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Comparative Analysis of Different Types of Bacterial Colonies from the Soils of Yusmarg Forest, Kashmir valley India

*Gowhar Hamid Dar[1,2], Suhaib A. Bandh[2], Azra N. Kamili[2], Ruqaya Nazir[2], Rouf Ahmad Bhat[1]**

1 - Department of Environmental Science, University of Kashmir, Srinagar-190006 INDIA
2 - Centre of Research for Development, University of Kashmir, Srinagar -190006 INDIA
* Corresponding autor: rufi.bhat@gmail.com

Abstract. The present work was carried out in the soils of Yusmarg forest to study about the bacterial load (density and diversity), to identify and isolate the bacteria from the soils. During the study a total of thirty six isolates were obtained, among thirty-six different isolates obtained at the four sites B_7 and B_8 were present at all the four sites, B_6 and B_9 were present only at site I in November, B_{16} and B_{17} were present only at site II in November, B_{19}, B_{22}, B_{23} and B_{24} were present only at site III in November, B_{32}, B_{33} and B_{34} were present only at site III in December and B_{35} was present only at site IV in December. Comparative analysis of different types of colonies found at the four sites during the study indicates that the bacterial load was dominant in the month of November.

Key words: Bacteria, Yusmarg, Soil, Isolate, Nutrient Agar.

Introduction

On our planet earth, we feel blessed to be surrounded by natural resources which benevolent God has provided us in abundance. Among all natural resources, one that is rendering its valuable support to sustain human race is soil. Soil, one of the greatest gifts of nature is a vital factor for life. It is the best medium for the growth of micro-organisms. Soil microbial population is the key element in the bio-geochemical cycling of nutrients in nature (PELCZAR *et al.*, 1993). The number and kind of bacteria found in different types of ecosystems vary and are influenced by the ecosystem processes maintaining plant primary productivity (GRIFFITHS *et al.*, 2003). Most of the soil bacteria are decomposers that consume simple organic compounds, such as root exudates and fresh plant litter. It has been estimated that there may be as many as 10^9 bacterial cells per gram of soil (KARATHANSIS & HARRIS, 1994) and it is widely accepted that the majority of soil bacteria possibly as many as 99% cannot be cultured using traditional media based techniques. Bacteria are vital in recycling nutrients with many steps in nutrient cycles depending on these organisms such as the fixation of nitrogen from the atmosphere and Putrefaction.

Material and Methods

Study Area. Yusmarg situated at an altitude of about 2743m a.s.l, lying in the Budgam district of Jammu and Kashmir, India, is a small idyllic meadow set in the heart of mountains to the South West of Srinagar. It is situated at a distance of 47 Km from Srinagar city. The destination is witnessed by grassy meadow ringed by forests of pine, towering beyond them awesome and

snow clad mountains. It is an emerging destination which is completely raw, pristine and still unspoiled, bandied by rivers and the backdrop of snow capped mountains. It mesmerizes tourists with its scenic meadows, sparkling reservoir and mountains comparable to European Alps. Situated amidst Sang Safed valley, it is reputed for having some unique spring flowers. The mighty river Doodh Ganga rises from these peaks and a distributory of the same flows into the reservoir. The climate of this beautiful area is very bracing and enjoys a subtropical climate. Here the precipitation normally occurs in the form of snowfall during the winters. Summers are mild and winters are very cold. The maximum temperature ranges around 30°C and the minimum temperature around 18°C during the summer months. Temperatures start coming down only from September. During winter months this area experiences a maximum temperature of 15°C to 8°C and a minimum temperature of around -2°C (Fig.1).

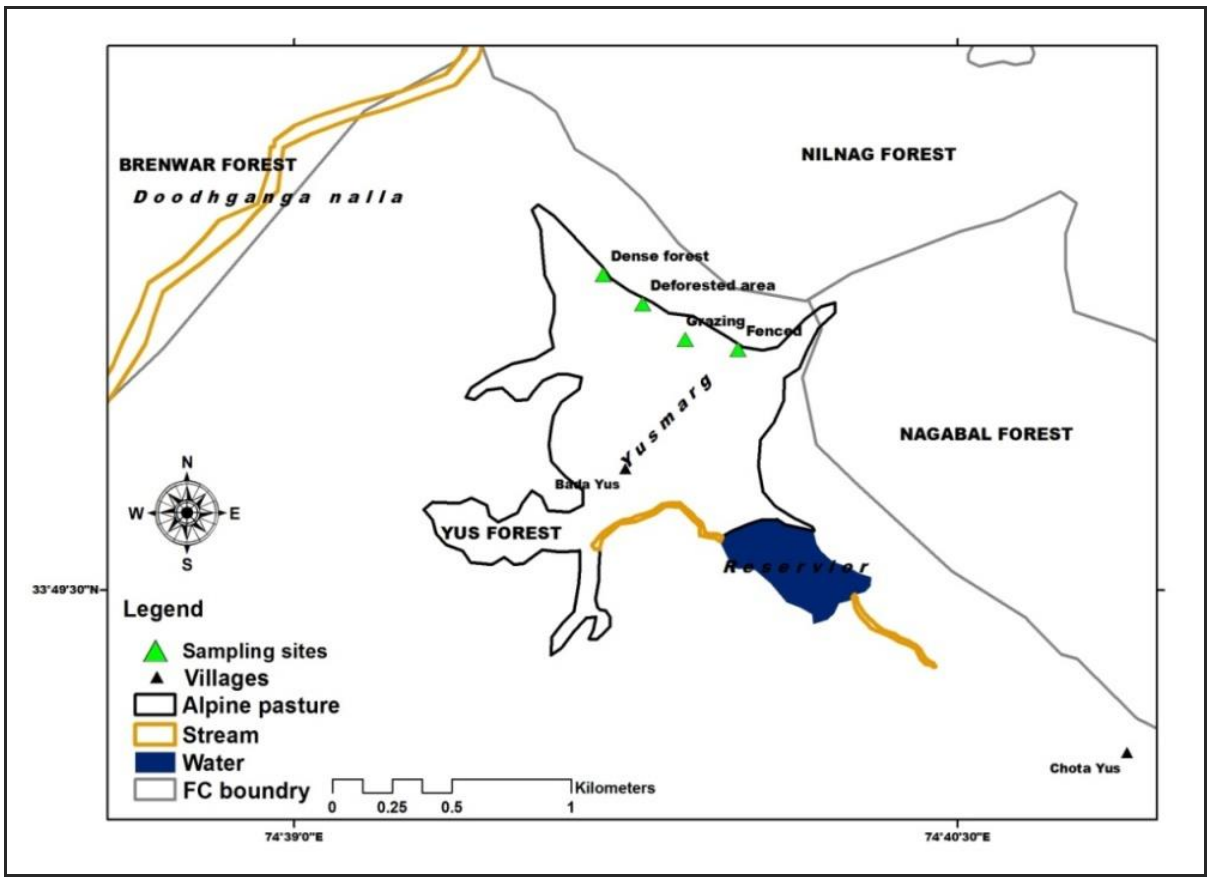

Fig. 1. Map showing the study area and study sites.

Study Sites. Four sites were selected to carry out the work and the brief site descriptions are given below:

Site I (Fenced Area) - The Site renowned for its green pasture least affected by human and animal activities and lies between the geographical co-ordinates of 74° 40′ 1.653″ E and 33° 50′ 0.665″ N, having an elevation of 2418 m. The area was fenced, which prevented it from grazing and anthropogenic impacts.

Site II (Grazing Area) - The site under high grazing pressure and was highly influenced by the human and animal activities, lies between the geographical coordinates of 74° 39′ 57.555″ E and 33°50 ′ 1.768″ N, having an elevation of 2411 m and was surrounded by coniferous forest and road on the other sides.

Site III: (Deforested Area) - The site is close to main forest and was marked by deforestation. It lies between the geographical coordinates of 74° 39′ 57.506″ E and 33° 50′

0.034'' N having an elevation of 2446 m. It was near the grazing area, having vegetation of conifers (*Pinus wallichiana, Abies, pindrow* and *Piscea smithiana*).

Site IV (Forested Area) - This site located in dense forest of conifers. It lies between geographical coordinates of 74° 39′ 56.262'' E and 33° 49′ 55.747'' N, having an elevation of 2451 m. The vegetation was dominated by *Pinus wallichiana*, with other conifers and relatively very small number of shrubs and herbs.

Collection Technique. Composite samples of soil from the four sites were collected during the study period, from a depth of 5 inches. Samples were collected in sterile polythene bags and carried to laboratory for bacteriological analysis. The samples were processed using the soil plate method (WARCUP, 1950) and Soil dilution plate Method (WAKSMAN, 1922).

Laboratory Analysis

Soil plate method - About 1g of soil was scattered on the bottom of a sterile Petri dish and molten cooled (40-45°C) agar medium (NA) was added, which was then rotated gently to disperse the soil particles in the medium. The plates were then incubated at 28±2°C for 24 hours.

Soil dilution plate method - The soil samples were mixed with sterile distilled water and a series of dilutions were made. From the dilutions, 0.1ml inoculum was poured onto Nutrient agar and incubated at 28±2°C for 24 hours. The number of colonies counted was expressed as cfu/g and were calculated by using the formula:

$$\text{cfu/gm} = n \times d$$

Where: n= number of colonies; d = dilution factor = 1/dilution.

Results

The recorded temperature and pH at the four sites during November and December 2010 are presented in Table 1.

Different types of colonies were obtained during the study period. Some colonies were circular in shape and some irregular, some rhizoid and some filamentous. A total of 36 colonies were obtained during the study and were assigned the names from B_1 to B_{36} (Table 2).

Table1. Temperature and pH recorded at four sites during November and December 2010.

Site	Temperature (^{0}C)		pH	
	Nov.	Dec.	Nov.	Dec.
I	12.5	2.5	6.26	6.48
II	11.5	2.3	6.6	5.9
III	10.0	1.2	5.25	4.5
IV	9.0	0.3	4.86	4.7
Average	**10.75**	**1.6**	**5.7**	**5.3**

Table 2. Comparative analysis of different types of colonies found at the four sites in the months of November and December 2010.

Isolate number	Site I		Site II		Site III		Site IV	
	Nov.	Dec.	Nov.	Dec.	Nov.	Dec.	Nov.	Dec.
B_1	+	-	+	-	-	-	-	-
B_2	+	-	-	-	+	-	-	-
B_3	-	-	-	-	-	-	+	-
B_4	+	-	-	-	-	-	-	-
B_5	-	-	-	-	+	-	+	-
B_6	+	-	-	-	-	-	-	-
B_7	-	+	+	+	-	+	+	+
B_8	+	+	-	+	+	+	+	+
B_9	+	-	-	-	-	-	-	-
B_{10}	+	-	+	-	-	-	-	-
B_{11}	+	-	+	-	-	+	-	-
B_{12}	-	-	+	-	-	-	+	-
B_{13}	-	-	+	-	-	-	-	-
B_{14}	-	-	+	-	+	-	-	+
B_{15}	-	-	+	+	-	+	-	-
B_{16}	-	-	+	-	-	-	-	-
B_{17}	-	-	+	-	-	-	-	-
B_{18}	-	-	+	-	+	-	-	-
B_{19}	-	-	-	-	+	-	-	-
B_{20}	-	-	-	-	+	-	-	-
B_{21}	-	+	-	+	+	+	-	+
B_{22}	-	-	-	-	+	-	-	-
B_{23}	-	-	-	-	+	-	-	-
B_{24}	-	-	-	-	+	-	-	-
B_{25}	-	+	-	+	+	-	+	-
B_{26}	-	-	+	-	-	+	+	-
B_{27}	-	+	-	-	-	-	-	+
B_{28}	-	+	-	-	-	+	-	-
B_{29}	-	+	-	-	-	-	-	+
B_{30}	-	+	-	-	-	-	-	+
B_{31}	-	+	-	-	-	+	-	+
B_{32}	-	-	-	-	-	+	-	-
B_{33}	-	-	-	-	-	+	-	-
B_{34}	-	-	-	-	-	+	-	-
B_{35}	-	-	-	-	-	-	-	+
B_{36}	-	-	-	-	-	-	-	+

The different colonies obtained during the study were tested for gram's reaction and subsequently were examined under microscope to determine the cell shape. Among the different isolates, a total of 8 strains of bacteria were isolated from site I, 12 from Site II, 12 from site III and 7 from site IV during the month of November 2010. During the month of December, 9 strains of bacteria were isolated from site I, 5 from site II, 11 from site III and 10 from Site IV.

Discussion

The increase in the bacterial population at site 1 from November to December may be attributed to the increase in pH from 6.26 to 6.48, the grazing rate also decreased from November to December at this site. A study was carried out by (KOHLER *et al.,* 2005) to study the effect of cattle grazing on bacterial communities in pastures and he showed that bacterial community changes due to simulated effects of cattle grazing. The cattle activities may induce changes in the bacterial community structure. During the study the temperature and pH was recorded at the four sites under consideration, the maximum soil temperature (12.5^0C) was at site I in November while as it was minimum (0.3^0C) at site IV in December. The average soil temperature decreased from 10.75^0C in November to 1.6^0C in December. Average pH also decreased from 5.7 to 5.3. This decrease in the count may be attributed to the difference in various biotic and abiotic factors that have been found to influence the density and diversity of soil bacterial communities. The results thus obtained in the present study are confirmed by the findings of PIAO *et al.* (2000) and FIERER & JACKSON (2006). However, the average variation in temperature and pH at the four sites in the months of November and December may also be attributed for the decrease in the bacterial population. The physical properties of soils like temperature and pH recorded at the four sites under consideration during the study period showed a variation of about 10°C in soil temperature from November to December and also a pH change of 0.3 (Table1). From the values of temperature and pH, it is quite clear that the average temperature varied from 10.75°C in November to 1.6°C in December, a variation of about 10°C. Similar results were shown by (MURPHY, 2000) who showed that the bacteria grow faster at higher temperatures; the growth rate slows dramatically at low temperatures. The present findings are also confirmed by (PETTERSSON, 2004) who reported that the soil bacterial community had an optimum temperature for growth and diversity. Another reason for the decrease of bacterial population from November to December may be attributed to the decrease in pH because the average pH varied from 5.7- 5.3.The present study is confirmed by the findings of (LAUBER *et al.,* 2009) who reported that the effect of soil pH on bacterial community composition is evident at even relatively coarse levels of taxonomic resolution. Similar results were also shown by (ROUSK *et al.,* 2010) who reported that the composition of the bacterial communities is closely defined by the soil pH, the apparent direct influence of pH on bacterial community composition is probably due to the narrow pH ranges for optimal growth of bacteria.

Conclusion

From the study it can be concluded that isolate B8 was present at all the four sites during the study period.

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