



Original Article

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Evaluation of physicochemical and microbiological quality of drinking water in the distribution system of Dharan, Nepal

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Abstract

Dharan has been facing drinking water-related problems for a very long time now and this research was conducted in order to determine whether the quality of water being distributed throughout the city was one of them. Hence, 31 samples were taken from the drinking water distribution system of Dharan in the spring of 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 National Drinking Water Quality Standards (NDWQS) for drinking water with temperatures ranging from 23.6 °C to 25.6 °C, pH 7.7 to 8.5, conductivity 38.2 to 38.7 µS/cm, Dissolved Oxygen (DO) 7.7 to 9.0 mg/L, Biological Oxygen Demand (BOD) 0.8 to 1.8 mg/L, chloride 29.82 to 34.08 mg/L, nitrite 10 mg/L and ammonia <0.5 mg/L. However, the coliform bacteria levels were significant, with the highest TCC (Total Coliform Count) being 137 CFU/100mL, the highest FCC (Fecal Coliform Count) being 85 CFU/100mL and the highest TPC (Total Plate Count) being TMTC (Too Many To Count). The water was found to be unsafe to drink without disinfection treatments. It may be necessary to carry out treatment procedures like chlorination as advised by WHO (World Health Organization) as soon as possible while also taking into account the proper application of filtration techniques for distributing safe drinking water to the residents of Dharan.

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1. Introduction

Water that is clean and safe is essential for good health and 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. According to the WHO (2022), 2.2 billion people still lack access to safe drinking water and more than half of the world's population lacks basic sanitation due to which the supply of safe drinking water has become a global concern.

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. In Nepal, 10.8 million people lack access to better sanitation and 3.5 million lack access to even the most basic water services (UNICEF, 2018). As of mid-2015, the Department of Water Supply and Sewerage Management (DWSSM) claimed that around 86% of Nepalese population have availability to reliable water supply facilities (DWSSM, 2015). The quality of the water provided, however, is questionable. The Department of Health Service (DoHS) reported 23,742 cases of water-borne illnesses among Nepalese patients in its 2016–2017 annual report, with 270 cases resulting in death (DoHS, 2016).

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). According to WHO (2007), inadequate sanitation, pollution, or a lack of water are responsible for up to 80% of all sicknesses and diseases in the world. Nepal, like many developing countries, has a slew of issues with both the quality and availability of drinking water (Warner et al., 2008). A large number of deaths and morbidities are caused by contaminated drinking water and water-borne diseases (Prasai et al., 2007). Water-borne diseases are one of the main causes of death for children under the age of five. Children lose their lives every day to diseases that might be avoided due to poor water quality, inadequate sanitation, and poor hygiene (UNICEF, 2018).

Water should be pathogen-free from a microbiological aspect because their presence in drinking water could be dangerous to people's health. The vast majority of the drinking water distribution system's sources and reservoirs were discovered to be seriously polluted with indicator species, pointing to a substantial problem with water pollution in Dharan area (Pant et al., 2016). Similarly, 47 samples from various drinking water projects were gathered in collaboration with the Department of Water Supply and Sewerage Management and WHO, Nepal, and 59% of the samples were confirmed to be contaminated with *Escherichia coli* (DoHS, 2022). Therefore, assessment of drinking water quality in terms of physicochemical and microbiological aspects can help to take effective management decisions to promote public health.

In Dharan, the drinking water distribution system supplies water through the pipelines. The source of water in the distribution system is the rivers. There are four rivers supplying drinking water to the city namely Shardu, Kharu, Shimle and Tamakham. The water from Shardu and Kharu are supplied directly through the pipelines whereas the water from Shimle and Tamakham rivers are first collected in a reservoir and then supplied through the pipelines. 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 primary objective of this study was to analyze the physicochemical and microbiological quality of water in the source and reservoirs of the drinking water distribution system of Dharan.

2. Materials and Method

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. 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 (26.82806°N, 87.284754°E). The approximate location of the study area is displayed in figure 1.

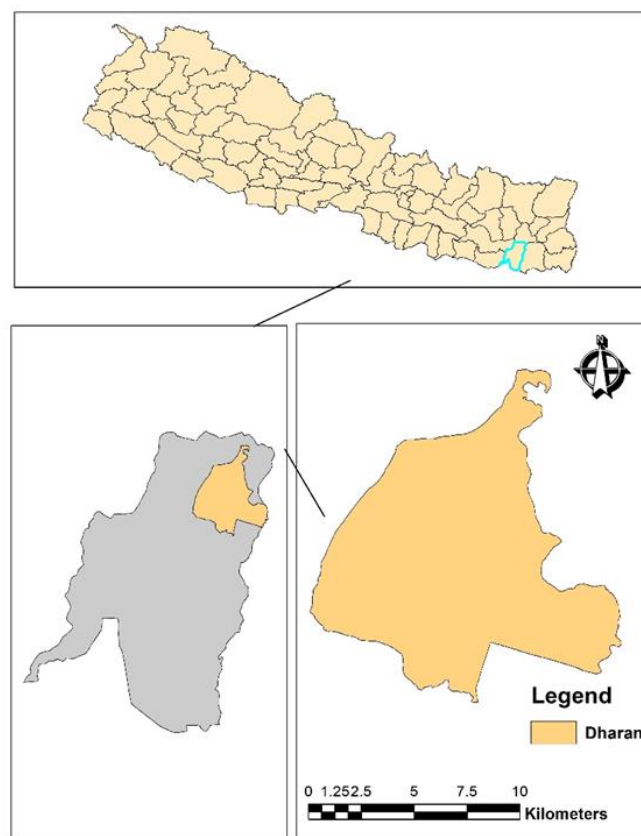


Figure 1: Study Area

2.2. 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 a cold chain within half an hour of collection and the samples were processed as soon as possible.

2.3. Physicochemical Analysis

2.3.1. Temperature

To measure the temperature of the water, it was collected in a beaker and a mercury-filled Celsius thermometer was placed inside it. After achieving a stable reading, the temperature was noted.

2.3.2. pH

Using an automatic digital pH meter (Hanna Instruments), pH was measured. By submerging the electrode in a pH 7 standard buffer solution, the pH meter was first calibrated. After being cleaned with distilled water and dried with a tissue, the glass electrode was once more dipped into the buffer solution with a pH of 10. The electrode was repeatedly cleaned with distilled water, dried with a tissue, and then submerged in the water sample-containing beaker until the reading stabilized at a particular value. The pH reading was then recorded.

2.3.3. Conductivity

Conductivity was tested using a conductivity meter (Spectronics India). The magnetic stirrer was first used to calibrate the conductivity meter by being placed in a beaker filled with 0.1M Potassium Chloride and adjusting its conductivity to 1412 $\mu\text{S}/\text{cm}$ at 30°C. The electrode was washed with distilled water and wiped with a tissue paper. Then, it was dipped in the beaker containing a water sample until the reading was stabilized at a certain point. Then, conductivity reading was noted down.

2.3.4. Dissolved Oxygen (Winkler's Method)

The APHA (2012) procedure was used to determine the DO. A 300 mL BOD bottle was used to gently collect the sample, preventing any bubbling or air bubbles from being trapped inside the bottle after the stopper was applied. Well below the surface from the bottle

well, 2 mL of manganese sulphate (MnSO_4) and 2 mL of alkaline iodide azide solution were added until a precipitate developed. The cork was then firmly fastened, and the container was stirred to ensure that the contents were well combined. It was left for a while to allow the precipitate to settle. Then, it was added with 2 mL of concentrated H_2SO_4 and shaken to completely dissolve the precipitate. The sample was then divided into 50 mL and put in a conical flask before being titrated against sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) of 0.025M with the aid of starch as an indicator. The initial blue color becomes colorless at the end point.

Calculation:

$$\text{DO}(\text{mg/L}) = \frac{(\text{mL} \times \text{M}) \text{ of titrant consumed} \times 8 \times 1000}{V_2 \times (V_1 - V)}$$

Where, V_1 = volume of sample bottle after placing the stopper, V_2 = volume of part of content titrated, V = volume of MnSO_4 and KI added, mL = volume of thiosulphate consumed, M = molarity of thiosulphate consumed.

2.3.5. Biological oxygen demand (BOD)

The quantity of biologically degradable organic matter that is present in a water sample is measured by the biochemical oxygen demand (BOD), which is also known as the amount of oxygen needed by microbes to stabilize biologically degradable organic matter under aerobic conditions. The method's basic idea is to compare the sample's dissolved oxygen levels after five days of incubation at 20 °C.

Calculation:

$$\text{BOD, mg/L} = (\text{D}_0 - \text{D}_5) \times \text{dilution factor}$$

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

2.3.6. Chloride

Chloride was measured by titration method. 50 mL of sample was taken in a conical flask. The sample solution was titrated against 0.02M silver nitrate using 2 mL of potassium chromate until a brick red color developed.

Calculation:

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

Where V is the volume of the sample in mL and A , B , and M are the volumes of the titrants (silver nitrate) for the sample and blank, respectively.

2.3.7. Nitrite

Nitrite content in the water sample was determined by using a nitrite test kit (HiMedia laboratories). For this, the aqua check jar was filled with 10 mL 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 of nitrite content was done as: NaNO_2 , $\text{mg/L} = 5 \times (\text{number of drops of 07B})$

2.3.8. Ammonia

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

2.4. Microbiological Analysis

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

2.4.1. Total Plate Count (TPC)

Total plate count was determined by spread plate technique using Nutrient Agar (HiMedia, India). 0.1 mL of water sample was pipetted out onto the center of the surface of the 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 °C for 24 hours.

2.4.2. 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 were 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 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 °C for 24-48 hours.

2.4.3. 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 °C for 24-48 hours.

2.4.4. Sub-culture

Colonies obtained in Eosine Methylene Blue (EMB) agar plates after 48 hours 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. Results and Discussion

3.1. 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 (µS/cm)	DO (mg/L)	BOD (mg/L)	Chloride (mg/L)	Nitrite (mg/L)	Ammonia (mg/L)
1	23.7	8.2	38.2	9.0	1.8	31.2	10	<0.5
2	23.6	8.2	38.2	8.8	1.7	31.2	10	<0.5
3	23.6	8.1	38.2	8.6	1.8	31.2	10	<0.5
4	24.8	8.4	38.6	7.7	0.8	31.2	10	<0.5
5	24.7	8.5	38.5	7.9	0.8	31.2	10	<0.5
6	24.8	8.4	38.6	7.7	0.7	31.2	10	<0.5
7	25.1	8.0	38.6	8.5	1.2	29.8	10	<0.5
8	25.3	8.0	38.7	8.5	1.2	29.8	10	<0.5
9	25.1	8.0	38.6	8.5	1.2	29.8	10	<0.5
10	25.2	7.8	38.5	8.5	1.5	34.1	10	<0.5
11	25.2	7.7	38.5	8.5	1.4	34.1	10	<0.5
12	25.1	7.8	38.4	8.5	1.4	34.1	10	<0.5
13	25.6	8.0	38.7	8.5	1.5	31.2	10	<0.5

14	25.5	8.0	38.7	8.5	1.4	31.2	10	<0.5
15	25.5	8.1	38.7	8.5	1.5	31.2	10	<0.5

Number of samples= 15

3.2. Microbiological analysis of water samples

coliform count obtained for all the water samples are listed in Table 2.

The total plate count, total coliform count and fecal

Table 2: Microbiological analysis of the water samples

Sampl e code	TPC (CFU/0.1mL)	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

Number of samples= 31 *TMTC: Too Many To Count (more than 300 colonies)

3.3 Data Analysis

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

3.3.1. 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.

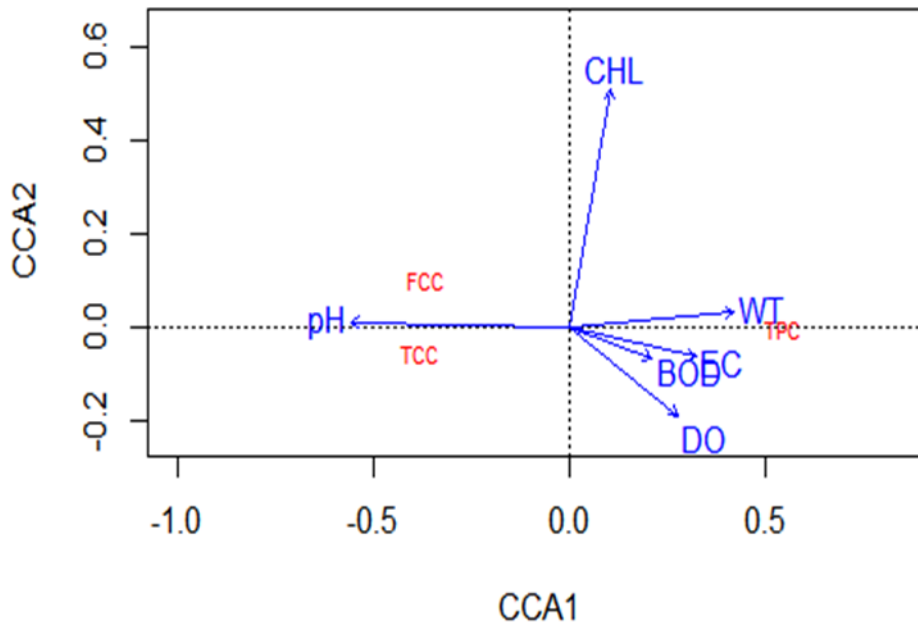


Figure 2: Canonical Correspondence Analysis (CCA)

Table 3: Test for equal means

	Sum of squares	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):

$$\text{Var}(\text{group}) = 893.165, \quad \text{Var}(\text{error}) = 685.852, \quad \text{ICC} = 0.565646, \quad \omega^2 = 0.3944$$

Levene's test for homogeneity of variance, from means

$$p(\text{same}) = 0.1746$$

Levene's test, from medians

$$p(\text{same}) = 0.2986$$

Welch F test in the case of unequal variances:

$$F = 20.53, \quad Df = 23.95, \quad p = 0.0001374$$

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.

Results from the current study were compared to those from earlier research on water quality for the same sources and reservoir conducted in 2016 (Pant et al., 2016). The parameters such as temperature and TPC were found higher, the pH was similar but TCC and

FCC were discovered to be lower than the prior record. Table 4 shows present (2022) and past (2016) findings

with the minimum and maximum values along with the NDWQS values.

Table 4: Present (2022), past (2016) and NDWQS values of parameters

S.N.	Parameters	2022 (Min)	2022 (Max)	2016 (Min)	2016 (Max)	NDWQS
1	Temperature (°C)	23.6	25.6	14	20	-
2	pH	7.7	8.5	6.9	8.7	6.5-8.5
3	EC (µS/cm)	38.2	38.7	-	-	1500
4	DO (mg/L)	7.7	9.0	-	-	-
5	BOD (mg/L)	0.7	1.8	-	-	-
6	Chloride (mg/L)	29.8	34.1	-	-	250
7	Nitrite (mg/L)	10	10	-	-	-
8	Ammonia (mg/L)	<0.5	<0.5	-	-	1.5
9	TPC (CFU/0.1mL)	14×10 ¹	TMTC	0	175	Nil
10	TCC (CFU/100mL)	29	137	0	1500	Nil
11	FCC (CFU/100mL)	17	85	0	850	Nil

The pH ranged from 7.7 to 8.5, the conductivity ranged from 38.2 to 38.7 µS/cm, chloride from 29.8 to 34.1 mg/L and ammonia less than 0.5 mg/L which were all within the World Health Organization standards and National Drinking Water Quality standards. The highest temperature observed was 25.6 °C and the lowest was 23.7 °C. Regarding water temperature and its effect on public health, there are no standard guidelines. The nitrite levels were found to be 10mg/L in all the collected water samples.

Dissolved oxygen (DO) values of the water samples ranged from 7.7 to 9.0 mg/L which was in accordance with the one observed in Sundarijal reservoir (Dhungana, 2019). Another parameter most frequently used to assess the level of organic pollution in water is the biological oxygen demand (BOD). BOD is used to determine how much decomposed organic matter is present in water. As a result, a low BOD denotes clean water, while a high BOD denotes unclean water. The Biological oxygen demand (BOD) values ranged between 0.8 to 1.8mg/L which were within WHO standards (<5mg/L) (WHO, 2004).

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 four samples and it ranged from 14×10¹ CFU/mL to 13×10² CFU/mL which was higher than the previous study. The Total Coliform Count (TCC) ranged from 29 CFU/100mL to 137 CFU/100mL and the Fecal Coliform Count (FCC) ranged from 24 CFU/100mL to 85 CFU/100mL which were higher than that of previous study (Pant et al., 2016).

The study showed conductivity to range within the WHO guidelines and National 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 by Jayana et al. (2009) and Maharjan (2018), 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. Chloride in drinking water comes from a

variety of sources, including natural sources, sewage and industry wastewater and saline intrusion. 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.

4. Conclusions

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) guidelines and National Drinking Water Quality Standards (NDWQS) in terms of pH, conductivity, ammonia and chloride levels. It was observed that most of the sources and reservoirs were heavily polluted with indicator species, indicating a serious water pollution problem in the area. Improving the microbiological viability of the water sources and storage facilities that provide drinking water to Nepal's Dharan sub-metropolitan city requires immediate attention. Strict rules should be made and implemented for regular monitoring and management of the quality of drinking water. The provincial authority should devote greater resources for the treatment and purification processes of drinking water.

The present study creates a baseline for monitoring the sources, reservoirs and overall the distribution system of drinking water in Dharan. The concerned authorities must make the necessary efforts for effective management and implement some achievable steps to protect water resources and develop water quality management plans for this area in order to increase the quality of the drinking water supply. People are at risk of developing a number of health problems if they consume unsafe drinking water. Thus, the findings of this research could be useful for fulfilling its objective of making people aware of the need of drinking water safety as well as for the

maintenance and sanitation of water resources.

Author's Contribution

Final approval of manuscript was done by all the authors.

Competing interests

The authors declare no competing interests.

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Ethical Approval and Consent

Verbal consent was obtained from all the respondents for the interview and further publication of the report.

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