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RESEARCH ARTICLE

BACTERIOLOGICAL ANALYSIS OF WATER FROM YUSMARG HEALTH RESORT OF KASHMIR VALLEY

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ABSTRACT

A study of aquatic bacteria along with some physical parameters like temperature and pH was carried out during the month of November and December 2010, in Yusmarg area of Kashmir valley at four sites differing from each other markedly in terms of biotic and abiotic factors, to assess the density and diversity of bacterial flora. During the study the bacterial flora showed variation in relation to the physical parameters. The colony count was maximum at Site IV Reservoir outlet (300) followed by Site III Reservoir Intlet (135), Site I Dudhganga (95) and site II Tank Area (75). The total bacterial population was maximum at Site IV Reservoir outlet (1.8×10^5 in Nov and 1.2×10^5 in Dec.) and minimum at Site II Tank Area during both the months (4.5×10^4 in Nov and 3.0×10^4 in Dec.). Among the strains of isolated bacteria 28% were Gram positive (GP) and 72% were gram negative (GN). Most dominant of the isolated strains 60% were Cocci followed by 24% bacilli, 12% Diplococci (DC), and 4% Streptococci (SC). It was also found that 12% of strains were Gram Positive Cocci (GPC), 48% were Gram Negative Cocci (GNC), 8% were Gram Negative Bacilli (GNB), 16% were Gram Positive Bacilli (GPB), 12% were Gram Negative Diplococci (GND) and 4% were Gram Negative Streptococci (GNS).

INTRODUCTION

Among all the planets of the solar system, earth is the only planet where life in all its forms thrives freely. One of the major reasons for this is the presence of a liquid that is indispensible for life on Earth, which is water. Hence the story of water is, in many ways, the story of life on earth. Next to air, water is the most important constituent of life supporting system. Water is the basis and origin of life. The importance of water is obvious, it's just about everywhere, and it's in everything. Water is essential to sustain life, and without it life becomes impossible (WHO, 1997). Water is an indispensable commodity, which should be easily accessible, adequate in quantity, free of contamination, safe, affordable and available throughout the year in order to sustain life (Al Khatib *et al*., 2003). Fresh water is a vital resource for agriculture, manufacturing, transportation and countless other human activities. Water also plays a key role in sculpting the earth's surface, moderating climate and diluting pollutants. In fact, without water life could not exist. Water is now more precious than ever before in history. Based on this growing demand for water, people have rightfully stated that water is the oil of the 21st century. Water serves as the second best natural medium for the growth of micro organisms. Micro organisms are ubiquitous. They are found in soil, air, water, food, sewage, and on body surfaces. In short, every area of our environment is replete with them. In the natural environments micro organisms have very specific jobs especially the recycling of material and purification of water. The study of microbes and microbial communities in water environments is broadly known as aquatic micro biology. Microbial communities of aquatic environments include viruses, bacteria, fungi, algae and other microbes. The aquatic systems are mostly dominated by bacteria and fungi. Increase in the human population has resulted in the decrease of both the quality and the quantity of the fresh water. This causes alteration in the physical, chemical and biological characteristics of water which in turn results in the alteration of biology of water. Pathogens are ubiquitous on earth (Agbonlahor, 1998; Cheesbrough, 2000). In the aquatic ecosystem, they are found in all the environmental compartments: water, sediment, aquatic plants and animals.

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MATERIALS AND METHODS

Location and Site Description

Yusmarg is a set of meadows surrounded by Pine trees and mountains. It is about 47Km away from Srinagar and lies in the district Budgam of Jammu and Kashmir. There were three main water sources: Dudhganga river (Site I), Tank Area (Site II), Reservoir, which was divided into two sites: Reservoir Inlet (Site III) and Reservoir Outlet (Site IV). Dudhganga lies between the geographic coordinates of 33°50' 34.30" N and 74°39' 46.63" E at an elevation of about 2275m a.s.l. This is a mighty river which makes a little white foam so it is called Dudhganga. The geographic coordinates of Tank Area are 33°49' 53.26"N and 74°40' 25.46" E with an altitude of 2407m a.s.l. The tank receives water from Kalnag and Fransnag (two tributaries of Dudhganga) through pipes. The tank remains twelve months operative and is a gravity based system. The Reservoir is fenced. The geographic coordinates of Reservoir Inlet are 33°49'30.56"N and 74°40'11.01"E with an elevation of 2364m a. s. l. The geographic coordinates of reservoir outlet are 33°49' 25.72" N and 74°40' 25.46" E with an elevation of about 2362 m a. s. l. The bottom of reservoir was lined by silt.

Collection of samples (Sampling)

Samples of water from all the sites under consideration were collected in 1 litre plastic bottles, which were previously cleaned and rinsed with ethanol and then three to four times with distilled water and then three to four times with the site water (A.P.H.A 1998). The samples of water were collected at the depth of 1-10 cm from the water

surface. During collection of samples, extreme care was exercised to avoid contamination of the parts of bottle. The collected samples were then processed for microbial analysis.

Laboratory Analysis

Microbiological analysis of water samples was done by plate count test using the methodology of APHA (1998). The most important method used for the measurement of microbial community is plate count technique which measures the number of viable cells. In this technique it is important that only a limited number of colonies, 30-300 should develop on a plate (APHA, 1998). Otherwise it may lead to in accuracies in the count.

Below 30 TFTC: Too few to be counted,
Above 300 TNTC: Too numerous to be counted.

Nutrient agar was used for enumeration and cultivation of bacteria and McConkey's broth was used for the detection of coliform. About 15 to 20 ml of medium was poured in each Petri plate on a laminar flow cabinet. The medium after cooling got solidified. The Petri plates were incubated over night to check the contamination if any, inside the media. McConkey's Broth was put in test tubes of big size, then tightly plugged with non absorbent cotton (Fig 1 a) and then sterilized in an autoclave for about 20 to 30 minutes. Before inoculation the samples were diluted to different levels, in order to get the approximate number and density of the bacteria easily. Spread plate technique was followed for inoculation of water samples. This technique involves distribution of water samples (0.1 ml) over surface of prepared agar plate. This technique allows the microbial colonies to grow over the surface of the medium and eventually counting becomes easier. After inoculation the culture plates were incubated in an incubator in inverted position at a temperature of 28ºC for 48 hours to assess the growth of colonies. The test tubes containing McConkey's Broth were incubated at 37ºC for 48 hours. Tubes with both colour change and gas production were taken as positive (Fig 1 c). Pure cultures were obtained by platinum loop through streaking technique (APHA, 1998). Micro-organisms were transferred from one medium to another for the preparation of pure cultures. Colonies that developed on agar plates were counted with unaided eyes as per key given by Johnson and Case, 1995. The counts were expressed as CFU/ml (Colony Forming Unit) of water sample. The number of colonies counted were expressed as CFU/ml and were calculated by using the following formula.

$$CFU/ml = n \times f$$

Where, n = number of colonies
f = dilution factor = 1/dilution (10^{-1}, 10^{-2}, 10^{-3}….)

Coliform

MPN/100ml was determined for the water samples from all the sites during both the months. The results obtained are given in Table 6. Test tubes with both color change and gas production were taken as positive. MPN in 100ml of water sample was calculated by comparison with Mc Cardy's Table (APHA-1998). MPN: Most Probable Number.

RESULTS AND DISCUSSION

A total of 25 types of colonies were obtained and were assigned the names from B1 to B25 as given in the Table 1

Table 1. Colony morphology and microscopic examination of isolates from four sites

S. No.	Appearance	Margin	Elevation	Colour	Grams reaction	Cell shape	Assigned name
1	Circular	Entire	Flat	White	+ve	C	B_1
2	Circular	Entire	Flat	Yellow	-ve	C	B_2
3	Circular	Entire	Flat	Orange	-ve	B	B_3
4	Circular	Entire	Flat	L. Orange	+ve	C	B_4
5	Circular	Entire	Flat	Cream	-ve	SC	B_5
6	Circular	Entire	Flat	BluishWatery	-ve	C	B_6
7	Circular	Entire	Flat	L. Yellow	-ve	C	B_7
8	Circular	Entire	Convex	Orange	-ve	C	B_8
9	Circular	Entire	Convex	Yellow	-ve	C	B_9
10	Circular	Entire	Convex	Pink	+ve	B	B_{10}
11	Circular	Entire	Convex	White	-ve	DC	B_{11}
12	Irregular	Undulate	Flat	Yellow	-ve	C	B_{12}
13	Irregular	Undulate	Flat	White	+ve	B	B_{13}
14	Irregular	Undulate	Flat	Orange	-ve	C	B_{14}
15	Irregular	Undulate	Flat	Cream	-ve	B	B_{15}
16	Irregular	Undulate	Flat	L. Yellow	-ve	C	B_{16}
17	Circular	Entire	Raised	L. Yellow	-ve	C	B_{17}
18	Circular	Entire	Raised	L. Orange	-ve	C	B_{18}
19	Circular	Entire	Raised	Orange	-ve	DC	B_{19}
20	Circular	Entire	Raised	Cream	-ve	C	B_{20}
21	Circular	Undulate	Flat	White	+ve	B	B_{21}
22	Circular	Undulate	Convex	Yellow	-ve	C	B_{22}
23	Rhizoid	Filamentous	Flat	White	-ve	DC	B_{23}
24	Irregular	Filamentous	Flat	Green	+ve	B	B_{24}
25	Irregular	Lobate	Flat	White	+ve	C	B_{25}

Table 2. Colony Count, number of isolates and CFU/ml at all the sites during both the months

Site	November			December			Grand Total
	Number of isolates	Colony count	CFU/ml	Number of isolates	Colony count	CFU/ml	
Site I	9	60	6.0×10^4	6	35	3.5×10^4	95
Site II	7	45	4.5×10^4	5	30	3.0×10^4	75
Site III	7	82	8.2×10^4	6	53	5.3×10^4	135
Site IV	9	180	1.8×10^5	6	120	1.2×10^5	300

The different isolates were tested for Gram's reaction and subsequently were examined under microscope to determine the cell shape. As presented in Table 2 the total monthly bacterial density decreased from November to December at all the Sites. This decrease in the bacterial count may be attributed to the difference in various abiotic and biotic factors that have been found to influence the density and diversity of bacterial communities in water. The variation in temperature and pH of water may also be attributed to the decrease in the bacterial population. There was a temperature difference of about 6.6°C between November and December (Table 3).

Table 3. Temp. and *p*H at all sites during the two months

	Temperature (°C)		pH	
	November	December	November	December
Site I	8.5	1.5	7.14	6.86
Site II	7.3	1.0	7.12	6.80
Site III	7.9	1.1	7.0	7.10
Site IV	9	2.5	7.1	6.83
Average	8.15	1.52	7.09	6.90

So the difference in the Bacterial count may be attributed to temperature. The present study is confined by the results of Murphy (2000) who reported that bacteria grow faster at higher temperature and the growth rate slows down dramatically at low temperature. The effect of pH may also be attributed to the changes in the bacterial count. The present study is also confirmed by the results of Alvarez (1981) who observed a decline in bacterial count in winter with decrease in pH and temperature in the North Florida. Among the different isolates, a total of 9 strains of bacteria were isolated from Site I, 7 from Site II, 7 from Site III and 9 from Site IV during the month of November 2010 (Table 2). During the month of December, 6 strains of bacteria were isolated from Site I, 5 from Site II, 6 from Site III and 6 from Site IV. The colony count was maximum at Site IV (300) followed by Site III (135), Site I (95) and Site II (75). The total bacterial population was maximum at Site IV (1.8×10^5 cfu/ml in Nov and 1.2×10^5 cfu/ml in Dec.) and minimum in tap water during both the months (4.5×10^4 cfu/ml in Nov and 3.0×10^4 cfu/ml in Dec.) as shown in Table 2. The results given in the Table 4 show that 28% isolates were Gram Positive (GP) and 72% were Gram Negative (GN).

Table 4. Percentage of gram+ve and gram –ve isolates

S. No.	Isolate type	Gram's reaction	Percentage	Cell shape
1	B_1	+ve		C
2	B_4	+ve	7 (28%)	C
3	B_{10}	+ve		B
4	B_{13}	+ve		B
5	B_{21}	+ve		B
6	B_{24}	+ve		B
7	B_{25}	+ve		C
8	B_2	-ve		C
9	B_3	-ve	18 (72%)	B
10	B_5	-ve		SC
11	B_6	-ve		C
12	B_7	-ve		C
13	B_8	-ve		C
14	B_9	-ve		C
15	B_{11}	-ve		DC
16	B_{12}	-ve		C
17	B_{14}	-ve		C
18	B_{15}	-ve		B
19	B_{16}	-ve		C
20	B_{17}	-ve		C
21	B_{18}	-ve		C
22	B_{19}	-ve		DC
23	B_{20}	-ve		C
24	B_{22}	-ve		C
25	B_{23}	-ve		DC
	Total =25			

C = Cocci, B = Bacilli, DC = Diplococci, SC = Streptococci.

It was also found that about 60% of the strains were Cocci, 24% were Bacilli, 12% were Diplococci (DC) and 4% were Streptococci (SC). Further it was also observed that 12% strains were Gram Positive Cocci (GPC), 48% were Gram Negative Cocci (GNC), 8% were Gram Negative Bacilli (GNB) and 16% were found to be Gram Positive Bacilli as shown in Table 5.

Table 5. Percentage of different bacterial strain

S.No.	Isolate type	Gram's reaction	Percentage		Cell shape
1	B_1	+ve			C
2	B_4	+ve	12%	60 %	C
3	B_{25}	+ve			C
4	B_2	-ve			C
5	B_6	-ve	48 %		C
6	B_7	-ve			C
7	B_8	-ve			C
8	B_9	-ve			C
9	B_{12}	-ve			C
10	B_{14}	-ve			C
11	B_{16}	-ve			C
12	B_{17}	-ve			C
13	B_{18}	-ve			C
14	B_{20}	-ve			C
15	B_{22}	-ve			C
16	B_3	-ve	8%		B
17	B_{15}	-ve		24 %	B
18	B_{10}	+ve			B
19	B_{13}	+ve	16%		B
20	B_{21}	+ve			B
21	B_{24}	+ve			B
23	B_{11}	-ve			DC
24	B_{19}	-ve	12%	12%	DC
25	B_{23}	-ve			DC
25	B_5	-ve	4 %	4%	SC

C = Cocci, B = Bacilli, DC = Diplococci and SC = Streptococci.

The water sources have the pressure of livestock's as the grazing is done on the upper reaches of Yusmarg by live stock animals. Similar results were shown by Okonko *et al*. (2008) who reported that illegal dumping of domestic wastes, livestock management, faecal deposit and waste dumps can affect bacterial concentration in runoff. Point sources for pathogens may include municipal wastewater discharges and heavily polluted tributaries within a river system. Diffuse sources, on the other hand, include urban, agricultural and forestry runoffs with microbial impact from livestock and wild animals in the catchment area. Furthermore, the microbial load to the raw water within the catchments is influenced by natural factors, such as climatological parameters (rain, sunlight and temperature), hydrology and topography (Taylor 2003; Kay *et al*. 2005). The water samples taken from reservoir outlet were muddy as the depth was very low and the distance between the bottom sediment and water surface was very less and thus the reservoir outlet showed maximum types and number of bacteria. Similar study was carried out by Niewolak and Opieka (2000) who reported that microorganisms in water and bottom sediments in the Czarna Hańcza River, Poland were found in smaller quantities in water, and in larger quantities in the bottom sediments. Same results were obtained in this study. MPN index was determined for the water samples and they showed that they were crossing the permissible limit of WHO (1997) standards (0/100ml).Thus the drinking water supply provided by the Tank was not potable. The positive reaction indicated that water samples were being contaminated by organic material of human or animal origin. Tanveer and Kamili (2004) have also found the presence of coliform in the Tap water supplied from Dudhganga (J&K, India). Same results were obtained in this study. Another reason for the increase of MPN index may be attributed to the inadequate sanitation and unhygienic conditions which were prevailing in the catchment of Yusmarg that affect the water sources in the area. Similar results were shown by Edema, *et al*. (2001) and Okonko, *et al*. (2008) who reported that the greatest danger associated with water used for food processing, drinking purposes and for human consumption is contamination by

Table 6. MPN/100ml at different sites during both months
TOTAL COLIFORM BACTERIA MPN/100 ml

	November					December			
	NUMBER OF POSITIVE TUBES			MPN/100		NUMBER OF POSITIVE TUBES			MPN/100 ml
	10 ml	1 ml	0.1 ml	ml		10 ml	1 ml	0.1 ml	
Site I	4	2	0	22	Site I	4	1	0	17
Site II	4	1	0	17	Site II	3	1	0	11
Site III	4	2	1	26	Site III	4	1	1	21
Site IV	5	1	1	50	Site IV	5	0	2	40

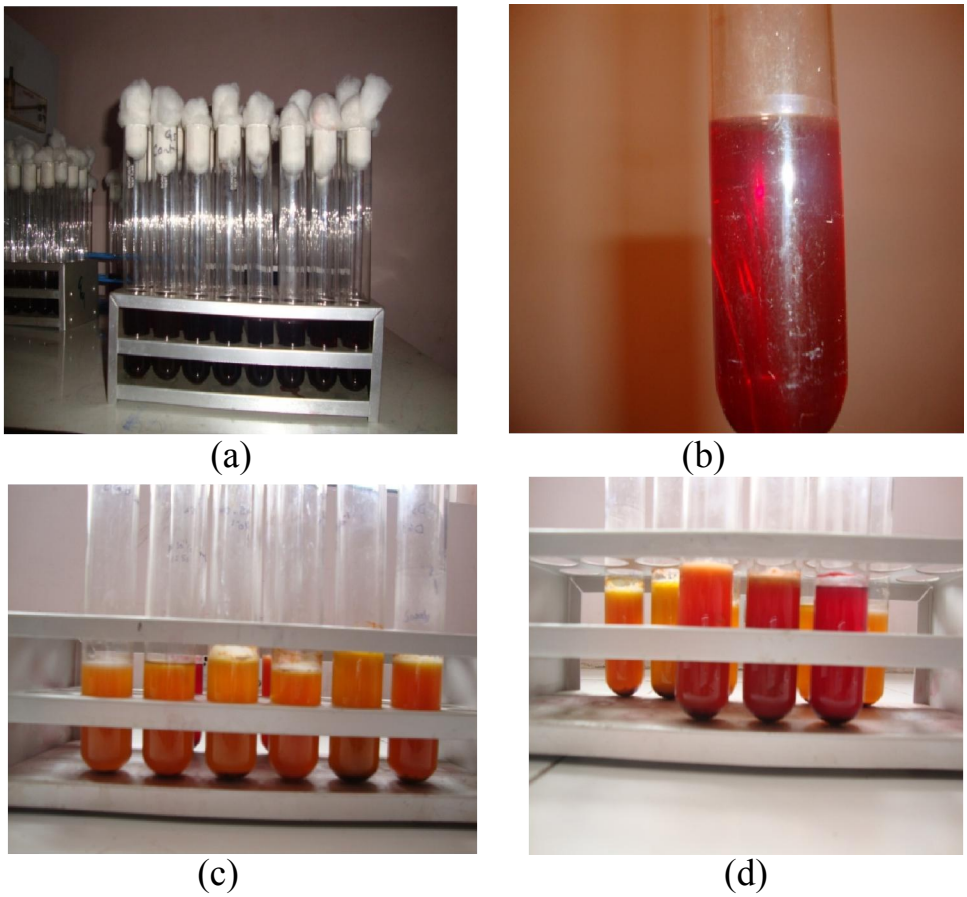

Fig. 1. (a) Test tubes containing McConkeys broth (un inoculated). (b) Test tube containing Durham's tube. (c) Tubes showing the presence of Coliform. (Colour change). (d) Both +ve & -ve tubes

human excrement. The present study is also confirmed by the results of Sahota (2005) who showed that in adequate sanitation and unhygienic practices account for the major source of microbial contamination of any portable water.Comparing MPN value of various sites, it is clear that the Reservoir outlet (Site IV) was most polluted followed by Reservoir inlet(Site III), Dudhganga (Site I), and the Tap water (Site II) was least polluted. The tap water was being tapped through pipes from the upper reaches of Yusmarg. The contamination and the human interference at that point would have been low as compared to the lower areas where grazing and human excrement is common. The canal feeding the reservoir was polluted by human interference and by grazing animals. Thus MPN value for Tap water was minimum and for reservoir outlet it was maximum.

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