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SEASONAL DYNAMICS OF COLIFORM CONTAMINATION IN GROUNDWATER ASSOCIATED WITH ONSITE SANITATION: A CASE STUDY OF PERI-URBAN VILLAGE NAWADA, PATNA, INDIA

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ABSTRACT: *Water is essential for the living world. Life cannot exist without water. For drinking purposes, water is obtained from many sources. Available sources of water on Earth are rivers, streams, groundwater, etc. In India, a major source of drinking water comes from groundwater through hand pumps, boring, or bore wells. Aquifers are the geographical formation of groundwater. An onsite sanitation system (OSS) is commonly used by the rural population of Bihar. Poorly designed or closely spaced OSS, like soak pits, lead to bacterial contamination of shallow aquifers, which poses a severe public health hazard in peri-urban and rural areas of Bihar. The present study investigates the microbial water quality of twenty different groundwater sources situated at variable lateral distances from the soak pit at Nawada village, Patna, Bihar, over three seasons, i.e., pre-monsoon (summer), monsoon, and post-monsoon (winter). By using the standard MPN technique, the most probable number of total coliforms per 100 ml of water sample was quantified. Based on the obtained result, it was found that groundwater was severely and widely contaminated with microbes during the monsoon season, due to a rise in water level, which accelerates the subterranean transport of bacteria. Most of the samples during the monsoon period show the presence of coliform, with the highest number of 1600. These results highlight the necessity of distance in sanitation-groundwater interaction, especially during the monsoon. Targeted intervention, development program, and public awareness campaign are recommended to reduce bacterial contamination in groundwater and improve the community health of Nawada.*

KEYWORDS: Aquifer, Coliform, Groundwater, MPN, OSS, Soak pit.

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INTRODUCTION

Water is essential for the survival of human beings. Approx 65% of total human body weight is water (Chandra, 2007). Millions of cells of the human body contain water (Yusuf, 2002). Water is used by humans for different activities like bathing, drinking, and cooking (Sunarti, 2016). For the growth of microorganisms, water serves as a natural medium (Ezemba et al., 2021). 50% of global domestic water requirement fulfilled by groundwater (UNESCO, 2022). Anthropogenic activities have increased over the past decade, and pollution levels have also elevated. Humans need water most importantly for drinking, which is available through rainwater, springs, surface water, and mainly groundwater. Water that does not pose any significant risk to human health after consumption is categorized as potable or safe water (Fewtrell & Colford, 2005). Groundwater is devoid of direct anthropogenic activity, so it is considered the most abundant and safest source of drinking water in comparison to surface water (Jupri et al., 2023). Groundwater may be contaminated due to a lack of sanitation, unplanned waste disposal, and faulty construction (Rai & Sharma, 1995). Coliforms, which are gram-negative, bacillus bacteria, can contaminate ground water. Coliforms are lactose-fermenting facultative anaerobes often used as an indicator microbe for contamination of water (Dadi et al., 2014; Tominaga, 2019). Contamination arises from different sources like leakage, infiltration, and seepage of sewage lines, septic tanks, and soak pits into shallow aquifers (Ananthimalini et al., 2018). In rural Bihar, OSS is identified as a major cause of coliform contamination in groundwater due to defects in the design and construction of onsite sanitation systems (Dey et al., 2022; Peal et al., 2020). Human and Animal excreta are major sources of coliform. Detection of these indicator coliforms in drinking water signifies the presence of pathogenic organisms, which are the cause of various waterborne diseases (Al-Khatib & Hassan, 2009). The fecal contamination flow from OSS to groundwater depends on the lateral distance between the sanitation system and the water source. Rainfall is an important risk factor for faecal contamination in groundwater (Guo et al., 2019; Kostyla et al., 2015). This study was conducted to analyze the microbial quality of the drinking water source near the OSS of Nawada village, Patna, Bihar. The outcome of this study offers insights to inform evidence-based sanitation policies in Bihar that deal with groundwater contamination and safeguard public health.

MATERIALS AND METHODS

Study area

Village Nawada lies at latitude 25.549, longitude 85.060, and is located in Panchayat Nohsha, Block Phulwari of District Patna, Bihar, in India, as shown in Figure 1.

Figure 1. The map of the village of Nawada.

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Sample collection

Groundwatersamples were collected from houses near OSS at 20 different locations within Nawada village. Samples were collected in a clean, sterile 100 ml sample container and sealed with parafilm, and further transported to the Department of Biotechnology, A. N. College, Patna laboratory inside an ice box. Sampling was done in all three seasons, pre-monsoon, monsoon, and post-monsoon for same 20 site.

MPN

Bacteriological studies were carried out by the most probable number (MPN) method. MPN technique, also known as the multiple tube fermentation technique, was used to detect total coliform. This test was carried out in three stages, i.e., presumptive, confirmed, and completed test (Singh et al., 2017).

Presumptive test

The presumptive test was carried out by using 3 sets of 5 tubes each (5-5-5); the first set of 5 tubes contains 10 ml each of double strength MacConkey broth (HiMedia, Mumbai, India). The second set and the third set each tube contains single strength 10 ml MacConkey broth media. Media prepared and autoclaved at 121°C, 15 lb. pressure; then put in tubes. Samples were inoculated in three different dilutions. In the 1st set of 5 tubes containing 10 ml of 2x media, 10 ml of sample was inoculated; the 2nd set of tubes was inoculated with 1 ml of sample, and 3rd set was inoculated with 0.1 ml of sample. For one sample, a total of 15 tubes were prepared with inverted Durham tubes. After inoculation, tubes were incubated at 37°C for 24-48 hrs. and observed for the gas bubble in the Durham tube or a change in colour of the media (acid production) (Kanth et al., 2018).

Figure 2. MPN tubes.

Figure 3. MPN tubes of 2x strength.

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Confirmed test

This test was carried out by preparing a culture tube containing 10 ml of Brilliant green bile lactose broth (BGLB HiMedia, Mumbai, India) equipped with a Durham tube. 1 ml of inoculum from the positive tube of the presumptive test was taken and inoculated into a 10 ml tube of BGLB. Again, incubated at 37°C for 24-48 hrs.

Completed test

Tubes with positive results were subculture to EMB agar plates and incubated at 37°C overnight. Colonies appeared and were identified based on their colour, texture, etc. *E. coli* appears with a metallic green sheen, as shown in Figure 4.

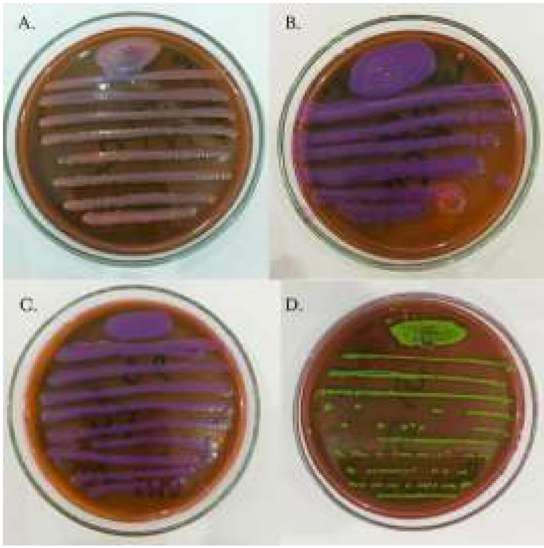

Figure 4. The culture plates of bacteria isolated from MPN tubes.

RESULTS

Multiple sets of tubes were observed for acid and gas production in Figures 2 and 3. For each volume of water sample added, i.e., 10 ml, 1 ml, and 0.1 ml (each volume in 5 tubes), the number of tubes with acid and gas production was precisely observed and noted. The noted result was subjected to comparison with the probability Table 4, for 20 water samples from three different seasons, the MPN index with total coliform value for all 20 samples of 3 seasons are shown in Table 1, Table 2, and Table 3. The mean deviation ± SD for the respective season i.e., pre-monsoon, monsoon, and post-monsoon was found to be 117.19 ± 347.98, 233.41 ± 332.53, and 71.46 ± 200.06 respectively.

Table 1. The pre-monsoon data for the MPN index and total coliform.

S.No	Sample code	MPN index	Total Coliform
1	NSII01	4 2 0	22
2	NSII02	5 4 4	350

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3	NSII03	1 1 0	4
4	NSII04	0 0 0	<1.8
5	NSII05	2 2 2	14
6	NSII06	0 0 0	<1.9
7	NSII07	4 3 0	27
8	NSII08	5 1 0	33
9	NSII09	3 4 1	24
10	NSII10	5 5 5	>1600
11	NSII11	3 3 2	24
12	NSII12	2 1 0	6.8
13	NSII13	5 3 1	110
14	NSII14	4 4 2	47
15	NSII15	4 3 2	39
16	NSII16	0 0 0	<1.10
17	NSII17	2 3 0	12
18	NSII18	3 1 1	14
19	NSII19	3 0 1	11
20	NSII20	0 0 0	<1.11

Table 2. The monsoon data for the MPN index and total coliform.

S.No	Sample code	MPN index	Total Coliform
1	NMII01	5 4 2	220
2	NMII02	5 5 1	350
3	NMII03	2 1 1	9.2
4	NMII04	4 1 1	21
5	NMII05	5 4 2	220
6	NMII06	4 1 1	21
7	NMII07	4 1 1	21
8	NMII08	5 3 1	110
9	NMII09	5 4 2	220
10	NMII10	5 5 4	1600
11	NMII11	5 5 2	540
12	NMII12	5 5 1	350
13	NMII13	5 4 0	130
14	NMII14	5 5 0	240

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15	NMII15	5 5 1	350
16	NMII16	5 1 0	33
17	NMII17	5 2 1	70
18	NMII18	5 2 0	49
19	NMII19	5 3 1	110
20	NMII20	1 0 1	4

Table 3. The post-monsoon data for the MPN index and total coliform.

S.NO	Sample code	MPN index	Total Coliform
1	NWII01	4 1 0	17
2	NWII02	5 4 2	220
3	NWII03	1 0 0	2
4	NWII04	0 0 0	<1.12
5	NWII05	2 2 1	9.2
6	NWII06	0 0 0	<1.13
7	NWII07	4 2 1	26
8	NWII08	5 1 0	33
9	NWII09	4 1 0	17
10	NWII10	5 5 3	920
11	NWII11	3 3 2	24
12	NWII12	5 2 0	49
13	NWII13	4 3 0	27
14	NWII14	4 3 0	27
15	NWII15	3 3 1	21
16	NWII16	0 0 0	<1.14
17	NWII17	2 1 1	9.2
18	NWII18	3 2 0	14
19	NWII19	2 1 1	9.2
20	NWII20	0 0 0	<1.15

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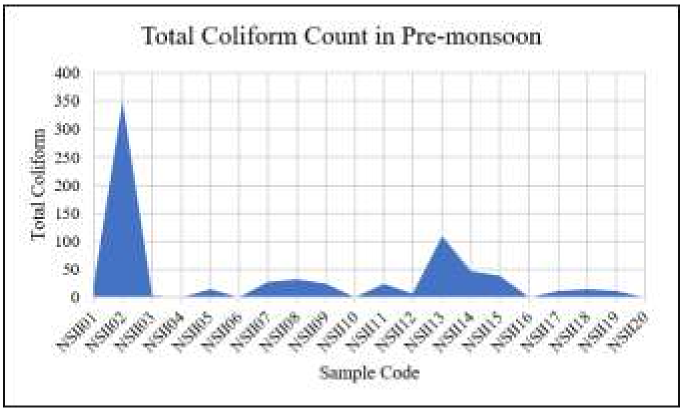

Figure 5. The graphical representation of the total coliform count for the pre-monsoon season.

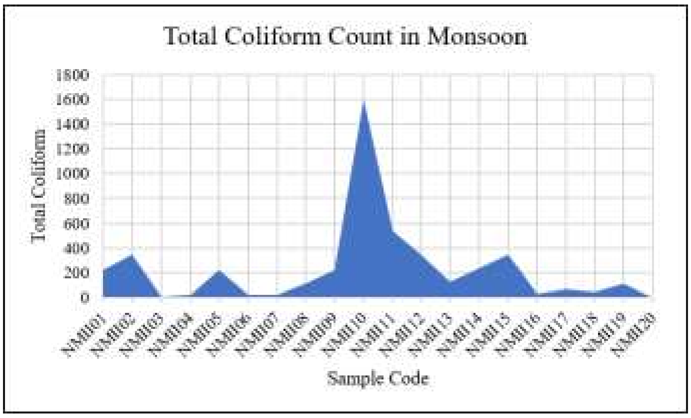

Figure 6. The graphical representation of the total coliform count for the monsoon season.

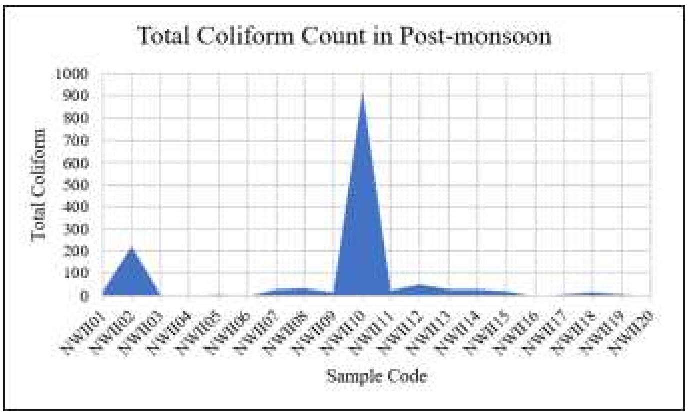

Figure 7. The graphical representation of the total coliform count for the post-monsoon season.

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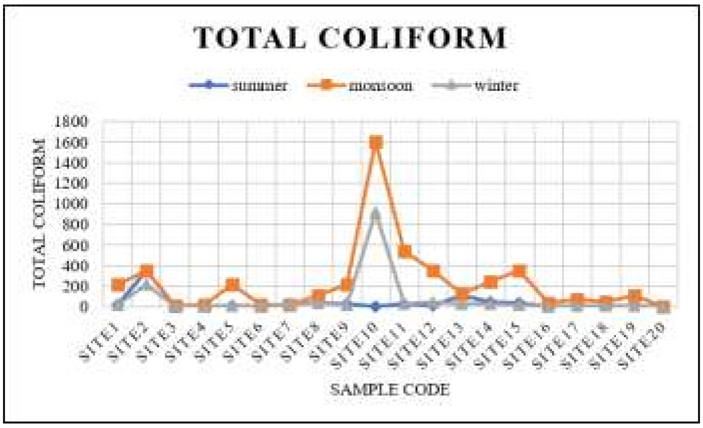

Figure 8. The graphical comparison of total coliform counts for all three seasons.

DISCUSSION

The data shows a significant uprising in total coliform value during the monsoon season compared to the summer and winter seasons. All 20 samples of groundwater sources exceeded the permissible limits prescribed by WHO and BIS during the monsoon season. Total coliform bacteria concentration in 100 ml of drinking water should be zero. The highest coliform concentration during monsoon observed for isolate NMII10 was 1600 per 100 ml, whereas NMII03 & NMII20 isolates recorded the lowest concentration of coliform.

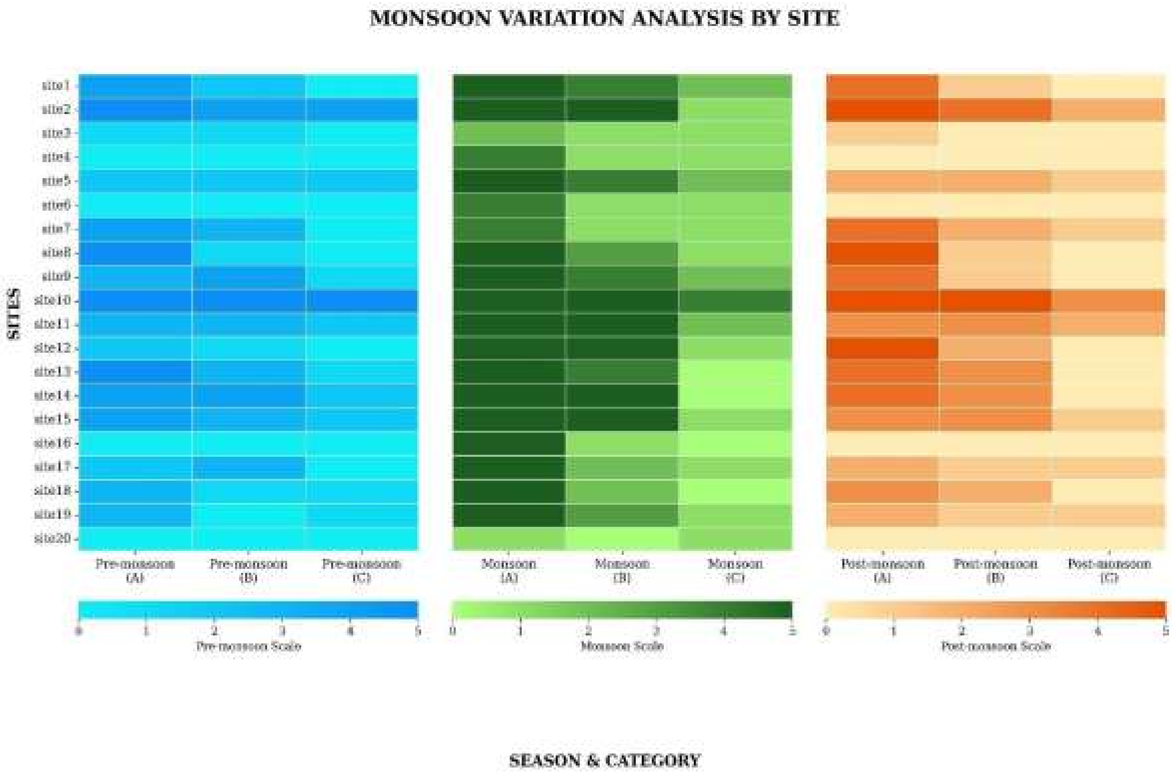

Figure 9. Heatmap of the MPN for the samples collected from different sites in the village of Nawada.

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Water table elevation during the monsoon leads to extreme contamination. Heavy monsoonal precipitation enhances the hydraulic loading of the soak pits, which accelerates the downward and lateral flow of liquid waste.

Coliform concentration for all 20 samples was recorded as low as compared to the monsoon because during summer, due to the temperature rise, the upper soil layer desiccates, leading to the death of bacteria. Lower water table during summer increased the mechanical filtration and absorption of bacterial cell walls onto clay and organic soil matrices. Due to this isolate number 4, 6, 16, and 20 show the least coliform concentration.

In the winter season, due to low temperatures and a lower water table, almost all samples show significantly lower concentrations of coliform compared to the summer and monsoon seasons. Again, samples number 4, 6, 16, and 20 show the least coliform concentration, similar to summer.

CONCLUSIONS

The present study indicates that the drinking water available to the residents of village Nawada is highly contaminated, given that ground water is the main source of water in village Nawada.

Higher MPN counts for groundwater samples were recorded because of the close distance between the water source and the OSS soak pit. It is recommended that groundwater for drinking purposes should be treated in the same manner as surface water. Water sources should be tested regularly for their microbiological quality. Early detection of contamination can prevent waterborne disease outbreaks. People should be educated about the treatment of water, such as boiling, chlorination, etc.

ABBREVIATIONS

OSS: On-site sanitation

MPN: Most Probable Number

WHO: World Health Organization

UNICEF: United Nations Children’s Fund

EMB: Eosin Methylene Blue

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