EVALUATION OF PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITY OF DRINKING WATER IN THE DISTRIBUTION SYSTEM OF DHARAN, NEPAL

A PROJECT WORK SUBMITTED TO THE

DEPARTMENT OF MICROBIOLOGY

CENTRAL CAMPUS OF TECHNOLOGY

INSTITUTE OF SCIENCE AND TECHNOLOGY

TRIBHUVAN UNIVERSITY

NEPAL

FOR THE AWARD OF

BACHELOR OF SCIENCE (B. Sc.) IN MICROBIOLOGY

BY

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T.U. REGISTRATION No: 5-2-8-147-2017

June 2022

RECOMMENDATION

This is to recommend that **Aasara Khatiwada,** Symbol no: 500080031**,** T.U. Registration no: 5-2-8-147-2017 , has carried out the project work entitled **"Evaluation of Physicochemical and Microbiological Quality of Drinking Water in the Distribution System of Dharan, Nepal"** for the requirement to the project work in Bachelor of Science (B.Sc.) degree in Microbiology under my supervision in the Department of Microbiology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

To my knowledge, this work has not been submitted for any other degree.

She has fulfilled all the requirements laid down by the Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal for the submission of the project work for the partial fulfillment of her Bachelor of Science (B.Sc.) degree.

…….

Mrs. Bijaya Laxmi Maharjan
Supervisor
Department of Microbiology
Central Campus of Technology
Tribhuvan University

[12, JUNE, 2022]

DECLARATION

This project work entitled **"Evaluation of Physicochemical and Microbiological Quality of Drinking Water in the Distribution System of Dharan, Nepal"** is being submitted to the Department of Microbiology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal for the partial fulfillment of the requirement to the project work in Bachelor of Science (B.Sc.) degree in Microbiology. This project work is carried out by me, under the supervision of Mrs. Bijaya Laxmi Maharjan in the Department of Microbiology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

This work is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

…….

Aasara Khatiwada

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[12, JUNE, 2022]

LETTER OF FORWARD

[Date: 12/June/2021]

On the recommendation of **Mrs. Bijaya Laxmi Maharjan**, this project work is submitted by Aasara Khatiwada, Symbol No. 500080031, T.U. Registration No 5-2-8-147-2017, entitled **"Evaluation of Physicochemical and Microbiological Quality of Drinking water in the Distribution System of Dharan, Nepal"** is forwarded by the Department of Microbiology, Central Campus of Technology, for the approval to the Evaluation Committee, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal.

She has fulfilled all the requirements laid down by the Institute of Science and Technology (IoST), Tribhuvan University (T.U.), Nepal for the project work.

……

Mr. Dhiren Subba Limbu
Head of Department
Department of Microbiology
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BOARD OF EXAMINATION AND CERTIFICATE OF APPROVAL

This project work (PRO-406) entitled **"Evaluation of Physicochemical and Microbiological Quality of Drinking Water in the Distribution System of Dharan, Nepal"** by Miss Aasara Khatiwada (Symbol No. 500080031 and T.U. Registration No. 5-2-8-147-2017) under the supervision of Mrs. Bijaya Laxmi Maharjan in the Department of Microbiology, Central Campus of Technology, Institute of Science and Technology (IoST), Tribhuvan University (T.U.), is hereby submitted for the partial fulfillment of the Bachelor of Science (B.Sc.) degree in Microbiology. This report has been accepted and forwarded to the Controller of Examination, Institute of Science and Technology, Tribhuvan University.

……

Mrs. Bijaya Laxmi Maharjan
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Ms. Aasara Khatiwada

Symbol No. 500080031

T.U. Registration No. 5-2-8-147-2017

June 2022

ABSTRACT

Water is an essential part of the ecosystem that supports life on the earth since it contains essential elements for human health. Studies of the water's constituents are essential to obtaining a precise picture of the water quality. Hence, 31 samples were taken from the drinking water distribution system of Dharan in 2022 for evaluating the physicochemical and microbiological quality of drinking water being distributed across the sub-metropolitan city. Though public knowledge and adequate management of watershed and reservoir premises were insufficient, the physicochemical characteristics were determined to be within the World Health Organization (WHO) and National Drinking Water Quality Standards (NDWQS) for drinking water with temperature ranging from 23.6°C to 25.6°C, pH 7.7 to 8.5, conductivity 38.2 to 38.7µmho/cm, DO 49.54 to 58.39 mg/l, BOD 2.61 to 5.22 mg/l, chloride 29.82 to 34.08 mg/l, nitrite 10 ppm and ammonia <0.5 ppm. However, the coliform bacteria levels were significant, with the highest TCC being 137 cfu/100ml, highest FCC being 85 cfu/100ml and highest TPC being TMTC. The water was found to be unsafe to drink without intensive disinfection treatments. It may be necessary to carry out treatment procedures like chlorination as advised by WHO as soon as possible while also taking in account the proper application of filtration techniques for distributing safe drinking water to the residents of Dharan.

Keywords: drinking water, microbiological quality, coliform, physicochemical, conductivity

शोधसार

पानी पारिस्थितिक प्रणालीको एक आवश्यक भाग हो जसले पृथ्वीमा जीवनलाई समर्थन गर्दछ किनभने यसमा मानव स्वास्थ्यको लागि आवश्यक तत्वहरु छन् । पानीको गुणस्तरको सटीक तस्वीर प्राप्त गर्न पानीको घटकहरुको अध्ययन आवश्यक छ । तसर्थ उपमहानगरपालिकामा वितरण भैरहेको खानेपानीको भौतिक र शूक्ष्मजैविक गुणस्तर मूल्याङ्कन गर्न सन् २०२२ मा धरानको खानेपानी वितरण प्रणालीबाट ३१ नमूना संकलन गरिएको थियो । सार्वजनिक ज्ञान, जलाशय र जलाशय परिसरको पर्याप्त व्यवस्थापन नभए तापनि भौतिक रसायनिक विशेषताहरु विश्व स्वास्थ्य संगठन र राष्टिय पेयजल गुणस्तर मापदण्ड भित्र रहेको पुष्टि भयो । संकलित नमूनाहरुको तापक्रम २३.६डिग्री सेल्सियसदेखि २५.६ डिग्रीसम्म, पी एच ७.७ देखि ८.५ सम्म, कन्डक्टिभिटी ३८.२ देखि ३८.७ सम्म, डि ओ ४९.५४ देखि ५८.३९ सम्म, वि ओ डि २.६१ देखि ५.२२ सम्म, क्लोराइड २९.८२ देखि ३४.०८ सम्म, नाइट्राइट १० पि पि एम र अमोनिया <०.५ पि पि एम भएको पाइयो । यद्यपि, कोलिफर्म ब्याक्टेरिया प्रसस्त मात्रामा देखियो जसमा उच्चतम टि सि सि १३७, एफ सि सि ८५ र टि पि सि टि एम टि सि रहेको थियो । सघन कीटाणुशोधन उपचार विना पानी पिउन असुरक्षित भएको पाइयो । धरानका बासिन्दाहरुलाई सुरक्षित पिउने पानी वितरण गर्न फिल्टर गर्ने प्रविधिको उचित प्रयोग तथा विश्व स्वास्थ्य संगठनको सुझाव अनुसारको क्लोरिनेशन जस्ता उपचार प्रक्रियाहरु जतिसक्दो चाँडो पूरा गर्न आवश्यक देखिन्छ ।

Keywords: drinking water, microbiological quality, coliform, physicochemical, conductivity

LIST OF ABBREVIATIONS

BOD	Biological Oxygen Demand
CFU	Colony Forming Unit
DFTQC	Department of Food Technology and Quality Control
DO	Dissolved Oxygen
DoHS	Department of Health Services
DWSS	Department of Water Supply and Sewerage
EMB	Eosine Methylene Blue
FCC	Fecal Coliform Count
ICMR	Indian Council of Medical Research
NDWQS	Nepal Drinking Water Quality Standards
TCC	Total Coliform Count
TMTC	Too Many To Count
TPC	Total Plate Count
TSIA	Triple Sugar Iron Agar
UNESCO	United Nations Educational, Scientific and Cultural Organization
WHO	World Health Organization
WWF	World Wide Fund

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CHAPTER 1

1. INTRODUCTION

1.1 General Introduction

Water is the most important resource for all forms of life on this planet and it is necessary for the ecosystems' integrity and sustainability (UNESCO, 2003). Rapid population growth, rising living standards in cities, and industrialization have resulted in increased demand for high-quality water on the one hand, while pollution of water sources has been continuously increasing on the other. Fresh water availability and access have become one of the world's most pressing natural resource concerns in recent years. Freshwater is vital to human health, agriculture, industry, and natural ecosystems, but it is becoming increasingly rare in many parts of the globe (WWF, 1998).

Water that is clean and safe is essential for good health and a productive life. Water has a significant impact on human health, and the quality of the water supplied is critical in determining individual and community health. Safe drinking water is a crucial concern in terms of public health, as the human race's health is inextricably linked to the quality of water utilized (Sharma, *et al.,* 2005).

According to the WHO, 1.15 billion people in developing countries do not have access to improved water supply. Because more than one billion people still lack access to safe drinking water and more than two-thirds of the world's population lacks basic sanitation, the supply of safe drinking water has become a global concern (WHO,2007).

Microbial contamination caused by the presence of human and animal excrement is the most prevalent reason for water being deemed unsuitable to drink due to the high likelihood of pathogenic organisms present. Furthermore, animal husbandry operations such as grazing can result in high concentrations of bacteria and nitrates released into water, posing health risks due to the presence of pathogens (Obasohan, *et al.,* 2010).

Water pollution is an issue that poses a significant threat to human life. Acquiring sufficient water is of higher concern for most Nepalese than obtaining safe water. As of mid-2015, the Department of Water Supply and Sewerage (DWSS) claimed that

roughly 86 percent of Nepalese people have access to basic water supply facilities (DWSS, 2015). The quality of the water provided, however, is questionable. According to a 2016/2017 report by the Department of Health Service (DoHS), there were 23,742 occurrences of water-borne infections among Nepalese patients, with 270 cases resulting in death (DoHS, 2016).

For the safe drinking water, its physicochemical and microbiological parameters should meet the minimum requirements of drinking water quality standards. This study will aim at evaluating the pathogenic bacteria along with the physicochemical and microbiological quality of drinking water in distribution system of Dharan.

1.2 Statement of problem

The overall goal of drinking-water distribution system is to promote public health by supplying safe water. The quality of water directly affects the incidence of water-borne diseases as the pathogens are mainly transmitted through drinking water. The physical parameters of water quality like color, temperature, odor, Total Dissolved Solid (TDS), conductivity etc. add to the aesthetic value of water, while parameters like ammonia, nitrite, nitrate etc. may cause adverse health effects. Higher or lower pH, higher turbidity, higher content of chloride, nitrate, ammonia, nitrite is questionable for drinking. From microbiological point of view, water should be free from any kinds of pathogens as their presence in drinking water may pose threat in human health. The majority of the sources and reservoirs of the drinking water distribution system were found to be heavily contaminated with indicator species, indicating a serious water pollution problem in Dharan area (Pant, *et al.*, 2016). Therefore, assessment of drinking water quality in terms of physicochemical and microbiological aspects can help to take effective management decision to promote public health.

1.3 Significance of study

In Dharan, the drinking water distribution system supplies water through the pipelines. The source of water in the distribution system is the rivers. Usually, the drinking water distribution system has the infrastructure for the treatment of the water or the chemicals are added to ensure the safety of water. But the water distribution system of Dharan has the reservoir facility but not the treatment system and the chemicals are added only during the rainy season. Since, the system supplies water directly without treatment, we are not sure regarding the safety of water. Hence, the main aim of this research is to analyse the physicochemical and microbiological quality of water in the source and reservoirs of drinking water distribution system of Dharan. The outcome of this work is believed to play a vital role in guiding for the improvement of distribution system if required.

1.4 Objectives

1.4.1 General Objective:

To evaluate the physicochemical and microbiological parameters of drinking water in the distribution system of Dharan.

1.4.2 Specific Objectives:

1. To analyze the physicochemical parameters of water.

2. To test the Total Plate Count (TPC) in water.

3. To test the Total Coliform Count (TCC) in water.

4. To test the Fecal Coliform Count (FCC) in water.

CHAPTER 2

2. LITERATURE REVIEW

Water is both a life-giving and a life-sustaining element. It is crucial natural resource and is necessary component for all living and non-living organisms (Pandey, 2012). The availability of a safe and dependable source of water is a necessary condition for the development of a stable society.

Water takes approximately one-third of the Earth's surface area. However, only 0.3% of it is safe to drink. Safe drinking water is described as water that meets WHO guidelines and national drinking water quality requirements in terms of microbiological and physicochemical characteristics (WHO, 2007).

According to WHO, inadequate sanitation, pollution, or a lack of water are responsible for up to 80% of all sickness and disease in the world. A large number of deaths and morbidities are caused by contaminated drinking water and water-borne diseases (Prasai, *et al.*, 2007). Hence, safe water quality is a major concern in terms of public health importance, as human health and well-being are inextricably linked to the quality of water used (Sharma, *et al.*, 2005).

Despite significant attempts to provide safe piped community water to the world's population, the reality remains that safe water sources will not be available to all people in the foreseeable future (Agarwal, 1981).

Growing supply and demand imbalances have led in pollution and environmental damage. Waterborne infections such as diarrhoea, dysentery, cholera, and gastroenteritis are common as a result of such unsanitary water quality. These diseases are widespread across the world, in both urban and rural locations. Contaminated water-borne diseases are among the top ten most common water-borne diseases in Nepal (DoHS, 1998).

Thousands of people die or get sick due to water and sanitation issues. As a result, water, the most important resource for all forms of life on this planet, may be exceedingly deadly when it serves as a vehicle for disease transmission (Sharma, *et al.*, 2005).

Nepal, like many developing countries, has a slew of issues with both the quality and availability of drinking water (Warner, *et al.*, 2008). The most important water quality issue in Nepal is fecal pollution of drinking water (ENPHO, 2001).

People in Nepal are subjected to serious health risks as a result of sewage, farm, and industry contamination of water. Typhoid, dysentery, and cholera are endemic every summer due to the influence of sewage (Khadka, 1993). The infections are spread primarily by human and animal excrement, particularly feces (WHO and UNICEF, 2004).

A study was performed to analyze the household drinking water in 39 localities of Kathmandu valley. Coliform tests were performed on water samples and results showed that all the working samples had some degree of fecal contamination. The study found coliforms ranging from 4 to 460 cfu per 100 mL (Sharma, 1978).

Water from Sundarijal (Sundarijal reservoir, Bagmati, Shyalmati and Nagmati) was analyzed on July 1982 which reported that reservoir had the lowest *E. coli* count (10MPN/100ml) and Bagmati had the highest (180MPN/100ml). However, all the sources contained equal number of total coliform (180MPN/100ml) (Yadav, *et al*., 1984).

The quality of water samples from different sources of Kathmandu and Lalitpur area were studied which found that the coliform concentration had significantly increased in nine years. Water samples were taken in dry summer, rainy and winter seasons. The coliform bacteria count ranged from 0 to 4800 during the rainy season, 0 to 75 in winter season and 0 to 460 per 100ml in the summer season (Sharma, 1986).

Bacteriological tests were carried out in drinking water sources of two villages of central Nepal: Chaubas (Shivapuri area) and Saibu (Langtang N.P. area) and it reported that pollution of drinking water was a problem in these villages. The coliform count 11 ranged from 5-100 cells/100ml of water. In Chaubas, contamination range was within 5-10 cells/100ml whereas in Saibu, it was within 20-100cells/100ml (Joshi, 1986).

DISVI (1989) conducted a study on the quality of drinking water of Kathmandu valley by taking 472 samples at 58 sampling points, 44 water taps, 7 storage, and 7 water

treatment plants which showed existence of bacterial contamination in most of the sampling points.

A study in 1989 analyzed 472 different water samples from treatment plant and reservoirs, hospital storage tanks and public water taps. Their result showed that the bacteriological contamination increased as the water travelled from the water treatment plant to the distribution systems. It also reported that all the water samples had coliform count far exceeding WHO standard (Bottino and Sharma, 1989).

Water quality testing experiment was conducted for chemical and bacteriological parameters in two rivers (Madri Khola and Bhote Khola), a reservoir and four public taps of city water supply of Pokhara. The study showed that all the tested physiochemical parameters were under WHO guidelines values with absence of iron and manganese in all samples (Shrestha, *et al*., 1991).

ENPHO/DIVSI (1992) conducted a one year monitoring on microbiological quality of water supply in Kathmandu. Water samples were collected from 39 taps and 6 treatment plants.18% of the treatment plants and 50% of public taps showed significant contamination.

A study on the drinking water quality of Kathmandu and Pokhara observed significant contamination where coliform counts were 2400/100 mL and 4800/100 mL respectively in the sampled areas (Sharma, 1993).

ENPHO (1997) on monitoring and assessment of water quality in the Shivapuri watershed from August 1996 to August 1997, found that among Bagmati, Shyalmati, Nagmati and Sundarijal reservoir, Shyalmati had F.C. count 2 to 1,200 col/100ml, Nagmati had 4 to 390 col/100ml, Bagmati had 96 to 3,800 cells/100ml and Sundarijal reservoir had 144 to 10, 900 col/100ml.

ENPHO (2001) carried out water quality analysis in Kavre, Parsa and Chitwan. The bacteriological water quality in Kavre indicated that about 76% water samples at Mahadevsthan, 86% in Kusadevi, 82% in Dhumkharku, 82% in Sathighar 12 and 64% in Shyampati were contaminated by fecal matter and unsafe for consumption. In Parsa, about 36% in Sakhuwa Parsami, 13% in Bageshwori Titrauna, 14% in Pancharuhhi, 55% in Belwa and 7% in Amarpati water samples were contaminated by fecal matter

and unsafe for consumption. In Chitwan, about 71% in Dibya Nagar, 63% in Geetanagar, 93% in Jutpani, 88% in Pithuwa and 79% in Kabilas water samples were contaminated by fecal matter and unsafe for consumption.

A study on the quality of drinking water of Kathmandu where samples were taken from various sources like well, stream and treatment plants, all of which showed contamination. Hence it was concluded that most drinking water supplies in Kathmandu are microbiologically contaminated (Brittner, *et al.*, 2000).

A study analyzed MPN of coliform in drinking water samples of handpumps, taps and ponds at Janakpur. The MPN of coliform bacteria varied from 10 to 1060per100ml. Tube well water samples contained 10 to 54 coliforms per 100ml of sample, corporation water contained 15 to 180 coliforms per 100ml of sample while the pond water samples contained 120 to 1060 coliforms per 100ml of sample. The study showed that all the fifteen water samples were found to contain total coliform bacteria exceeding the WHO water quality standard (Das and Jha, 2002).

A study was conducted on quantitative and qualitative determinants of drinking water in the Tulkarem district in Palestine. Five hundred drinking water samples were collected from different sources in the district. Out of these samples, 34% and 92% were contaminated with total coliforms and fecal coliforms, respectively (Khatib, *et al*., 2003).

Water in Kathmandu valley was sampled from over 100 sources including municipal taps, dug wells, shallow and deep aquifer tube wells and stone spouts. It was found that the most problematic were total coliform and E. coli which was present in 94% and 72% of all water samples respectively. Contamination by nitrate, ammonia and heavy metals was more limited (Warner, *et al*., 2007).

A study on microbial and chemical quality of water available in Kathmandu with the samples of tap and river from Sundarighat upstream found that the physicochemical parameters were below WHO standards except chloride. Also, bacteriological contamination was 900 cfu/100 mL in average (Gyawali, 2007).

According to a study carried out in the samples of drinking water supply in Morocco, even though, temperature, pH, DO and ammonia results revealed all sampled sources of water to be safe to drink according to World Health Organization (WHO) criteria, turbidity and NO_3 levels were found to be greater than the allowed limits and fecal contamination (total coliforms, *E. coli* and intestinal enterococci) was found in all sources at different times, according to the microbiological study (Barakat, *et al*., 2018).

A study in different drinking water sources in Gujarat, India showed great deviations in pH, hardness, and DO levels from the standard WHO guidelines. *E. coli* and *Enterobacter* were found in the majority of water samples and the total number of aerobic bacteria was also high (Vyas, *et al*., 2015).

61.4 percent (70/114) of water samples had total coliforms, with 15.7 percent (11/70) having thermotolerant coliforms in the drinking water supplied by Kathmandu metropolitan city. Ten distinct enteric bacteria were recovered from collected samples, with *E. coli* being the predominant (Shakya, *et al.,* 2012).

In 76 and 92 percent of the samples of drinking water, fecal coliform and total coliform bacteria were detected respectively at >300 CFU/100 mL. The bacterial population exceeded the Department of Food Technology and Quality Control's (DFTQC) guideline of zero CFU per 100 mL of water. Chemical parameters for ammonia (1.5 mg/L) were found to be higher than DFTQC values, with a maximum concentration of 4.66 mg/L. The majority of the analyzed drinking water exceeded the E. coli and TC bacteria threshold standards, posing a risk if consumed (Burlakoti, *et al*., 2020).

According to a study in water samples of Ratuwa river of Damak, Jhapa, all of the measured parameters, including color, pH, TSS, TDS, DO, sulphate, chloride, total hardness, total phosphorus, calcium, magnesium etc were within permissible ranges, but turbidity and BOD were higher than the standard values set by the Nepal Drinking Water Quality Standard (NDWQS) and the Indian Council of Medical Research (ICMR), respectively (Shrestha and Basnet, 2018).

Heterotrophic bacteria, total coliforms, and fecal coliforms were discovered in abundance in the majority of the sources and reservoir tanks in the drinking water

distribution system of Dharan. It was found to be contaminated by more than one species of indicator organism (Pant, *et al.,* 2016).

A study in Kathmandu valley showed temperature and nitrate levels to be within WHO guidelines, however pH, conductivity, turbidity, chloride, iron, arsenic, ammonia, and Coliform bacteria levels were found to be higher. Turbidity, iron, ammonia, and conductivity were all problematic factors in various sources of drinking water. Coliform bacteria were detected in 36% of treated water samples and 80% of tap water samples (Koju, *et al.*, 2014).

In 2009, a study was conducted to assess the drinking water quality of Madhyapur-Thimi which found pH (1.9%) and conductivity (34.28%) of water samples to be above the WHO and national standard permitted guideline thresholds. All of the samples had nitrate levels that were within the WHO permitted limit as well as the national standard, but chloride (2.85%) and ammonia (11.42%) above the WHO guideline value. The total coliform count revealed that 64.76 percent of samples exceeded WHO guidelines (Jayana, *et al.*, 2009).

In 2003 and 2004 AD, water samples from Sundarijal reservoir and its main feeding streams: Bagmati, Nagmati, and Shyalmati were studied for physico-chemical (pH, conductivity, dissolved oxygen, alkalinity, hardness, nitrogen, heavy metals) and bacteriological (coliform bacteria) characteristics. The physicochemical characteristics were within World Health Organization standards, despite a lack of public knowledge and effective management of watershed and reservoir premises. However, the coliform bacteria levels were significant, and the water was unsafe to drink without intensive disinfection treatments (Bhattarai, *et al.*, 2008).

In a sample taken from the Sundarijal reservoir in January 2018, physico-chemical parameters and bacteriological features of water were investigated. The physico-chemical characteristics were determined to be within the World Health Organization (WHO) and National Drinking Water Quality Standards (NDWQS) for drinking water. However, the coliform bacteria levels were high and the water was unsafe to consume (Dhungana, 2019).

A study of drinking water in Tokha, Kathmandu observed the pH, total hardness, chloride, nitrate, and arsenic content of the samples to be all within acceptable

guidelines, while the calcium hardness and ammonia content of the samples above Nepal standard values. All of the water samples had high total viable counts, exceeding the water limit (1.0×102 cfu/ml). Coliforms and fecal organisms were identified in abundance in all of the water samples, exceeding the WHO/FAO water guidelines. On M-endo agar plate, fecal coliform colonies ranged from 143 to 152, and total coliforms varied from 110 to 248 per 100 ml water, both exceeding the standard limit for water (Shidiki, *et al.*, 2016).

In the physicochemical and microbiological analysis of 116 water samples collected from four different sources in the Bhaktapur Municipality area, conductivity, turbidity, iron, and chloride levels were found to be higher in 57 (49.14%), 9 (7.76%), 56 (48.28%), and 1 (0.87%) of water samples, respectively. Total coliform was found in 96 (82.76 %) of water samples according to bacteriological analysis (Diwakar, *et al.*, 2008).

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Materials

The materials, equipment, media and reagents used in this study are listed in Appendix.

3.2 Methods

3.2.1 Study Design and study area

The research was laboratory based cross sectional study. All the laboratory procedures were conducted in the microbiology laboratory of Central Campus of Technology, Hattisar, Dharan, Sunsari, Nepal.

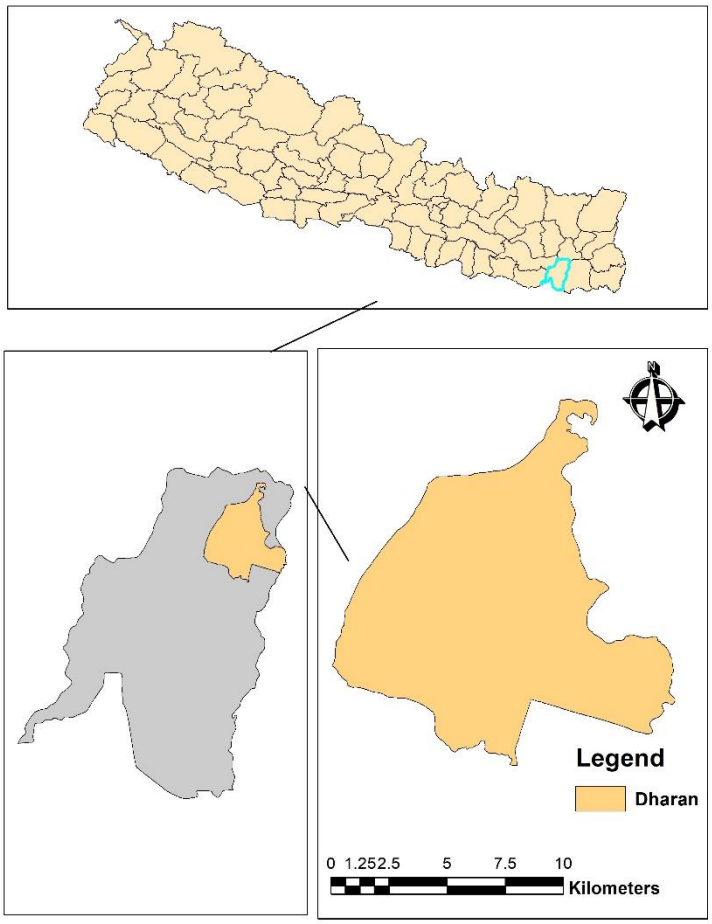


Figure 1: Study area

3.2.2 Sample size and types

For the study, a total of 31 water samples were collected from the sources (12), reservoirs (3) and taps (16) of drinking water distribution system of Dharan, Province no. 1, Nepal.

3.2.3 Laboratory set up

Laboratory setting was done in Microbiology laboratory, Central Campus of Technology, Dharan-14.

3.2.4 Sample collection, transportation and processing

The samples were collected aseptically in sterile BOD bottles for the physicochemical and microbial quality analysis. The BOD bottles were directly dipped into the water body in the reachable sources and wherever the water level was low, the bottles were tied in a string and dipped into the water body for the collection of water samples from the sources and the reservoirs. The tap water samples were collected directly in the BOD bottles from the taps and brought to the laboratory. For the microbial quality analysis, the bottles were filled up to their necks and capped leaving some air space inside the bottles so that the strict aerobes if present were not killed. The water samples for the physicochemical analysis were collected in the bottles without leaving space from the water sources, reservoir and taps of the distribution system. The samples were carried to the microbiology laboratory of Central Campus of Technology, Dharan maintaining cold chain within half an hour of collection and the samples were processed as soon as possible.

3.2.5 Physicochemical Analysis

3.2.5.1 Temperature

For the determination of temperature, water was collected in a beaker. Mercury filled Celsius thermometer was inserted into the beaker and reading was noted after constant reading was obtained.

3.2.5.2 pH

pH was measured by automatic digital pH meter. The pH meter was first calibrated with a standard buffer solution. The glass electrode was washed with distilled water. Then, glass electrode was dipped in the beaker containing water sample until the reading was stabilized at a certain point. Then, pH reading was noted down.

3.2.5.3 Conductivity

Conductivity is a measure of water's capability to pass electrical flow. This ability is directly related to the concentration of ions in the water. It was measured with conductivity meter. The conductivity meter was first calibrated by placing the magnetic stirrer in a beaker containing 0.1N Potassium Chloride and adjusting its conductivity to 14.12millisiemens/ cm at 30°C. The electrode was washed with distilled water and wiped with a tissue paper. Then, it was dipped in the beaker containing water sample until the reading was stabilized at a certain point. Then, conductivity reading was noted down.

3.2.5.4 Dissolved Oxygen (Winkler's Method)

DO was measured by using APHA, (1998) method. The sample was collected in a 300 mL BOD bottle carefully, avoiding any kinds of bubbling and trapping of air bubbles in the bottle after placing the stopper. 2 ml of Manganese sulphate (MnSO4) and 2 ml of alkaline iodide azide solution were added well below the surface from the wall of the bottle till a precipitate appeared. Then, the stopper was placed tightly and the bottle was shaken by inverting the bottle repeatedly to insure proper mixing of the contents. The bottle was kept for some time to settle down the precipitate. 2ml of conc. H_2SO_4 was added to it and shaken well to dissolve all the precipitate. Then, 50 ml of sample was

taken in a conical flask and titrated against sodium thiosulphate ($Na_2S_2O_3$) of 0.025N using starch as an indicator. At the end point, the initial blue color changes to colorless.

Calculation:

$$DO_{(\text{mg/L})} = \frac{(ml \times N)_{\text{of titrant consumed}} \times 8 \times 1000}{\frac{V_2 \times (V_1 - V)}{V_1}}$$

Where, V_1= volume of sample bottle after placing the stopper, V_2= volume of part of content titrated, V= volume of MnSO4 and KI added, ml= volume of thiosulphate consumed, N= normality of thiosulphate consumed

3.2.5.5 Biological oxygen demand (BOD)

Biochemical oxygen demand (BOD) is the measure of the degradable organic material present in a water sample and can be defined as the amount of oxygen required by the microorganisms in stabilizing the biologically degradable organic matter under aerobic conditions. The principle of the method involves measuring the difference of the dissolved oxygen of the sample after incubating it for 5 days at 20^0C.

Calculation:

BOD, mg/L = (D_0 -D_5) × dilution factor

Where, D_0 = Initial D_0 in the sample, D_5 = DO after 5 days

3.2.5.6 Chloride

Chloride was measured by titration method. 50 ml of sample was taken in a conical flask. 2 ml of Potassium chromate was added to the sample solution and titrated against 0.02N silver nitrate until a persistent brick red color appeared.

Calculation:

$$\text{Chloride}_{(\text{mg/L})} = \frac{(a - b) \times N \times 35.5 \times 1000}{V}$$

Where, a = Volume of titrant (silver nitrate) for sample, b= Volume of titrant (silver nitrate) for blank, V = Volume of the sample in ml, N = normality of silver nitrate

3.2.5.7 Nitrite

Nitrite content in the water sample was determined by using nitrite test kit (HiMedia laboratories). For this, the aqua check jar was filled with 10ml water sample and 2 drops of reagent 07A was added and mixed well. After that, reagent 07B was added dropwise counting the number of drops till the pale bluish color appeared.

Calculation as ppm $NaNO_2$: 5× (number of drops of 07B)

3.2.5.8 Ammonia

Ammonia content in the water sample was determined by using ammonia test kit (Prerana laboratories). For this, 10ml of water sample was taken in test jar, 5drops of reagent AM-1 was added and mixed well by capping it properly and inverting several times. After 5minutes, the water was transferred into empty compartment of color ladder comparator and the color was compared and reading was noted accordingly.

3.2.6 Microbiological Analysis

Microbiological analysis of water samples from the sources, reservoirs and taps of drinking water distribution system of Dharan were processed for standard total coliform count (TCC), fecal coliform count (FCC) and total plate count (TPC).

3.2.6.1 Total Plate Count (TPC)

Total plate count was determined by spread plate technique using Nutrient Agar (Himedia, India). 0.1ml of water sample was pipetted out onto the center of the surface of NA plate. The L-shaped glass spreader was dipped into alcohol and flamed over a Bunsen burner. The sample was then evenly spread over the surface of NA using the sterile glass spreader, rotating the Petri dish underneath at the same time and the plate was incubated at 37^oC for 24 hrs.

3.2.6.2 Total Coliform Count (TCC)

Total Coliform count was done by Membrane filtration method using Eosine Methylene Blue Agar (EMB). For membrane filtration technique, the funnel and the apparatus were sterilised and the forceps was flamed at first. The membrane filter was removed from the sterile package and placed into the funnel assembly. The water

sample was poured into the funnel and the vacuum was turned on to allow the liquid to draw completely through the filter. The forceps was flamed again and the membrane filter was removed from the funnel. The membrane filter was placed into the prepared EMB agar plate and incubated at 37^{o}C for 24-48 hrs.

3.2.6.3 Fecal Coliform Count (FCC)

Fecal Coliform count was done by Membrane filtration method using Eosine Methylene Blue Agar (EMB). The plates were incubated at 44.5^{o}C for 24-48 hrs.

3.2.6.4 Sub-culture

Colonies obtained in Eosine Methylene Blue (EMB) agar plates after 48hrs of incubation were sub-cultured onto Nutrient Agar (NA) for pure culture. Isolated bacteria were identified on the basis of their colonial characteristics, morphological characteristics and biochemical properties according to Bergey's Manual of Determinative Bacteriology, 1994.

3.2.6.5 Methodology of Biochemical Test for the Identification of Bacteria

A. Gram Staining:

Gram staining is a differential bacterial staining technique used to differentiate bacteria into Gram Positive and Gram Negative according to their cell wall composition. It is generally the first test performed on bacteria during their identification and observation process.

Procedure: In a clean glass slide, a loop full of bacterial culture was taken with the help of a sterilized inoculating loop and a smear was made. It was air-dried and heat fixed. Then, it was flooded with crystal violet for 30-60 secs. It was rinsed with running water and flooded with Gram's iodine solution for 30-60 secs. It was rinsed again with running water and then flooded with decolorizing solution for 15-20 secs. Then, it was flooded with safranin for 30-60 secs and rinsed off. It was air-dried, a drop of immersion oil was added and observed under the microscope in 100X.

B. Catalase test:

This test is performed to detect the presence of catalase, an enzyme that catalyses' the release of oxygen from hydrogen peroxide. During aerobic respiration, in the presence of oxygen, microorganisms produce hydrogen peroxide, which is lethal to the cell itself. The enzyme catalase splits hydrogen peroxide to water and oxygen. The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria, the main exception being *Streptococcus* spp.

Procedure: A small amount of a culture from Nutrient Agar plate was taken in a clean glass slide and about 2-3 drops of 3% H_2O_2 was put on the surface of the slide. The positive test is indicated by the formation of active bubbling of the oxygen gas.

C. Indole Production test:

This test detects the ability of the organisms to produce an enzyme Tryptophanase. Tryptophan is oxidized by some bacteria by the enzyme Tryptophanase resulting in the formation of indole, pyruvic acid and ammonia.

Procedure: The bacterial colony was inoculated on tryptone broth and then incubated ad 37°C for 24 hours of incubation, 1ml of Kovac's Reagent was added. Appearance of Red color (red ring) on the top of the medium indicates Positive Indole Test.

D. Methyl Red test:

This test is performed to test the ability of an organism to produce and maintain stable acid end product from the fermentation of glucose to give a red color with the indicator methyl red and to overcome the buffering capacity of the system. Medium used in the study was Clark and Lubs medium (MR/VP broth, pH 6.9). Methyl red is an indicator which is already acid and will denote changes in degree of acidity by color reactions over a pH range of 4.4- 6.0.

Procedure: A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of methyl red reagent was added and mixed well. The positive test was indicated by the development of bright red color, indicating acidity.

E. Voges-Proskauer (VP) test:

The principle of this test is to determine the ability of some organisms to produce an acetyl methyl carbinol, a neutral end product (acetoin) or its reduction product 2,3-butanidiol during fermentation of carbohydrates. An organism of the Enterobacteriaceae group is usually either methyl red positive and Voges- proskauer-negative or methyl red negative and VogesProskauer positive. The Vogesproskauer test for acetoin is used primarily to separate E. coli from Klebsiella and Enterobacter species.

Procedure: A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of Barritt's reagent was added and shaken well for maximum aeration and kept for 15 minutes, positive test is indicated by the development of pink red color.

F. Citrate Utilization test:

This test is performed to detect whether an organism utilizes citrate as the sole source of carbon. The utilization of citrate depends on the presence of an enzyme citrase produced by the organisms that breaks down the citrate to oxalo acetic acid and acetic acid which later converted to pyruvic acid and carbon dioxide. Simmons Citrate Agar is used for this test, where sodium citrate is the only source of carbon and energy. Once the CO_2 is generated, it combines with sodium and water to form Sodium Carbonate an alkaline product, which changes the color of the indicator (Bromothymol Blue) from green to blue.

Procedure: A loopful of test organism was streaked on the slant area of Simmon's Citrate Agar medium and incubated at 37°C for 24 hours. A positive test was indicated by the growth of organism and change of media by green to blue, due to alkaline reaction. Bromothymol blue is green when acidic (P^H 6.8 and below) and blue when alkaline (P^H 7.6 and higher).

G. Oxidative- fermentative test:

The oxidative-fermentative test is performed to determine if certain gram-negative rods metabolize glucose by fermentation or aerobic respiration (oxidatively). During the

anaerobic process of fermentation, pyruvate is converted to a variety of mixed acids depending on the type of fermentation. The high concentration of acid produced during fermentation will turn the bromthymol blue indicator in OF media from green to yellow in the presence or absence of oxygen.

Procedure: A pure colony of the test organism was inoculated into duplicate tubes of 2 ml of OF medium, sterile paraffin was applied to one of them and cap was tightened. Then, they were incubated at 37°C for 24 hours. Fermentative result was indicated by acid production on both (open and covered) tubes changing the color from green to yellow. Oxidative result was indicated by acid production in the open tube (aerobic) and not the oil-covered tube (anaerobic). Non-fermenting bacteria that metabolize glucose via oxidative metabolism give an oxidative result.

H. Urease test:

This test demonstrates the urease activity present in certain bacteria which decomposes urea, releasing ammonia and carbon dioxide. Ammonia thus produced changes the color of indicator (phenol red) incorporated in the medium.

Procedure: The test organism was inoculated in a medium containing urea and the indicator phenol red. The inoculated medium was incubated at 37°C for overnight. Positive organism shows pink red color due to the breakdown of urea to ammonia. With the release of ammonia, the medium becomes alkaline as shown by a change in color of the indicator to pink.

I. Triple Sugar Iron Agar Test:

This test is performed to determine whether a Gram-negative bacilli ferments glucose and lactose or sucrose and forms hydrogen sulfide (H2S) and to differentiate members of the Enterobacteriaceae family from other Gram-negative rods.

Procedure: TSI agar was inoculated with well-isolated test organism by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant. It was incubated at 37°C for 24 hours. Following incubation, color change was examined in slant and butt, blackening and cracks in the medium.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Physicochemical analysis of water samples

The temperature, pH, conductivity, dissolved oxygen (DO), biological oxygen demand (BOD), chloride, nitrite and ammonia of the water samples observed are listed in Table 1.

Table 1. Physicochemical analysis of water samples

S.N.	Temperature (°C)	pH	Conductivity (µmho/cm)	DO (mg/l)	BOD (mg/l)	Chloride (mg/l)	Nitrite (ppm)	Ammonia (ppm)
1	23.7	8.2	38.2	57.36	2.61	31.24	10	<0.5
2	23.6	8.2	38.2	58.39	2.76	31.24	10	<0.5
3	23.6	8.1	38.2	57.36	2.61	31.24	10	<0.5
4	24.8	8.4	38.6	49.54	5.21	31.24	10	<0.5
5	24.7	8.5	38.5	50.14	5.11	31.24	10	<0.5
6	24.8	8.4	38.6	49.54	5.21	31.24	10	<0.5
7	25.1	8.0	38.6	54.76	5.22	29.82	10	<0.5
8	25.3	8.0	38.7	54.76	5.22	29.82	10	<0.5
9	25.1	8.0	38.6	54.76	5.22	29.82	10	<0.5
10	25.2	7.8	38.5	54.76	5.22	34.08	10	<0.5
11	25.2	7.7	38.5	54.76	5.22	34.08	10	<0.5
12	25.1	7.8	38.4	54.76	5.22	34.08	10	<0.5
13	25.6	8.0	38.7	54.76	2.61	31.24	10	<0.5
14	25.5	8.0	38.7	54.76	2.61	31.24	10	<0.5
15	25.5	8.1	38.7	54.76	2.61	31.24	10	<0.5

No of samples= 15

4.1.2 Microbiological analysis of water samples

The total plate count, total coliform count and fecal coliform count obtained for all the water samples are listed in Table 2.

Table 2: Microbiological analysis of the water samples

Sample code	TPC (cfu/ml)	TCC (cfu/100 ml)	FCC (cfu/100 ml)
1	52×10^1	121	60
2	73×10^1	98	57
3	65×10^1	137	69
4	17×10^1	107	49
5	16×10^1	118	64
6	14×10^1	126	85
7	54×10^1	81	24
8	TMTC	73	32
9	85×10^1	96	39
10	TMTC	103	72
11	121×10^1	97	59
12	50×10^1	111	60
13	42×10^1	53	28
14	45×10^1	29	17
15	71×10^1	46	31
16	24×10^1	79	41
17	29×10^1	68	37
18	130×10^1	95	55
19	111×10^1	82	58
20	50×10^1	84	37
21	53×10^1	60	31
22	44×10^1	91	52
23	40×10^1	72	50
24	22×10^1	57	43
25	28×10^1	53	46
26	87×10^1	76	60
27	71×10^1	59	41
28	53×10^1	63	44
29	55×10^1	71	38
30	TMTC	101	79
31	TMTC	92	66

No of sample= 31

4.1.3 Biochemical characteristics of isolates

Table 3: Biochemical test of the isolates obtained from water samples

S. N.	Catalase	Oxidase	Indole	MR	VP	Citrate	Urease	TSI	O/F
1	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Y/Y, Hn, Gp	F
2	+ve	-ve	-ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gn	F
3	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gp	F
4	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gp	F
5	+ve	-ve	-ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gn	F
6	+ve	-ve	-ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gn	F
7	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gp	F
8	+ve	-ve	-ve	+ve	-ve	+ve	-ve	Y/Y, Hp, Gp	F
9	+ve	-ve	-ve	+ve	-ve	+ve	-ve	Y/Y, Hp, Gp	F
10	+ve	-ve	-ve	-ve	-ve	+ve	-ve	R/R, Hp, Gn	F
11	+ve	-ve	-ve	+ve	-ve	+ve	-ve	Y/Y, Hp, Gp	F
12	+ve	-ve	-ve	+ve	-ve	+ve	-ve	Y/Y, Hp, Gp	F
13	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gp	F
14	+ve	-ve	-ve	+ve	-ve	+ve	-ve	Y/Y, Hp, Gp	F
15	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gp	F
16	+ve	-ve	-ve	-ve	-ve	+ve	-ve	R/R, Hp, Gn	F
17	+ve	-ve	-ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gn	F
18	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gp	F
19	+ve	-ve	-ve	-ve	-ve	+ve	-ve	R/R, Hp, Gn	F
20	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gp	F
21	+ve	-ve	-ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gn	F
22	+ve	-ve	-ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gn	F
23	+ve	-ve	-ve	+ve	-ve	+ve	-ve	Y/Y, Hp, Gp	F
24	+ve	-ve	-ve	+ve	-ve	+ve	-ve	Y/Y, Hp, Gp	F
25	+ve	-ve	+ve	-ve	+ve	+ve	-ve	Y/Y, Hp, Gp	F
26	+ve	-ve	-ve	+ve	-ve	+ve	-ve	Y/Y, Hp, Gp	F
27	+ve	-ve	-ve	-ve	-ve	+ve	-ve	R/R, Hp, Gn	F

Note: +ve: positive

-ve: negative

F: fermentative

Y/Y: yellow / yellow

R/R: red / red

Hp: H_2S positive; Hn: H_2S negative

Gp: gas positive; Gn: gas negative

4.2 DATA ANALYSIS

The data was analysed with the help of MS Excel 2013 and further analysis was done using R.

4.2.1 Physicochemical VS Microbiological Quality

The result obtained after the Canonical Correspondence Analysis (CCA) was plotted in figure 2. One way analysis of variance on canonical correspondence analysis (CCA) showed that among the selected parameters, water temperature, pH, conductivity and chloride were the influencing factors (P<0.05) of drinking water quality.

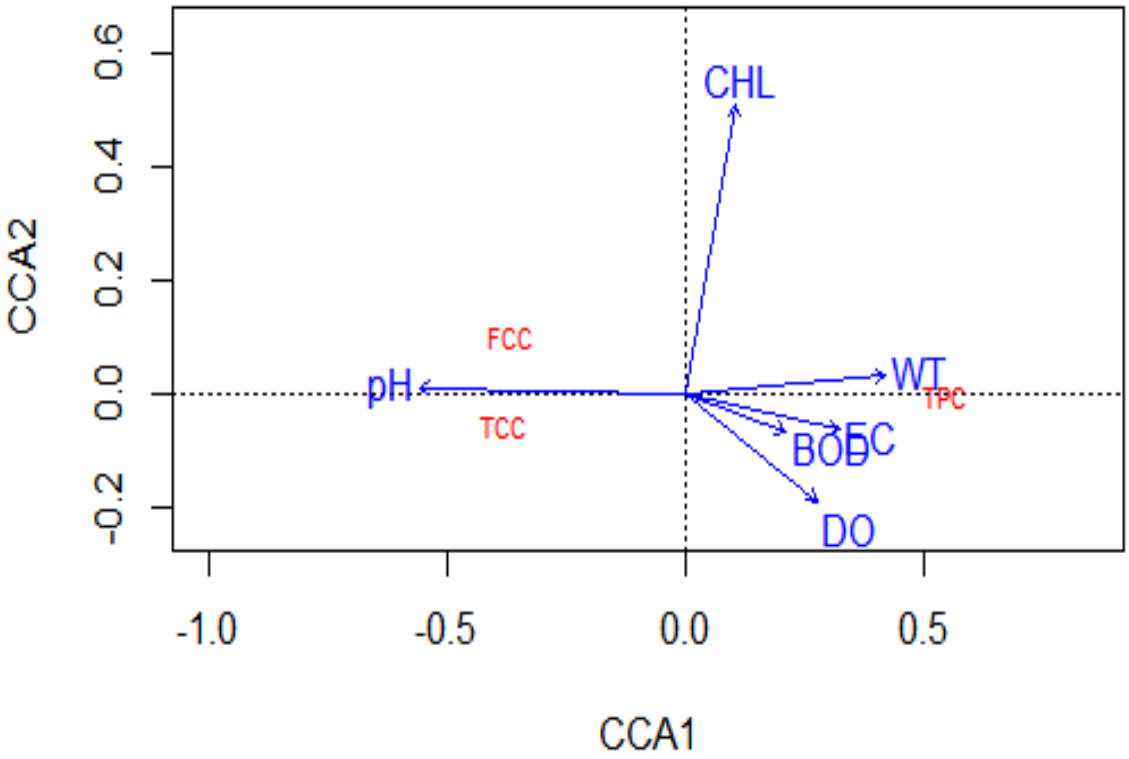


Figure 2: Canonical Correspondence Analysis (CCA)

Table 4: Test for equal means

	Sum of sqrs	df	Mean square	F	P (same)
Between groups	14083.3	1	14083.3	20.53	9.973E-05
Within groups	19203.9	28	685.852	Permutation p (n=99999)	
Total	33287.2	29	0.00013		

Components of variance (only for random effects):

Var(group): 893.165 Var(error): 685.852 ICC: 0.565646

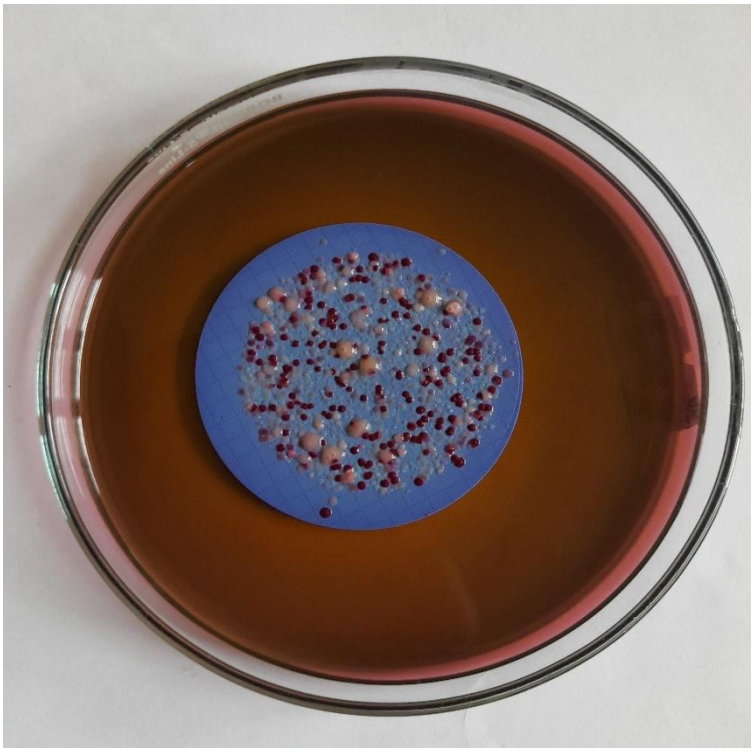
omega2: 0.3944

Levene´s test for homogeneity of variance, from means p (same):0.1746

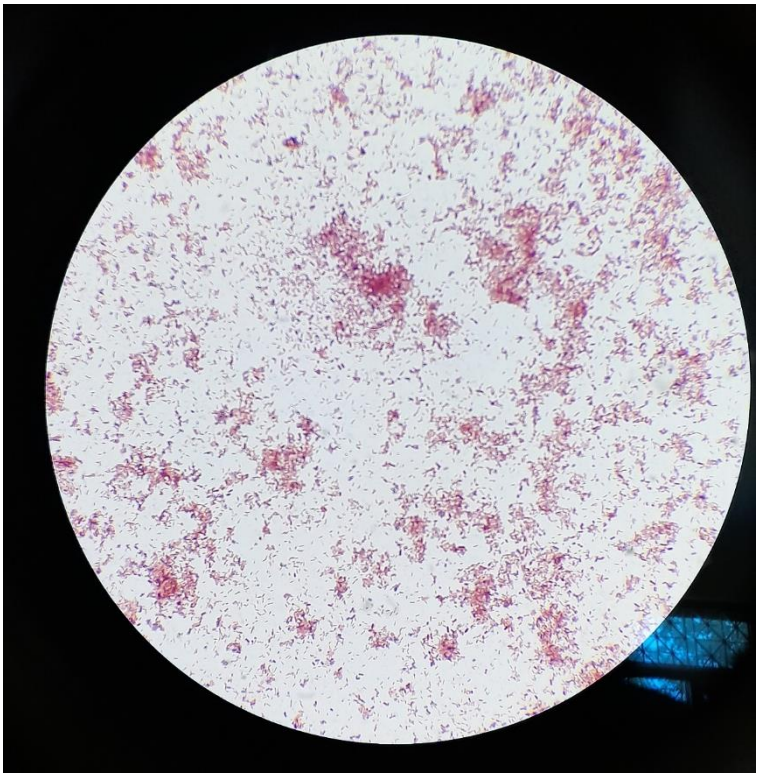
Levene´s test, from medians p (same):0.2986

Welch F test in the case of unequal variances: F=20.53, df=23.95, p=0.0001374

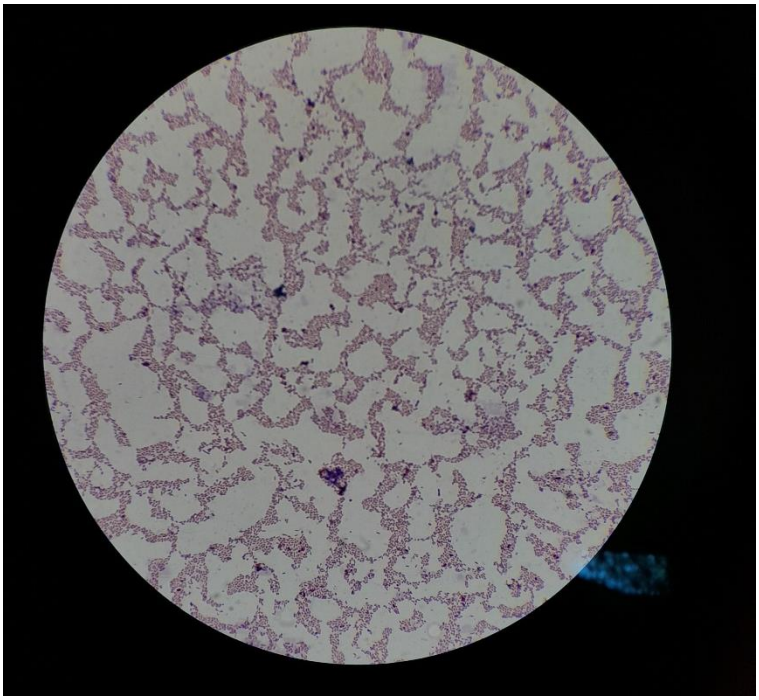
Photographs



Photograph 1: Growth of microorganisms in EMB agar after24 hrs incubation at 44.5^{o}C.



Photograph 2: Microscopic view of Gram staining of bacterial isolate



Photograph 3: Microscopic view of Gram staining of bacterial isolate

Photograph 4: Biochemical test of bacterial isolate

4.3 DISCUSSION

A total of 31 water samples were collected from the distribution system of Dharan, Nepal; out of which 12 were from sources, 3 from reservoirs and 16 from the taps. They were analyzed for the determination of physicochemical (temperature, pH, conductivity, dissolved oxygen, biological oxygen demand, chloride, nitrite and ammonia) and microbiological (total plate count, total coliform count and fecal coliform count) quality. The physicochemical quality of samples from the tap water was not analyzed.

In this study, the pH ranged between 7.8-8.4, the conductivity ranged between 38.2-38.7µmho/cm, chloride from 29.82 to 34.08 mg/l and ammonia less than 0.5ppm which were all within the World Health Organization standards and Nepal Drinking Water Quality standards. The highest temperature observed was 25.6°C and the lowest was 23.7°C. The nitrite levels were found to be 10ppm in all the collected water samples.

Dissolved oxygen (DO) values of the water samples ranged between 49.54-57.36mg/l which was higher compared to the study by (Dhungana, 2019). The difference in the concentration of DO in water sample of a particular area might be explained by a combined effect of temperature, photosynthesis, respiration, organic waste, aeration and sedimentation concentration (Badge, *et al*., 1985).

The biological oxygen demand (BOD) is one of the most often used indicators of organic pollution in water. The amount of putrescible organic matter in water is measured by BOD. As a result, a low BOD indicates good water quality, whereas a high BOD suggests dirty water. The Biological oxygen demand (BOD) values ranged between 2.61-5.22mg/l showing three of the samples unacceptable according to WHO standards (<5mg/l) varying from the study by (Dhungana, 2019).

The Total Plate Count (TPC), Total Coliform Count (TCC) and Fecal Coliform Count (FCC) obtained from all the water samples are listed in table 2. The TPC was TMTC in three samples and it ranged from 14×10^{1} cfu/ml to 13×10^{2} cfu/ml. The Total Coliform Count (TCC) were found to range from 29 cfu/100ml to 137 cfu/100ml and the Fecal Coliform Count (FCC) ranged from 24 cfu/100ml to 85 cfu/100ml.

The study showed conductivity to range within the WHO guidelines and Nepal Drinking water Quality Standards varying greatly from the results of study in 2009

showing the conductivity of 34.28 percent of the samples exceed the WHO and national standard permitted guideline limit (Jayana, *et al.*, 2009). Although the research conducted thus far have not revealed any direct health effects, high conductivity frequently indicates the presence of contaminants.

According to two different studies, (Jayana, *et al.*, 2009) and (Maharjan, 1998), only 2.85% of the water samples had chloride levels that exceeded the WHO's recommended limit which was higher than that of our current study. Natural sources, sewage and industrial effluents, urban runoff carrying de-ionizing salts, and saline intrusion are all sources of chloride in drinking water. Water and beverages with a high chloride percentage have a salty taste. Depending on the alkalinity of the water, excessive chloride concentrations accelerate the corrosion of metals in the distribution system.

pH, conductivity, turbidity, total hardness, iron, arsenic, ammonia, and total Coliform are the most problematic parameters in Kathmandu valley drinking water sources. By using the MF technique, 80 percent of tap water samples had Coliforms (max. 300 CFU/100 ml), indicating probable faecal contamination (Koju, *et al.*, 2014) which was higher than that of our study.

Our study was in accordance with the previous study conducted in Dharan in 2016 by (Pant, *et al.*, 2016) where indicator species were detected in the majority of the sources and reservoirs, pH of all the sources and reservoirs was within acceptable limits, four of the five sources were found to be heavily polluted with heterotrophic bacteria and similarly, total coliforms and fecal coliforms were found to be contaminated in three sources.

CHAPTER 5

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Physicochemical and microbiological examination of water from the distribution system of Dharan, Nepal demonstrates that the water being distributed to the sub-metropolitan city was mostly compatible with the World Health Organization (WHO) standards and Nepal Drinking Water Quality Standards (NDWQS) in terms of pH, conductivity, ammonia and chloride levels. The majority of the sources and reservoirs were found to be substantially contaminated with indicator species, indicating a serious water pollution problem in the area. Improving the bacteriological quality of the water sources and reservoirs supplying drinking water to Nepal's Dharan sub-metropolitan city requires immediate attention.

5.2 Limitations of the work

1. Due to the time constraint, seasonal variation of the physicochemical and microbiological quality was not studied.

2. Pathogen-specific study was not carried out.

5.3 Recommendations

1. Strict rules should be made and implemented for regular monitoring and management of the quality of drinking water.

2. The provincial authority should devote greater resources for the treatment and purification processes of drinking water.

REFERENCE

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APPENDIX 1

List of materials

Equipments used:

Autoclave	Weighing balance
Hot air oven	Incubator
Microscope	Refrigerator
Digestion apparatus	Micropipette
Thermometer	pH meter
Ammonia test kit	Nitrite test kit

Microbiological media and Biochemical media:

Eosin Methylene Blue Agar	Simmon's Citrate Agar
Nutrient Agar	Triple Sugar Iron Agar
MR-VP Broth	Urea Agar Base
Tryptophan Broth	Hugh and Leifson's medium

Chemicals and Reagents:

Catalase reagent (3% H2O2)	Kovac's Reagent
Potassium hydroxide	Methyl Red
Alpha-napthol	Urea
Crystal violet	Lysol

- Sulfuric acid
- Gram’s Iodine
- Potassium chromate
- Potassium dichromate
- Ferroin
- Manganous sulphate
- Silver nitrate
- Ethanol
- Safranin
- Paraffin
- Ferrous ammonium sulphate
- Sodium thiosulphate
- Sodium azide

Glasswares:

- Test tubes
- Pipettes
- Conical flasks
- Petriplates
- Beakers
- Glass rods and glass tubes
- BOD bottles
- Slides
- Measuring cylinders

Miscellaneous:

- Aluminum foils
- Inoculating loop/ needles
- Forceps
- Cotton plugs
- Labeling tape
- Measuring scale
- Blotting paper
- Test tube holder

APPENDIX 2

Preparation of media and reagents

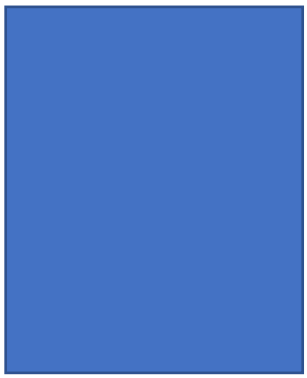
1. **Urea Agar Base**

Dextrose	1.0gm/L
Peptic digest of animal tissue	1.5gm/L
Sodium chloride	5.0gm/L
Monopotassium phosphate	2.0gm/L
Phenol red	0.012gm/L
Agar	15.0gm/L
Urea	40%

2. **Hugh and Leifson's Medium**

Peptone	2.0gm/L
Sodium chloride	5.0gm/L
Dipotassium phosphate	0.30gm/L
Glucose (Dextrose)	10.0gm/L
Bromothymol blue	0.030gm/L
Agar	3.0gm/L

EVALUATION OF PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITY OF DRINKING WATER IN THE DISTRIBUTION SYSTEM OF DHARAN, NEPAL



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