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Evaluation of Coliform and Faecal Coliform Bacteria in the Lakes of Broknes and Grovnes Peninsula, East Antarctica

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ABSTRACT

More than 150 lakes on different peninsulas and islands are situated in the Larsemann Hills. The Larsemann Hills is an ice-free area and are located halfway between the Vestfold Hills and the Amery Ice Shelf on the southeastern coast of Prydz Bay, Princess Elizabeth Land, and East Antarctica. During 34th Indian Scientific Expedition to Antarctica (ISEA) from 2014 to 2015, twenty lake water samples in triplicates were collected from the Broknes & Grovnes peninsula. Coliform and fecal coliform bacteria were analyzed in these samples. Out of twenty, eleven lake water samples were found to be contaminated with coliform bacteria. However, fecal coliform bacteria were absent in all the lake water samples. Coliforms are found in the lakes of Broknes peninsula (P2 Lake & P3 Lake) and Grovnes peninsula (L1C NG, L1D NG, L1E NG, L7 NG, L7A NG, L7B NG, L2 SG, L4 SG & L5 SG). Antarctic lakes water is being polluted due to anthropogenic activities caused by various research activities and tourism. The present study confirms the presence of coliform bacteria in the lakes of East Antarctica which indicates an alarming situation and needs to be investigated further.

INTRODUCTION

Antarctica is the world's most remote and unspoiled continent (Bhardwaj & Pawan 2022). It is the largest pristine wilderness in the world. Lenihan (1992) & Bhardwaj et al. (2021) stated that it is a continent with a pristine environment. Approx. 53 research stations are now located in Antarctica. The population in winter and summer is around 1000 to 4000 people respectively. It is increasing over time due to more researchers taking an interest in research on Antarctica. Antarctic lakes are polluted by sewage waste released from research stations and commercial fishing vessels. Sewage waste which contains food waste and human waste is discharged untreated from research stations to Antarctica and affects its environment. Some local wildlife populations such as seals and sea birds are also releasing their fecal waste in Antarctica. Lenihan et al. (1990) stated that human sewage can be the dominant source of fecal microorganisms in Antarctica and have a significant impact on its environment.

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Waterhouse (2001) studied the presence of coliforms and fecal coliforms bacteria in Antarctic water and stated that it is due to the activities of the local vertebrates and human populations. These bacteria are nonpathogenic, but their presence indicates the possibility of finding pathogens (Harmon et al. 2014). Mishra et al. (2018) stated that coliforms can be divided into total coliforms and fecal coliforms. Fecal coliform bacteria such as *Escherichia coli* (*E. coli*) are found in feces and their presence in drinking water indicates fecal contamination (Bhardwaj & Sharma 2021). *E. coli* can also be a pathogen itself, so if *E. coli* is found in the water then there is a chance that other pathogens will be present. Fecal coliform is a rod-shaped, gram-negative, non-sporulating, and facultative anaerobic bacterium. It generally originated in the intestine of warm-blooded animals. Coliforms are commonly used microbiological markers of sewage pollution in Antarctica (Hill et al. 1996, Hughes & Blenkharn 2003, Khan & Gupta 2020). Cowan et al. (2011) stated that some strains of coliform bacteria which were found in Antarctica are not indigenous to Antarctica and are transported by anthropogenic activities. Green et al. (1992) studied the different sewage indicators from sewage around Antarctic research stations.

Several researchers studied the different physical factors such as solar radiation, temperature, and ice condition which

affect the survival and distribution of fecal coliform bacteria in Antarctica (Patrick & Bernard 1991, Šolić & Krstulović 1992, Smith et al. 1994). The high level of Ultra-Violet (UV) radiation is also responsible to reduce the viability of sewage microorganisms in Antarctic water and air (Statham & McMeekin 1994, Sinton 1994). The survival rate of fecal bacteria can vary from a few minutes to many days and depends upon the environmental conditions (Statham & McMeekin 1994). Sewage microorganisms can remain viable for prolonged periods in the Antarctic environment while the terrestrial environment is potentially less hospitable due to desiccation stress and wide diurnal temperature ranges (Smith et al. 1994, Upton et al. 1997). Sewage microbes have the potential to infect people and become part of the gut of local wildlife populations in Antarctica (Gardner et al. 1997, Edwards et al. 1998).

In Antarctica, the number of fecal coliform bacteria was found high in early winter due to increased fecal input by migrant wildlife and low doses of solar radiation, while in summer, despite the high population on the research station, the number of fecal coliform bacteria was found low due to the biologically damaging effects from the radiation of solar. According to several scientists after the first human expedition to Antarctica, the disposal of fecal waste has generally been into the sea and either buried in snow or discharged into the Antarctic lakes (McFeters et al. 1993, Parker & Martel 2002, Hughes & Blenkharn 2003, Hughes 2003). Parker & Martel (2002) stated that once the fecal waste is buried in the snow, it remained the same for a long time due to low temperature, it undergoes relatively little degradation and could become a long-term pollution problem in the future in Antarctica. The purpose of this study was to determine the occurrence of coliform and fecal coliform bacteria in the lake water samples of Broknes and Grovnes peninsula of Larsemann Hills, East Antarctica.

MATERIALS AND METHODS

Study Area: Broknes & Grovnes peninsula of Larsemann Hills, East Antarctica was selected as a study area. The location map of the study area is shown in Fig. 1.

Sampling Sites and Collection: The sampling of the lake water samples was carried out in the month of Dec-Feb of 2014-2015. A total of 15 samples were collected randomly from P1 Lake, P2 Lake, P3 Lake, P4 Lake, and Reid Lake from the Broknes peninsula. While 45 samples were collected from L1C NG, L1D NG, L1E NG, L3 NG, L5 NG, L6 NG, L7 NG, L7A NG, L7B NG, Murk Water Lake NG, L1 SG, L2 SG, L3 SG, L4 SG, L5 SG from Grovnes peninsula. Thirty samples from Northern Grovnes and fifteen samples from the Southern Grovnes peninsula were collected respectively. Before taking samples from each site, dark amber color sterile polyethylene (PET) bottles were rinsed twice with the lake water. Three replicates of each sample (1 L) were collected from easily accessible inner areas of each lake. All samples were immediately stored in an ice chest with ice at 4°C and transported to the laboratory after completion of the expedition for fecal coliform and coliform analysis. The sampling sites are shown in Fig. 2, 3 and 4, and Tables 1 and 2.

Requirements (Equipment and Culture Media): Autoclave (Laczene Biosciences Solutions), laminar airflow

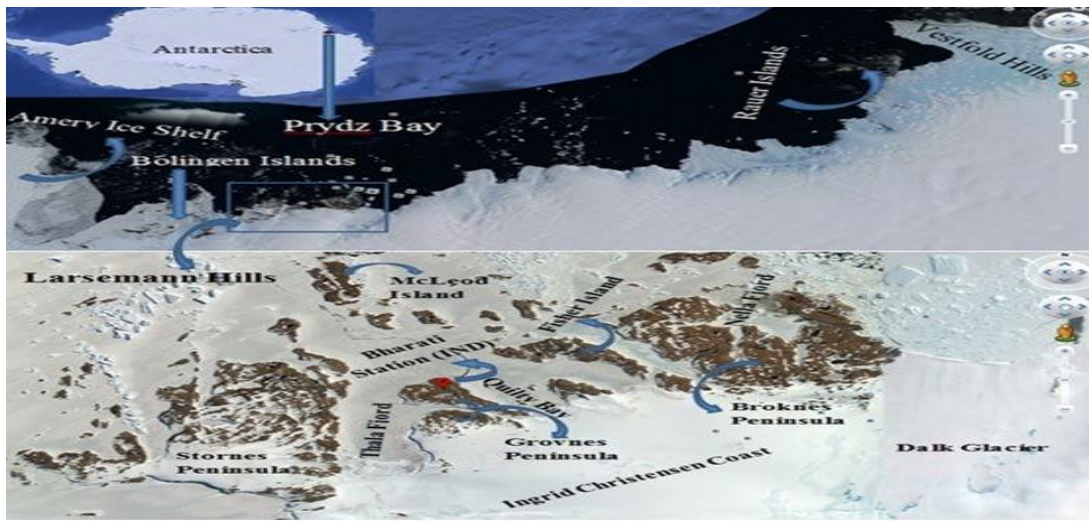

Fig. 1: Location map of the study area in Larsemann Hill (downside), Prydz Bay in East Antarctica marked on the continental map of Antarctica (upper side).

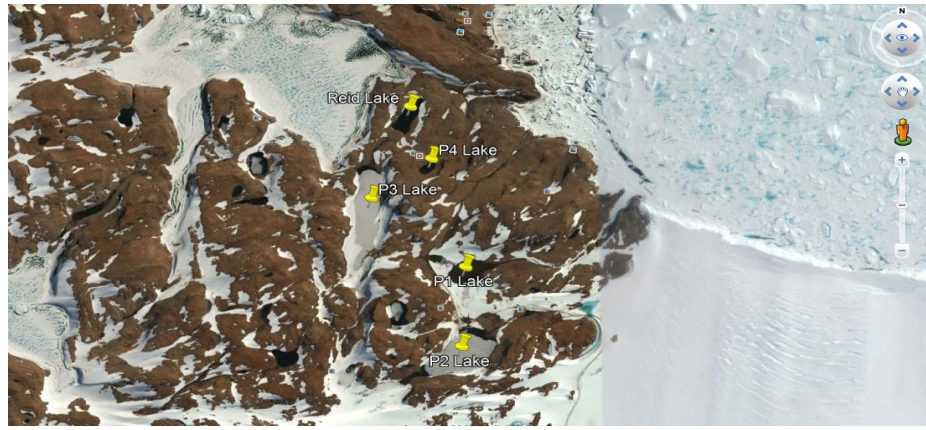

Fig. 2: location of sampling sites mark with pinpoints on Broknes Peninsula, East Antarctica.

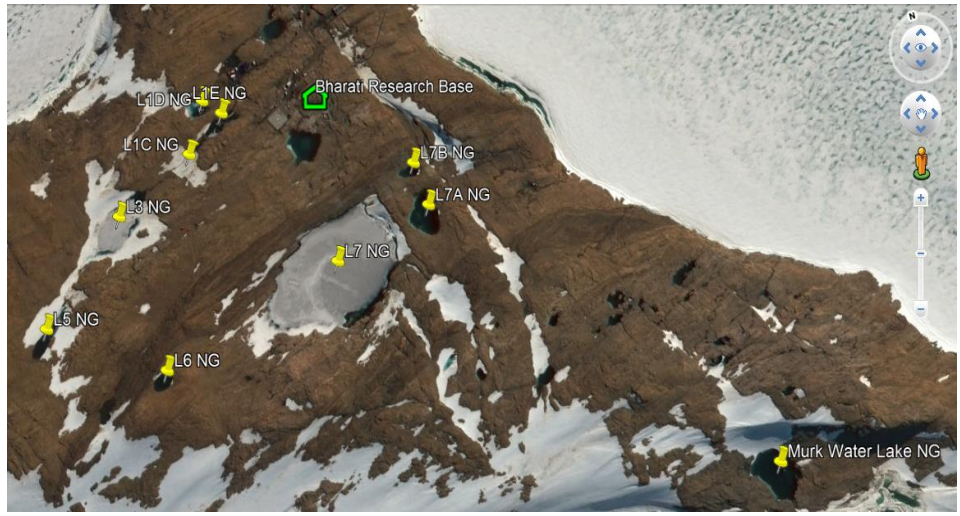

Fig. 3: Location of sampling sites mark with pinpoints on Northern Grovnes Peninsula, East Antarctica.

Fig. 4: Location of sampling sites mark with pinpoints on Southern Grovnes Peninsula, East Antarctica.

Table 1: Sample details with geographic coordinates, date of sampling, sample quantity, and name of the lakes at Broknes Peninsula, East Antarctica.

S. No.	Sample ID (Lake Name)	Latitude	Longitude	Replicates	Date of Sampling	Sample Quantity
1	P1 Lake	69°23'49.54" S	76°23'17.43" E	P1-1	17-02-2015	1 L
				P1-2	17-02-2015	
				P1-3	17-02-2015	
2	P2 Lake	69°24'09.005" S	76°23'15.00" E	P2-1	18-02-2015	1 L
				P2-2	18-02-2015	
				P2-3	18-02-2015	
3	P3 Lake	69°23'32.17" S	76°22'17.78" E	P3-1	19-02-2015	1 L
				P3-2	19-02-2015	
				P3-3	19-02-2015	
4	P4 Lake	69°23'22.19" S	76°22'55.82" E	P4-1	20-02-2015	1 L
				P4-2	20-02-2015	
				P4-3	20-02-2015	
5	Reid Lake	69°23'8.83" S	76°22'42.06" E	Reid Lake-1	21-02-2015	1 L
				Reid Lake-2	21-02-2015	
				Reid Lake-3	21-02-2015	

Table 2: Sample details with geographic coordinates, date of sampling, sample quantity, and name of the lakes at Northern and Southern Grovnes Peninsula, East Antarctica.

S. No.	Sample ID (Lake Name)	Latitude	Longitude	Replicates	Date of Sampling	Sample Quantity
1	L1C NG	69°24'25.43" S	76°11'17.66" E	L1C NG-1	25-12-2014	1 L
				L1C NG-2	25-12-2014	
				L1C NG-3	25-12-2014	
2	L1D NG	69°24'22.41" S	76°11'22.26" E	L1D NG-1	25-12-2014	1 L
				L1D NG-2	25-12-2014	
				L1D NG-3	25-12-2014	
3	L1E NG	69°24'23.51" S	76°11'25.20" E	L1E NG-1	26-12-2014	1 L
				L1E NG-2	26-12-2014	
				L1E NG-3	26-12-2014	
4	L3 NG	69°24'27.72" S	76°11'2.70" E	L3 NG-1	26-12-2014	1 L
				L3 NG-2	26-12-2014	
				L3 NG-3	26-12-2014	
5	L5 NG	69°24'32.83" S	76°10'45.75" E	L5 NG-1	27-12-2014	1 L
				L5 NG-2	27-12-2014	
				L5 NG-3	27-12-2014	
6	L6 NG	69°24'37.30" S	76°11'5.13" E	L6 NG-1	27-12-2014	1 L
				L6 NG-2	27-12-2014	
				L6 NG-3	27-12-2014	
7	L7 NG	69°24'34.32" S	76°11'39.41" E	L7 NG-1	28-12-2014	1 L
				L7 NG-2	28-12-2014	
				L7 NG-3	28-12-2014	

S. No.	Sample ID (Lake Name)	Latitude	Longitude	Replicates	Date of Sampling	Sample Quantity
8	L7A NG	69°24'32.78" S	76°11'57.96" E	L7A NG-1	28-12-2014	1 L
				L7A NG-2	28-12-2014	
				L7A NG-3	28-12-2014	
9	L7B NG	69°24'30.05" S	76°11'57.38" E	L7B NG-1	29-12-2014	1 L
				L7B NG-2	29-12-2014	
				L7B NG-3	29-12-2014	
10	Murk Water Lake NG	69°24'53.37" S	76°12'46.16" E	Murk Water Lake NG-1	30-12-2014	1 L
				Murk Water Lake NG-2	30-12-2014	
				Murk Water Lake NG-3	30-12-2014	
11	L1 SG	69°25'13.70" S	76°13'18.33" E	L1 SG-1	02-01-2015	1 L
				L1 SG-2	02-01-2015	
				L1 SG-3	02-01-2015	
12	L2 SG	69°25' 5.10" S	76°12'45.05" E	L2 SG-1	02-01-2015	1 L
				L2 SG-2	02-01-2015	
				L2 SG-3	02-01-2015	
13	L3 SG	69°25'09.07" S	76°12'36.1" E	L3 SG-1	03-01-2015	1 L
				L3 SG-2	03-01-2015	
				L3 SG-3	03-01-2015	
14	L4 SG	69°25'04.46" S	76°12'19.93" E	L4 SG-1	03-01-2015	1 L
				L4 SG-2	03-01-2015	
				L4 SG-3	03-01-2015	
15	L5 SG	69°25'08.65" S	76°11'53.9" E	L5 SG-1	04-01-2015	1 L
				L5 SG-2	04-01-2015	
				L5 SG-3	04-01-2015	

(Laczene Biosciences Solutions), weighing balance (0.01 mg to 220 g, Sartorius), micropipettes, test tubes, Petri discs, inoculation loop, conical flask, Durham's tubes, and spirit lamp were used. MacConkey broth (MCB, M007) Brilliant Green Bile Lactose (BGBL, M121S), Eosin Methylene Blue (EMB, M317) media were procured from HiMedia Laboratories Pvt. Ltd.

Enumeration of Coliform and Faecal Coliform Bacteria: The most probable number (MPN) of coliform and fecal coliform (coliform/100 mL) in lake water samples was determined as per the Indian Standard (IS):1622 (1981). The enumeration was carried out in triplicate and included three phases.

Presumptive Test: Three sets of test tubes were taken for every sample and each set contained five test tubes. 10 mL of double-strength MacConkey broth (MB) was inoculated in each tube of the first set. On the other hand, for the second and third sets of test tubes, 10 mL of single-strength MB was inoculated in each tube. Durham's tube (small tube) was placed inside each tube in an inverted position. Homogenized lake water samples (10 mL, 1 mL & 0.1 mL) were inoculated in the first, second, and third sets of test tubes respectively. All tubes were incubated at 37 ± 1°C for 24-48 h (at 44.5°C for 24 h for fecal coliform). After incubation, the observation was recorded for the gas production (i.e. bubble formation) in Durham's tube. If bubbles were present, then consider that tube positive. If no gas was observed in any test tube, then discontinue the test and record the result as less than 2 organisms/100 mL. This test is shown in Fig. 5.

Confirmative Test: Three sets of test tubes were taken for each positive tube and each set contained five tubes. 10 mL Brilliant Green Bile Lactose (BGBL) broth was inoculated in each test tube. Durham's tube was placed inside each tube in an inverted position. A loopful inoculum from each positive tube was inoculated into three sets of tubes. All tubes were Incubated at 37 ± 1°C for 24-48 h. After incubation, the observation was recorded for gas production in Durham's

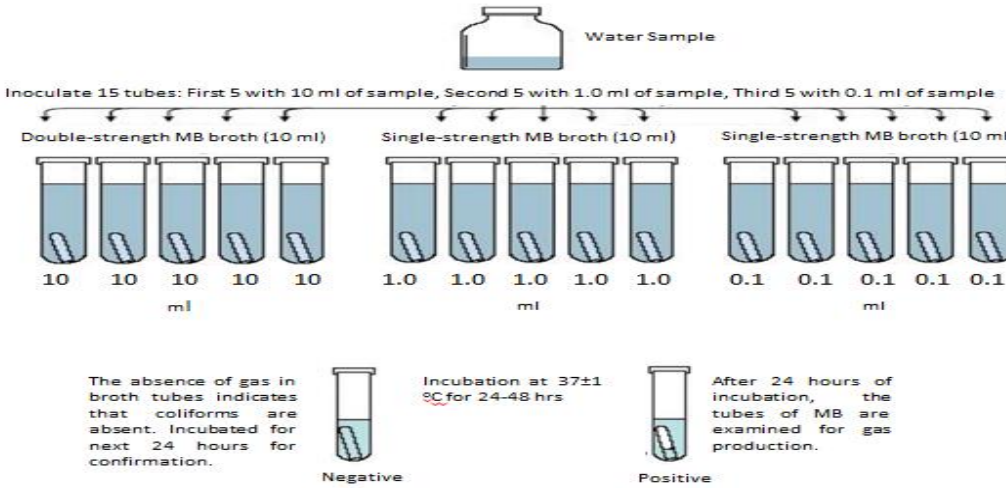

Fig. 5: Presumptive test.

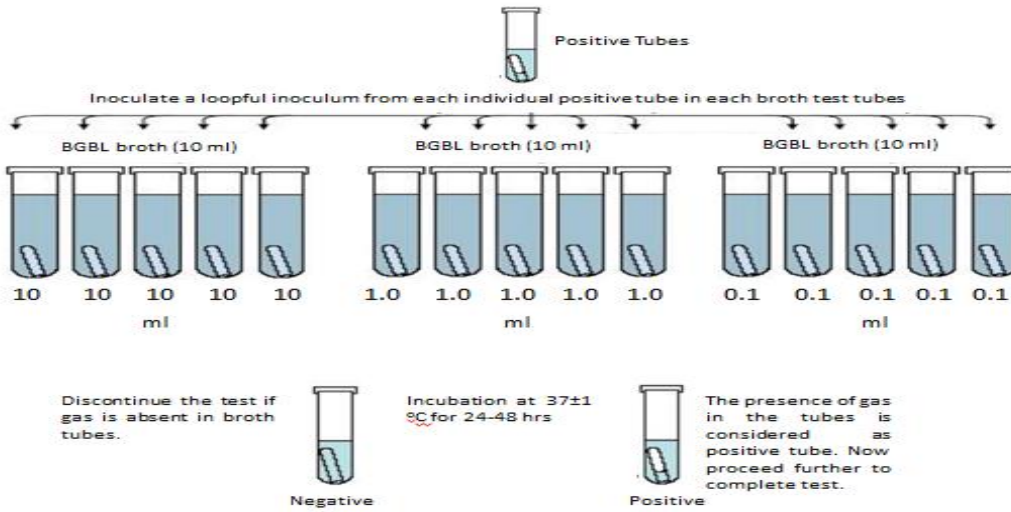

Fig. 6: Confirmative test.

tube. The presence of gas in the tubes was considered a positive tube and record the number of these positive tubes then further proceed to the completed test. In case gas production is not observed in any of the tubes, discontinue the test and record the result as less than 2 organisms/100 mL. This test is shown in Fig. 6.

Completed Test: Eosin Methylene Blue (EMB) media were prepared according to the positive tubes of the confirmative test and then poured into the Petri discs. Three Petri discs were taken for each positive tube. A loopful inoculum from each positive tube was streaked on EMB media plates and these plates were incubated at 37°C for 24 h in an inverted position. The presence of green metallic sheen colonies confirmed the presence of E. Coli. These isolates were further confirmed by Gram's staining for E. Coli as per IS:5887 (1976). This test is shown in Fig. 7.

Quality Assurance/Quality Control (QA/QC): Each sample was analyzed in triplicate. All dilutions and media were prepared in double-distilled water. Based on the number of positive tubes as recorded in a confirmed test calculate the probable number of coliform and fecal coliform/100 mL of lake water sample by using the MPN table. MPN of the organism present per 100 mL of sample is shown in Table 3.

RESULTS

An Occurrence of Coliform and Faecal Coliform Bacteria in the Broknes Peninsula, East Antarctica: No growth was

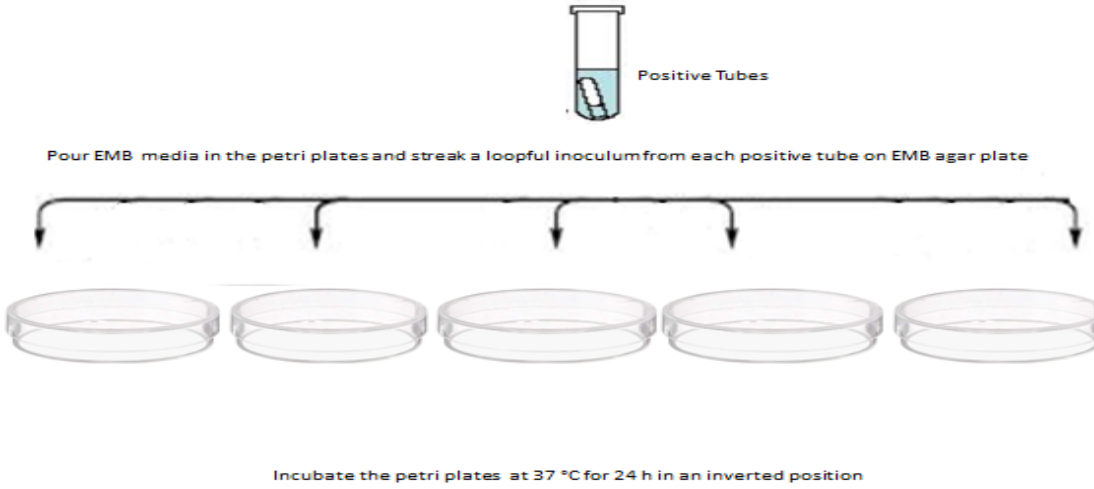

Fig. 7: Completed test.

Table 3: MPN of organism present per 100 mL of sample and confidence limits using 5 tubes of 10 mL, 5 tubes of 1 mL, and 5 tubes of 0.1 mL.

S. No.	Combination of Positive Tubes	MPN Index per 100 mL	95 % Confidence Limits	
			Lower	Upper
1	0-0-0	<2	-	-
2	0-0-1	2	1.0	10
3	0-1-0	2	1.0	10
4	0-2-0	4	1.0	13
5	1-0-0	2	1.0	11
6	1-0-1	4	1.0	15
7	1-1-0	4	1.0	15
8	1-1-1	6	2.0	18
9	1-2-0	6	2.0	18
10	2-0-0	4	1.0	17
11	2-0-1	7	2.0	20
12	2-1-0	7	2.0	21
13	2-1-1	9	3.0	24
14	2-2-0	9	3.0	25
15	2-3-0	12	5.0	29
16	3-0-0	8	3.0	24
17	3-0-1	11	4.0	29
18	3-1-0	11	4.0	29
19	3-1-1	14	6.0	35
20	3-2-0	14	6.0	35
21	3-2-1	17	7.0	40
22	4-0-0	13	5.0	38
23	4-0-1	17	7.0	45
24	4-1-0	17	7.0	46
25	4-1-1	21	9.0	55
26	4-1-2	26	12	63
27	4-2-0	22	9.0	56
28	4-2-1	26	12	65
29	4-3-0	27	12	67
30	4-3-1	33	15	77
31	4-4-0	34	16	80
32	5-0-0	23	9.0	86
33	5-0-1	30	10	110
34	5-0-2	40	20	140
35	5-1-0	30	10	120
36	5-1-1	50	20	150
37	5-1-2	60	30	180
38	5-2-0	50	20	170
39	5-2-1	70	30	210
40	5-2-2	90	40	250
41	5-3-0	80	30	250
42	5-3-1	110	40	300
43	5-3-2	140	60	360
44	5-3-3	170	80	410
45	5-4-0	130	50	390
46	5-4-1	170	70	480
47	5-4-2	220	100	580
48	5-4-3	280	120	690
49	5-4-4	350	160	820
50	5-5-0	240	100	940
51	5-5-1	300	100	1300
52	5-5-2	500	200	2000
53	5-5-3	900	300	2900
54	5-5-4	1600	600	5300
55	5-5-5	□1600	-	-

observed of fecal coliform in all different lake water samples while coliform was present in P2 Lake and P3 Lake. No growth was observed of coliform in P1 Lake, P4 Lake, and Reid Lake. Maximum MPN coliform was found in P2 Lake followed by P3 Lake. An observation of MPN coliform and fecal coliform/100 mL in different lake water samples of Broknes peninsula is shown in Table 4.

An Occurrence of Coliform and Faecal Coliform Bacteria in the Grovnes Peninsula, East Antarctica: No growth was observed of fecal coliform in all different lake water samples while coliform was present in L1C NG, L1D NG, L1E NG, L7 NG, L7A NG, L7B NG, L2 SG, L4 SG, and L5 SG. No growth was observed of coliform in L3 NG, L5 NG, L6 NG, Murk Water Lake NG, L1 SG, and L3 SG. Maximum MPN coliform was found in LIENG followed by L7A NG, L4 SG, L7B NG, L1D NG, L1C NG, L2 SG, and L7 NG. An observation of MPN coliform and fecal coliform/100 mL in different lake water samples of the Grovnes peninsula is given in Tables 5 and 6.

DISCUSSION

In the present study, we assessed the environmental impact of the different sewage markers such as coliform and fecal coliform bacteria. Antarctica is the hub of freshwater lakes (Bhardwaj et al. 2019, Bhardwaj & Jindal 2020). Now, these lakes have been contaminated through anthropogenic activities and wildlife populations, such as seals and penguins. The release of sewage waste in Antarctica can produce microbiological pollution in the lake water. Fecal coliform is a more suitable bacteria for the investigation of the anthropogenic impact on the Antarctic environment. Edwards et al. (1998) & Hughes (2003) studied that some strains of fecal coliforms, such as fecal enterococci and spore-forming bacillus can survive in Antarctica for 30-40 years. Fujioka et al. (1981) and Kapuscinski & Mitchell (1981) have described

Table 4: An occurrence of coliform and faecal coliform bacteria in the lake water samples of Broknes Peninsula, East Antarctica.

S. No.	Sample ID/Lake Name	Altitude [mt]	MPN Coliform/100 mL	MPN Faecal Coliform/100 mL
1	P1 Lake	54	No Growth Observed (<2)	No Growth Observed (<2)
2	P2 Lake	3	40 organisms	No Growth Observed (<2)
3	P3 Lake	24	23 organisms	No Growth Observed (<2)
4	P4 Lake	29	No Growth Observed (<2)	No Growth Observed (<2)
5	Reid Lake	54	No Growth Observed (<2)	No Growth Observed (<2)

Table 5: An occurrence of coliform and faecal coliform bacteria in the lake water samples of Northern Grovnes Peninsula, East Antarctica.

S. No.	Sample ID/Lake Name	Altitude [mt]	MPN Coliform/100 mL	MPN Faecal Coliform/100 mL
1	L1C NG	24	26 organisms	No Growth Observed (<2)
2	L1D NG	22	27 organisms	No Growth Observed (<2)
3	L1E NG	25	60 organisms	No Growth Observed (<2)
4	L3 NG	39	No Growth Observed (<2)	No Growth Observed (<2)
5	L5 NG	30	No Growth Observed (<2)	No Growth Observed (<2)
6	L6 NG	91	No Growth Observed (<2)	No Growth Observed (<2)
7	L7 NG	53	17 organisms	No Growth Observed (<2)
8	L7A NG	51	42 organisms	No Growth Observed (<2)
9	L7B NG	40	32 organisms	No Growth Observed (<2)
10	Murk Water Lake NG	5	No Growth Observed (<2)	No Growth Observed (<2)

Table 6: An Occurrence of coliform and faecal coliform bacteria in the lake water samples of Southern Grovnes Peninsula, East Antarctica.

S. No.	Sample ID	Altitude (mt)	MPN Coliform/100 mL	MPN Faecal Coliform/100 mL
1	L1 SG	8	No Growth Observed (<2)	No Growth Observed (<2)
2	L2 SG	3	21 organisms	No Growth Observed (<2)
3	L3 SG	24	No Growth Observed (<2)	No Growth Observed (<2)
4	L4 SG	28	34 organisms	No Growth Observed (<2)
5	L5 SG	25	11 organisms	No Growth Observed (<2)

the effect of sunlight on E. Coli. Statham & McMeekin (1994) studied the effect of solar radiation on the survival of E. Coli at Davis Research Station, Antarctica, and stated that fecal bacteria were rapidly inactivated when exposed to sunlight in the Antarctic water.

Several scientists have reported the presence of coliform and fecal coliform in the sewage outfall of the Antarctic research stations (Delille 1987, Green et al. 1992, Edwards et al. 1998). Coliforms are less able to survive in Antarctic environmental conditions than spore-forming bacteria. Nedwell et al. (1994) stated that coliform bacteria can survive <50 years while spore-forming bacteria can survive >80 years in Antarctica. The fecal Streptococcus strain was more resistant to the effects of radiation than the gram-negative strains (Statham & McMeekin 1994). Fox Cooper (1998) reported that in some areas of Antarctica, regional warming has caused a decrease in permanent snow cover around nunataks and coastal regions with the result that previously buried toilet pits, depots, and food dumps are now melting out. Green et al. (1992) reported a similar amount of coliform bacteria in the outfall of sewage from the Davis research station, which was reported in the sewage outfall of the McMurdo by Edwards et al. (1998).

In our finding, fecal coliform bacteria were absent in all different lakes of Broknes & Grovnes peninsula, Larsemann Hills, East Antarctica. While coliform bacteria were present in two lakes (P2 Lake and P3 Lake) of Broknes peninsula and nine lakes (L1C NG, L1D NG, L1E NG, L7 NG, L7A NG, L7B NG, L2 SG, L4 SG, and L5 SG) of Grovnes peninsula. The present study confirms that lake water contamination is limited to the immediate vicinity of the sewage outfall and will be useful in the future for the assessment of microbiological pollution in Antarctica.

Hughes & Blenkharn (2003) studied the bacterial reproduction in untreated sewage which was released from the Rothera Research Station, Antarctica. Goldsworthy et al. (2003) reported the presence of feces coliform bacteria in intertidal pools adjacent to sewage effluent from the research stations at Laersemann Hills, East Antarctica. Bruni et al. (1997) reported a high concentration of fecal bacteria (Sediment 94 CFU/100 mL; Water 800 CFU/100 mL) in the sewage outfall from the Italian Research Station, Terra Nova Bay. Hughes and Nobbs (2004) studied the viable fecal coliform bacteria in 30-40 years old human feces which were dumped at Fossil Bluff Field Station, Alexander Island, Antarctic Peninsula. The previous methods of human waste disposal on land are now starting to produce environmental pollution as well as potential health and scientific problems.

CONCLUSIONS AND RECOMMENDATION

Coliform and fecal coliform bacteria are mainly reached Antarctica through anthropogenic activities and are useful indicators of sewage waste. However, other factors may lead to coliform contamination in Antarctica such as the migration of microbes through birds and the transport of food items from the ship to the research station. The presence of coliform bacteria in Antarctic lakes may be due to the human population, which is living at research stations. Our study confirms the presence of coliform bacteria in the lakes of East Antarctica, as reported earlier in other Antarctic regions. Untreated sewage waste released from the research stations is the main cause of the presence of coliform in the Antarctic environment. Due to climate change the rate of snow and ice melting increased and has resulted in previously buried fecal material becoming exposed.

Before release sewage discharge should be fully treated; otherwise, it will leave pathogenic contaminants in the pristine environment of Antarctica. We should give priority to maintaining a relationship between actual human presence and the purification capacity of the plant systems, to reduce as much possible anthropogenic inputs into the Antarctic ecosystem. We need regular monitoring at research stations by which we can control the release of microbiological contaminates. Otherwise, these contaminants can cause several diseases in humans and other animal species. Research on the presence of fecal coliform bacteria in Antarctic Lake water could be helpful to provide a better estimate of the human impact.

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