

DETECTION AND ENUMERATION OF COLIFORMS IN TUBE WELL WATER COLLECTED FROM UNIVERSITY OF YANGON CAMPUS

Mya Phyo Nandar¹, Khine Zar Ni²

Abstract

The aim of this study was to determine the hygienic status of the tube well water supply from Yangon University campus. A total of ten water samples were collected from the tube wells in the campus. Microbiological analysis was carried out at the Microbiology laboratory, Department of Zoology, University of Yangon and water quality parameters were examined at the Water and Soil Examination Laboratory, Thaketa Township. Water samples were analyzed bacteriologically for total coliform and fecal coliform counts by the MPN method. The highest total coliform counts of >1100 MPN/100mL were observed in the water samples of V, VI, VII and X. The highest fecal coliform counts of >1100 MPN/100mL were found in the water samples of V, VI and VII. Water samples were then treated with calcium hypochlorite to reduce the contamination of bacteria. After chlorination, the highest total coliform counts of 4 MPN/100mL were detected in water samples I and II. But the fecal coliform counts could not be found in all the water samples after the treatment. Regarding the identification of bacteria, five groups of bacterial species were isolated, namely *Escherichia coli*, *Klebsiella* sp., *Salmonella* sp., *Enterobacter* sp. and *Pseudomonas* sp. After chlorination, these genera of bacteria could not be isolated from all water samples. Analysis of water physico-chemical parameters revealed that pH ranged from 5.0 to 7.3; temperature ranged from 25°C to 28.1°C; total dissolved solids (TDS) ranged from 8mg/L to 12mg/L. Temperature and TDS values were normal according to WHO (2011), but pH values were out of the WHO guideline values for drinking water. According to the present study, tube well water in University of Yangon campus should be chlorinated to kill and reduce bacteria levels and to get safe water for public utilization.

Keywords Total coliform counts, fecal coliform counts, chlorination, physico-chemical parameters

Introduction

Water is very important for survival and growth of all living organisms. Actually, 70 percent of the human body is made up of water. The body is helped to metabolize fat by water that also helps us maintain our body temperature through perspiration. Dehydration is tended to give rise by lack of water in the body, thereby posing hurdles for the blood to circulate (Skinner and Carr, 1976).

Nowadays, increased human population, industrialization, use of fertilizers in the agriculture and man-made activities make natural water highly polluted with different harmful contaminants. So, water pollution caused by harmful microorganisms, is a global problem. It is difficult and expensive to test water for each of these germs. Instead, public health workers measure coliform bacteria levels in order to know water quality. When coliforms are present in drinking water, it is suggested that there may be feces and disease-causing agents in the water. The most commonly used indicators of fecal pollution in water and food are fecal coliform bacteria. Direct person to person contact, contaminated food, and contaminated water transmit the diseases (Pleczar *et al.*, 1974).

Tube well water is commonly used and very useful in our daily life. But, adequate construction and well protection is vital to get clean tube well water. Tube well water may have several pathogenic bacteria. Some of these may be *Escherichia coli*, *Klebsiella* species, *Salmonella* species, *Enterobacter* species, and *Pseudomonas* species, etc. Most of these bacteria are harmful to humans.

¹ Department of Zoology, University of Yangon

² Department of Zoology, University of Yangon

One of the most commonly used water purifying methods is chlorination. Chlorine is added to water either as elemental chlorine (chlorine gas), or chlorinating chemicals such as calcium hypochlorite (tablets or granules) or solutions of sodium hypochlorite (liquid bleach). In chlorination of a piped distribution system, it is desirable to maintain free chlorine residues of concentration 0.2-0.5mg/liter throughout, to reduce the risk of microbial regrowth and the health risk of recontamination WHO (1997). For domestic water use, the WHO safe water system for household disinfection in developing countries allows a dosage of 1.875 or 3.75 mg/L of sodium hypochlorite with a contact time of 30 minutes.

In University of Yangon, tube well water is used primarily as a source for drinking, food preparation, showering, washing and gardening. People in University of Yangon campus who use tube wells assumed them as safe water source. Since there is paucity of information concerning microorganisms in the tube well water, the present research was conducted to assess the coliform levels in the tube well water, to isolate and identify the bacteria from the tube well water before and after treatment with calcium hypochlorite and to examine the effectiveness of calcium hypochlorite for control of pathogenic bacteria contaminated in the tube well water.

Materials and Methods

Study areas and sites

University of Yangon campus (16°49'44"N and 96°08'15"E) and Fisheries and Aquaculture compound (16°50'05"N and 96°08'13"E), University of Yangon were selected as study areas (Fig.1). A total of ten water samples were collected from tube wells (110-200 ft) near the departments and hostels at the University of Yangon. Samples from the sites designated as I, II, III, V, VI, VII, VIII, IX and X were collected from water taps of Zoology Department, Inya Hostel, Shwebo Hostel, University Laboratory Building, Professors' Houses, International Cooperation Office, Thiri Hostel, University of Yangon Research Center and Fisheries and Aquaculture building respectively. Sample-IV was directly collected from the tube well near Staffs' Housing.

Study period

The study period was from February to August, 2022.

Sampling methods

Water samples were collected in three sterile glass bottles with caps per sample with the amount of approximately 1000 ml per bottle and packed inside black plastic bags to prevent from light. Two glass bottles were carried to the Microbiology Laboratory, Department of Zoology, University of Yangon. After arrival at the laboratory, one of the collected two glass bottles was treated with calcium hypochlorite (2mg/L). And these two glass bottles (one with calcium hypochlorite and another one without calcium hypochlorite) were studied in two steps: first step for MPN (Most Probable Number) after Brown (2007) and second step for isolation and identification of bacteria, which was done after Atlas (1995) and, Dubey and Maheshwari (2002). And then, the third collected glass bottle of water was sent to the Water and Soil Examination Laboratory, Freshwater Aquaculture Research, Aquaculture Division, Department of Fisheries, Ministry of Agriculture, Livestock and Irrigation, Thaketa Township for measurement of the physico-chemical parameters (except temperature) according to WHO (1993). Temperature was measured at the sampling sites by using a mercury thermometer (Plate 1).

Characterization of the isolated bacteria

Isolated bacteria were characterized by means of colonial morphology, Gram-staining reaction, motility test, and biochemical reactions (Hucker and Conn, 1923).

Colonial morphology

The characteristic features of colony morphology such as colour, shape, surface, elevation and edges of colonies were determined after methods of Bisen and Verma (1998).

Gram-staining

The most widely used bacteriological stain, the differential Gram-stain, was used to observe the Gram-staining nature and shapes of the bacterial cells.

Biochemical tests

Confirmation of bacteria by biochemical reactions was based on the methods as described by Collins and Lyne (1995), Bisen and Verma (1998) and HiMedia (1998).

Identification of bacterial isolates

To determine the identification of the isolates, colonial and cell morphology, Gram-nature and biochemical properties recorded during the work were compared to those described in Bergey’s Manual of Determinative Bacteriology by Breed, Murry and Smith (1994) and Cowan and Steel’s Manual for the Identification of Medical Bacteria (Cowan, 2009).



A. University of Yangon campus



B. Fisheries and Aquaculture compound, University of Yangon

Figure 1 Map showing locations of University of Yangon campus and Fisheries and Aquaculture compound (Source: from Google Earth, 2021)



A. House near tube well (Department of Zoology)



B. Decaying leaves on storage reservoir (Inya Hostel)



C. Toilet near tube well
(University Laboratory Building)



D. Toilet near storage reservoir
(University Laboratory Building)

Plate 1 Surrounding conditions of some studied tube wells and water storage reservoirs

Results

Most Probable Number of total and fecal coliforms in the water samples before treatment with calcium hypochlorite

Total coliforms and fecal coliforms were detected using MPN method for the water samples of ten sampling sites. Sample-I (near Department of Zoology) had the total coliform count of 150 MPN/100mL and the fecal coliform count of 93 MPN/100mL. Sample-II (near Innaya Hostel) had higher total coliform count of 460 MPN/100mL and the fecal coliform count of 460 MPN/100mL. Sample-III (near Shwe-bo Hostel) also had higher total coliform count of 460 MPN/100mL and the fecal coliform count of 460 MPN/100mL. Sample-IV (near Staffs' Housing) had the lowest total coliform count of 23 MPN/100mL and the lowest fecal coliform count of 23 MPN/100mL. Sample-V (near University Laboratory Building) had the highest total coliform count of >1100 MPN/100mL and the highest fecal coliform count of >1100 MPN/100mL. Sample-VI (near Professors' Housing) also had the highest total coliform count of >1100 MPN/100mL and the highest fecal coliform count of >1100 MPN/100mL. Sample-VII (near International Cooperation Office) also had the highest total coliform count of >1100 MPN/100mL and the highest fecal coliform count of >1100 MPN/100mL. Sample-VIII (near Thiri Hostel) had the low total coliform count of 43 MPN/100mL and the low fecal coliform count of 43 MPN/100mL. Sample-IX (near University of Yangon Research Center) had the total coliform count of 210 MPN/100mL and the fecal coliform count of 93 MPN/100mL. Sample-X (Fisheries and Aquaculture building compound) had the highest total coliform count of >1100 MPN/100mL and the fecal coliform count of 1100 MPN/100mL (Fig. 2).

Most Probable Number of total and fecal coliforms in the water samples after treatment with calcium hypochlorite

After treatment with 2mg/L calcium hypochlorite with a contact time of 30 minutes, total coliform counts and fecal coliform counts were considerably lowered in all water samples. After treatment, samples-III, IV, V, VI, VII and VIII had no total coliform and fecal coliform counts. All samples had no fecal coliform counts. Sample-I and II had very low total coliform counts of 4 MPN/100mL respectively. Sample-IX and X had total coliform counts of 3 MPN/100mL (Fig. 3).

Characterization and identification of bacteria isolates

A total of five different colony types were detected from the culture media (Table 1). Morphology of the colonies of the isolates were found as follow:

Colonial morphology of identified genera of bacteria

Colony shape, edge, elevation, texture, and colour were observed in the following isolates.

Escherichia coli

Escherichia coli colonies on selective agar were detected as circular in shape with smooth edge, flat and glistening surface and a metallic sheen.

Klebsiella sp.

Klebsiella sp. as pure isolate had pink to purple colonies, circular in shape with entire edge, convex and smooth.

Salmonella sp.

Salmonella sp. as pure culture on EMB agar gave colonies pink in colour, circular in shape with entire margin, convex, smooth and gummy.

Enterobacter sp.

Enterobacter sp. as pure isolate had colonies circular in shape with entire margin, convex and pink in colour on EMB agar.

Pseudomonas sp.

Pseudomonas sp. as pure colony was purple in colour, circular in shape, entire margin, convex and smooth on EMB agar (Table 1).

Gram staining reactions and cell shapes of identified genera of bacteria

After recording the colonial morphology of the isolates, gram-staining properties and cell shapes were observed using Gram-staining method. All isolates were Gram-negative rod shape bacteria that showed pink to purple colour under oil immersion magnification (Table 1).

Biochemical tests

Indole production test, Methyl red test, Voges-Proskauer test, Citrate utilization test, Carbohydrate formation test (TSI) and H₂S production test were performed to identify the isolated bacteria. Detailed results of the biochemical tests of the five identified genera of the isolated bacteria are described and shown in (Table 1 and Plate 2).

Motility test for identified genera of bacteria

Escherichia coli, *Salmonella* sp., *Enterobacter* sp. and *Pseudomonads* sp. showed motility by spreading growth in the Sulphide Indole Motility (SIM) semisolid medium. But *Klebsiella* sp. did not show any motility in the SIM semisolid medium (Table 1 and Plate 2).

Occurrence of genera of bacteria isolates in the water samples before treatment with calcium hypochlorite (2mg/L)

Five genera of bacteria, *Escherichia coli*, *Klebsiella* sp., *Salmonella* sp., *Enterobacter* sp. and *Pseudomonads* sp. were discovered in the water samples. *Escherichia coli* was found in the samples I, IV, VI and VIII. *Klebsiella* sp. was found in the samples V, VI, VII and X. *Salmonella* sp. was found in the samples II and VII. *Enterobacter* sp. was found in the samples I and X. *Pseudomonads* sp. was found in all samples I, II, III, IV, V, VI, VII, VIII, IX and X. Unidentified spp. were found in the water samples IV, VI and VII. After treatment with 2 mg/L calcium hypochlorite, the five genera of bacteria isolates were no longer detected in all water samples.

The physico-chemical parameters of the water samples

The pH of water samples ranged from 5.0 to 7.3. Temperature value ranged from 25°C to 28.1°C. The total dissolved solids ranged from 8mg/L to 12mg/L. The dissolved oxygen ranged

from 4.5mg/L – 7.3mg/L. The biochemical oxygen demand (BOD) ranged from 0mg/L to 2.5mg/L. The chemical oxygen demand (COD) ranged from 0.73mg/L to 11.04mg/L. (Table 2).

Table 1 Morphological characters and biochemical properties of the identified bacteria isolates from the water samples

Sr no.	Morphological Tests			Biochemical Tests									Identified bacteria isolates
	Colony Morphology	Gram Stain	Shape	TSI			Cit	MR	VP	SIM			
				butt	slant	gas				H ₂ S	I	M	
1.	Metallic sheen, circular, entire, flat and glistening on EMB agar	-	Rods	A	A	+	-	+	-	-	+	+	<i>Escherichia coli</i>
2.	Pink, circular, entire, convex, smooth on EMB agar	-	Rods	V	V	-	+	V	V	-	V	-	<i>Klebsiella</i> sp.
3.	Pink, circular, entire, convex, smooth and gummy on EMB agar	-	Rods	A	K	-	+	+	-	V	-	+	<i>Salmonella</i> sp.
4.	Pink, circular, entire, convex, smooth on EMB agar	-	Rods	A	A	-	+	V	+	-	-	+	<i>Enterobacter</i> sp.
5.	Purple, circular, entire, convex, smooth on EMB agar	-	Rods	V	K	+	+	V	-	-	-	+	<i>Pseudomonas</i> sp.

TSI = Triple Sugar Iron

Cit = Simmon's citrate

MR = Methyl Red

VP = Voges-Proskauer

SIM = Sulphide Indole Motility

I = Indole

(+) = acid formation or positive reaction

(-) = no change

A = acid

K = alkaline

V = variable (positive or negative)

M = motility

Table 2 Physico-chemical parameters of the water samples collected from ten sampling sites

Parameter	I	II	III	IV	V	VI	VII	VIII	IX	X	WHO Drinking Water Standard*
pH	6.0	5.0	5.5	5.0	6.0	5.5	5.2	5.5	7.3	6.5	6.5-8.5
Temperature (°C)	25.6	27.5	26	27.4	26.1	28.1	26.4	25	27	27	20-30
TDS (mg/L)	8	10	10	8	12	10	8	10	10	8	< 600
DO (mg/L)	4.5	5.0	5.5	5.0	6.0	5.5	5.2	5.5	7.3	6.5	NG
BOD ₅ (mg/L)	0.5	0	0.75	2.5	1.5	1.75	2.5	0.5	0.5	0.75	NG
COD (mg/L)	1.84	5.88	4.78	3.68	3.68	3.68	6.25	5.88	0.73	11.04	NG

*Guidelines for Drinking Water Quality, 4th ed. World Health Organization, 2011

TDS = Total Dissolved Solids

DO = Dissolved Oxygen

BOD₅ = Biochemical Oxygen Demand

COD = Chemical Oxygen Demand

NG = No Guideline

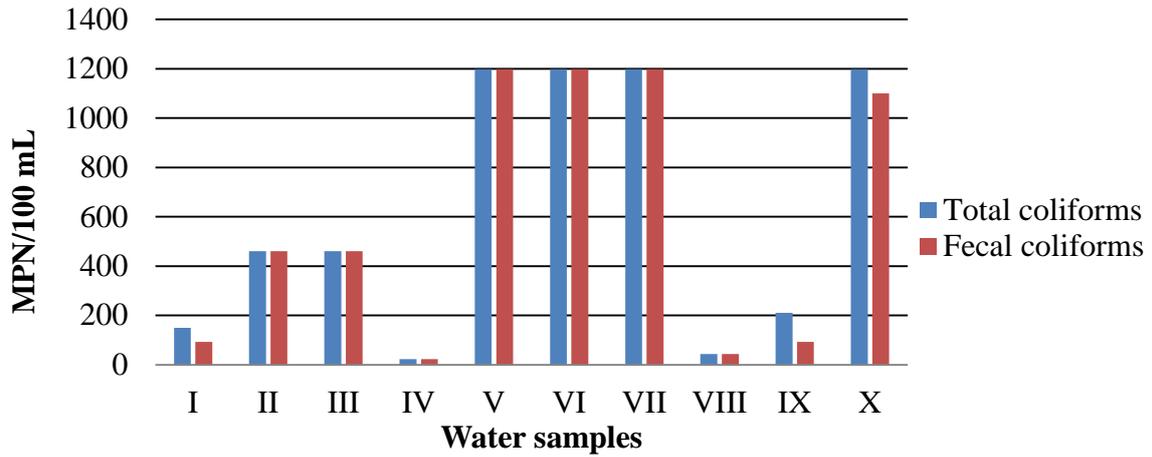


Figure 2 Total coliform and fecal coliform counts of the water samples before treatment with calcium hypochlorite

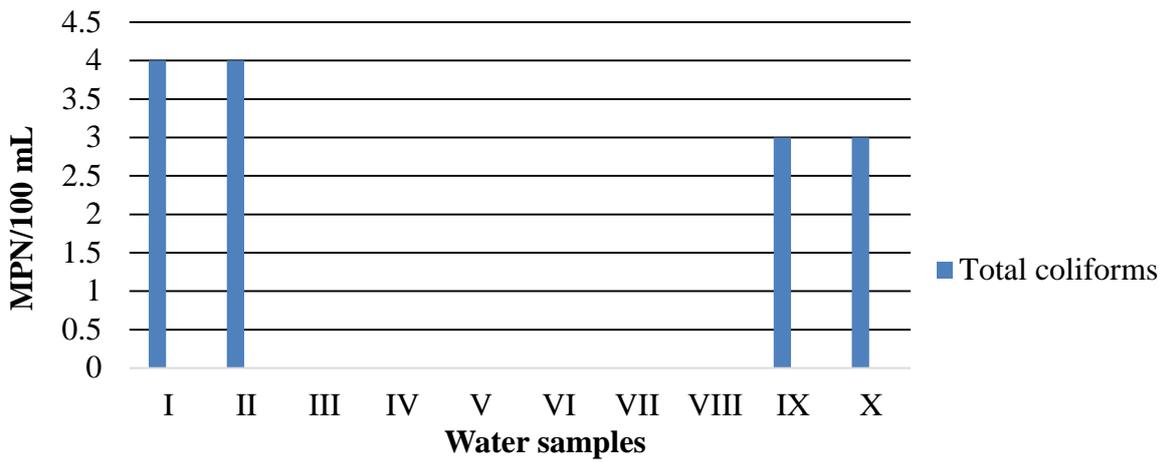


Figure 3 Total coliform counts of the water samples after treatment with calcium hypochlorite (2mg/L)



A. *Escherichia coli* colonies on EMB agar

B. Biochemical tests for *E. coli*

C. *Pseudomonas* sp. colonies on EMB agar

D. Biochemical tests for *Pseudomonas* sp.

1. TSI = Triple Sugar Iron
2. Simmon's Citrate
3. MR = Methyl Red
4. VP = Voges-Proskauer
5. SIM = Sulphide Indole Motility

Plate 2 Some morphological and biochemical characteristics of some bacteria isolates

Discussion

In the present study, a total of 10 water samples from different tube wells in University of Yangon campus were collected and analyzed to assess the levels of total coliform and fecal coliform contamination. The water samples were then treated with 2 mg/L calcium hypochlorite with a contact time of 30 minutes. Before treatment with calcium hypochlorite, the total coliform counts of samples I, II, III, IV, V, VI, VII, VIII, IX and X are 150 MPN/100mL, 460 MPN/100mL, 460 MPN/100mL, 23 MPN/100mL, >1100 MPN/100mL, >1100 MPN/100mL, >1100 MPN/100mL, 43 MPN/100mL, 210 MPN/100mL and >1100 MPN/100mL respectively. And the fecal coliform counts of samples I, II, III, IV, V, VI, VII, VIII, IX and X are 93 MPN/100mL, 460 MPN/100mL, 460 MPN/100mL, 23 MPN/100mL, >1100 MPN/100mL, >1100 MPN/100mL, >1100 MPN/100mL, 43 MPN/100mL, 93 MPN/100mL and 1100 MPN/100mL respectively. The highest total coliform counts and fecal coliform counts were found in the water samples of S-V (University Laboratory Building), S-VI (Professors' Housing), S-VII (International Cooperation Office) and S-X (Fisheries and Aquaculture Building). The tube well and storage reservoir of S-V is very near to the toilet. There are trees and shrubs around the tube well of S-VI and it seems people also take bath near the tube well because of the existence of a shower facility. And the tube well of S-VII is near to a house and there are some rubbish around the tube well. Furthermore, the storage reservoir of S-VII tube well is very near to the house. Moreover, the tube well of S-X is near a garbage dump.

The second highest total coliform and fecal coliform counts were found in the water samples of S-II (Inya Hostel) and S-III (Shwebo Hostel). It might be due to the presence of decaying leaves on the storage tank of S-II or due to contamination of water pipe and water tap. And, in S-III, the tube well is near to a house. The third highest total coliform and fecal coliform counts were found in the water sample of S-IX (University of Yangon Research Center) because the tube well of S-IX is located between two houses. The fourth highest total coliform and fecal coliform counts were found in the water sample of S-I (Department of Zoology) because the tube well of S-I is near a house.

After treatment with calcium hypochlorite (2mg/L), the highest total coliform counts were recorded in the water samples of S-I (Department of Zoology) and S-II (Inya Hostel) which were 4 MPN/100mL but fecal coliform counts were not observed. Fecal coliform bacteria in the drinking water samples should be 0 counts MPN/100 mL according to WHO (2004). After treatment with chlorine (5mg/L), the highest coliform count recorded was 210 MPN/100mL but fecal coliform counts were not observed (San Thaw Tar Aung, 2017). Total coliforms and fecal coliforms were highest in untreated pond water samples from a Thaketa pond (Su Mon Win, 2014). The global standard for *E. coli* count was 0-1 MPN/100mL in drinking water (Atlas, 1993).

In the present study, five groups of bacteria were identified in all water samples before treatment such as *Escherichia coli*, *Klebsiella* sp., *Salmonella* sp., *Enterobacter* sp., and *Pseudomonas* sp. Among all bacteria isolates, *Pseudomonas* sp. had highest frequency of occurrence in the water samples and it causes from mild symptoms such as ear and eye pain to severe symptoms such as diarrhea, pneumonia and urinary tract infections. *Escherichia coli* causes diarrhea, bloody diarrhea and hemolytic uremic syndrome. *Klebsiella* sp. causes meningitis, endophthalmitis, liver and splenic abscesses and bacteremia. *Salmonella* sp. causes typhoid fever and *Enterobacter* sp. causes respiratory infections, soft tissue infections, osteomyelitis and endocarditis (Ramirez and Giron, 2022). So, all groups of the identified bacteria in the present work are harmful. The isolates of *Escherichia coli* had highest frequency of occurrence in the water samples from Hlawga Reservoir (San Thaw Tar Aung, 2017).

The isolates of *Escherichia coli*, *Salmonella* sp., *Enterobacter* sp., *Bacillus* sp., and *Pseudomonas* sp., were found in pond water of all sites in all seasons (Su Mon Win, 2014).

In this research, pH values of the tube well water samples were observed from 5.0 to 7.3 in which some were quite acidic. The WHO drinking water standard of pH was between 6.5 to 8.5 (WHO, 2011). The pH can also fluctuate because of precipitation, rain water and wastewater (Fondriest Environmental Learning Center, 2013). Temperature value was found between 25°C-28.1°C in all study sites which was within the WHO limit of 20-30 °C. Total dissolved solids (TDS) was found at value of 8-12mg/L. Standard TDS value was <600mg/L according to WHO (2011).

Although there are many harmless *Escherichia coli* strains, the presence of *E. coli* in water indicates that the water is unsafe and may contain other fecal coliforms bacteria. In the present study, fecal coliforms counts before treatment were unacceptable according to WHO (1993) guideline. The presence of fecal coliforms may be because of dirty environment, pipes and tanks. Temperature and total dissolved solids in all water samples were normal but some of the pH values were under the limits. So, these water supplies from the tube wells were not suitable to drink without chlorination or boiling. A regular chlorine treatment should be performed before consumption.

Conclusion

In the present study, total coliform and fecal coliform counts were high in all the water samples tested. Indication of the presence of bacteria in the water samples suggested that the water was not fit for drinking without proper processing. The physico-chemical parameters of temperature and total dissolved solids in all water samples were normal according to WHO guidelines. But some of the pH values were lower than the WHO standard limits. It was suggested that some pH levels were acidic or declining because of precipitation of rain water and contamination by wastewater. After treatment with 2mg/L calcium hypochlorite, total coliform counts were considerably lowered and fecal coliform counts were not observed. But the fecal coliform counts before treatment was unacceptable according to WHO (1993) guideline and they were harmful to humans. It might be due to some tube wells being very near to the toilets, houses and garbage dump. In addition, trees, shrubs and decaying leaves were also not far enough from the tube wells and the storage tanks. It might also be due to dirty water pipes and contaminated water taps. So, these water supplies are unsafe to drink without chlorination or boiling or other suitable processing.

Acknowledgements

We would like to express our gratitude to Dr. Kay Lwin Tun, Professor and Head of Zoology Department, University of Yangon for providing laboratory facilities of the department and her permission to conduct the research with the chosen topic.

References

- Altas, R.M., 1993. *Handbook of Microbiological Media*. CRC Press, Inc. London.
- Altas, R.M., 1995. *Microorganisms in Our World*. Mobsy Year Book, Inc.
- Bisen, P.S., and Verma, K., 1998. *Handbook of Microbiological Media*. CBS Publishers and Distributors, New Delhi – 110002 (India).
- Breed, R.S., Murry, E.G.D and Smith, N.R., 1994. *Bergey's Manual of Determinative Bacteriology*. The Williams and Wilkins Company. USA.

- Brown, A. E., 2007. *Laboratory Manual in General Microbiology*, Short version, Tenth edition. Published by Mc Graw-Hall, New York.
- Collins, C.H., and Lyne, P.M., 1995. *Microbiological Methods*. Butterworth-Heinemann Ltd, Oxford, London.
- Cowan, S.T., 2009. *Cowan and Steel's Manual for the Identification of Medical Bacteria*. Cambridge University Press, London.
- Dubey, R.C. and Maheshwari, D.K., 2002. *Practical Microbiology*. S. Chand and Company Ltd. Ram Nagar, New Delhi – 110 055.
- Fondriest Environmental, Inc. 2013, "pH of Water", Fundamentals of Environmental Measurements. <<https://www.fondriest.com/environmental-measurements/parameters/water-quality/pH/>>
- HiMedia, 1998. *The HiMedia Manual for Microbiology Laboratory Practice*. HiMedia Laboratories Pvt. Ltd., India. <http://whqlibdoc.who.int/publications/2009/9789241547871-eng.pdf>.
- Hucker, G.J. and Conn, H.J., 1923. Methods of Gram Staining. *Tech. Bull. N. Y. St. agric. Exp.Sta.no.93*.
- Pelczar, M.J., Reid, J.R. and Nohvac, M., 1974. *Microbiology*. Ta Ta McGraw Hill Publishing Comp Ltd., New Delhi.
- Ramirez, D. and Giron, M., 2022. *Enterobacter Infections*. Stat Pearls Publishing, Treasure Island (FL).
- San Thaw Tar Aung, 2017. Bacteriological analysis of water supply from Hlawga reservoir to some Yangon areas, *M.Sc. Thesis*, Zoology Department, University of Yangon.
- Skinner, F.A. and Carr, J.G., 1976. *Microbiology in Agriculture, Fisheries and Food*. The White Fairs Press Ltd., London and Cambridge, England.
- Su Mon Win, 2014. Investigation of effective microorganisms, chlorine and lime on the decontamination of bacteria in pond water for common use, *Ph.D. Thesis*, Zoology Department, University of Yangon.
- WHO, 1993. *Guidelines for Drinking Water Quality*, Volume 1, Recommendation. 2nd edition. World Health Organization, Geneva.
- WHO, 1997. *Guidelines for Drinking Water Quality*, 2nd ed. Vol.3, Surveillance and Control of Community Water Supplies. WHO, Geneva.
- WHO, 2004. *Guidelines for Drinking Water Quality*, 2nd ed, Vol. 1, Recommendations, WHO, Geneva.
- WHO, 2011. *Guidelines for Drinking-Water Quality*, 4th ed. *Addendum: Microbiological agents in drinking water*. Geneva, World Health Organization, Switzerland.