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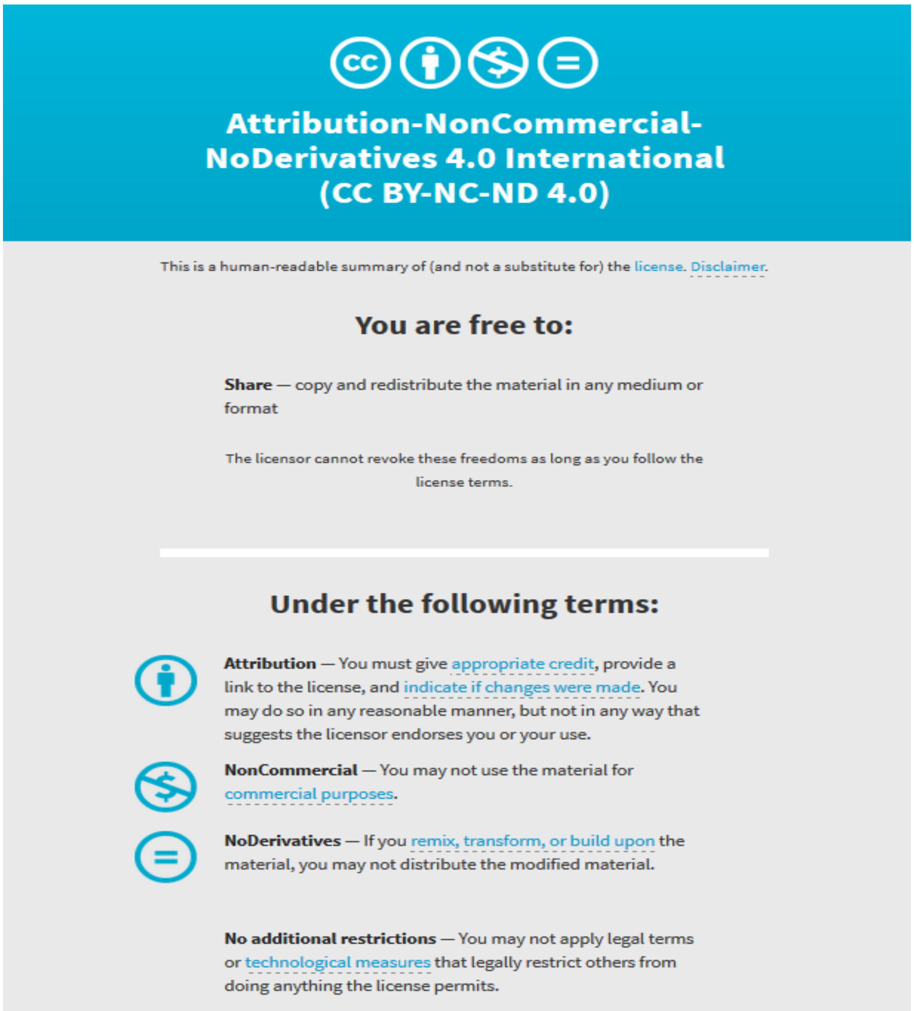

Determination of fecal indicator bacteria in shallow and deep groundwater sources in the Kathmandu valley, Nepal

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***Abstract* – Groundwater is a crucial source of water for the residents of the Kathmandu valley, with an estimated 50% of total inhabitants dependent on groundwater supply for daily use. Hence, microbial evaluation of groundwater is a paramount exigency to evaluate the impact on public health of fecal contamination of the groundwater. The purpose of this study was to compare the distribution of fecal indicator bacteria in both shallow and deep groundwater sources of the Kathmandu valley as well as to evaluate their microbial quality. A total of nine groundwater sources within the Kathmandu valley including four shallow water sources and five deep-bore wells were sampled during the study period (May-July 2015) and tested for *Escherichia coli* and total coliforms by the most probable number (MPN) method using Colilert reagent (Idexx Laboratories). Coliform bacteria were detected in 100% (9/9) of the groundwater samples tested and *E. coli* was detected in 89% (8/9) of the samples tested. The occurrence of *E. coli* in the shallow groundwater sources was found to be 100% (4/4) and in the deep sources was 80% (4/5), with the average value ranging from 2 to 629.4 MPN/100 ml and <1 to 3 MPN/100 ml, respectively. Similarly, the distribution of coliforms was found to be 100% in both the shallow and deep groundwater sources, with the average value ranging from 960.6 to >2419.6 MPN/100ml and 18.7 to 155.3 MPN/100ml. respectively. Deep groundwater sources were found to be comparatively less contaminated with fecal indicator bacteria compared to shallow groundwater sources. None of the groundwater samples analyzed met the drinking water quality standard recommended by World Health Organization, a finding which warrants the implementation of an effective treatment measure prior to use.**

Keywords – Groundwater, Coliform, *E. coli*, Kathmandu

1. Introduction

Groundwater is a crucial source of water for the residents of the Kathmandu valley. Currently, approximately 50% of the water supply (estimated to be 59.06 million liters a day) that is used for drinking and other domestic requirements in the Kathmandu valley are derived from groundwater [1]. Several groundwater sources including dug wells, shallow tube wells, stone sprouts and deep tube wells are used in Kathmandu [2-3] with water from the deep tube wells comprising about 32% (37.49 million liters a day) of the water used in the Kathmandu valley [1].

Contextually relevant, recent studies have reported that the groundwater in the Kathmandu valley has significant microbial contamination that exceeds the drinking water quality standard recommended by the World Health Organization (WHO) guidelines [2, 4].

Waterborne pathogens are present in high concentration in the feces of infected patients and they are transmitted primarily through fecal-oral route, especially through consumption of contaminated food and water [5]. In addition to the risk of diarrhea from drinking water, extensive use of groundwater for bathing purposes can be implicated as an important transmission pathway through accidental water ingestion leading to diarrhea [6]. Knowledge regarding the prevalence of waterborne pathogens is essential to evaluate the risk of infection from pathogens transmitted via contaminated water. Hence, microbial evaluation of groundwater is a paramount exigency to evaluate the impact of fecal contamination of groundwater on public health. As revealed by this milieu, the present study was undertaken to compare the contamination intensity of shallow and deep groundwater sources of the Kathmandu valley by fecal indicator bacteria.

2. Material and Methods

2.1 Study Site and Sample Collection

Three study sites in the Kathmandu valley, (Kathmandu, Bhaktapur and Lalitpur), are located in the upper part of the Bagmati River Basin in central Nepal. The watershed has a total surface area of 665 km^2. During the months of May-July 2015 (i.e., mid-monsoon), groundwater samples were collected from four shallow groundwater sources [including two stone spouts located at Sinamangal (Gw-Sw1), Kupandole-Lalitpur (Gw-Sw2) and two shallow dug wells located at Nala-Bhaktapur (Gw-Sw3), Kapan (Gw-Sw4)] and five deep groundwater sources [including five deep bore wells located at Sukedhara (Gw-Dw1), Gaushala (Gw-Dw2), Jagati-

Bhaktapur (Gw-Dw3), Maitidevi (Gw-Dw4) and Sitapaila (Gw-Dw5)]. The average depth of the shallow groundwater sources ranged between 10 to 15 ft and those of the deep groundwater sources ranged between 40 to 110 ft.

Water samples were collected in 100ml sterile polyethene bottles and transported to the Public Health Research Laboratory, Institute of Medicine within 2 hours of collection for microbial analysis. The bottles were thoroughly rinsed before sample collection.

2.2 Microbial Analysis

Total coliform and *E. coli* were measured by the most probable number (MPN) method using Colilert reagent (Idexx Laboratories) following the manufacturer's protocol. Briefly, 100 ml of sample water from a sterile polythene bottle was thoroughly mixed with Colilert powder and transferred in Quanti-tray. After removing the bubbles inside the Quanit-tray, it was sealed using the Quanti-tray sealer by placing the tray onto a Quanty tray rubber insert of the sealer. Finally, the tray was incubated overnight at 37 °C and following the incubation, large and small positive wells (yellow colored) were counted for total coliform. For *E. coli* detection the tray was observed under UVlamp and blue fluorescence in the wells was observed. Ultimately, MPN was determined by referring to the Quanti-tray MPN table.

3. Results and Discussion

Total coliforms were detected in all nine groundwater sample tested with an average value ranging from 960.6 to >2419.6 MPN/100 ml and 18.7 to 155.3 MPN/100 ml respectively, in shallow and deep groundwater sources. *E. coli* was detected in 89% (8/9) of the samples tested. The occurrence of *E. coli* in the shallow and deep groundwater sources was found to be 100% (4/4) and 80% (4/5), with an average value ranging from 2 to 629.4 MPN/100 ml and <1 to 3 MPN/100 ml, respectively. Table 1 shows the results of detection of total coliforms and *E. coli* in the shallow and deep groundwater sources.

In the study, the entire groundwater samples tested were found to be positive for total coliforms and 89% of the sources were contaminated with *E. coli*. This compares closely to several previous studies [4, 7, 8] in which severe fecal contamination and microbial pollution of groundwater in the Kathmandu valley have been documented. According to the WHO drinking water guidelines, *E. coli* should not be present in 100 ml of drinking water [9]. Out of the nine groundwater samples tested, only one of the deep bore wells in our study met the WHO drinking water guidelines for *E. coli*.

Fecal pollution of groundwater can lead to health problems because of the presence of infectious microorganisms which may be derived from human sewage or animal sources. The intestine of an infected vertebrate may harbor various harmful microbes, including bacteria, viruses, and protozoa, which may contaminate the water source as indicated by the presence of fecal coliforms derived from human sewage and animal droppings [10]. This is why coliform bacteria are considered "indicator organisms"; their presence warns of the potential presence of disease causing organisms. The *E. coli* test is especially useful as an indicator organism because these microbes are only related to fecal wastes. Therefore, while total coliforms are a useful indicator of bacteria in the water, *E.coli* is much more useful for determining whether water has been contaminated from fecal wastes or not [15].

For the purpose of this study, groundwater sources (with depth <20ft) were considered as shallow water sources and those (with depth ⩾20 ft) were considered as deep water sources. Both the contamination intensity as well as the burden of total coliforms and *E. coli* was found to be higher in shallow water sources compared to deep water sources in this study. Bacterial contamination of groundwater is usually due to the mixing of surface runoff passing through urban areas and pastures, leakage of sewage disposal systems and septic tanks, overloaded sewage treatment plants, disposal systems, and raw sewage deep well injection [12]. In the Kathmandu valley, sewers are leaky because of improper construction [13]. Most of the contaminated water sources in our study were found to be located closer to septic tanks, pit latrines and sewage drains. Hence, it is likely that a huge amount of wastewater is leaking into the subsurface of these water sources. In the case of the Kathmandu valley storm drains and sewer lines carry mixtures of sewage and storm water. The frequent overflowing of such drains through manholes pollute the land surface and subsequent seepage of the water from such polluted surface results in contamination of subsurface aquifers [14]. Likewise, infiltration of contaminants and fecal materials generated from anthropogenic and zoonotic sources may contribute in the contamination of shallow groundwater sources [6].

In contrast to the shallow water sources, the deep groundwater sources were found to be less contaminated with both fecal coliforms and *E. coli* which is in accordance with the findings of other studies conducted in Kathmandu [5, 6]. However, total coliforms were detected in all the deep water sources tested with an average value ranging from 18.7 to 155.3 MPN/100 ml and *E. coli* was also detected in 80% of the deep bore wells tested, although in minimal quantity. Industrial and household waste is discharged into the deep groundwater in the Kathmandu valley [1], which could be one possible explanation for the detection of total coliforms and *E. coli* in the deep water sources tested.

Table 1 The distribution of total coliforms and *E. coli* in the groundwater samples tested

No.	Sample ID	Source type	Depth of the source (ft)	Location	Distance from septic tank/sewage drainage (m)	Total coliforms (MPN/100 ml)	*E. coli* (MPN/100 ml)
1	GW-SW_1	Stone spout	10	Sinamangal	NA*	1011.2	629.4
2	GW-SW_2	Dug well	12	Nala	20	>2419.6	16
3	GW-SW_3	Dug well	15	Kapan	8	2419.6	2
4	GW-SW_4	Stone spout	15	Kupandole	6	960.6	23.5
5	GW-DW_1	Deep bore well	110	Sukedhara	5	79.4	<1
6	GW-DW_2	Deep bore well	100	Gaushala	NA	18.7	1
7	GW-DW_3	Deep bore well	50	Jagati	8	36.4	2
8	GW-DW_4	Deep bore well	40	Kupandole	2	101.2	2
9	GW-DW_5	Deep bore well	60	Sitapaila	2	155.3	3

* NA- not available

4. Conclusions

This study found that deep groundwater sources of the Kathmandu valley are less contaminated with fecal indicator organisms than the shallow groundwater sources. Most of the groundwater samples tested in the study were found to be placed or constructed without fulfilling the criteria laid down by the WHO which requires the source of groundwater to be located more than 50 feet from likely sources of contamination. This failure to observe the criteria has led to the contamination of groundwater with the fecal material due to seepage and percolation, resulting in higher contamination of *E. coli*. Since more than 50% of the population of the Kathmandu valley is dependent on groundwater sources, the legal authority should facilitate an effective treatment measure of the groundwater prior to use. An immediate action with regard to preventing drinking water sources from contamination is needed so as to prevent the waterborne health problems and related morbidity and mortalities in future

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