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Physicochemical and bacteriological analyses of groundwater in the periphery of the Bagmati river in Kathmandu Valley

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Abstract

The objective of this study was to assess the physicochemical and bacteriological quality of groundwater samples. A total of 34 samples were taken during the time period of June 6 to June 21 of 2016 from the groundwater sources in Kathmandu, located in the periphery of Bagmati river, from Chobhar to Sinamangal with approximate distance of 10 km. Site for study, sample collection, transportation, preservation and processing (bacteriological and physicochemical analyses) of samples were carried out following standard protocols issued by [1, 2]. Membrane filtration technique was employed for enumeration of indicator organism (coliform and thermotolerant coliform) and biochemical tests for its identification.

The temperatures of water sample ranged from 19.6°C to 24.6°C and pH from 6.9 to 7.8 during early afternoon of different sampling days. Electrical conductivity (EC) varied from 20 μS/cm to 890 μS/cm showing a positive correlation with the Total Dissolved Solids (TDS) values. The samples were found to contain higher turbidity that ranged from 5 NTU to 14 NTU. The Dissolved Oxygen (DO) values were within the WHO limit, ranging from 5 mg/L to 7.9 mg/L. The water samples did not have problems regarding the chloride, nitrate or phosphate levels. The levels of ammonia and iron were found to be higher than the standard values. Out of 34 samples, only three samples (9%) were coliform free and the rest of the samples showed contamination. *Escherichia coli* (*E. coli*) isolates were obtained from six of the samples (18%), ranging from 2 cfu/100 mL to 5 cfu/100 mL. Thus, 31 samples were found to be unfit for drinking having tested positive for coliforms. Overall, samples from Chobhar to Balkhu, specifically nearby Manjushree area were found to have better quality than other water samples but still none of the samples have unquestionable quality.

Keywords: coliform; contamination; groundwater; membrane filtration

1. Introduction, definitions and standard results

Nepal is known as the second richest country in the world regarding the opulence of water resources. However, not more than two-thirds of the populations have provision of safe drinking water [3]. Having no proper provision of safe drinking water, the residents (mostly the slum dwellers) in the periphery of the Bagmati river depend on alternative sources of drinking water like dug wells, tube wells and stone spouts. Not all of them are aware of the methods of filtration and sterilization of water for drinking. In fact, most people directly consume such water and use them for washing rice or vegetables every day, relying solely to the clarity of such water and unaware of the hidden microbes and chemicals. The water borne diseases that come after such unwary consumption of such water has been bothering the residents in the periphery of the Bagmati river in the valley.

It is well known that more than 88% of the global diarrheal diseases are water-borne infections caused by drinking unsafe and dirty water [4]. Nepal faces a serious crisis of potable drinking water in both urban cities and rural areas. Every summer, water borne epidemics (diarrhea, cholera, typhoid, etc.) hit different parts of the country including Kathmandu valley and cause a heavy death toll. Most of the water sources in Kathmandu valley do not comply with the guidelines provided by the WHO [5, 6].

This study is aimed at assessing the microbiological and physicochemical parameters of drinking water that come from underground sources in the periphery of the Bagmati river. Since, the water quality of the Bagmati river is already in an almost declining state; the quality of water from tube wells and spouts near the river is vividly questionable. These underground water resources in the periphery of the river may share water contamination by pathogenic bacteria, pesticides, nitrate, industrial effluents, and domestic sewage through seepage and alter the overall quality of groundwater which has been tested in this study. It is must that the water is taken every few months, for assessment of essential parameters of drinking water so that the consumers of such water can know if they are drinking safe water. If the water quality is objectionable, appropriate measures can be taken for making those people cognizant of sterilization techniques of water.

2. Materials and methods

2.1. Study design and site

A cross-sectional study was carried out in Kathmandu Valley, Nepal as shown in Fig. 1, to assess the bacteriological and physicochemical quality of groundwater in the periphery of the Bagmati River, one that is quite often consumed or consumed on a daily basis. The study was conducted in Microbiology Laboratory of Kathmandu College of Science and Technology from Jestha 23, 2073 (June 6, 2016) to Asaadh 7, 2073 (June 21, 2016). Samples were taken from groundwater sources such as tube wells, dug spouts, boring water, etc., from Chobhar to Sinamangal. Altogether, 34 samples were collected from different places lying inside the aforementioned range during early afternoon and samples were classi-

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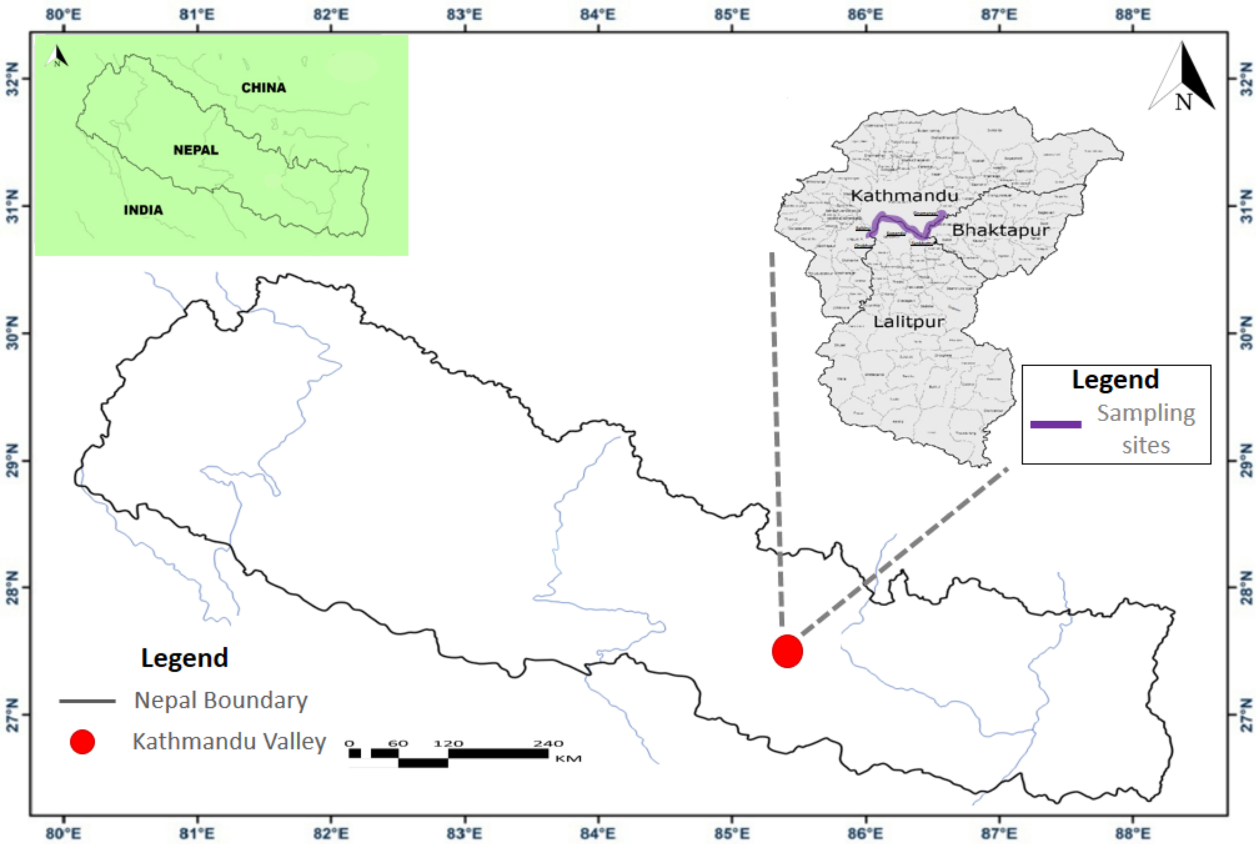

Figure 1: Location map of study area (Kathmandu Valley, Nepal).

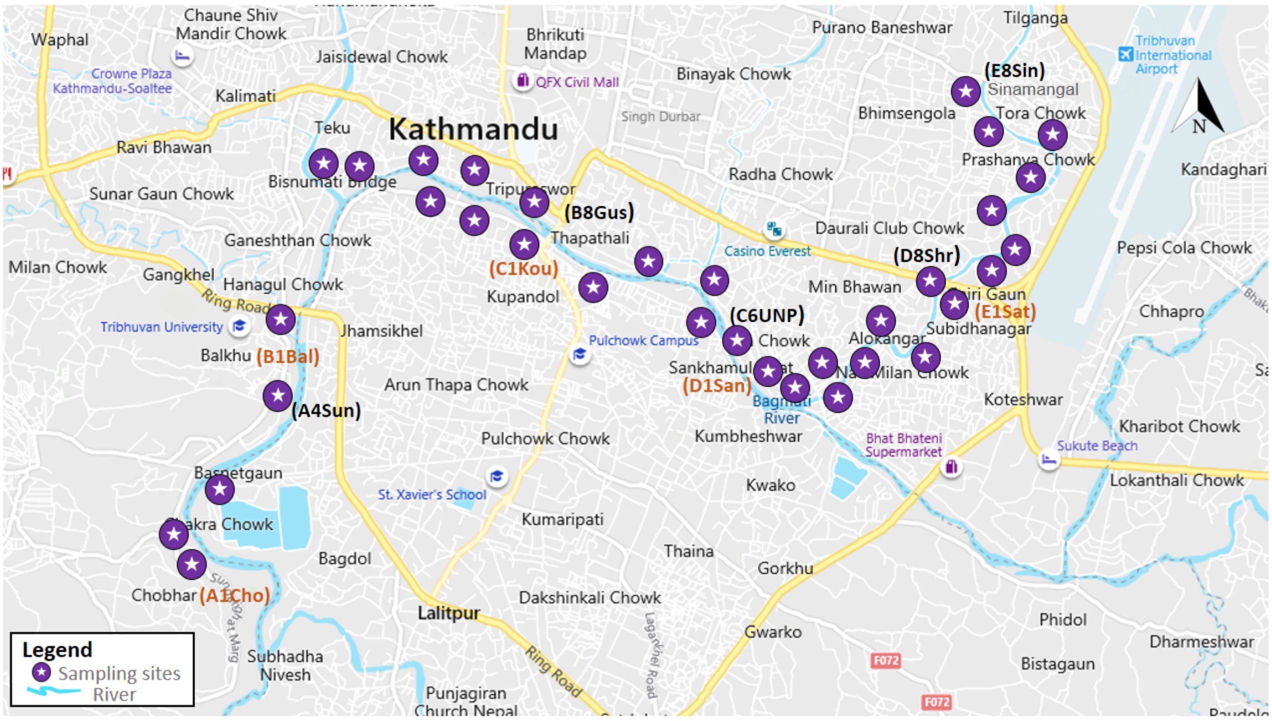

Figure 2: Sample collection sites.

fied into five groups based on their location-ranges starting from Chobhar (GPS: 27°40'14.06"N, 87°17'28.19"E) to Sinamangal (GPS: 27°41'56.27"N, 85°20'46.94"E) as highlighted in Fig. 2. The following table summarizes the group classification.

2.2. Sample size and sampling points

Geographical positioning system (GPS) was used to locate the sampling points with the help of GPS in an Android phone. A total of 34 samples were collected from Chobhar to Sinamangal with an approximate distance between every pair of samples being around 300 m (Fig. 2).

Inclusion criteria: Underground water samples that were used for any or all of the purposes such as drinking or washing rice and/ or vegetables were included in the study.

Exclusion criteria: Underground water samples that were used for purposes other than drinking or washing rice and/ or vegetables were excluded from the study. Water samples that could not be timely transported to lab or those which could not be properly collected were excluded from study.

2.3. Collection of water samples

The water samples were collected for bacteriological analysis as given by [2]. The water samples were collected in pre-sterilized glass bottles (15 lbs at 121°C for 15 minutes) of 300 mL capacity and transported to the laboratory in an ice box containing freezer ice packs. From each sampling point, 300 mL samples were taken for the analyses. And for the chlorinated water samples (a reservoir of bored water was chlorinated), sodium thiosulphate was added to stop the chlorination process during transportation. Three milliliter (3 mL) sodium thiosulphate was added into each sampling bottle. The bacteriological tests were undertaken within 6 hours after collection to avoid the growth or decay of microorganisms in the sample [1]. With regard to the physicochemical analysis, 200 mL water samples were collected in clean bottles, washed and rinsed 3-4 times with water to be tested.

2.4. Transportation and preservation of samples

After collection, they were labeled and transported to the Microbiology Laboratory of Kathmandu College of Science and Technology (KCST), Kamalpokhari - Kathmandu, holding in an ice box at 4°C. Water samples were examined as soon as possible on arrival or within 6 hours of collection. In some cases when immediate analysis was not possible, the samples were preserved at 4°C.

2.5. Analysis of physicochemical parameters

Standard methods for the examination of water and wastewater [7] were followed to analyze physicochemical parameters of water.

Temperature

It was measured in the site by dipping the bulb of a standard mercury thermometer in water samples collected in the beaker and the temperature was noted. The unit of measurement used was degree centigrade (°C).

pH

pH values were measured by a digital pH meter which was first calibrated with a standard buffer solution. The glass electrode was washed with distilled water and then dipped in the beaker containing water sample until the reading stabilized at a certain point. Then pH readings were obtained and noted down for the study.

Conductivity (EC) and total dissolved solids (TDS)

Water samples were collected separately in beakers and a low range pH/conductivity/TDS tester (HI98312 EC/TDS/Temperature tester; HANNA Instruments) was used to measure the electrical conductivity (μS/cm) and TDS (mg/L). The temperature was again cross checked by this device.

Turbidity

This was measured by the comparison of the interference of light rays passing through the collected water sample with that of standard samples using the spectrophotometer. The unit of measurement was Nephelometric Turbidity Unit (NTU).

Dissolved oxygen (DO)

For this measurement, Winkler's method was followed [8]. Then the quantity of DO was calculated in mg/L by using the following equation:-

$$\text{DO (mg/L)} = \frac{(\text{mL} \times N) \text{ of } \text{Na}_2\text{S}_2\text{O}_3 \times 8 \times 1000}{\frac{V_2\,(V_1 - V)}{V_1}}$$

Where, V_1 = Volume of sample bottle after placing the stopper
V_2 = Volume of the part of contents titrate
V = Volume of $MnSO_4$ and alkaline KI added.

Chloride (Cl^-)

Chloride level was determined by titration with silver nitrate ($AgNO_3$) solution using potassium chromate (K_2CrO_4) as an indicator [9].

Nitrate (NO^{3-})

Nitrate was analyzed using Brucine Absorptivity method [7].

Phosphate (PO_4^{3-})

Phosphate was analyzed using Ammonium Molybdate method [7].

Ammonia (NH_3)

Nessler's Reagent method was used for analysis of ammonia [7].

Total Iron

Iron content in the water sample was determined by colorimetric method. In this method, 50 mL of the sample was taken in a conical flask. Two mL of concentrated HCl and 1 mL of hydroxylamine hydrochloride solutions were added. Then some glass beads were put in the flask and boil till the content is reduced about half. It was cooled and 10 mL of acetate buffer solution and 2 mL of Phenanthroline (Phen) solution was added, then orange red color was appeared. Distilled water was added to make the volume 100 mL in a volumetric flask. Let it stand for 10 minutes, and the absorbance of the color was measured by using spectrophotometer at 510 nm using distilled water blank with the same amount of chemical. The same procedure was repeated for standard solution of different concentrations. Then the concentration was determined with the help of standard curve.

2.6. Bacteriological analysis of water samples

In order to evaluate the bacteriological quality, the presence of two indicator bacteria, total coliforms (TC) and thermotolerant coliforms were tested from the water sources, distribution points, and distribution networks (tap water) from different residential areas in periphery of the Bagmati river.

Total coliform and thermotolerant coliform count: In this study, total coliform and thermotolerant coliform were enumerated by the membrane filtration (MF) technique [10].

Isolation and Identification of E. coli: Greenish metallic sheen colonies (indicative of thermotolerant coliform - *E. coli*) in the EMB

Table 1: Classification of sampling sites.

Locations	Places	Approximate length (km)	No. of Samples	Codes
A	Chobhar-Balkhu	1.5	4	A1Cho- A4Sun
B	Balkhu- Koupondole	2.2	8	B1Bal- B8Gus
C	Koupondole- Sankhamul	1.8	6	C1Kou- C6UNP
D	Sankhamul- Subidhanagar	2	8	D1San- D8Shr
E	Subidhanagar- Sinamangal	2	8	E1Sat- E8Sin
Total	Chobhar- Sinamangal	9.5	34	A1Cho- E8Sin

agar plates were sub-cultured onto MacConkey Agar (MA) and incubated at 44.5°C for 24 hours. All lactose fermenting colonies were sub-cultured on Nutrient agar (NA) for pure culture. Identification of *E. coli* was done based on colony characteristics, morphological characteristics and biochemical properties on respective media [11]. Cell morphology was studied using gram's reaction under oil immersion.

Study of biochemical tests: Biochemical tests are based on the ability of microorganisms to produce enzymes. The isolated pure colonies were inoculated into different biochemical media for different tests [12].

2.7. Statistical application

All laboratory data were analyzed using MS Excel 2013. Associations among variables were assessed using SPSS v.21 software, whenever required.

3. Results

The results of the study of 34 samples of groundwater are given under respective headings of their analyses.

3.1. Physicochemical Parameters

The water samples collected from Sankhamul to Subidhanagar (location D) were found to have the highest temperatures, turbidity, chloride, and phosphate and iron level and the lowest mean pH. Water samples from Balkhu to Koupondole (location B) were found to have the highest conductivity, TDS, ammonia and phosphate levels and the lowest average DO value as mentioned in Table 2. Samples from Chobhar to Balkhu (location A) were found to have the highest average pH and DO values and the lowest of most of the parameters. Samples from Koupondole to Sankhamul (location C) were found to have the highest average nitrate level.

Temperature

The water samples showed a variation of temperatures, the highest being 24.6°C from D2San and D3San (both from Sankhamul) and the lowest being 19.6°C from Aditi Marg (E3Adi). Taking the averages of the temperatures from samples of five locations, the highest mean temperature (23.075°C) was seen from Sankhamul to Subidhanagar (location D) and the lowest mean temperature (20°C) was seen from Chobhar to Balkhu (location A) as shown in Table 2. The temperature values of samples from Chobhar to Balkhu showed the lowest deviation from the mean temperature. Previous studies have also shown a variation in temperature of drinking water of Kathmandu. Studies conducted by [13, 14, 15, 16] are in agreement with this study in terms of temperature variation. Water in the temperature range of 7°C to 11°C has a pleasant taste and is refreshing [17]. Microbial pathogens, organic matters, turbidity, pollution, route of flow, water current, flow rate, human and environmental factors of water may have contributed to the high temperature records of water, samples we collected hence did not meet the WHO standard of raw drinking water temperature which is to be less than 15°C [18].

pH

The highest pH (7.8) was recorded from sample Chobhar (A1Cho) and the lowest (6.9) from Sankhamul (D1San). Taking the averages of pH of different places classifying them into five location ranges A to E, the highest mean pH (7.675) was obtained for Chobhar to Balkhu (location A) and the lowest mean (7.062) for Sankhamul to Subidhanagar (location D). Location B and location D have higher deviation of pH values from their averages while location A and C have the least deviation. They did not show significant difference in pH, the slightly lower pH recorded may be due to swampy surrounding of the area that enhances microbial activity that release microbial leachate into water source. Similar results were obtained by [5, 6, 16, 19, 20, 21] which explained that the pH of the water samples lie within the permissible limit. Over all pH records of water sample from the sources were found to be slightly basic. Hence, the pH status was within the recommended standard limits of 6.5-8.5 [18].

Conductivity

The values of conductance ranged from 890μS/cm in Sankhamul (sample D4San) to 20μS/cm in Chobhar (sample A2Cho). The mean conductivity was highest (503.125 μS/cm) for Balkhu to Koupondole (location B) and lowest (63.75 μS/cm) for Chobhar to Balkhu (location A). The conductivity of water depends on the presence of ionic substances. Conductivity of distilled water ranges from 1-5μS/cm. Pressure of salts and contamination with wastewaters increase the conductivity [7].

Total Dissolved Solids (TDS)

The lowest TDS was measured to be 612 mg/L from Gairigaun (E5Gai) and the highest to be 1302 mg/L from Sankhamul (D4San). The average TDS was found to be the highest (1016.875 mg/L) in Balkhu to Koupondole (location B) and the lowest (661.75 mg/L) in Chobhar to Balkhu (location A). There is no Primary drinking water standard for total dissolved solids. TDS in drinking-water originate from natural sources, sewage, urban run-off, industrial wastewater, and chemicals used in the water treatment process. In general, the total dissolved solids concentration is the sum of the cations (positively charged) and anions (negatively charged) ions in the water. Cations combined with Carbonates $CaCO_3$, $MgCO_3$; Associated with hardness, scales formation, bitter taste. Cations combined with chlorides NaCl, KCl give salty or brackish taste and increase corrosiveness.

Turbidity

The highest turbidity was found to be 14 NTU in two samples from Sankhamul (samples D1San and D2San) and the lowest was found to be 5 NTU in 17 samples. Taking the averages of the turbidity values of the water samples, the lowest turbidity (5.250 NTU) was recorded in Chobhar to Balkhu (location A) and the highest

Table 2: Physico-chemical parameters (averages) of water samples.

Parameters	Locations					Standard parameters	
	A	B	C	D	E	WHO	NDWQS
Mean Temperature (°C)	20.6	22.712	22.53	23.075	21.562	>15	-
Mean pH	7.675	7.537	7.516	7.062	7.2	6.5-8.5	6.5-8.5
Mean Conductivity (μS/cm)	63.75	503.125	353.33	378.75	395.625	-	-
Mean TDS (mg/L)	661.75	1016.875	885.5	903.75	917.833	600	1000
Mean Turbidity (NTU)	5.25	6.375	5.833	8.625	6.125	>5	-
Mean DO (mg/L)	7.4	6.025	6.1	6.187	6.375	-	-
Mean Chloride (mg/L)	38.09	43.75	48.35	87.01	77.27	200	250
Mean Nitrate (mg/L)	0.13	0.26	0.465	0.305	0.328	10	50
Mean Phosphate (mg/L)	0.14	0.47	0.301	0.437	0.32	-	-
Mean Iron (mg/L)	0.3	0.33	0.383	0.475	0.425	0.3	0.3
Mean Ammonia (mg/L)	0.05	0.125	0.116	0.125	0.1	1.5	1.5

(8.625 NTU) in Sankhamul to Subidhanagar (location D). The turbidity of drinking water should not be more than 5 NTU, and should ideally be below 1 NTU [1].

Dissolved Oxygen (DO)

Dissolved oxygen is the oxygen dissolved in water. DO in potable water is >5mg/L [1]. The highest DO value (7.9 mg/L) was seen in water sample from Gairigaun (sample E5Gai) and the lowest (5 mg/L) in water sample from Teku (sample B3Tek). Highest average DO was found in Chobhar to Balkhu (location A) and the lowest in Balkhu to Koupondole (location B). DO is important water quality parameter and is also index of physical and biological processes going on water. Oxygen saturated waters have a pleasant taste while the waters lacking oxygen have an insipid taste. The oxygen demanding waste depletes oxygen and decreases DO. At low DO, aquatic lives cannot survive and low DO is generally due to organic waste. The DO level of water indicates pollution with oxygen demanding waste in the water.

Chloride

The highest chloride concentration was determined to be 153.06 mg/L in the water sample from Sankhamul (D3San) and the lowest to be 8.52 mg/L from Chobhar (A1Cho). The highest average chloride concentration was found in Sankhamul to Subidhanagar (location D) and the lowest in Chobhar to Balkhu (location A).The concentration of chloride when increases to more than 250 mg/L , it gives rise to detectable taste in water and there is no any health-based value of chloride in drinking water [10].

Nitrate

The highest nitrate was recorded in Sati Marg (sample E1Sat) as 0.61 mg/L, whereas the lowest was 0.06 mg/L in Gusingal (sample B8Gus). Koupondole to Sankhamul (location C) displayed the highest average nitrate concentration while Chobhar to Balkhu (location A). The detection of nitrate is an important water quality indicator that shows organic matter pollution due to microbial activity, or the downward-leaching and accumulation of nitrate from the surface [22]. In the present study, no other physicochemical parameter showed significant variations in water samples like nitrate contents. A maximum contaminant level of 50mg/L of nitrate has been established for drinking water [1, 18].

Phosphate

The phosphate level ranged from 0.11 mg/L in Gairigaun (sample E4Gai) to 0.87 mg/L in Teku (sample B3Tek). Taking the averages of phosphate concentrations in five locations, Balkhu to Koupondole (location B) and Sankhamul to Subidhanagar (location D) showed higher values of averages, whereas Chobhar to Balkhu (location A) had comparatively very low and the lowest average value (0.14 mg/L).The SD does not show much difference between the average and the obtained values. Although there is no guideline value for phosphate content in drinking water, phosphate levels greater than 0.64mg/L [22] could interfere and induce coagulation in water treatment.

Iron

The iron level ranged from 0.3 mg/L in most of the water samples to 0.8 mg/L in a few samples. Sankhamul to Subidhanagar (location D) showed the highest average of the obtained values while Chobhar to Balkhu (location A) showed the lowest as shown in Table 2. All the locations showed similar level of standard deviation from their respective averages. Most of the samples showed an iron level of 0.3 mg/L and none of the samples were found to be brown in color. In anaerobic ground water iron is found in the form of Iron (II), concentration will be 0.5 to 10mg/L and the concentrations of iron in drinking water are normally around 0.3 mg/L [10]. Thus, Most of the samples were within permissible limit.

Ammonia

The ammonia level measured in the water samples showed a range of 0 to 0.2 mg/L. Taking the averages of the ammonia levels of all water samples classified in five locations, Balkhu to Koupondole (location B) and Sankhamul to Subidhanagar (location D) showed the highest average (0.125 mg/L) while Chobhar to Balkhu (location A) had a very low average (0.05 mg/L) as mentioned in Table 2. All locations showed clustered values. Ammonia may be present in drinking-water as a result of disinfection with chloramines and the natural levels 0f ammonia in groundwater are usually below 0.2 mg/L [10]. The presence of ammonia at higher than geogenic levels is an important indicator of fecal pollution. Cement mortar used for coating the insides of water pipes may release considerable amounts of ammonia into drinking-water and compromise disinfection with chlorine. The presence of elevated ammonia levels in raw water may interfere with the operation of manganese-removal filters because too much oxygen is consumed by nitrification, resulting in moldy, earthy-tasting water.

3.2. Bacteriological analyses of water samples

Total samples positive and negative for coliforms

Thirty one samples out of 34 tested positive for coliforms which is 91% of the total as explained in Fig. 3. Only 3 samples (9%) were in accordance to the value of 0 cfu/100mL [18].

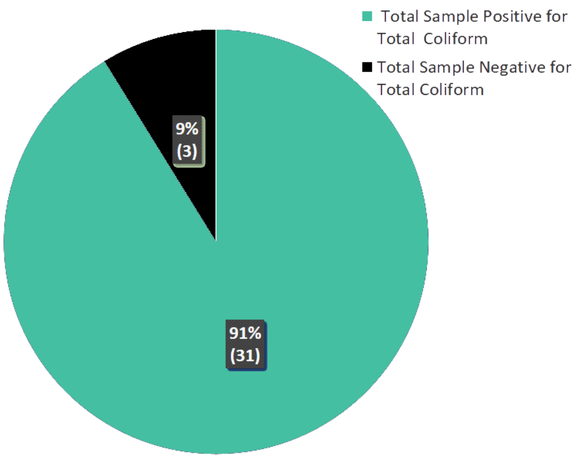

Figure 3: Distribution of total coliform samples.

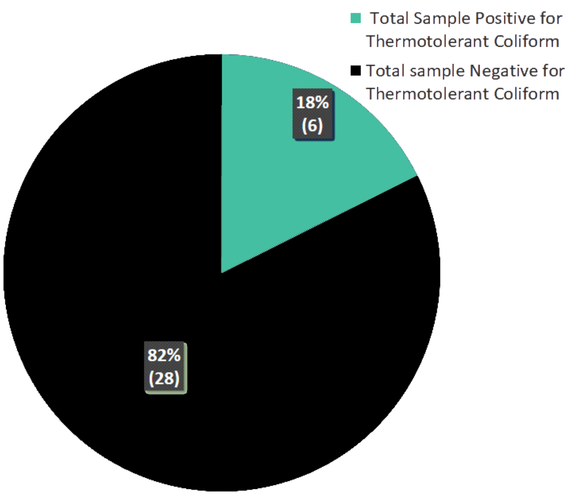

Figure 4: Distribution of thermotolerant coliform samples.

Total samples positive and negative for thermotolerant coliforms

Six samples (18%) tested positive for thermotolerant coliforms while 28 samples (82%) were found to have no thermotolerant coliforms as shown in Fig. 4. Potable water should have 0 cfu/100mL of thermotolerant coliforms [18].

This study along with the previous studies carried till date has shown that the drinking water quality of Kathmandu is deteriorating with time in all types of sources. The result of this study in terms of microbial quality of drinking water is in agreement with the studies carried out by [6, 14, 15, 16, 19, 20, 21, 23, 24, 25, 26, 27] all of which indicate that the public water supply is far from satisfactory in almost all localities. [19, 28] both isolated *E. coli* as predominant bacteria from water samples.

There are many sources of groundwater contamination. These includes agricultural pesticides and fertilizers, underground fuel storage tanks, industrial solvents, land disposal of agricultural and municipal wastes, landfills, septic tanks and hazardous waste disposal sites. This result underscores the deteriorating quality of water which may result in outbreak of water-borne diseases. The seemingly pure water was found to be contaminated with a heavy density of coliforms in most of the samples owing to many reasons, the most significant one being the infiltration of sewage from the river flowing viscously in the vicinity. People unaware of such contamination are consuming the water every day, which may trigger an outbreak of water borne diseases.

4. Conclusions and recommendations

Water borne diseases are extant in Nepal because of the fecal contamination and pollution of water sources and unhygienic practices or lack of sanitation. Groundwater from sources like tube wells, spouts, etc. are not as reliable as common people take it to be. The findings of this study show a heavy coliform contamination in most (91%) of the samples and on the other hand, *E. coli* was isolated from six of the samples (18%). Overall, the parameters such as temperature, turbidity, TDS, iron and ammonia were the ones that didn't comply with the WHO standards in most of the samples taken. Parameters like pH, DO, electrical conductivity, concentration of chloride, nitrate and phosphate were close to the WHO standards.

This study draws a conclusion that water samples from Chobhar to Balkhu (location A) were comparatively better in physicochemical and bacteriological aspects than other water samples, but still none of the samples have an unquestionable quality.

This study aims to deliver the following recommendations:

1. Periodic bacteriological quality assessment and disinfection of all drinking water sources should be planned and conducted.

2. Common and easy methods of safe household water management like boiling, filtration, solar disinfection of water (SODIS), chlorination and using metal vessels for their oligodynamic action or combination of these techniques should be practiced.

3. Drinking water sources should be protected from potential seepage of sewage and wastewater and other pollutants.

4. If possible, squatter settlement should be displaced to other places and a safe margin from the river bank should be promulgated to discourage such settlement so that the river does not get more polluted and the polluted water does not have much impact on people.

5. Untreated waste waters and sewers must be treated before discharging into the river.

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