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Application of the Presence/Absence (H_2S) Test Vial for Coliforms Detection in Rural Drinking Water of Nepal

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Abstract

Background: Access to safe drinking water remains a significant public health challenges in rural regions of Nepal. This study aimed to assess fecal contamination in drinking water sources across eight rural municipalities (RM) of four districts (Dhankuta, Jajarkot, Morang, and Surkhet), using a cost-effective method for detecting fecal coliform in low-resource settings. **Method:** A total of 160 water samples from diverse sources were analyzed for fecal coliform contamination using the Coliform Presence/Absence (H_2S) test vial. The samples were incubated for 48 hours at ambient temperatures ranging from 22°C to 44°C. By collecting water samples from the identical sources and locations across pre-monsoon and post-monsoon seasons, we directly measured the impact of seasonal variations on contamination. **Results:**

Coliforms contamination was found to be more prevalent during the pre-monsoon season i.e. 72.5% compared to the post-monsoon (57.5%), indicating seasonal variation in water quality. During the pre-monsoon season, contamination was highest in pipe water (78.3%), followed by household-stored water (77.8%), spring water (73.9%), and tube well (56.2%). Post-monsoon results indicate a reduction in contamination for most sources: tube wells dropped to 42.9%, household-stored water to 50%, and spring water to 63.6%, whereas pipe water decreased slightly to 69.6%. Chi-square analysis revealed a significant association between water source type and coliform contamination in both seasons ($p = 0.033$), as well as a significant association between season and coliform contamination ($p = 0.047$), suggesting that contamination levels differ between the pre- and post-monsoon seasons **Conclusion:** This study demonstrates that the presence/absence (H_2S) test is a reliable, low-cost, and practical tool for detecting fecal contamination in drinking water. Its simplicity, affordability, and proven effectiveness make it particularly well suited for rural and resource limited settings where laboratory infrastructure is scarce. The method's ease of application supports its use in routine water quality monitoring and makes it especially valuable during high-risk seasons, enabling timely public health interventions and improving community protection. **Novelty:** This is one of the first studies in Nepal to apply the presence/absence (H_2S) test vial across multiple rural municipalities of four districts and a variety of water sources. It provides new insights into regional and seasonal patterns of contamination and demonstrates the feasibility of using the H_2S test in large-scale, decentralized water quality assessments.

Keywords: Presence/absence (H_2S) test, fecal coliform, water contamination, Nepal

Introduction

In Nepal, different people and regions have varying degrees of access to safe drinking water. While some areas have access to treated and piped water systems, many people rely on various alternative sources for drinking water. As per the population census conducted in Nepal in 2021, the majority of the population depends on various sources of water such as piped water, water from covered wells (known as "Kuwa"), uncovered wells, spout water, and water from rivers or streams (National Population and Housing Census, 2021). Recent data from the Department of Water Supply and Sewerage Management reported that 51.69% of the populations have access to improved drinking water sources, while the remaining 48.31% rely on un-piped locally and privately managed systems like private tube wells (DWSSM, 2019). Overall, Nepal faces challenges in ensuring safe drinking water due to issues like contamination from agricultural runoff, industrial pollutants, inadequate sanitation facilities, and natural sources of contamination. Among these, the most common cause of epidemic outbreaks is fecal contamination of drinking water. The presence of fecal coliform bacteria can be used as an indicator of potential danger to human health, as fecal contamination poses significant health risks. Further according to WHO (1996), for drinking water to be safe, a 100 ml sample should be free of coliform bacteria.

In Nepal, water-testing facilities are limited to a few commercial and public laboratories, predominantly situated in major urban centers. Rural areas lack any provisions for microbial

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quality assessment of drinking water. Therefore, we choose to conduct the coliform test on different drinking water sources in several locations around Nepal. Consequently, we used the coliform presence/absence (H_2S) test vial for qualitative detection of fecal contamination in drinking water in remote areas of Nepal. The hydrogen sulfide (H_2S) presence/absence test is an inexpensive, easy-to-use, and portable alternative field-based water quality test (Manja et al., 1982). It has gained popularity as a low-cost test for determining fecal contamination (Gandhi, 2006) and has been use for more than 20 years worldwide (Gupta et al., 2008). The H_2S test is intend to detect bacteria of fecal origin, some of which are able to reduce organic sulfur to sulfide as H_2S gas. This reacts with the reagents in the test vial to form a black precipitate and allows visual detection of fecal contamination by examining the color of the water in the test vial.

Research Objective

To evaluate the effectiveness of the presence/absence (H_2S) test as a simplified method for detecting fecal contamination in rural drinking water sources across different districts and seasons in Nepal.

Materials and Methods

Study site

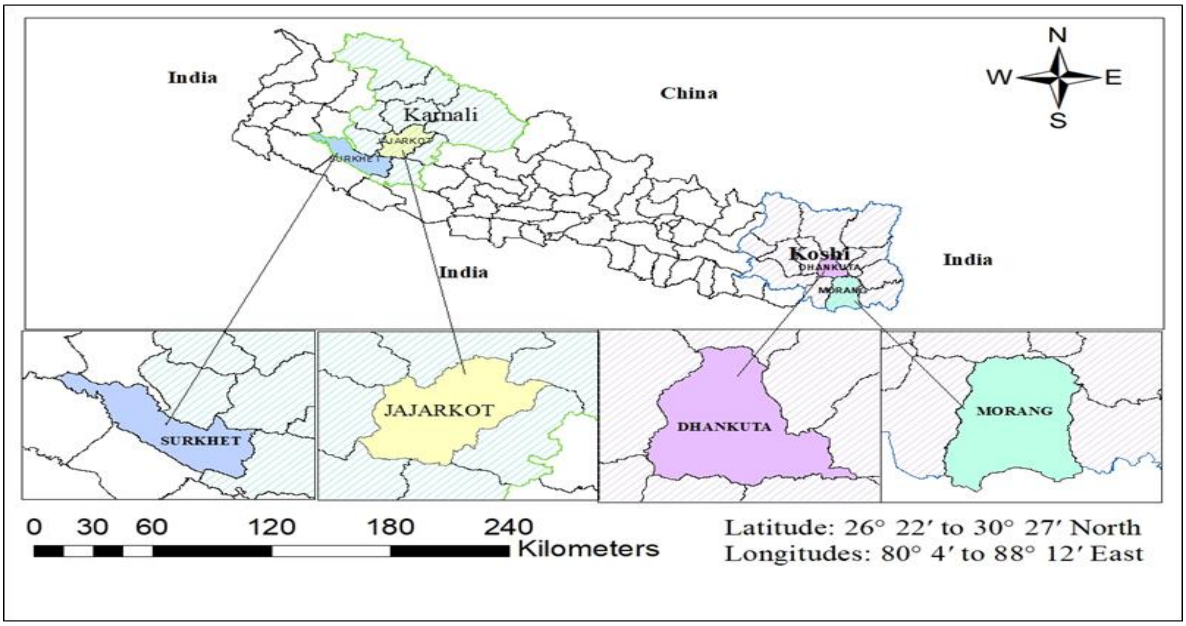

Figure 1: Study area in color represent Dhankhuta, Morang, Surkhet and Jajarkot district

Water samples were collected from selected study sites in two provinces: Koshi Province and Karnali Province. In Koshi Province, the sites included Kanephokhari and Kerabari Rural Municipalities (RM) in Morang district, and Sangurigadhi and Chaubise RMs in Dhankuta district. In Karnali Province, samples were collected from Chingad and Simta RMs in Surkhet district, and Shiwalaya and Junichande RMs in Jajarkot district. They show wide vertical cross sections of Nepal's two main development regions. With a subtropical climate, Province 1 spans 25,905 km^2 and has an elevation range of 60 m to 8000 m asl (Figure 1). However, province 6, with an overall area of 27, 984Km^2 is the largest province in Nepal, spans an

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altitudinal range of 738 m to 4,790 m asl and encompasses tundra, arctic, and sub-tropical climates. These locations were chosen based on factors like availability of potable water, availability of toilets, and accessibility to automobiles. While the rural area of koshi province is populate more densely and has greater access to transportation and water supplies, the Karnali province under consideration is primarily rural and has fewer people living there.

Study design and Sample size

This study is part of a Ph.D. research project investigating the impacts of climate change on water-borne diseases particularly diarrheal diseases in Nepal. It is a descriptive cross-sectional study design to assess fecal coliform contamination in drinking water sources across eight rural municipalities' of four districts. Water samples were collected in the pre-monsoon and post-monsoon seasons to capture potential seasonal variations in contamination levels and differences in contamination of the sources in two seasons. Water samples collected from various sources, include tube wells, natural springs, piped water systems (sourced from rivers and water stored in community collection tanks), and household-stored drinking water. Additionally, samples were obtained from household bio-sand filters, designed by Dr. David and primarily used in the Morang and Dhankhuta districts, to evaluate their effectiveness in improving water quality. Water sampling was systematically conducted during two seasons: pre-monsoon (May) and post-monsoon (November–December). In each season, 10 water samples were randomly collected from identical sources across eight rural municipalities, resulting in a total of 80 samples per season and 160 samples overall. This consistent sampling strategy ensured comparability between seasons and across sources.

Water sampling equipment and procedure

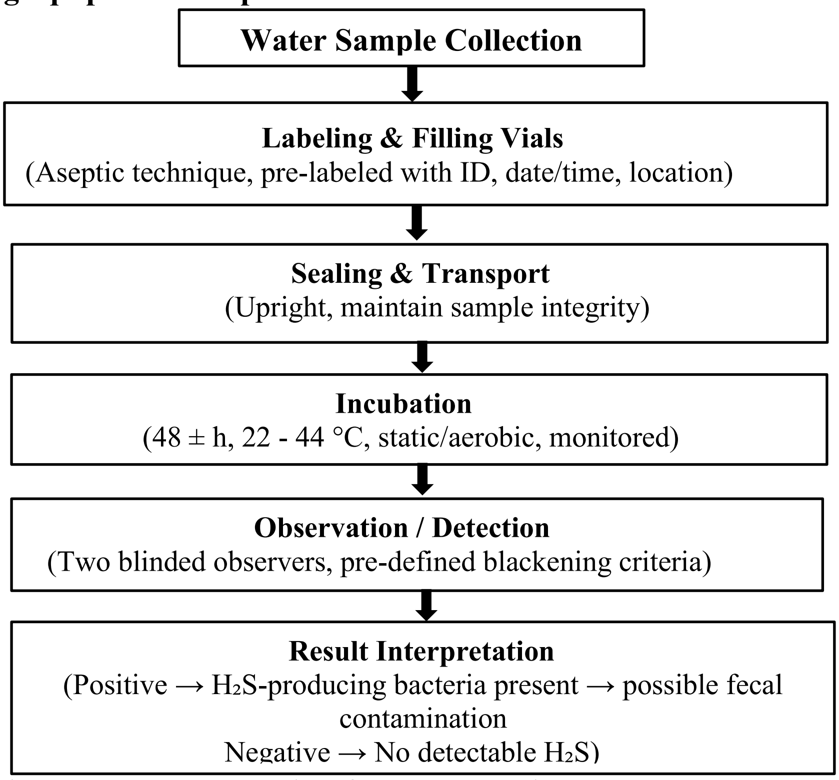

Figure 2: H_2S Test Procedure for Water Samples

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Water samples from multiple sources were analyzed using Coliform Presence/Absence (H_2S) Test Vials, developed by the Environment and Public Health Organization (ENPHO). The vials (Batch No. 6) were manufactured and distributed by Eco Concern Pvt. Ltd., based in Kathmandu, Nepal. Each vial was pre-labeled with a unique sample identification code, collection date and time, location, and source type. Upon arrival at the testing site, vials were filled to the manufacturer's designated mark using aseptic technique. Sterile gloves were worn at all times to prevent any contact between the water sample and external surfaces. Vials were sealed immediately to maintain sample integrity and transported in an upright position. The vials were placed upright for 48 ± hours at 22 - 44 °C in a monitored environment, with temperature recorded using a calibrated thermometer. Incubation was conducted under static, aerobic conditions, with consistent light and humidity. Following incubation, the vials were examined by two blinded observers for blackening of the medium in accordance with pre-defined criteria. A positive result was interpreted as the presence of hydrogen sulfide–producing bacteria, suggesting possible fecal contamination. Whereas, negative results were defined as the absence of detectable hydrogen sulfide–producing bacteria.

Figure 3: Coliform presence/absence (H_2S) test vials after 48-hour incubation. Left: Negative result (no color change), Right: Positive result (black coloration indicating fecal contamination).

Statistical analysis

This study was based on simple descriptive studies in which sophisticated statistical and econometrical tools have not been incorporated to analyses the outcomes. The gathered water sample observation was entered into a customized database in a Microsoft Excel and data were presented in table, figures and heat map. A chi-square test was performed to assess the statistical significance of differences in contamination levels across seasons and sources.

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Ethical approval

The Ethical Review Board (ERB) of the Nepal Health Research Council (NHRC) approved the protocol of this study (registration no. 574/2022). Water samples were collected concurrently with the questionnaire survey. Prior to data and water sample collection, all participants were fully informed about the purpose of the study, their right to withdraw at any time, the significance of their participation, the confidentiality of their responses, and the fact that no financial incentives were provided. Written informed consent was obtained from each participant before data collection and water sample commenced. All research procedures were conducted in accordance with relevant ethical guidelines and regulations.

Results

Table 1: Distribution of water source types in study districts during the pre-monsoon season

Study District	No of samples	Spring water	Tube well water	Piped water	Water stored at household
Chinghad RM, Surkhet	10	4	0	4	2
Simta RM, Surkhet	10	4	0	4	2
Shiwalaya RM, Jajarkot	10	4	0	4	2
Junichaande RM, Jajarkot	10	4	0	4	2
Kanephokhari RM, Morang	10	0	7	0	3
Kerabari RM, Morang	10	0	5	2	3
Sanguregadi RM, Dhankhuta	10	3	3	2	2
Chaubesi RM, Dhankhuta	10	4	1	3	2
Total	80	23	16	23	18

The table 1 presents the sources of water samples collected during the pre-monsoon period from the eight RM of the study districts. A total of 80 samples were collected of which 23 samples were collected from springs, 16 from tube wells, 23 from piped water systems sourced from rivers or water stored at community tank, and 18 from drinking water stored in households.

Table 2: Distribution of water source types in study districts during the post-monsoon season

Study District	No of samples	Spring water	Tube well water	Piped water	Bio-sand filter water	Water stored at household
Chinghad RM, Surkhet	10	4	0	4	0	2
Simta RM, Surkhet	10	4	0	4	0	2
Shiwalaya RM, Jajarkot	10	4	0	4	0	2
Junichaande RM, Jajarkot	10	4	0	4	0	2
Kanephokhari RM, Morang	10	0	6	0	1	3
Kerabari RM, Morang	10	0	4	2	1	3
Sanguregadi RM,Dhankhuta	10	2	3	2	1	2
Chaubesi RM, Dhankhuta	10	4	1	3	0	2
Total	80	22	14	23	3	18

The table 2 summarizes the sources of water samples collected during the post-monsoon period across the study districts. A total of 80 samples were collected of which 22 samples were

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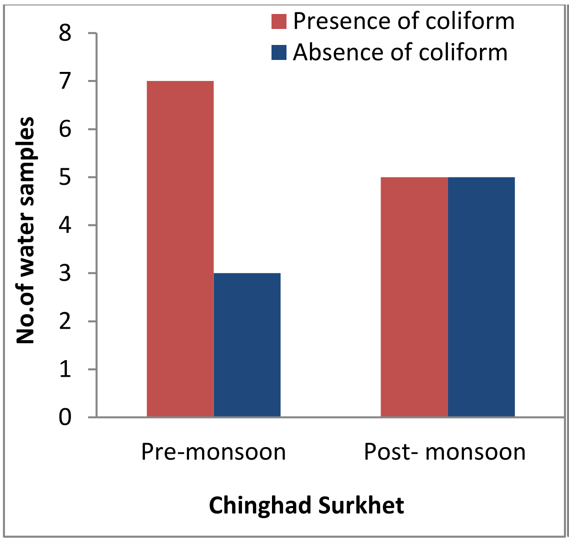
obtained from springs, 14 from tube wells, 23 from piped water systems, 3 from bio-sand filters, and 18 from household-stored drinking water.

Comparison of water sample collected in pre-monsoon and post-monsoon in Surkhet



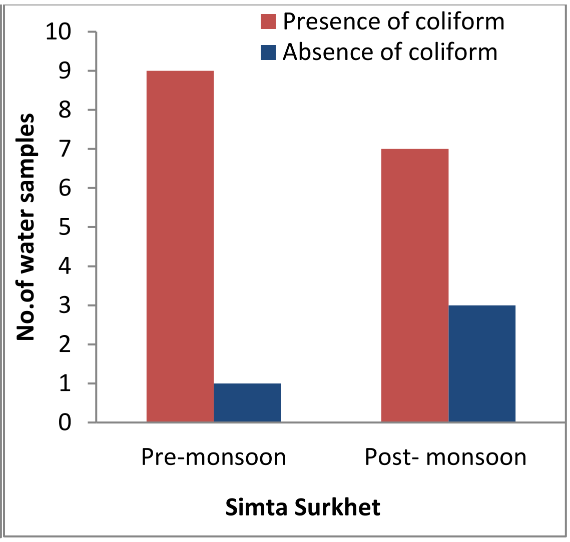
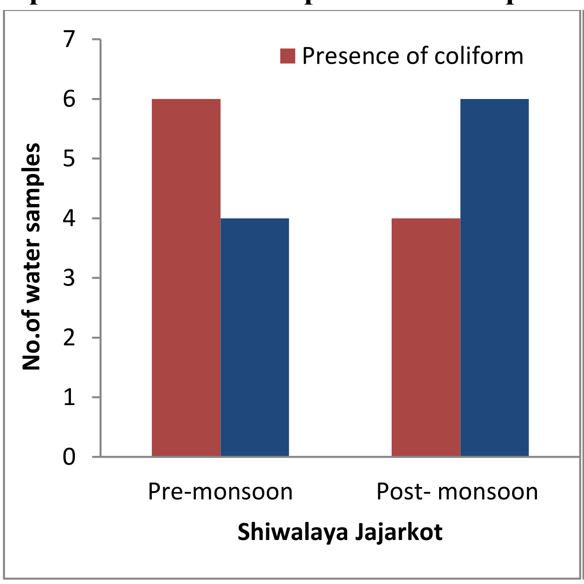


Figure 4: water sample collected in Chinghad Surkhet

Figure 5: water sample collected in Simta Surkhet

Figures 4 and 5 compare the presence of coliform in water samples collected during the pre-monsoon and post-monsoon seasons from the same sources in the rural municipalities of Chinghad and Simta located in Surkhet district. In Chinghad, coliform was detected in 7 out of 10 water samples during the pre-monsoon seasons, while only five samples tested positive for coliform in the post-monsoon. In contrast, Simta showed coliform presence in nine water samples during the pre-monsoon and in seven samples during the post-monsoon, as illustrated in figure 5.

Comparison of water sample collected in post-monsoon and pre-monsoon in Jajarkot



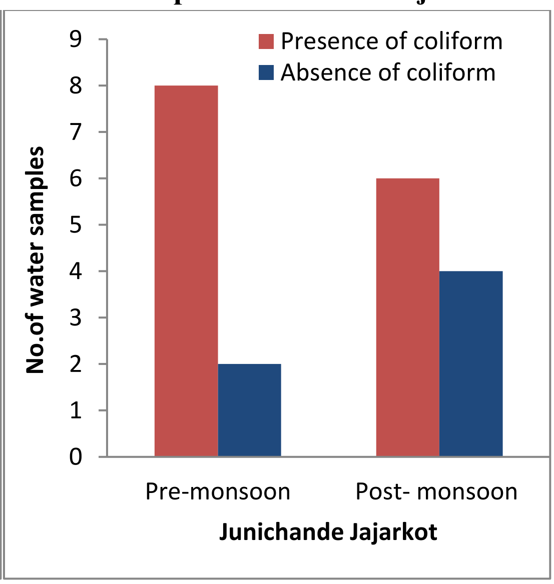


Figure 6: water sample collected in shiwalaya Jajarkot Figure 7: water sample collected in
Junichande Jajarkot

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Figures 6 and 7 compare the presence of coliform bacteria in water samples collected during the pre-monsoon and post-monsoon seasons in the rural municipalities of Shiwalaya and Junichande, located in the Jajarkot district. In Shiwalaya (Figure 6), coliform was detected in six water samples during the pre-monsoon, while only four samples showed contamination in the post-monsoon. Similarly, in Junichande (Figure 7), eight water samples tested positive for coliform in the pre-monsoon season, compared to six samples in the post-monsoon.

Comparison of water sample collected in post-monsoon and pre-monsoon in Morang

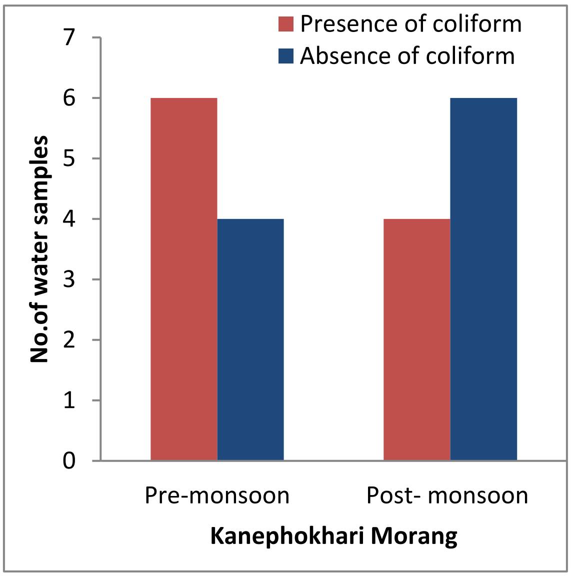

Figure 8: water sample collected in Kanephokhari Morang

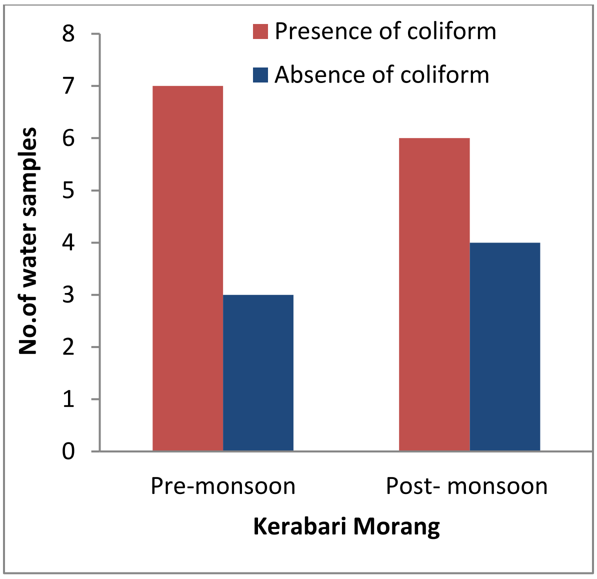

Figure 9: water sample collected in Kerabari Morang

Figures 8 and 9 illustrate the comparison of coliform presence in water samples collected during the pre-monsoon and post-monsoon seasons in the rural municipalities of Kanephokhari and Kerabari located in Morang district. In Kanephokhari, six water samples tested positive for coliform during the pre-monsoon, while only four samples showed contamination in the post-monsoon. In contrast, Kerabari RM (figure 9) exhibited higher coliform presence, with seven contaminated samples in the pre-monsoon and six in the post-monsoon.

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Comparison of water sample collected in post-monsoon and pre-monsoon in Dhankuta

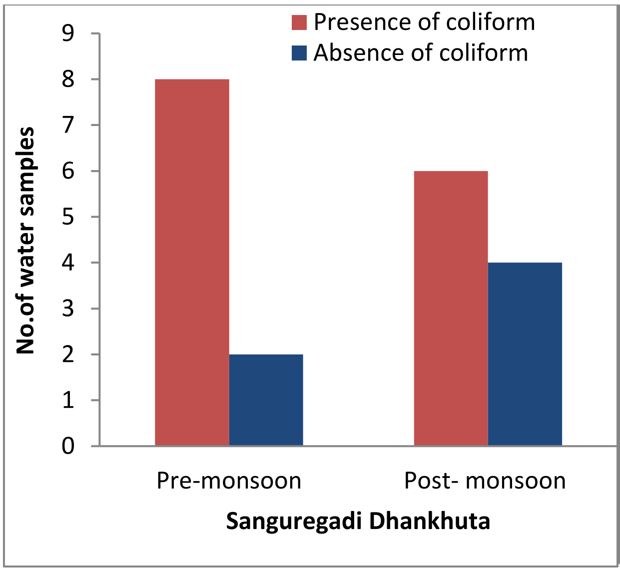

Figure 10: water sample collected in Sanguregadi Dhankhuta

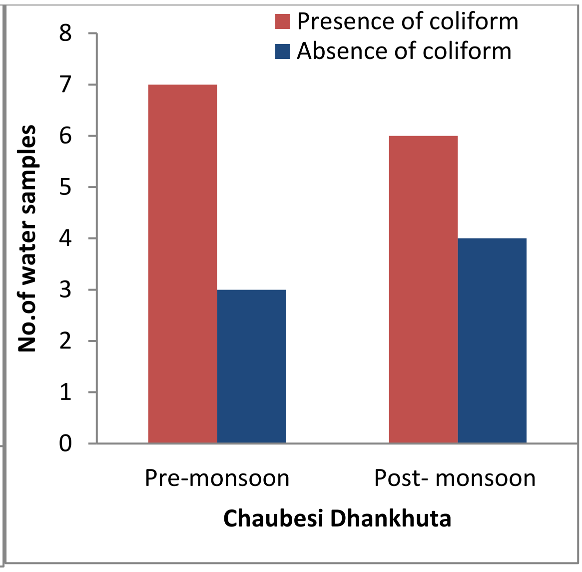

Figure 11: water sample collected in Chaubesi Dhankhuta

Figures 10 and 11 compare the presence of coliform in water samples from the rural municipalities of Sangurigadhi and Chaubise in Dhankuta district across pre-monsoon and post-monsoon seasons. In Sangurigadhi, coliform contamination was found in eight samples before the monsoon, decreasing to six after the monsoon. Similarly, in chaubise (Figure 11), seven samples were positive for coliform in the pre-monsoon period, while six samples showed contamination post-monsoon.

Comparison of water sample collected in pre-monsoon from different sources

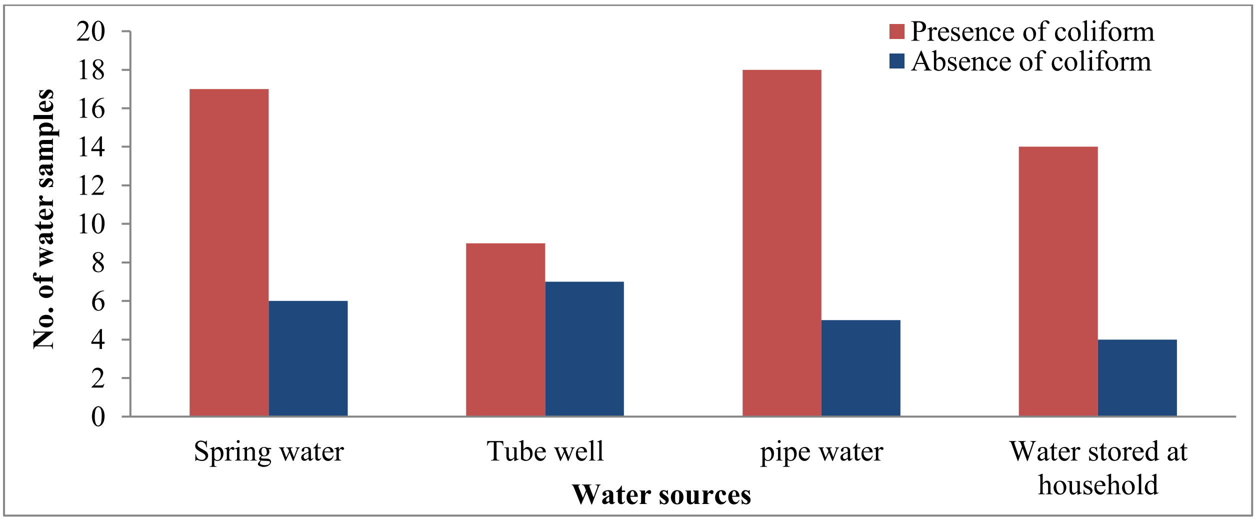

Figure 12: water samples collected in pre-monsoon from different water sources

Figure 12 illustrates the presence of coliform contamination in water samples collected from various sources during the pre-monsoon season. Overall, coliform levels were higher in the pre-monsoon samples compared to the post-monsoon samples. Coliforms were detected in 18

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piped water samples intended for drinking. Likewise, 17 out of 23 spring water samples tested positive for coliform contamination. Additionally, 14 household-stored drinking water samples and 9 tube well samples were also found to contain coliforms.

Comparison of water sample collected in post-monsoon from different sources

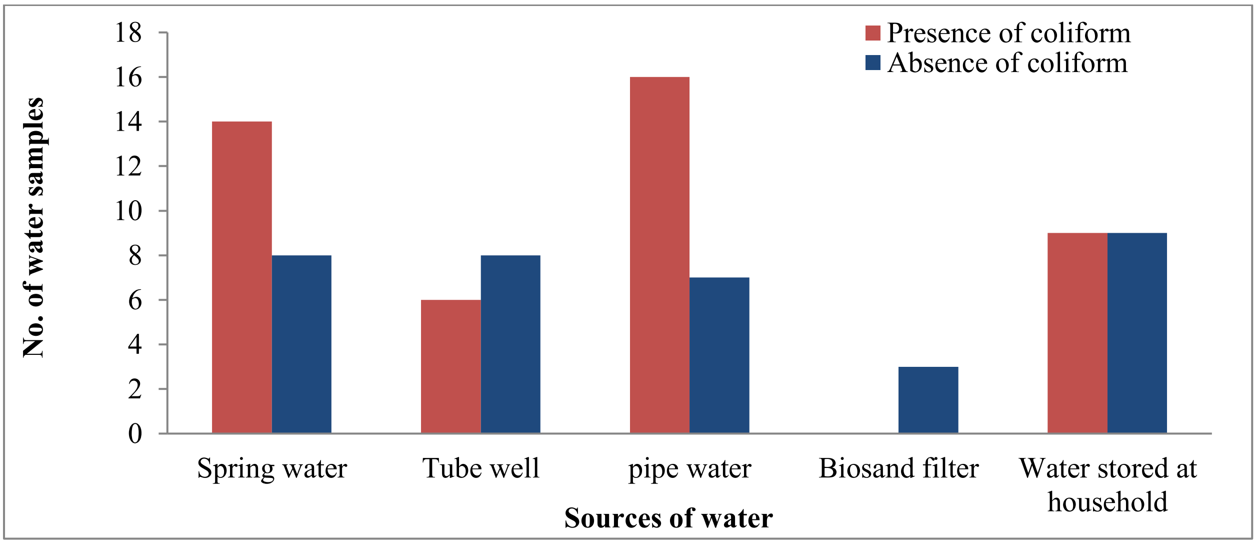

Figure 13: water samples collected in post-monsoon from different water source

Figure 13, presents the distribution of coliform contamination in drinking water samples collected during the post-monsoon season from various sources in the study areas. Piped water showed the highest level of contamination, with coliforms detected in 16 samples, the largest proportion among all sources. Of the 22-spring water samples tested, 14 were found to be contaminated. Similarly, coliforms were present in six samples from tube wells and nine samples from household water storage used for drinking.

Heat map of Coliform Contamination in Pre- and Post-Monsoon seasons

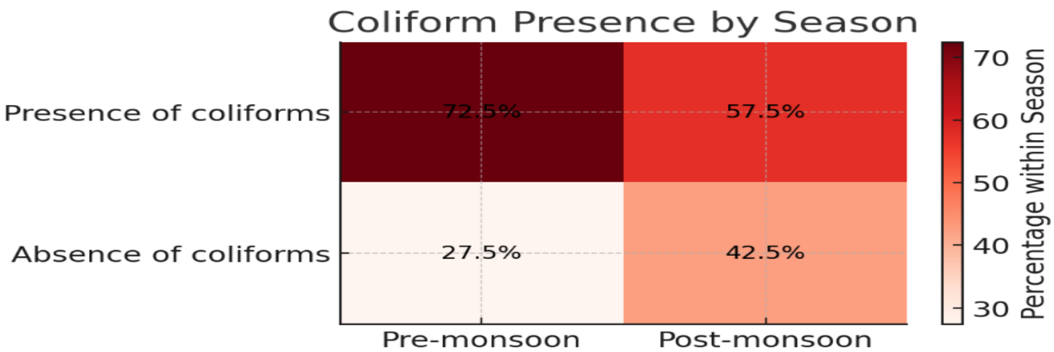

Figure 14: Heat map showing the presence and absence of coliforms during pre-monsoon and post-monsoon seasons.

Note: Darker shades indicate higher percentages within each season.

The heat map representing the Coliform Presence by Season. It illustrates the percentage of water samples with or without coliform bacteria during pre-monsoon and post-monsoon seasons. In the pre-monsoon Season, 72.5% of samples showed the presence of coliforms,

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while 27.5% showed their absence. Similarly in the post-monsoon Season, 57.5% of samples showed the presence of coliforms, and 42.5% showed their absence. The chi-square test revealed a statistically significant association between season and coliform contamination (p = 0.047), indicating that the level of coliform contamination differed between the pre-monsoon and post-monsoon seasons.

Heat map of coliform contamination in water sources (Pre- and Post-Monsoon)

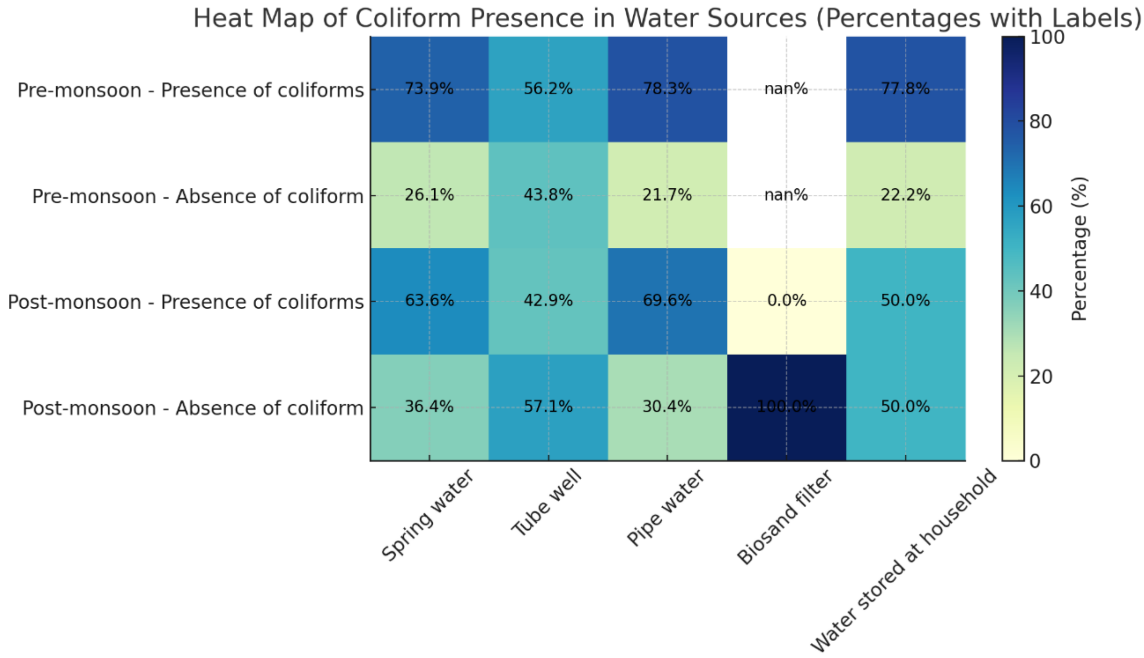

Figure 15: Seasonal variation in coliform presence across water sources

Note: Percentages represent the proportion of samples from each water source that tested either positive or negative for coliform during pre- and post-monsoon periods. Values are calculated per source, so each column totals 100% within a season. Warmer colors indicate higher percentages, making contamination patterns easier to compare across seasons and water sources. nan% represent not a number or N/A

The heat map illustrates the percentage of coliform contamination in five water sources: spring water, tube wells, pipe water, bio-sand filters, and household-stored water during pre- and post-monsoon seasons. The data's are presented for both the presence and absence of coliforms, highlighting seasonal and source-specific variations. In the pre-monsoon period, contamination was highest in pipe water (78.3%), household-stored water (77.8%), and spring water (73.9%) and tube wells with 56.2%. Post-monsoon results show reductions in most sources: tube well contamination dropped to 42.9%, household-stored water to 50%, and spring water to 63.6%, while pipe water showed a slight decrease to 69.6%. The Chi-Square test showed a significant association between water source type (spring water, tube wells, piped water, bio-sand filters, and household-stored water) and coliform contamination across pre- and post-monsoon seasons (p = 0.033), confirming that contamination levels vary significantly by source in both seasons.

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Discussion

The coliforms are indicative of the general hygienic quality of the water and potential risk of infectious diseases from water. The first study of E. coli and coliform bacteria in the Sagarmatha National Park was conducted between 2008 and 2010 (Ghimirie et al., 2013). Their study focused entirely on the major rivers within the park, and they concluded that all of the rivers contained E. coli and coliform bacteria, although the upper ranges of most rivers did not. However, we analyzed coliform contamination across different water sources during both the pre- and post-monsoon seasons. Overall, our study found that water samples collected during the pre-monsoon season (May) had a higher percentage of coliform contamination compared to those collected in the post-monsoon season (November–December). During the pre-monsoon season, elevated ambient and water temperatures, often ranging between the optimal growths ranges of fecal coliforms (30-37 °C), enhance their metabolic activity and reproduction, resulting in higher contamination levels. In contrast, during the post-monsoon period, cooler temperatures and increased dilution from rainfall reduce the rate of bacterial growth, leading to comparatively lower fecal coliform concentration. Similar, study conducted on Gangetic river system also found that total coliform and thermo tolerant coliforms were at a maximum during summer (followed by monsoon and winter) (Bahghel et al., 2005).

In the study area, spring water exhibited consistently high levels of fecal coliform contamination during both the pre-monsoon and post-monsoon seasons. Beyond seasonal variation and the effects of temperature fluctuations, hydrological processes, and contamination dynamics, human activities, such as agriculture, domestic waste disposal, and land-use practices further exacerbate the contamination. Observations of animal feces near several spring sources suggest that livestock are a significant contributor to direct contamination. Such contamination is likely to worsen during the rainy season, when surface runoff can carry fecal matter into the springs, increasing microbial load. Additionally, human activities such as bathing and laundry conducted in and around the springs further contributed to fecal coliforms and microbial pollution. Similar findings were reported in a study of the Thulokhola watershed, where high levels of fecal coliform and E. coli were detected (Poudel and Duex, 2017). These results strongly suggest that the microbial quality of spring water in the region is significantly compromised by human and animal interference. A similar study by (Daghara et al., 2019) reported a wide variation in microbial levels among spring water samples. Several other studies have also confirmed that the presence of coliform bacteria indicates fecal contamination, likely resulting from sewage intrusion and intensified human activity, especially in springs located near settlements (Haruna et al., 2005, Batool, 2008 and Sarkar et al., 2022). These findings highlight the susceptibility of spring water to both seasonal fluctuations and human activities, emphasizing the urgent need for effective management strategies. Measures such as controlling livestock access, regulating human activities near water sources, and implementing regular water quality monitoring are essential to protect public health.

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Piped water sources showed the highest levels of fecal coliform contamination during the pre-monsoon season compared to the post-monsoon season. Contamination is typically greater before the monsoon because accumulated fecal matter remains in the environment, whereas the heavy rains of the monsoon season flush it away, reducing its concentration. Beyond seasonal variation, multiple factors contribute to coliform contamination in rural Nepal. Inadequate infrastructure poses a major barrier to effective water treatment and sanitation. Aging and deteriorating pipelines, insufficient sanitation facilities, and poorly designed cross-connections between sewage and water supply systems substantially elevate the risk of fecal contamination in rural drinking water networks. A study by (Bartram et al., 2010), reported that many piped water systems in developing and middle level income generating countries work only for few hours per day and often deliver unsafe water. Similarly, the WHO and UNICEF's water supply and sanitation global assessment report further highlighted that, in Asia, more than one out of five water supplies does not meet the national water quality standards (WHO and UNICEF, 2000). In addition, a study by (Maharjan, 2013) reported frequent instances of fecal contamination in piped drinking water in Nepal with outbreaks of waterborne diseases. To mitigate these risks, communities must adopt household measures such as boiling or filtering drinking water and preventing livestock access near water sources. At the same time, authorities should prioritize repairing damaged infrastructure, ensuring complete separation of water and sewage networks, and enforcing routine water quality monitoring.

In our study area, water storage was common among rural households due to the lack of a consistent piped water supply. However, most stored drinking water samples were highly contaminated with fecal coliforms (77.8%) during the pre-monsoon, likely because accumulated fecal matter in the environment are not washed away until the monsoon rains, which reduce contamination levels. However, beyond seasonal influences, studies have shown that contamination can arise both at the source and during household water handling (Alexander and Blackburn, 2013 and mintz et al., 1995). Studies from other developing countries also confirm that microbial contamination often increases between the source and point of use (Badowski et al., 2011). Many households transported and stored drinking water in uncovered containers (Hsan et al., 2019), increasing the risk of microbial invasion. Another study mention household storage itself emerged as a key contamination point (Clasen and Bastable, 2003 and Ngasala et al., 2019). Similar behaviors of was observed in the study area such as not covering the water storage container, which may significantly contribute to household water contamination and potential pathogen transmission. In addition, we also observed that inadequate protection of water sources, including exposure to agricultural runoff containing manure and fertilizers along with poor site selection and limited hygiene awareness. Further, a study indicates that poorly constructed infrastructure (e.g., unlined wells, unsealed tanks, and faulty pipe connections) further elevate contamination risks (Esrey et al., 1991). Therefore, to reduce contamination, households should consistently cover storage containers, use clean utensils for water handling, and treat drinking water through boiling, filtration, or chlorination before consumption. During the pre-monsoon season, when the risk of

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contamination is highest, community-level interventions are critical. These include improving source protection, constructing properly lined wells and sealed tanks, maintaining water distribution infrastructure, promoting hygiene awareness, and preventing agricultural runoff from entering water sources. Such measures are essential to safeguarding water quality and protecting public health.

Limitations

While the H_2S test provided a practical and low-cost means of detecting potential fecal contamination, its qualitative nature and the possibility of occasional false positives or negatives should be consider when interpreting results. The method detects hydrogen sulfide–producing bacteria, which may include some fecal coliforms and other fecal-associated organisms, but it does not capture all fecal coliform species. As a result, negative results may not indicate the complete absence of fecal contamination, and positive results may sometimes reflect the presence of non-fecal sulfur-reducing bacteria. Furthermore, the lack of quantitative microbial counts limits the ability to fully characterize contamination levels or conduct detailed risk assessments.

Conclusion and Recommendation

This study demonstrates consistently high levels of fecal contamination in rural drinking water sources across seasonal variations. The findings underscore the urgent need for regular microbial testing of springs and piped water systems in rural Nepal, particularly during seasons of elevated contamination risk. Furthermore, rigorous research is required to better elucidate seasonal contamination dynamics and to identify the predominant sources of bacterial pollution across rural Nepal, thereby informing targeted interventions and policy measures. Most importantly, establishing consistent water quality monitoring programs and upgrading local water infrastructure are critical measures to ensure safe drinking water and prevent waterborne diseases.

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