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Seasonal Fluctuation of *Escherichia coli* in Nainital Lake, Uttarakhand State, India

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Abstract: The season has a significant impact on the presence of microorganisms in water sources. Various contaminants and bacteria are transported into water sources by heavy rains. Because of the poor microbiological condition of such water bodies, regular monitoring is required. Routine monitoring, on the other hand, considers the microbial quality of the water column.The objective of this research was to investigate the microbiological quality of Nainital Lake in Uttarakhand, using *Escherichia coli* as a faecal indicator organism, and examine how seasonal fluctuations (dry and rainy seasons) affected its abundance. During 2018 and 2019, samples were taken from Nainital Lake sampling sites during the summer (dry) and rainy (wet) seasons and presence of *E. coli* was examined using various media, IMViC and catalase tests. *E. coli* was discovered at all of the locations and its concentration was influenced by seasonal variations. In both years, the cfu/ml count during the rainy season was observed higher in comparison to count during the summer. The transmission of this bacteria into the water column as a result of events such as floods or human activities might harm the health of those who consume untreated lake water for recreation and other domestic reasons. As a result, community education for health care practitioners and the general public about the proper use of lake water is essential. In order to preserve human health and other aquatic life, regular water quality monitoring is also required.

Keywords: Nainital Lake, *Escherichia coli*, Seasonal variation, IMViC test, Catalase test

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Introduction

The majority of human infections are acquired through the consumption of contaminated foods and water, both directly and indirectly (Acheson, 2009; Haider *et al.*, 2020). Water bodies, including groundwater, used to be much cleaner and uncontaminated, but modern civilization is reversing the trend. Water bodies are becoming polluted as a result of rapid industrial development, increased human population, animal wastes, untreated sewage, chemical effluents, and other factors (Nwachcuku and Gerba, 2004; Evans *et al.*, 2018). Drinking or cooking with contaminated water can induce diseases and infections such as amoebiasis, giardiasis, hepatitis,

cholera, and diarrhoea (Pal *et al.*, 2018).

Increased bacterial contamination is associated with increased rainfall and runoff (Ackerman and Weisberg, 2003; Haack *et al.*, 2003). Precipitation processes can promote pollutant mobilization, migration, and release from land to water bodies (VanWormer *et al.*, 2016). Precipitation causes an increase in the concentration and load of microorganisms in the faeces. *E. coli* in the catchment areas of surface and groundwater systems increase the risk of human exposure to faecal contaminated water (Buckerfield *et al.*, 2019).

Water-borne pathogen contamination in water bodies, as well as related diseases, are major water quality concerns around the world. Pathogen contamination is a serious problem for almost all types of water bodies, so recognising and understanding it is critical (Fawell and Nieuwenhuijsen, 2003). Waterborne disease is a global burden that is estimated to kill over 2.2 million people each year (WHO, 2015). Many outbreaks have been caused by water-borne diseases caused by various bacteria, viruses, and protozoa (Craun *et al.*, 2006).

E. coli is a gram-negative bacteria with a rod shape (Huang *et al.*, 2008). For the past 60 years, *E. coli* has been extensively researched. It is the most extensively studied prokaryotic model organism and is regarded as a critical species in biotechnology and microbiology (Taj *et al.*, 2014; Blount, 2015). The presence of *E. coli* in environmental waters has long been thought to be a sign of faecal pollution (Edberg *et al.*, 2000; Giri *et al.*, 2021). *E. coli* is a type of faecal coliform. The majority of *E. coli* are harmless and are abundant in the intestines of humans and warm-blooded animals. Some *E. coli* strains are pathogenic and cause disease (Niyoyitungiye *et al.*, 2020). Pathogenic *E. coli* strains cause a wide range of human diseases such as diarrhoea, resulting in more than 2 million deaths each year (Kaper *et al.*, 2004). Pathogenic *E. coli* strains have been implicated in numerous waterborne outbreaks and have been blamed for waterborne outbreaks worldwide (Nataro and Kaper, 1998; Nguyen *et al.*, 2005; Chandran and Mazumder, 2015).

The specific aims of present study were to determine seasonal patterns in *E. coli* abundance over a two-year period.The current study sought to determine the presence of *E. coli* in Nainital Lake water. Because there has not been much research done on this bacteria in Nainital Lake, it is important to study it because of its pathogenic activity.

Materials and Methods

Lake Nainital is a high-altitude natural kidney-shaped lake located at 29°24' N latitude and 79°28' E longitude in Uttarakhand's Kumaun region (Jain *et al.*, 2007; Choudhary *et al.*, 2009). The lake receives water from springs, rain, and 22 inlet nullahs(Purushothaman *et al.*, 2012). There are two designated areas at the lake's extreme ends (northwest and southwest). The lake is split into two subbasins (Mallital and Tallital). Water pump stations are located in each of these regions to supply water to the city of Nainital (via lake bank filtration) (Jain *et al.*, 2007). The lake supports approximately 40,000 local residents in its catchment area. Anthropogenic activities such as surface runoff, domestic sewage, construction activities, and agricultural activities have significantly altered the water (Sharma, 2014).

Sample Collection:

Lake water samples were collected at a depth of 0.5 m at each site in lake during the summer and rainy seasons during 2018 and 2019. Lake water samples were collected from eight different sites, two from Thandi Sadak (TSS site), two from Mallital (MS site), two from Mall Road (MR site) and two from Tallital (TS site) (Table1). Water samples were collected from different locations in 500 ml sterile polypropylene bottles (Genaxy, India). Within 2 h of being processed, the water samples were transported to the laboratory.

Isolation and identification of bacterial isolates:

Water samples (1 ml) were directly plated into Eosin Methylene Blue (EMB) agar (selective agar for *E. coli*) plates (HiMedia, India). Furthermore,

water samples (1 ml; undiluted) were directly plated into HiCrome agar, Nutrient agar and MacConkey agar (HiMedia, India) plates using the pour plate technique and incubated overnight at 37 C. IMViC (indole, methyl red, Voges-Proskauer, and citrate utilisation) and catalase tests were used to further identify and confirm *E. coli*.

Table 1: Detail of sampling location

S. No.	Sites	Sampling location
1.	TSS1	Near Hanuman Mandir (Thandi Sadak Site)
2.	TSS2	PashadMandir (Thandi Sadak Site)
3.	MS1	Near Gurudwara (Mallital Site)
4.	MS2	Lake view point (Mallital Site)
5.	MR1	Library (Mall Road Site)
6.	MR2	Near Grand Hotel (Mall Road Site)
7.	TS1	Main boat stand (Tallital Site)
8.	TS2	Sinz cafe (Tallital Site)

IMViC test: IMViC test was performed by using kit method (SRL, India).

Indole test: Bacterial sample was inoculated into tube containing Tryphone broth and was incubated for 1 day at 37 C. After 1-2 days incubation few drops of Kovac's reagent was added to the test tube. Presence of red colour indicated the positive results.

Methyl red (MR) test: A bacterial sample was inoculated into Methyl Red-Voges Proskeuer (MRVP) medium. For 1-2 days, the culture was incubated at 37 C. Added 5 drops of methyl-red reagent. The colour red denoted positive outcomes.

Voges-Proskeuer (VP) test: In MRVP media, a bacterial sample was inoculated. After that, it was incubated for 1-2 days at 37 C. The culture was supplemented withBarritt reagent A and then added Barritt reagent B. The presence of VP-positive organisms was indicated by a deep rose colour after 15 min.

Citrate test: A Simonn Citrate medium slant was created in a tube and then inoculated by bacteria. Incubated for 1-2 days at 37 C after inoculation. The presence of deep blue coloration indicated positive results, while the presence of green coloration indicated negative results.

Catalase Test: When a small amount of the bacterial isolate was added to hydrogen peroxide (H_2O_2), it quickly formed oxygen bubbles. A lack/ insufficient bubble formation indicated catalase deficiency.

Results

Identification and biochemical test:

E. coli contamination was detected in all water sampling sites. In EMB agar, all samples showed blue sheen colonies. The samples that showed a positive result in EMB agar were identified further. Selected isolates were inoculated in Nutrient agar, HiChrome agar, and MacConkey agar for further identification testing. In these media, all isolates showed positive results. Isolates showed white colonies in Nutrient agar, blue colonies in HiChrome agar and pink colonies in MacConkey agar (Table 2).

Table 2: Microbiological and Biochemical test result of all isolated *E. coli* strains

S. No.	Name of Test	*E. coli* isolates
1.	EMB Agar Test	+
2.	HiCrome Agar Test	+
3.	MacConkey Agar Test	+
4.	Catalase test	+
5.	Indole Test	+
6.	Methyl Red Test	+
7.	VogusProskauer Test	-
8.	Citrate test	-

'+' = Positive, '-' = Negative

After isolation, the isolates were subjected to the IMViC and catalase tests for further confirmation (Table 2). In both tests, all isolates tested positive for *E. coli*. In the Indole test, isolates produced a positive result with a red layer at the top of the tube after adding Kovac reagent,

Table 3: Average bacterial plate count (CFU/ml) at different MS, TSS, MR and TS sampling sites of Nainital Lake during summer and rainy seasons in 2018 and 2019

Year	Season	Bacterial count (CFU/ml) at different Sampling sites							
		TS1	TS2	MR1	MR2	TSS1	TSS2	MS1	MS2
2018	Summer	1.68×10^2	3.6×10^1	9.16×10^1	9.5×10^1	4.2×10^1	4.6×10^1	6.63×10^1	5.2×10^1
	Rainy	2.65×10^2	7.43×10^1	1.16×10^2	2.57×10^2	1.17×10^2	7.76×10^1	`1.95×10^2	2.05×10^2
2019	Summer	1.77×10^2	2.5×10^1	9.5×10^1	1.16×10^2	5.46×10^1	2.23×10^1	8.63×10^1	7.73×10^1
	Rainy	2.98×10^2	6.6×10^1	1.66×10^2	2.91×10^2	1.09×10^2	7.06×10^1	1.56×10^2	1.84×10^2

Where MS- Mallital sites; TSS- Thandi Sadak sites; MR- Mall Road sites; TS- Tallital sites

demonstrating *E. coli* ability to decompose the amino acid tryptophane to indole (Li and Young, 2013). The formation of red colour after the addition of methyl red reagent indicated a positive test for *E. coli* because it first metabolised glucose to pyruvic acid and then produced the stable acid (Abedi and Hashemi, 2020). The acid produced reduces the pH to 4.5 or lower, as indicated by a change in the colour of methyl red from yellow to red.

In contrast, *E. coli* gave negative result for both VP and citrate tests (Pant *et al.*, 2015). Negative VP test indicated by a lack of colour change after the addition of Barritt's A and B reagents because *E.coli* does not produce acetyl methyl carbinol (acetoin). The citrate test evaluates an organism's ability to grow solely on citrate, resulting in alkalinity and a positive result by showing blue colour, whereas *E. coli* showed green colour, indicating a negative test.

In the present study all isolates yielded positive catalase test. Catalase is an enzyme that aids in the breakdown of hydrogen peroxide into oxygen and water (Nandi *et al.*, 2019). The presence of catalase was indicated by the production of bubbles. *E. coli* was used as a model for catalase-positive bacteria (Serra *et al.*, 2008).

Seasonal Variation:

Eight different sites were selected for the study i.e. TS1, TS2, MR1, MR2, TSS1, TSS2, MS1 and MS2, during 2018 and 2019. The comparative study of two consecutive years has been illustrated in Table 3. Figures 1 and 2 depict the bacterial count in summer and rainy season in 2018 and 2019, respectively.

Discussion

For many years, coliform bacteria, particularly *E. coli*, have been used to assess the microbiological quality of surface and ground water. Thus, the presence of faecal indicator *E. coli* in water sources is due to contamination by material of human and animal origin, which is of public health concern because these organisms have been widely reported as the cause of gastroenteritis in humans (Nkansah *et al.*, 2010; McLellan and Eren, 2014). Petit *et al.*, (2017) discovered a link between the density of *E. coli* microbes in water and the occurrence of water-related gastroenteritis.

In the present study, *E. coli* was reported from all study sites with variation in the number of count. MR, TS and MS sites have more *E. coli* load than TSS sites. In comparison to TSS sites, MR, TS and MS sites have more human interference, more

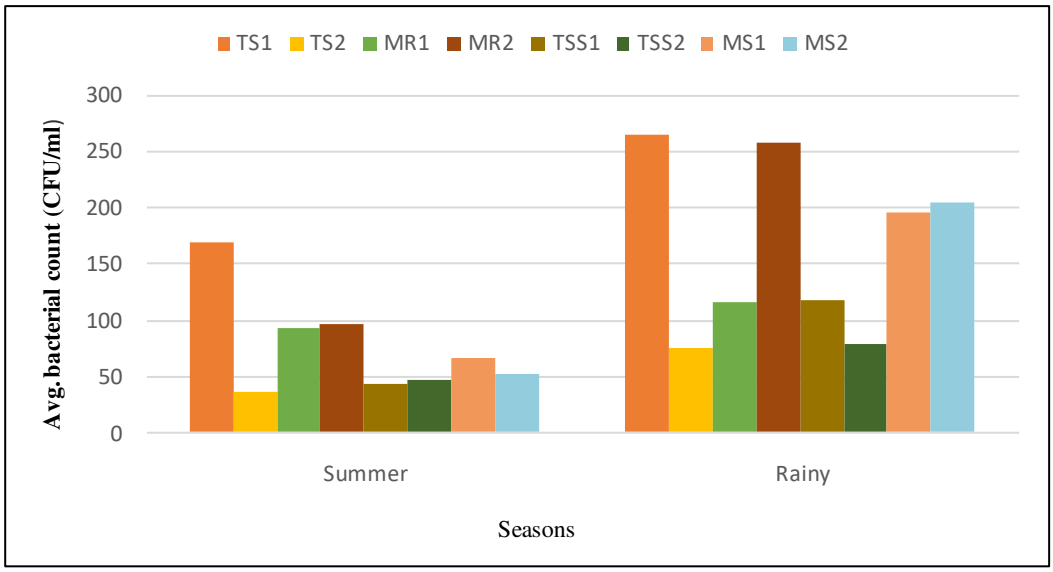

Fig. 1: Bacterial count (CFU/ml) during summer and rainy season at different sites in 2018.

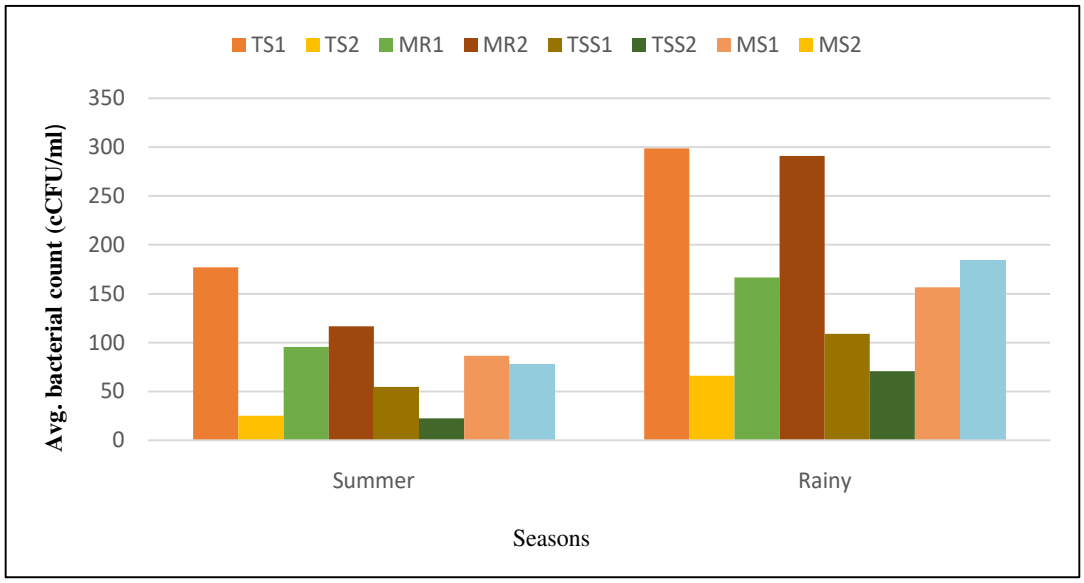

Fig. 2: Bacterial count (CFU/ml) during summer and rainy season at different sites in 2019.

tourist disturbance, more population and more hotels construction (Mishra *et al.*, 1983; Shah *et al.*, 2009). Nainital serves as a base for other tourist destinations in the surrounding area, and according to the 2011 Census, it provides permanent shelter to approximately 41,377 people. Natural lakes along with springs, water tanks, municipal water, and other sources of water supply are major sources of water supply for over 40,000 local residents in the city. Presence of such concentration of bacteria will directly and indirectly affect health and life of these people (Bisht *et al.*, 2019). Previous study which reported high *E. coli* count from extracted surface water samples such as Surveillance data from the United States of America (USA) showed that during 2009 and 2010, there were twenty four recreational water disease outbreaks associated with the use of

natural waters. Microbial agents implicated included *Campylobacter jejuni*, *E. coli* O157:H7, *Giardia intestinalis*, norovirus, *Shigella sonnei,* Cryptosporidium spp., and avian schistosomes (Hlavsa *et al.*, 2014). Sampson *et al.* (2005) measured *E. coli* at twenty seven beaches along Lake Superior, Wisconsin by defined substrate analysis. Vaiyapuri *et al.* (2021) reported *E. coli* from 77% of water samples drawn from 35 different stations of the Vembanad Lake in India.

In the present study in both year (2018-2019) rainy season impact on *E. coli* count was found to be greater than summer season. Regardless of quantity, any amount of rain can impact *E. coli* levels. Significant effects of rainfall on *E. coli* concentrations were observed in both years, and we conclude that rainfall is associated with increased risks of introducing *E. coli* into lake water in both years. This is consistent with the findings of Tryland *et al.*, (2002) and Amirat *et al.*, (2012), who found high levels of pollution in their studied water bodies during the rainy season. Whitman *et al.*, (2008) studied a section of Dunes Creek, a small coastal stream in southern Lake Michigan, and discovered that *E. coli* levels increased several-fold during both rainfall and snowmelt events compared to summer. Similarly Kisteman *et al.* (2002) and Odonkor and Mahami (2020) found that *E. coli* counts were significantly higher in the wet season than in the dry season.

Conclusion

Lake Nainital is a popular tourist destination in Uttarakhand, India. It is located in the heart of Nainital. Over the last few decades, there has been widespread concern about the lake's pollution and water quality, which has increased the likelihood of microbial contamination in Nainital Lake. The findings of current study lead us to the conclusion that the water in Nainital Lake is not safe for human consumption in terms of *E. coli*. *E. coli* identification is simple and inexpensive, so they can serve as useful risk indicators for the presence of faecal waste and associated pathogens. Because little information on bacterial contamination in freshwater lakes has been published, the goal of this study was to collect baseline data for counts of total *E. coli* indicator bacteria in Nainital Lake in order to address public concerns about bacterial contamination in Nainital Lake which may increase in future. Therefore, it is recommended that the water quality of the lake, its routine cleaning, anthropogenic activities and public awareness should be regularly monitored.

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