Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 3, Issue, 09, pp.053-056, September, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BACTERIOLOGICAL ANALYSIS OF SOIL FROM YUSMARG HEALTH RESORT OF KASHMIR VALLEY

[1]Gowhar H. Dar., [2*]Suhaib A. Bandh and [1]Azra N. Kamili

[1]Department of Environmental Science, University of Kashmir, Srinagar-190006, India
[2] Centre of Research for Development, University of Kashmir, Srinagar-190006, India

ARTICLE INFO

Article History:
Received 15th June, 2011
Received in revised form
17th July, 2011
Accepted 18th August, 2011
Published online 17th September, 2011

Key words:
Soil bacteria,
Yusmarg,
Diversity,
Density,
Cocci,
Bacilli,
Diplococci,
Streptococci.

ABSTRACT

A study of soil 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 highest viable count of bacteria was observed at site III (Deforested area) with a cfu/g of 1.8 x 10^4 in the month of November and the lowest viable count at site IV (Dense forest) with a cfu/g of 0.4 x 10^4 in the month of December. Among the isolated strains of bacteria 61% were gram negative (GN) and 39% were gram positive (GP). Most dominant of the isolated strains 58% were Cocci followed by 36% Bacilli, 3% each *Diplococci* (DC) and Streptococci (SC). It was also observed that 33% of strains were Gram Negative Cocci (GNC), 25% were Gram Positive Cocci (GPC), 11% were Gram Positive Bacilli (GPB), 25% were Gram Negative Bacilli (GNB) and 3% each were Gram Negative Diplococcic (GND) and Gram Positive Streptococci (GPS).

INTRODUCTION

Soil, one of the greatest gifts of nature is such a vital factor for life on which the survival and development of living organisms including man depends. It is the best medium not only for the growth of plants but the microorganisms as well. Soil is a natural body consisting of layers (soil horizons) of mineral constituents of variable thicknesses, which differ from the parent materials in their morphological, physical, chemical and mineralogical characteristics. Soil microbial population is the key element in the bio-geochemical cycling of nutrients in nature (Pelczar *et al.,* 1993). The cycling of carbon, oxygen, sulphur and nitrogen that take place in terrestrial and aquatic ecosystems are made possible by the different types of microbes dwelling in these ecosystems. More recently microbial diversity (community structure) has also been recommended as biological indicator of soil quality, microorganisms are thus a source of nutrients at the base of all ecological food chains and webs. 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).

Corresponding author: suhaibbandh@hotmail.com

The growth of bacteria in soil, like other microbes, is influenced by factors like the amount and type of nutrients available, moisture, degree of aeration, temperature and *p*H etc. Furthermore the existence and extensiveness of the root system in soil also influence the numbers and kinds of bacteria. Bacteria are the most numerous component of the soil microbial population. It has been estimated that there may be as many as 10^9 bacterial cells per gram of soil (Harris, 1994). Soil has a complex nutritional availability and is the natural habitat for highly diverse microbial flora. Torsvik *et al.,* (1990) found that above 4,000 differently sized microbial genomes are present per gram of soil representing roughly 13,000 different species.

MATERIAL AND METHODS

Location and Site Description

Yusmarg-situated at an altitude of about 2743m a.s.l, lying in the Budgam District of Jammu and Kashmir state, 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 Fence Area, free of human and animal activities lying between the geographical co-ordinates of 74°40′ 1.653″ E and 33° 50′ 0.665″ N, and an elevation of

Table 1: Colony morphology and Microscopic examination of different isolates

S. No.	Appearance	Margin	Elevation	Color	Grams reaction	Cell shape	Isolated designation
1	Irregular	Undulate	Flat	Cream	+ve	C	B_1
2	Irregular	Lobate	Flat	Cream	+ve	B	B_2
3	Irregular	Lobate	Flat	White	-ve	B	B_3
4	Irregular	Entire	Flat	Cream	-ve	C	B_4
5	Irregular	Undulate	Flat	White	-ve	C	B_5
6	Irregular	Filamentous	Flat	Cream	+ve	C	B_6
7	Circular	Entire	Flat	Cream	+ve	C	B_7
8	Circular	Entire	Flat	White	-ve	B	B_8
9	Rhizoid	Filamentous	Flat	Cream	-ve	B	B_9
10	Filamentous	Filamentous	Flat	Cream	+ve	C	B_{10}
11	Rhizoid	Lobate	Flat	Cream	-ve	C	B_{11}
12	Circular	Curled	Flat	Cream	-ve	B	B_{12}
13	Irregular	Lobate	Flat	Dark	+ve	C	B_{13}
14	Irregular	Filamentous	Flat	White	-ve	C	B_{14}
15	Circular	Undulate	Flat	White	-ve	B	B_{15}
16	Circular	Curled	Flat	White	+ve	B	B_{16}
17	Irregular	Entire	Flat	White	+ve	C	B_{17}
18	Irregular	Filamentous	Flat	White	-ve	C	B_{18}
19	Rhizoid	Filamentous	Flat	Yellow	+ve	B	B_{19}
20	Circular	Filamentous	Flat	White	-ve	C	B_{20}
21	Filamentous	Filamentous	Flat	White	-ve	C	B_{21}
22	Irregular	Undulate	Flat	Yellow	+ve	C	B_{22}
23	Irregular	Undulate	Flat	Green	-ve	B	B_{23}
24	Circular	Filamentous	Flat	White	-ve	B	B_{24}
25	Circular	Entire	Flat	Yellow	-ve	C	B_{25}
26	Circular	Curled	Convex	White	+ve	B	B_{26}
27	Rhizoid	Filamentous	Flat	White	-ve	C	B_{27}
28	Circular	Undulate	Raised	Cream	+ve	C	B_{28}
29	Irregular	Undulate	Raised	Cream	-ve	DC	B_{29}
30	Irregular	Filamentous	Flat	White	-ve	B	B_{30}
31	Rhizoid	Entire	Convex	White	-ve	C	B_{31}
32	Circular	Entire	Flat	Orange	+ve	SC	B_{32}
33	Circular	Entire	Raised	Cream white	+ve	C	B_{33}
34	Circular	Lobate	Flat	Yellow	-ve	B	B_{34}
35	Irregular	Entire	Flat	Light yellow	-ve	C	B_{35}
36	Circular	Entire	Flat	Pink	-ve	C	B_{36}

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

Site	November			December			Grand total
	Number of isolates	Colony count	Cfu/g	Number of isolates	Colony count	Cfu/g	
Site I (Fenced Area)	7	70	0.7×10^4	10	104	1.0×10^4	174
Site II (Grazing Area)	12	101	1.0×10^4	11	97	0.9×10^4	198
Site III (Deforested Area)	12	178	1.8×10^4	11	78	0.8×10^4	256
Site IV (Dense forest)	8	100	1.0×10^4	10	44	0.4×10^4	144

Table 3: Temperature and *p*H recorded at four sites

Site	Temperature (^{0}C)		*p*H	
	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

2418 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°39′ 57.555″ E and 33°50′ 1.768″ N, and an elevation of 2411 m a.s.l, the third site was a Deforested area, close to main forest marked by deforestation lying between the geographical coordinates of 74° 39′ 57.506″ E and 33° 50′ 0.034″ N and an elevation of 2446 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° 39′ 56.262″ E and 33° 49′ 55.747″ N, and an elevation of 2451 m a.s.l.

Collection of Samples

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).

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.

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

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

RESULTS AND DISCUSSION

A total of 36 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 B_1 to B_{36} (Table 1).

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

S. No.	Isolate type	Gram's reaction	Percentage	Cell shape
1.	B_1	+ve	14(39%)	C
2.	B_2	+ve		B
3.	B_6	+ve		C
4.	B_7	+ve		C
5.	B_{10}	+ve		C
6.	B_{13}	+ve		C
7.	B_{16}	+ve		B
8.	B_{17}	+ve		C
9.	B_{19}	+ve		B
10.	B_{22}	+ve		C
11.	B_{26}	+ve		B
12.	B_{28}	+ve		C
13.	B_{32}	+ve		SC
14.	B_{33}	+ve		C
15.	B_3	-ve	22(61%)	B
16.	B_4	-ve		C
17.	B_5	-ve		C
18.	B_8	-ve		B
19.	B_9	-ve		B
20.	B_{11}	-ve		C
21.	B_{12}	-ve		B
22.	B_{14}	-ve		C
23.	B_{15}	-ve		B
24.	B_{18}	-ve		C
25.	B_{20}	-ve		C
26.	B_{21}	-ve		C
27.	B_{23}	-ve		B
28.	B_{24}	-ve		B
29.	B_{25}	-ve		C
30.	B_{27}	-ve		C
31.	B_{29}	-ve		DC
32.	B_{30}	-ve		B
33.	B_{31}	-ve		C
34.	B_{34}	-ve		B
35.	B_{35}	-ve		C
36.	B_{36}	-ve		C
	Total		36 (100%)	

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 the four sites, except site I where the population increased.

Table 5: Percentage of different bacterial strains

S. No.	Isolate type	Gram's reaction	Percentage		Cell shape
1.	B_1	+ve			C
2.	B_6	+ve			C
3.	B_7	+ve			C
4.	B_{10}	+ve			C
5.	B_{13}	+ve			C
6.	B_{17}	+ve	9(25%)		C
7.	B_{22}	+ve			C
8.	B_{28}	+ve			C
9.	B_{33}	+ve			C
10.	B_4	-ve			C
11.	B_5	-ve			C
12.	B_{11}	-ve		21(58%)	C
13.	B_{14}	-ve			C
14.	B_{18}	-ve			C
15.	B_{20}	-ve			C
16.	B_{21}	-ve	12(33%)		C
17.	B_{25}	-ve			C
18.	B_{27}	-ve			C
19.	B_{31}	-ve			C
20.	B_{35}	-ve			C
21.	B_{36}	-ve			C
22.	B_2	+ve			B
23.	B_{16}	+ve			B
24.	B_{19}	+ve	4(11%)		B
25.	B_{26}	+ve			B
26.	B_3	-ve			B
27.	B_8	-ve			B
28.	B_9	-ve		13(36%)	B
29.	B_{12}	-ve			B
30.	B_{15}	-ve	9(25%)		B
31.	B_{23}	-ve			B
32.	B_{24}	-ve			B
33.	B_{30}	-ve			B
34.	B_{34}	-ve			B
35.	B_{29}	-ve	1(3%)	1(3 %)	DC
36.	B_{32}	+ve	1(3%)	1(3 %)	SC
	Total		36 (100%)		

This decrease in the bacterial 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 variation in temperature and *p*H (table 3) of soil may also be attributed to the decrease in the bacterial population. Similar results were found by Murphy, 2000 who showed that the bacteria grow faster at higher temperatures and the growth rate slows dramatically at lower 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. The results are further conformed by the findings of Rousk *et al.,* 2010 who reported that the composition of the bacterial communities is closely defined by the soil *p*H, the apparent direct influence of *p*H on bacterial community composition is probably due to the narrow *p*H ranges for optimal growth of bacteria. The increase in the bacterial density at site I from November to December may be attributed to the increase in *p*H from 6.26 to 6.48, since the grazing rate was negligible at this site. The results are in consonance with a study carried out by Kohler *et al.,* 2005 to study the effect of cattle grazing on bacterial communities in pastures, showing that bacterial community changes due to simulated effects of cattle grazing. Among the different isolates, a total of 7 strains of bacteria were isolated from site I, 12 from Site II, 12 from site III and 8 from site IV in November. In December, 10 strains of bacteria were isolated from site I, 11 from site II, 11 from site III and 10 from Site IV.

The total colony count as presented in table 3, was maximum at site III (256) followed by site II (198), site I (174) and site IV (144). The total bacterial population was maximum at site III with a cfu/g of 1.8 x 10^4 and minimum at site I with a cfu/g of 0.7 x 10^4 in the month of November and in December the maximum bacterial population was found at site 1 with 1.0 x 10^4 cfu/g and minimum at site IV with 0.4 x 10^4 cfu/g. The results given in table 4 show that 61% isolates were gram negative (GN) and 39% were gram positive (GP). Most dominant of the isolated strains 58% were Cocci followed by 36% Bacilli, 3% each Diplococci (DC) and Streptococci (SC). It was also observed (table 5) that 33% of strains were Gram Negative Cocci (GNC), 25% were Gram Positive Cocci (GPC), 11% were Gram Positive Bacilli (GPB), 25% were Gram Negative Bacilli (GNB) and 3% each were Gram Negative Diplococcic (GND) and Gram Positive Streptococci (GPS). The abundance of the gram negative bacteria 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). However, in tropical environments there are evidences that the enteric bacteria can survive as well as multiply (Rivera *et al*., 1988).

REFERENCES

Cappuccino, J.C. and Sherman, N. 1992, *Microbiology:* A Laboratory Manual. Addison Wesley Pub. Co.

Feachem, R.G., Bradley, B.J. and Garelick, H. 1983, Sanitation and Disease, Health Aspects of Excreta and Wastewater Management, Chichester. John Willey and Sons.

Griffiths, R.L.ss Whitely, A.S., O'Donnell, A.G. and Bailey, M.J. 2003, Influence of Depth and Sampling Time on Bacterial Community Structure in an Upland Grassland Soil. *FEMS Microbiol. Ecol.,* 43:35-43.

Harris, W.G. and Karathanasis, A.A. 1994, Quantitative thermal analysis of soil minerals, 360-411.

Jhonson, T.R. and Case, C.L. 1995, A Laboratory Experiments in Microbiology, 4th edn. The Benjamin/Cumming Publishing Company, Inc.

Kohler, F., Hamelin, J., Gillet, F., Gobat J.M. and Buttler, A. 2005, Soil microbial community changes in wooded mountain pastures due to simulated effects of cattle grazing. *Plant and Soil*., 278(1-2): 327-340.

Murphy, R.Y. 2000, Pathogenic lethality validation in ready-to-eat meat products. *Presented at advanced foods company*. Enid, OK.

Pelczar, J.R., Michael J., Chan, E.C.S. and Kreig, N.R. 1993, *Microbiology*. Tata McGraw-Hill Publishing Company Limited. New Delhi.

Pelczar, M.J., Chan, E.C.S. and Krieg, N.R., 1993, Microbiology Concepts and Applications, In: *Inheritance and Variability Chapter 13*, McGraw-Hill, 370.

Pettersson, M. 2004, Factors affecting rates of change in soil bacterial communities. *Doctoral Thesis.*

Rivera, S.C., Hazen, T.C. and Toranzos, G. A. 1988, Isolation of Fecal coliform from Pristine Sites in a tropical rain forest. *Applied Environmental Microbiology*. 54: 513-517.

Rousk, J., Baath, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R. and Fierer, N. 2010, Soil Bacterial and Fungal Communities Across a pH Gradient in an Arable Soil. *The ISME Journal*, 1–12

Taylor, R.H., Allen, M. J. and Geldreich, E.E. 1983, Standard Plate Count: A Comparison of Pour Plate and Spread Plate Methods. *Jour. American Water Works Association.,* 75: 35-37.

Torsvik, V., Goksoyr, J. and Doae, F.L. 1990, High diversity in DNA of soil bacteria. *Appl. Environ. Microbial.* 56: 782-787.

Waksman, S.A. 1922, A method of counting the number of fungi in the soil. *J. Bact*., 7: 339-341.

Warcup, J.H. 1950, The soil plate method for isolation of fungi from soil. *Nature*, 166: 117-118.

****