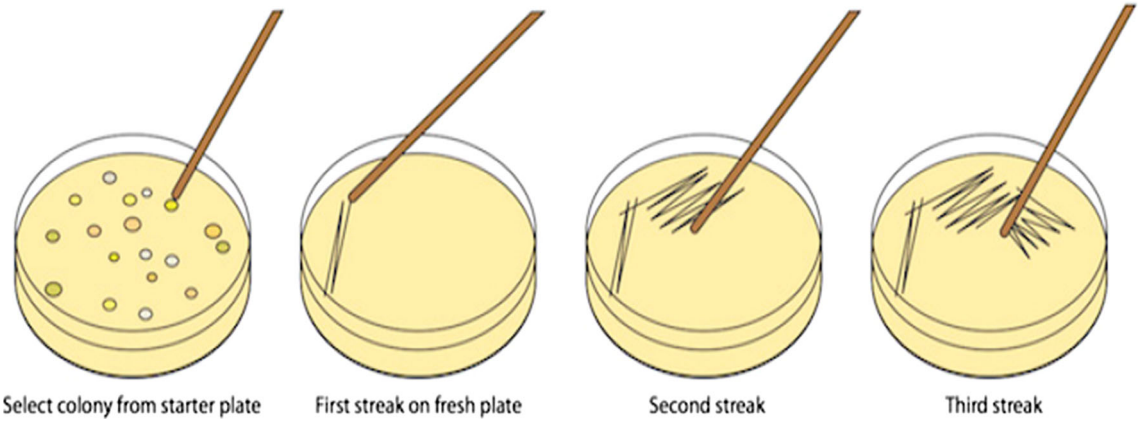
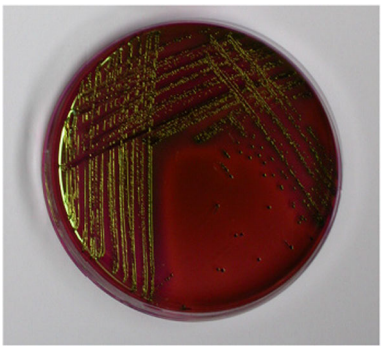


Fig. 5 Streaking pattern

Fig. 6 E. Coli on EMB Agar used



4.9 Transfer bacteria to nutrient agar

Preparation of Nutrient Agar must be done earlier, following the procedure as described in Sect. 4.3 for EMB Agar. Sterilization of inoculating loop was the same as mentioned in Sect. 5.1. Then, pick a bacteria colony on the membrane filter, and following the procedures of the Streak Plate Technique, do streaking three times.

Then sealed the Petri dishes, label them, and place them in an incubator for 24 h. Repeated these steps for bacteria colonies with different colours. After the incubation, bacteria could be observed clearly on the nutrient agar plates (see Figs. 7 and 8 for examples). Different bacteria would have different appearances on Nutrient Agar.

4.9.1 Transfer bacteria from nutrient agar to Mac Conkey agar

Mac Conkey Agar was a selective agar used to differentiate coliform bacteria and non-coliform bacteria. The Mac Conkey Agar used in this project was branded as "*Oxoid*". The preparation of Mac Conkey Agar was the same as described in Sect. 4.2. Again, bacteria grown on Nutrient Agar could be transferred to Mac Conkey Agar using Streak Plate Technique. Sterilized the inoculating loop and picked a bacterium from the nutrient agar plate. Following the procedures stated above streaking was done three times.

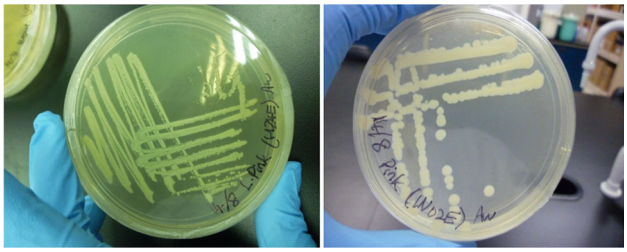


Fig. 7 Bacteria can be observed clearly on the nutrient agar plates

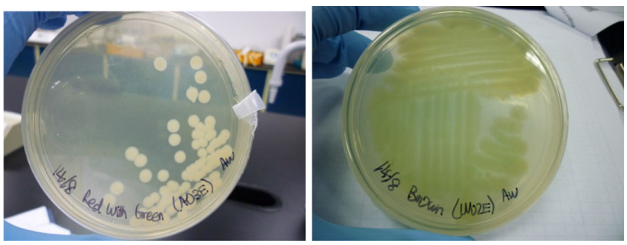


Fig. 8 Colonies of different bacteria grew on Nutrient Agar plates

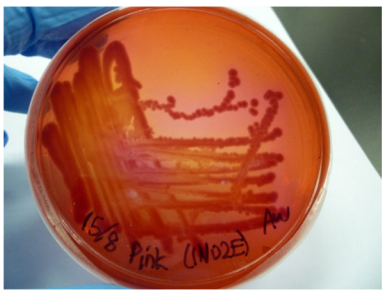


Fig. 9 Colonies of Enterobacter Aerogenes appeared on Oxoid Mac Conkey Agar

Sealed the Petri dishes, labelled them and placed them in the incubator for 24 h. Repeated these steps for different types of bacteria. The observation was done after incubation. Observed the colours and appearance of colonies presented on Mac Conkey Agar plates (see Fig. 9 for example) and the colonial characteristics of the agar.

5 Results and discussion

There were three colours of colonies presented on EMB agar plates. After the tests, the types of bacteria could be identified. If the bacterium types did not vary during the test, they should still be classified under similar categories.

5.1 Identification of bacteria

The three types of bacteria detected their colour and their appearance are given in Table 1. Enterobacter aerogenes, Staphylococcus Epidermidis and Klebsiella were the three types found in the test.

5.2 Enterobacter aerogenes

Enterobacter aerogenes appeared as hot pink colour on MacConkey Agar while E. Coli appeared as red colour on MacConkey agar. Enterobacter aerogenes causes in general, a wide range of illnesses. Common illnesses include bacteremia, septicemia, osteomyelitis, and pneumonia. It may also cause infections in the respiratory tract, gastrointestinal tract, urinary tract, and skin. It is resistant to

Table 1 Appearance of three types of bacteria on Oxoid MacConkey Agar

Colour of colonies on EMB agar	Appearance on Mac Conkey agar	Bacteria type
Red	Hot Pink colour, Shinny, Raised, smaller size	Enterobacter Aerogenes
Pink	Pale Pink Colour, dull, dry, bigger size of colonies	Staphylococcus Epidermidis
Brown	Pale Pink with brown colour colonies, Watery, Shinny, Smooth	Klebsiella

Table 2 Types of bacteria detected

Types of bacteria	Colour of appearance	Shape	Health risk	Gram reaction (±)	Surface
Enterobacter aerogenes	Hot Pink Colour under Oxoid Mac Conkey Agar	Rod	Bacteremia, septicemia, osteomyelitis and pneumonia, infection in the urinary tract and respiratory system	Gram-Negative	Smooth and Shiny
Staphylococcus epidermidis	Pale Pink Colour under Oxoid Mac Conkey Agar	Spherical	Septicemia, endocarditis, infections with intravascular devices, prosthetic joints, catheters, and large wounds	Gram-Positive	Dry and Dull
Klebsiella	Pale Pink with Brown Colour under Oxoid Mac Conkey Agar	Rod	Invasive infections, destructive pneumonia	Gram-Negative	Smooth and Shiny

most antibiotics. Hence this must be removed from the water supply. The much worrying part is that they are found even after the water is well treated and disinfected and then supplied to the consumers. The tests were conducted on the samples taken from the university campus.

5.3 Staphylococcus epidermidis

Staphylococcus Epidermidis which were also found in the treated water supply. They appeared to be spherical. It was not classified as Coliform Bacteria. But it is still dangerous for humans. It causes some infections in intravascular devices, joints, catheters, and large wounds. The effects due to the presence of these bacteria were summarized in Table 2.

5.4 Klebsiella

Klebsiella was also appearing as a little bit pale pink colour on the MacConkey Agar plate. Klebsiella had been identified with a colonising hospital patient, where the spread was associated with the frequent handling of patients. Patients with impaired immune systems are at the highest risk. For example, patients who are the elderly, with blisters or excessive deep cuts, and those who are undergoing immunosuppressive therapy. Colonization may lead to invasive infections. It may also cause serious infections such as destructive pneumonia.

5.5 Total coliform in water samples

The complete data obtained from laboratory works regarding the number of bacteria counted on EMB agar plates were tabulated. Table 3 shows the number of coliform bacteria observed in water samples collected in three weeks, which was sorted by the approximate distance measured from the water supply inlet.

5.6 Variation of total coliform with distance

Firstly, variation of total coliform bacteria with the approximate distance measured from the water supply inlet was observed. The experimental results in the 3rd Week were more reliable and consistent, as all the values were the geometric mean value of the two specimens. Therefore, the data on the 3rd week was considered in priority. In this section, water samples from 12 locations were analysed, the other 8 locations with water coolers were discussed in a separate section. Three sets of data were selected and plotted, viz., 23/7 Morning, 29/7 Morning, and 30/7 Evening (see Fig. 9).

From Fig. 10, it was observed that the coliform bacteria counts were directly proportional to the distance that water travels through. This might be governed by other factors. Generally, most of the locations had coliform bacteria less than 20 CFU/100 ml. There were two locations with an extremely high value than others. The highest one was 527 m (Block A Ground Floor Male Toilet Wash Basin, Code: A03), with 54 CFU/100 ml. The second highest was located at 613 m distance in Block C water tanks. They showed a value of 33 CFU/100 ml. According to "Malaysian National Drinking Water Quality Standards", the number of coliform bacteria should be less than 10 CFU/100 ml. Location A03 recorded a high value on average and throughout the entire research period. It might be due to the uncleanness of the water tank or pipe system in the Block A building itself. It might not be the main pipe problem or the head end.

For the case of Block C reservoir, the other two values were under 20 CFU/100 ml. The high value occurred in the evening section on a sunny day. This might be the cause as the opened-air water tank was located on the roof floor without shelter and exposed to the hot sun as in Fig. 11. The water temperature was the highest among other samples, so it might have caused bacteria to be active.

Table 3 Summary of laboratory results (sorted by distance)

No.	Sample Code	Distance from Inlet (m)	Total Coliform Bacteria Presented on Petri Dishes (CFU/100ml)											
			1st Week				2nd Week				3rd Week			
			16/7 M	16/7 E	17/7 M	17/7 E	22/7 M	22/7 E	23/7 M	23/7 E	29/7 M	29/7 E	30/7 M	30/7 E
01	IN02	0	52	27	34	≠	15	11	11	30	16	11	3	12
02	H22	304	18	24	51	≠	10	30	6	33	3	3	8	3
03	H23	316	20	16	43	≠	10	2	1	TNTC	20	20	2	12
04	H24	323	11	15	/	≠	7	9	2	24	1	1	1	5
05	A03	527	33	23	29	≠	30	27	15	23	54	TNTC	TNTC	26
06	F214	570	51	38	32	≠	10	12	4	17	6	22	6	12
07	F215	579	15	31	44	≠	≠	≠	≠	≠	≠	≠	≠	≠
08	F216	589	18	12	8	≠	17	9	16	18	7	2	3	6
09	B05	605	37	14	22	≠	5	8	5	8	4	11	9	7
10	B04	608	32	53	32	≠	≠	≠	≠	≠	≠	≠	≠	≠
11	G20	609	4	40	55	≠	2	TNTC	2	33	2	24	16	13
12	E10	612	23	12	49	≠	≠	≠	≠	≠	≠	≠	≠	≠
13	G21	612	18	12	22	≠	12	3	1	10	5	8	4	3
14	C07	613	22	32	45	≠	16	13	1	11	13	13	16	33
15	E11	614	15	15	/	≠	6	8	1	10	12	8	13	13
16	C08	630	38	29	41	≠	TNTC	15	4	6	5	8	16	16
17	B106	644	8	17	13	≠	5	6	2	18	1	0	4	9
18	F113	646	17	19	37	≠	5	2	4	2	3	2	1	4
19	F112	648	17	25	/	≠	≠	≠	≠	≠	≠	≠	≠	≠
20	F317	650	25	23	15	≠	5	3	3	14	3	2	7	12
21	D09	657	17	27	11	≠	8	3	7	13	1	1	2	5
22	N25	670	28	33	57	≠	9	TNTC	9	39	19	19	12	11
23	F319	681	8	7	/	≠	12	5	14	18	3	1	1	5
24	F318	684	27	24	TNTC	≠	≠	≠	≠	≠	≠	≠	≠	≠
25	N26	733	1	1	/	≠	12	16	1	2	2	2	1	9
26	CON01	/	/	/	/	/	/	/	/	/	1	/	/	0
	* Average value (Mean) is recorded in table if two samples are tested instead of one sample.													
	^ Total Coliform Bacteria includes *Enterobacter Aerogenes* and *Klebsiella*.													

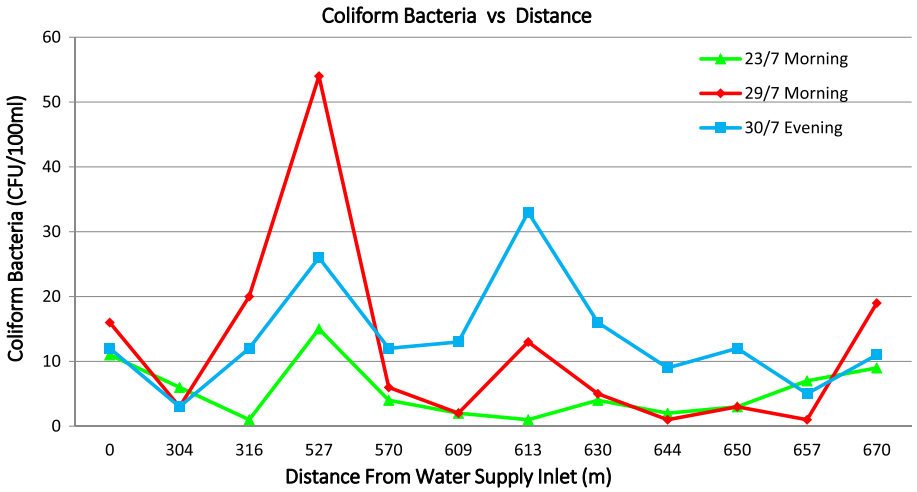


Fig. 10 Comparison figure on day 23/7, 29/7 and 30/7

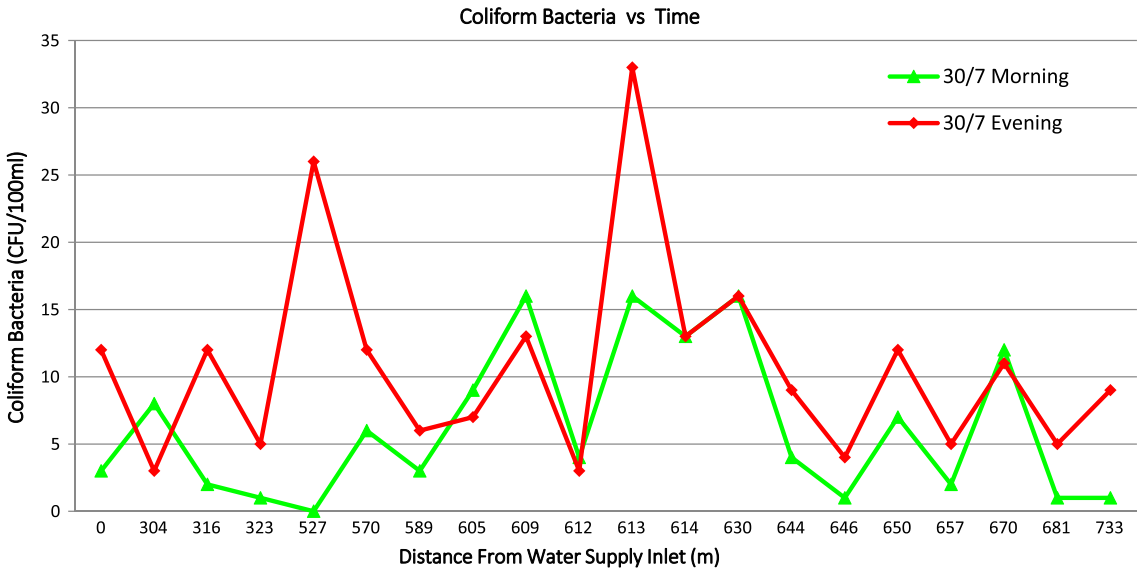


Fig. 11 Coliform bacteria (CFU/100 ml) vs Time on day 30/7

5.7 Variation of total coliform with time

Water samples were collected in the morning section and evening section to observe the variation of total coliform bacteria in a single day. In this section, water samples from 20 locations were analysed. Data on days 23/7 and 30/7 are selected and the relationships were plotted in Figs. 12 and 13 respectively.

From both figures, it could be observed that the red colour lines were slightly higher than the green colour lines, meanwhile maintaining the overall trend of fluctuating. This trend showed that there were more coliform bacteria found in water samples during the evening section compared to the morning section. The temperature plays a role to affect the number of coliforms.

The temperature was one of the important parameters that influenced the regrowth processes of bacteria. According to LeChevallier (1990), water temperature above 15 °C significantly increases bacterial growth. Another study by Fransolet et al. (1985) stated that the temperature of water not only influences the growth rate of microorganisms, but also the affecting lag phase and cell yield. More coliforms occurrences were found during the summer period compared to other months.

5.8 Total coliform in drinking water

In this section, water samples collected from water coolers were analysed. The samples are from 8 water coolers around campus. Data on day 29/7 were plotted in Fig. 13, with coliform bacteria (CFU/100 ml) against distance.

Water coolers were operating independently, so there was no trend showing their relationships with distance or time. However, a comparison could be made between water coolers and water tanks associated. For instance, 733 m was a water cooler at Block N

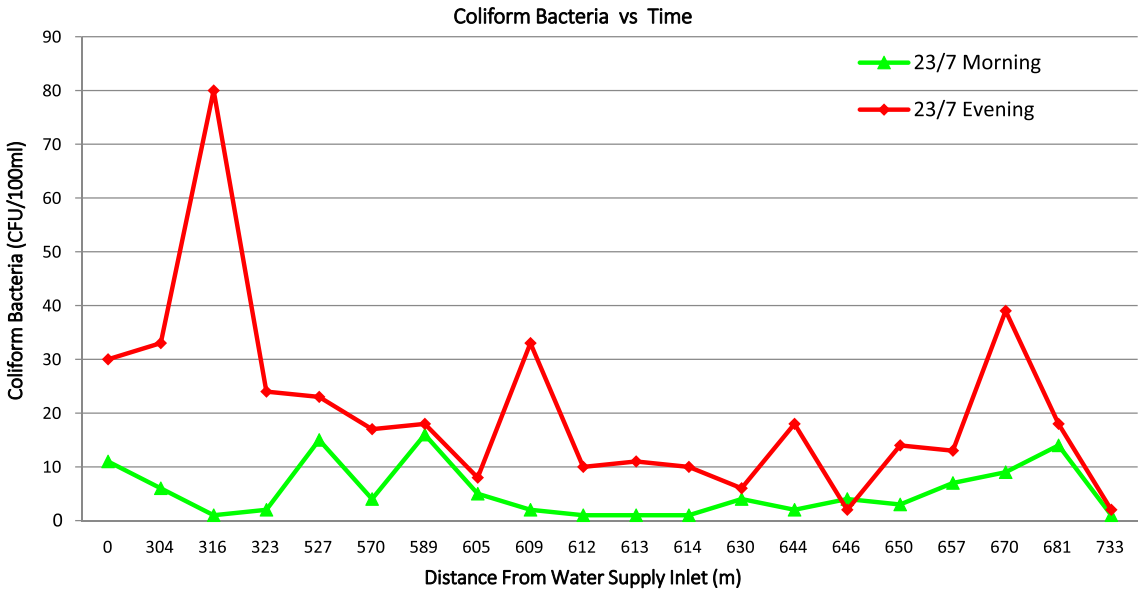
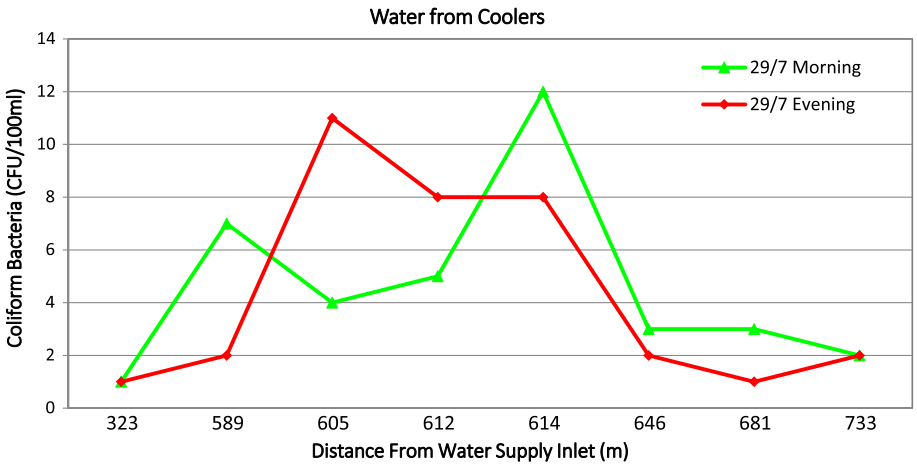


Fig. 12 Coliform bacteria (CFU/100 ml) vs Time on day 23/7. *80 denoted

Fig. 13 Samples from water coolers collected on 29/7



Research Building. The coliform bacteria were recorded as 2 CFU/100 ml for morning and evening on 29/7. Meanwhile, the water tank at Block N (670 m) had a value of 19 CFU/100 ml for both morning and evening on the same day. This showed that a water cooler serves its function to filter and trap microorganisms to provide safe drinking water.

The comparison of the number of coliform bacteria with Malaysian Nation Drinking Water Quality Standards expressed the following. It is stated that coliform organisms should be less than 4 CFU/100 ml for two consecutive samples. On the University campus, some water from coolers were within the limits while some others were beyond the standards. For example, water coolers at Block E which was located at 614 m and at Block B which was located at 605 m had hit 12 CFU/100 ml and 11 CFU/100 ml. It was recommended to have proper cleaning of water coolers to ensure that they function properly.

5.9 Possible sources of total coliform occurrence

It was very crucial to understand the occurrence of total coliform in the treated water supply, including the water distribution system. This section will indicate some of the possible causes of total coliform occurrence in the treated water supply.

5.9.1 Soil and water surrounding the pipes

Soil and water surrounding the pipes could be possible sources of contamination. The contaminant sources might be due to natural soil, animal waste, human waste, dead animals, incidental dirty water, or trash and rubbish. During the installation or maintenance work of pipelines, there were some potential contamination sources such as unsanitary human contact, sewage water, or agricultural runoff in the trench.

During construction, pipes were usually stored at the construction site without protective caps, and that would have provided a chance for them to be contaminated with soil and mud. This would lead to violations of water quality issues such as high heterotrophic bacteria counts and the occurrence of coliform bacteria.

5.9.2 Sediments

Sediments could be the possible cause of contamination as sediment accumulation will provide a habitat for microbial growth in the water distribution system. This will eventually provide microorganisms with protection against disinfectants. (USEPA, 1992).

5.9.3 Biofilm and microbial growth

There are many pathogens found in these biofilms, which had shown that they are protected from disinfectants. Due to long-term effects, these biofilms-related pathogens would have caused persistent detection leading to waterborne diseases. Biofilms are described as a complex mixture of organic, inorganic material, and microbes that accumulated on microbially-produced organic polymer matrix attached to the inner surface of the pipe distribution network (USEPA, 2002).

5.9.4 Finished water storage facilities

It was found that one of the possible pathways for coliform bacteria to enter the water distribution system was the storage tank deficiencies. For example, inadequate hatches and vents without a screen would have caused microbial proliferation. Microorganisms might have entered the system from surface runoff, especially at those ground storage tanks. For instance, the surface runoff from the roof of the water tank might seep into water tanks during heavy run under the overflow of the vent.

5.9.5 Cross connections

There are further causes that due to the connection of potable water systems network that was lead through industries and commercial processing yards. EPA has proof that this was one of the pathways for bacteria to enter the water distribution system and caused violations.

5.9.6 Hydraulic conditions

Hydraulic conditions were classified as one of the driving forces that allowed total coliforms to proliferate in the water distribution system, providing a source of contaminants. Contamination intrusion was most likely to occur when there was very low pressure occurred within the pipeline at the leakage point, while external pressure was higher. Low-pressure conditions in the water distribution system may be dangerous. Sometimes, due to backflow occurrences. That means whenever the outside source got pressurised by air or gas, it would have resulted in higher temperatures compared to the inner pressure.

5.9.7 Others

Other causes might be the occurrences of total coliform bacteria at locations such as operations and maintenance practices of routine water main flushing and cleaning, the retention time of water in the water distribution network, treatment breakthrough, and weather-related events such as flood and drought. Hence these locations were chosen for samples collection and study. Moreover, laboratory testing was done to differentiate coliform and non-coliform bacteria. The possible sources of contamination and causes were analysed and presented here. Thus, these problems should be overcome in the campus so that the contamination can be minimised, and the water quality is improved to fulfil Malaysia's drinking water quality standards.

6 Recommendations

Water coolers in UNMC must be cleaned thoroughly and maintained properly to make sure that it provides safe drinking water which fulfils Malaysian standards. Besides, the filter in the water supply inlet must be enhanced, as all the water entering campus will pass through the water filter.

Water tanks of each building must be cleaned at a frequent interval of time. Also, check the pipeline regularly to make sure there is no pipe burst along with the distribution system. A further test should be carried out to determine the type of bacteria accurately. In microbiology, some other tests are available such as Coagulase Test, Urease Test, Methyl Red Test, and so on. Hence, further sources can be found to safeguard the water supplies and public health.

7 Limitations in the study

Firstly, the water samples collected are limited to 25 locations, this may not be sufficient to cover the water supply on the entire campus. Although overall water quality could be observed through the data available, it is good to have more detailed and accurate experimental data by considering more locations in the water collection and sampling stage itself.

The information obtained from Estate Office is only the material used for the pipeline on campus, which is Galvanized Iron (GI) pipe. There is inadequate information about the distribution network such as positions of the pipeline (no provision of M&E drawing). Hence, the distance measured from the water supply inlet is only an approximate value, without knowing the exact position of the water pipe.

Furthermore, there is a chance of contamination of samples during laboratory procedures. The requirements stated were followed strictly with standard methods, but it cannot guarantee that no contamination had happened during the process. For the additional test to differentiate the type of bacteria, more testing should be conducted in the microbiology lab to identify the bacteria more accurately. The types of bacteria detected might be varied as only the agar method was used in this research. Besides, there might be other bacteria existing in the water supply of campus, but not being found or observed in the current study. This is due to the limitation of agar used as well. The agar used is a selective medium so not all bacteria can grow on a particular medium. An extensive microbiological study would reveal the causes and effects due to Total coliform violations in the complied NH2CL, O3, and UV treated municipal water supply system.

8 Conclusions

At the end of this research, the presence of total coliform bacteria in the treated water supply to UNMC is investigated, with daily and weekly results for comparison. Data is analysed together with plots to observe the variation of total coliform bacteria in water samples collected on campus, with consideration of the distance that water travel through pipe and time.

Water is an important resource for everyone's life, as it serves many purposes, such as washing, cleaning, bathing, and drinking, and thus for UNMC. Water quality becomes crucial, especially the water coolers which provide direct drinking water and laboratory testing. Hence, the test and analysis included in this project are significant and worthy to run the education industry [7].

In addition, differentiation of bacteria was done in the laboratory. The three types of bacteria detected are Enterobacter aerogenes (coliform), Staphylococcus Epidermidis (non-coliform), and Klebsiella (coliform). E. Coli is absent in findings but doesn't mean that it does not exist in the water supply. Data interpretation and discussion are done based on the number of total coliform bacteria which are more significant.

Possible sources of total coliform violations are studied and listed in the previous section. In the UNMC case, unclean water tanks with sediments are found to be one of the causes. The indoor water tanks are located in rooms that are warm and lack ventilation, which may ease the growth of bacteria. Besides, only one filter is available at the water inlet, and it is cleaned once a day. It may not be sufficient as water demand on campus is extremely high.

Moreover, the investigation also focused on direct drinking water from water coolers on campus. Data is plotted and compared to "Malaysian National Drinking Water Quality Standards". The number of coliforms is higher than standards at some water coolers but overall, they show a consistent trend. planned in Although this is an inexpensive method, the main advantage lies in the effectiveness of the system in reducing the effects of unmeasured disturbances. However, this requires the measurement of all state variables [16]. Future research should combine different tools to study and link microbial diversity and activity to better understand the relationship between microbes and system function.

Data availability This manuscript has no associated data or the data will not be deposited. [Authors'comment: There are no associated data available.]

References

1. N.H. Martin, A. Trmcic, T. Hsieh, K.J. Boor, M. Wiedmann, The evolving role of coliforms as indicators of unhygienic processing conditions in dairy foods. Front. Microbiol. **7**, 1549 (2016). https://doi.org/10.3389/fmicb.2016.01549
2. Bahagian A S (2019) Malaysia. Environmental quality report. Department of Environment, Ministry of Science. Technology and the Environment
3. R. Assefa, R. Bai, S. Leta, H. Kloos, Nitrogen removal in integrated anaerobic-aerobic sequencing batch reactors and constructed wetland system: a field experimental study. Appl. Water Sci. **9**(5), 1–11 (2019)
4. A.A. Mohd, S. Salmiati, M. Marpongahtun, R.S. Mohd, J.A. Lolo, S. Achmad, Biointerface Res. Appl. Chem. **10**, 6253 (2020)
5. N. Elfikrie, Y.B. Ho, S.Z. Zaidon, H. Juahir, E.S.S. Tan, Occurrence of pesticides in surface water, pesticides removal efficiency in drinking water treatment plant and potential health risk to consumers in Tengi River Basin Malaysia. Sci. Total Environ. **712**, 136540 (2020)
6. B. Petrie, R. Barden, B. Kasprzyk-Hordern, A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas, and recommendations for future monitoring. Water Res. **72**, 3–27 (2015)
7. B.G. Ramani, R.T. Mohamed, Water quality in health care. Int. J. Environ. Technol. Manage. **9**(1), 125–140 (2008)
8. M. Lee, E. McBean, C. Schuster, J. Huang, Use of a fuzzy logic model to investigate potential failures of drinking water systems. Proc. World Environ. Water Resour. Congr. **9**, 1–9 (2007)
9. M. Polit, A. Genovesi, B. Claudet, Fuzzy logic observers for a biological wastewater treatment process. Appl. Numer. Math. **39**(2), 173–180 (2001)

10. V. Ramani Bai, G. Vanitha, A.R.Z. Ariff, Effective hospital waste classification to overcome occupational health issues and reducing waste disposal cost infect control hospital. Epidemiology **34**, 1234 (2013). https://doi.org/10.1086/67346
11. B.V. Ramani, S. Mohan, R. Kabiri, Towards a database for an information management system on climate change an online resource, in *Climate change and the sustainable use of water resources climate change management*. ed. by F.W. Leal (Springer, 2011)
12. T. Mitra, R. Rjmandi, M. Shayeghi, S.M. Monavari, A. Karbassi, Application of multivariate statistical methods to optimize water quality monitoring network with emphasis on the pollution caused by fish farms. Iran J. Publ. Health **46**(1), 89–92 (2017)
13. A. Afshar, M.A. Marino, M. Ebtehaj, J. Moosavi, Rule-based fuzzy system for assessing groundwater vulnerability. J. Environ. Eng. **133**(5), 532–540 (2007)
14. W. Ocampo-Duque, N. Ferre-Huguet, J.L. Domingo, M. Schuhmacher, Assessing water quality in rivers with fuzzy inference systems: a case study. Environ. Int. **32**(6), 733–742 (2006)
15. Y. Lee, M. Dahab, I. Bogardi, Nitrate-risk assessment using fuzzy-set approach. J. Environ. Eng. **121**(3), 245–256 (1995)
16. P. Teppola, P. Minkkinen, Possibilistic and fuzzy C-means clustering for process monitoring in an activated sludge waste-water treatment plant. J. Chemo.: J. Chemo. Soc. **13**(3–4), 445–459 (1999)
17. E.F. Carrasco, J. Rodriguez, A. Punal, E. Roca, J.M. Lema, Rule-based diagnosis and supervision of a pilot-scale wastewater treatment plant using fuzzy logic techniques. Expert Syst. Appl. **22**(1), 11–20 (2002)
18. I. Douterelo, J.B. Boxall, P. Deines, R. Sekar, K.E. Fish, C.A. Biggs, Methodological approaches for studying the microbial ecology of drinking water distribution systems. Water Res. **65**, 134–156 (2014)
19. B.V. Ramani, R. Bouwmeester, S. Mohan, Fuzzy logic water quality index and importance of water quality parameters. Air, Soil Water Res. **2**, 51–59 (2009)
20. Ramani Bai V., Tkalich P., Mohan S., WebGIS based Database Information and Management System (DIMS) for India, Malaysia and Singapore. Junral Teknologi, **78**(8.6), 53–59, (2016). https://jurnalteknologi.utm.my/index.php/jurnalteknologi/article/view/9639
21. T.N. Cheema, M.A.Z. Raja, I. Ahmad, S. Naz, H. Ilyas, M. Shoaib, Intelligent computing with Levenberg–Marquardt artificial neural networks for nonlinear system of COVID-19 epidemic model for future generation disease control. Eur. Phys. J. Plus **135**(11), 1–35 (2020)
22. R. Sadiq, Y. Kleiner, B. Rajani, Water quality failures in distribution networks-risk analysis using fuzzy logic and evidential reasoning. Risk Anal.: Off. Publ. Soc. Risk Anal. **27**, 1381–1394 (2007)
23. Cotruvo, J. A (2017) WHO guidelines for drinking water quality first addendum to the fourth edition. J Am Water Works Assoc **109**(7): 44–51
24. World health organization vector-borne diseases, media center: Accessed March 2020. https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases, (2016).