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ANALYSIS OF BACTERIAL FLORA OF SOIL FROM GULMARG HEALTH RESORT OF KASHMIR VALLEY

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ABSTRACT

A study of soil bacteria along with some physical parameters like temperature and pH was carried out during the months of May, June, July, October and December in Gulmarg 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 highest viable count of bacteria was observed at site III (Deforested area) with a cfu/g of 1.0 x 10^4 in the month of June and the lowest viable count at site IV (forest area) with a cfu/g of 0.1 x 10^4 in the month of December. Among the different strains it was found that about 56% of strains isolated were Gram –ve and about 44% of strains were Gram +ve. Most dominant of the isolated strains 59% were Cocci followed by 35% Bacilli, 3% each Diplococci (DC) and Streptococci (SC). It was also observed that 37.5% of strains were Gram Negative Cocci (GNC), 22% were Gram Positive Cocci (GPC), 12.5% were Gram Positive Bacilli (GPB), 22% were Gram Negative Bacilli (GNB) and 3% each were Gram Negative Diplococcic (GND) and Gram Positive Streptococci (GPS).

Key words: cocci, density, diversity, gram negative, gram positive, soil bacteria

I.INTRODUCTION

Soil is the region on the earth's crust where geology and biology meet, the land surface that provides a home to plant, animal and microbial life. Soil teems with microscopic life (bacteria, fungi, algae, protozoa and viruses) as well as macroscopic life such as earthworms, nematodes, mites, and insects, and also the root systems of plants (Pelczar *et al*., 1993). The numbers and kinds of microorganisms present in soil depends on many environmental factors, for instance amount and type of nutrient availability, available moisture, degree of aeration, pH, temperature, etc (Prescott *et al*., 1999). Soil microorganisms also influence above-ground ecosystems by contributing to plant nutrition, plant health, soil structure and soil fertility (O'Donnell *et al.*, 2001). Soil is generally a favourable habitat for the proliferation of microorganisms, with micro colonies, developing around soil particles. Numbers of micro organisms in soil habitats normally are much higher than those in fresh water or marine habitats (J. C. Cappuccino and Sherman, 1992).

Bacteria are the most numerous component of the soil microbial population. Soil bacteria play pivotal roles in various biochemical cycles and are responsible for the recycling of organic compounds (Wall and Virginia, 1999). Soil bacteria often show morphological and physiological adaptations that allow the bacteria to utilize the soil habitat effectively. The soil bacterial community is under the constant influence of its environment. Changing any of the factors affecting the bacterial community will induce a selection pressure which, with time, will change the community (Hackl *et al.*, 2004). It has been estimated that there may be as many as 10^9 bacterial cells per gram of soil (Horner *et al.*, 2004) and a million bacterial cells in a millilitre of fresh water, in all, there are approximately five billion (5×10^{30}) bacteria on Earth, forming a biomass that exceeds that of all plants and animals. 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. This is due to the complex nature of the environments that soil provides which can't be simulated in the laboratory.

II.MATERIAL AND METHODS

2.1 Study area

Gulmarg-situated at an altitude of about 2690m a.s.l, lying in the Baramulla District of Jammu and Kashmir state, at 34°03′N 74°23′E 34.05°N 74.38°E is a small idyllic meadow set in the heart of mountains to the South West of Srinagar. Four (4) sites were selected for the present study with one in the protected Area, free of human and animal activities lying between the geographical co-ordinates of 74° 21′ 58.6″E and 34° 03′28″N, having an elevation of 2647 m a.s.l, second site was selected in the Grazing Area highly influenced by the human and animal activities lying between the geographical coordinates of 74° 22′ 59″ E and 34° 03′ 28.28″ N**,** having an elevation of 2648 m a.s.l, the third site was deforested area close to main forest and was marked by deforestation lying between the geographical coordinates of 74° 24′ 25.1″ E and 34° 02′ 04.0″ N having an altitude of 2328 m a.s.l and finally the fourth site, a Forested Area, a dense forest of conifers dominated by Pinus sp. Lying between geographical coordinates of 74° 18′ 47.0″ E and 34° 04′ 28.0″ N, having an elevation of 2783m a.s.l.

2.2 Collection of Samples

Composite samples of soil from four sites under consideration were collected in the months of May, June, July, October and December, by digging upto a depth of 5 inches with the help of spade. 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).

2.3 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.

2.4 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^{o}C for 24 hours. The number of colonies counted was expressed as cfu/g and were calculated by using the formula.

$$Cfu/g = n \times d$$

Where n= number of colonies; d = dilution factor = 1/dilution (10-1 , 10-2 etc)

III.RESULTS

A total of 32 different types of colonies, some circular in shape and some irregular, some rhizoid and some filamentous were obtained during the study and were assigned the names from B1 to B32 (Table 1).

Table 1: Colony morphology and Microscopic examination of different isolates

S. No.	Appearance	Margin	Elevation	Color	Grams reaction	Cell shape	Assigned name
1	Irregular	Lobate	Flat	White	-ve	B	B_1
2	Irregular	Entire	Flat	Cream	-ve	C	B_2
3	Circular	Entire	Flat	Cream	+ve	C	B_3
4	Rhizoid	Filamentous	Flat	Cream	-ve	B	B_4
5	Rhizoid	Lobate	Flat	Cream	-ve	C	B_5
6	Irregular	Lobate	Flat	Dark	+ve	C	B_6
7	Irregular	Filamentous	Flat	White	-ve	C	B_7
8	Irregular	Undulate	Flat	Cream	+ve	C	B_8
9	Irregular	Undulate	Flat	White	-ve	C	B_9
10	Circular	Entire	Flat	White	-ve	B	B_{10}
11	Filamentous	Filamentous	Flat	Cream	+ve	C	B_{11}
12	Circular	Undulate	Flat	White	-ve	B	B_{12}
13	Irregular	Lobate	Flat	Cream	+ve	C	B_{13}
14	Irregular	Filamentous	Flat	Cream	+ve	C	B_{14}
15	Circular	Curled	Flat	Cream	-ve	B	B_{15}
16	Circular	Filamentous	Flat	White	+ve	B	B_{16}
17	Circular	Filamentous	Flat	White	-ve	B	B_{17}
18	Circular	Curled	Flat	White	+ve	B	B_{18}
19	Irregular	Entire	Flat	White	+ve	C	B_{19}
20	Circular	Entire	Flat	Yellow	-ve	C	B_{20}
21	Irregular	Filamentous	Flat	White	-ve	C	B_{21}
22	Rhizoid	Filamentous	Flat	Yellow	+ve	B	B_{22}

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23	Filamentous	Filamentous	Flat	White	-ve	C	B_{23}
24	Irregular	Undulate	Flat	Green	-ve	B	B_{24}
25	Irregular	Undulate	Flat	Yellow	+ve	C	B_{25}
26	Irregular	Entire	Flat	Light yellow	-ve	C	B_{26}
27	Rhizoid	Entire	Convex	White	-ve	C	B_{27}
28	Circular	Entire	Raised	Cream white	+ve	C	B_{28}
29	Circular	Curled	Convex	White	+ve	B	B_{29}
30	Circular	Entire	Flat	Orange	+ve	SC	B_{30}
31	Rhizoid	Filamentous	Flat	White	-ve	C	B_{31}
32	Irregular	Undulate	Raised	Cream	-ve	DC	B_{32}

Table 2: Colony count, number of isolates and cfu/g at the four sites

Sites	May			June			July			October			December			Grand total
	no. of isolates	colony count	cfu/g	no.of isolates	colony count	cfu/g	no. of isolates	colony count	cfu/g	no. of isolates	colony count	cfu/g	no.of isolates	colony count	cfu/g	
Site I (protected area)	8	84	0.8×10^4	6	55	0.5×10^4	4	32	0.3×10^4	4	40	0.4×10^4	4	28	0.2×10^4	239
Site II (grazing area)	4	31	0.3×10^4	7	82	0.8×10^4	7	53	0.5×10^4	2	20	0.2×10^4	4	25	0.2×10^4	211
Site III (deforested area)	4	28	0.2×10^4	9	102	1.0×10^4	3	41	0.4×10^4	3	22	0.2×10^4	7	63	0.6×10^4	256
Site IV (forested area)	2	13	0.1×10^4	7	40	0.4×10^4	3	19	0.2×10^4	4	31	0.3×10^4	1	11	0.1×10^4	104

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Table 3: Temperature and pH recorded at four sites

Sites	Temperature(^{0}C)				
	May	June	July	October	December
Site I	10.1	15.2	20	11.5	2.0
Site II	8.9	16.2	18.2	12.5	2.5
Site III	9.3	17.3	19.6	12.7	2.6
Site IV	13.7	15.6	18.4	9.5	1.0
Average	10.5	16.1	19.1	11.5	2.0

Table 4: pH recorded at four sites during May, June, July, October and December 2012.

Sites	Ph				
	May	June	July	October	December
Site I	5.2	5.2	6.7	6.0	5.7
Site II	4.6	5.0	6.8	5.7	6.0
Site III	5.0	5.2	6.4	7.1	6.4
Site IV	5.2	5.0	6.2	6.6	6.2
Average	5.0	5.1	6.5	6.3	6.0

Table 5. Percentage of gram +ve and gram –ve isolates.

S. No.	Isolate type	Gram's reaction	Percentage	Cell shape
1.	B_3	+ve		C
2.	B_6	+ve		C
3.	B_8	+ve		C
4.	B_{11}	+ve		C
5.	B_{13}	+ve		C
6.	B_{14}	+ve		C
7.	B_{16}	+ve		B
8.	B_{18}	+ve		B
9.	B_{19}	+ve		C
10.	B_{22}	+ve		B
11.	B_{25}	+ve		C
12.	B_{28}	+ve		C
13.	B_{29}	+ve		B
14.	B_{30}	+ve	**14(44%)**	SC
15.	B_1	-ve	**(5 6 %**	B

16.	B_2	-ve		C
17.	B_4	-ve		B
18.	B_5	-ve		C
19.	B_7	-ve		C
20.	B_9	-ve		B
21.	B_{10}	-ve		B
22.	B_{12}	-ve		B
23.	B_{15}	-ve		B
24.	B_{17}	-ve		C
25.	B_{20}	-ve		C
26.	B_{21}	-ve		C
27.	B_{23}	-ve		B
28.	B_{24}	-ve		C
29.	B_{26}	-ve		C
30.	B_{27}	-ve		C
31.	B_{31}	-ve		DC
32.	B_{32}	-ve		B
Total			**32 (100%)**	

Table 6. Percentage of different bacterial strains

S. No.	**Isolate type**	**Gram's reaction**	**Percentage**		**Cell shape**
1	B_2	-ve	**12(37.5%)**	**19(59%)**	C
2	B_5	-ve			C
3	B_6	-ve			C
4	B_7	-ve			C
5	B_8	-ve			C
6	B_9	-ve			C
7	B_{20}	-ve			C
8	B_{21}	-ve			C
9	B_{23}	-ve			C
10	B_{26}	-ve			C
11	B_{27}	-ve			C
12	B_{31}	-ve			C
13	B_3	+ve	**7(2** **2%**		C

14	B_{11}	+ve			C
15	B_{13}	+ve			C
16	B_{14}	+ve			C
17	B_{19}	+ve			C
18	B_{25}	+ve			C
19	B_{28}	+ve			C
20	B_1	-ve	**7(22%)**	**11(35%)**	B
21	B_4	-ve			B
22	B_{10}	-ve			B
23	B_{12}	-ve			B
24	B_{15}	-ve			B
25	B_{17}	-ve			B
26	B_{24}	ve			B
27	B_{16}	+ve	**4(12.5%)**		B
28	B_{18}	+ve			B
29	B_{22}	+ve			B
30	B_{29}	+ve			B
31	B_{31}	ve		**3%**	DC
32	B_{32}	+ve		**3%**	SC
Total			**32(100%)**		

IV.DISCUSSION AND CONCLUSION

The different isolates were tested for Gram's reaction and subsequently were examined under microscope to determine the cell shape. During the study period the total bacterial population showed variations from May to December at the four sites. During the study period a total of 32 different types of isolates were obtained. All the isolates obtained were of different morphological characteristics (Table 1).The total colony count was maximum (max.) at site III (256) with max. in the month of June 102 and minimum (min.) 22 in the month of October. The second highest colony count was at site I (239) with maximum in the month of May (84) and min. 28 in the month of December. The third highest colony count was at site II (211) with max. colony count in the month of June (82) and min. 20 in the month of October. The site IV was found with minimum colony count (104) among all the sites with max. colony count in the month of June (40) and min. 11 in the month of December(Table 2). The results given in table 5 show that 56% isolates were gram negative (GN) and 44% were gram positive (GP). Most dominant of the isolated strains 59% were Cocci followed by 35% Bacilli, 3% each Diplococci (DC) and Streptococci (SC). It was also observed (table 6) that 37.5% of strains were Gram Negative Cocci (GNC), 22% were Gram Positive Cocci (GPC), 12.5% were Gram Positive Bacilli (GPB), 22% were Gram Negative Bacilli (GNB) and 3% each were Gram Negative Diplococcic (GND) and Gram Positive Streptococci (GPS). The

overall bacterial isolates were mostly gram negative in nature. The abundance of the gram negative bacteria (Gram Negative Cocci) observed at different sites may be attributed to the increased addition of the excretory substances to the soil by means of the ruminants including sheep, goat, horses, buffalos and cows etc. as the gram negative bacteria have a reservoir in the intestines of man and other warm blooded animals, are excreted in feces and are known to survive in the environment but do not reproduce (Feachem *et al*., 1983). The results of our study are in consonance with a recent Kashmiri study on the bacteriological analysis of soils of Yousmarg health resort which also reports the dominance of gram negative cocci in the Yousmarg soils.

The bacterial count was max. during the summer months and min. was in winter months (Table 2).The maximum bacterial count was at site III (256) as this site was having maximum human interference and minimum was at site IV(104) as there was not any type of human disturbance (Table 2). This variation 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 (Bartlett *et al*., 2007). The variation in bacterial count may also be attributed to the average variation in temperature at the four sites in the months of May, June, July, October and December (Table 3) (Murphy, 2000; Fierer and Jackson, 2006; Dar *et al*., 2011). The study conducted by Pettersson *et al.,* 2004 in Karst Areas of Southwest China, reported that the soil bacterial community had an optimum temperature for growth and diversity.

Rousk *et al.,* 2010 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. Our study also depicts the similar kind of results where we have found that the change of bacterial population from May to December at different sites may be attributed to the fluctuations in pH because the average pH varied from 5- 6.5 at the four sites in the Gulmarg area (Table 4).

From the present study it may be concluded that.

- The bacteria isolated from the study sites are mostly Gram –ve in nature.
- The Gram -ve Cocci strains are found in relatively higher density.

The dominance of Gram –ve bacteria in the soil of study area is of concern because most of the gram –ve bacteria are pathogenic in nature and if they come in contact with the wounds they cause a number of diseases in humans.

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