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Mapping of Microbial Diversity of Gautala Reserve Forest in Aurangabad (District) (M.S.), India

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Abstract Microorganisms help in the fertility and endurance of soil. Additionally supporting the growth of several biological systems, soil and soil microorganisms fill in as the best mode for plant growth. Soil microbes are essential for recycling old plant material and decaying organic matter. In the upkeep of the Environment microbial diversity plays a dominant role. The current paper is focused on the seasonal variations in the microbial count, which are increased or decreased (Impact on counts) in the Gautala reserve forest. In the present study, soil samples from Gautala Reserve Forest were collected from 15 different sites during the rainy, winter, and summer seasons. The present study was attempted to cover the microbial diversity of the whole Gautala forest through covering maximum sampling spots. The average total microbial count (TMC) in the rainy season for plate 1 and plate 2 was 36.73 and 35.46 respectively and the final count was 36.4×10^6. The average TMC in the winter season for plate 1 and plate 2 was 32.4 and 31.93 respectively and the final count was 32.46×10^5. The average TMC in the summer season for plate 1 and plate 2 was 37.13 and 36.4 and the final count was 37.6×10^4. The bacterial colony at sampling points 1, 4, and 7 exhibits presence of Gram-Negative Bacteria; whereas the rest of the sampling spots showed presence of Gram - Positive Bacteria. There were three types of Gram-negative and 12 were found to be Gram-positive bacteria with three genera i.e. Pseudomonas spp., Bacillus spp., and micrococcus spp. This paper discusses seasonal fluctuations in microbial counts and associated laboratory culture techniques and statistical analysis methods.

Keywords: *microbial diversity, soil microbes, Gautala, reserve, forest*

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1. Introduction

Soil is one of the most significant biological factors, developed naturally by the rock weathering process [1]. Soil is an essential component of the terrestrial ecosystem, as it supports immense biodiversity in terms of species diversity and functionality [2,3]. It's a vivacious habitat for the enormous diversity of life forms. It covers various animals from invertebrates such as worms and insects to mammals like rabbits, badgers, and rodents. It is also a habitation of microorganisms [4]. Microbial populaces in soil are determined by various factors such as soil depth, organic matter, bulk density, soil pH, etc. The microbial populace in soil counts for a large mass of organic matter on this planet. The soil microorganisms consist of bacteria, archaebacteria, yeast, fungi, algae, and protozoa [5]. Microorganisms can remain in the surroundings with humans, hot springs, internal rocks and very cold temperatures, and severe conditions that include very cold temperatures [6]. Soil microorganisms are the most diverse organisms that play an important role in biological cycles. However, environmental conditions that affect the number and composition of soil microbial communities prevent the role of soil biodiversity in ecosystem services [7].

The present study was attempted to study the gram negative and gram positive bacteria from the study area with the prime intention to observe the present status of biota with respect to existence of biodiversity. Mapping of microbial diversity of soil has numerous effects on the number and composition of soil communities. Present study was also aimed to study the colony characteristics and microscopic observation of isolates where priority was given to colony counts from the study area. Soil generally contains 109 to 1010 microorganisms per gram (dry weight), which can represent more than one million species of bacteria [8]. The characterization of microorganisms allows a glimpse of their potential physiological capacities and their impact on soil ecosystems [9]. Tree cover of forest can be increased by allowing space for the overall vegetation cover, planting, and natural regeneration [10]. The present study is focused on the seasonal variations in microbial counts and the identification of the microbial populace of Gautala Autramghat Wildlife Sanctuary.

2. Materials and Methods

2.1. Study Area

Gautala Reserve Forest, also known as 'Gautala Autramghat wildlife sanctuary', is situated in the west-north direction of the Aurangabad District, Marathwada region. It is expanded in about 261 sq. km, at longitude E 740, 55', latitude N 190, 54', and at altitude 1904 ft. [11,12,13]. Gautala is a Tropical Dry Deciduous Forest receiving an average annual rainfall of 600 mm. The average maximum temperature is about 42°C and the average minimum temperature is about 8°C.

2.2. Sample Collection

The Soil samples were collected from 15 different sites maintaining an average km distance among each other (Figure 1 and Figure 2). A sterile spatula was used for soil sample collection and the samples were stored in a sterile zip-lock pouch with proper labeling. All the samples were then without delay transferred to the laboratory.

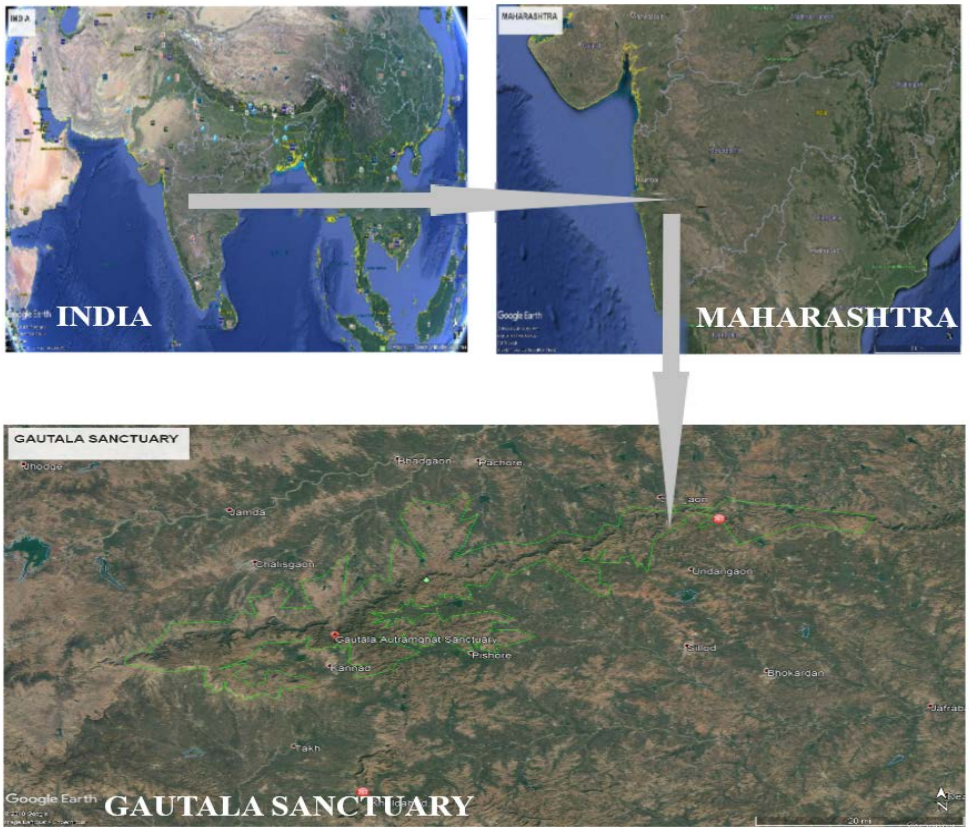

Figure 1. Study area

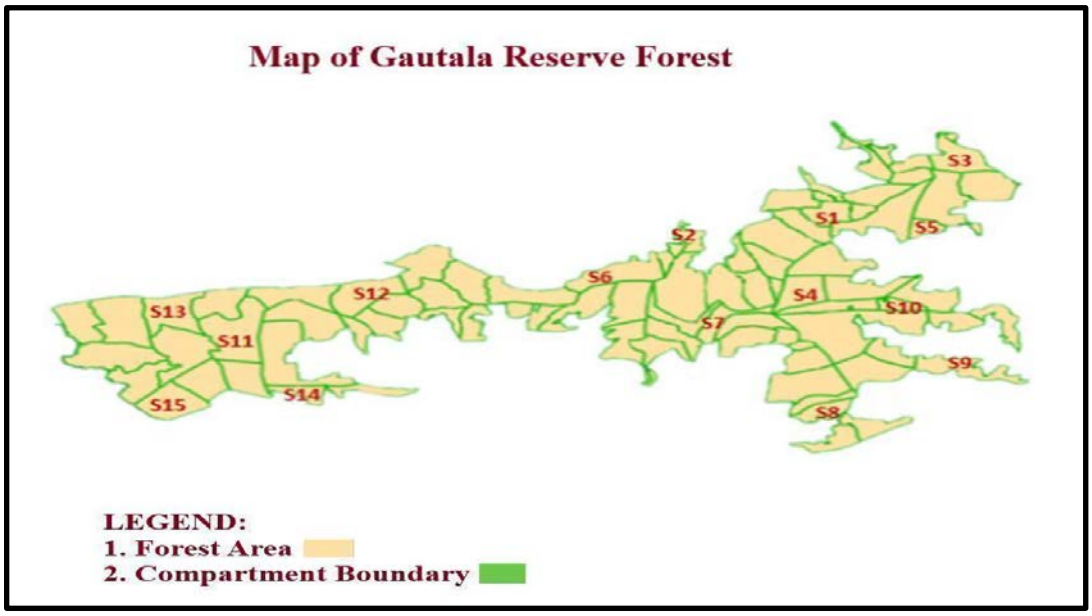

Figure 2. Study area with sampling points

2.3. Isolation of Microorganisms

2.3.1. Colony-forming Units (CFU)

Microbial growth was determined in terms of Colony-forming Units (CFU) in each soil sample, using the pour plate method. 10^{-4}, 10^{-5}, and 10^{-6} series of soil sample suspensions in distilled water were prepared [14,15]. 1ml of these stock solutions was transferred to the labeled sterile Petri dishes. A molten agar medium (40-45°C) was then transferred to the plate containing soil suspension and blended well by slightly rotating the plate. After the cooling and hardening of the agar, the plates were incubated at room temperature for 3-5 days. Pour plates for all the samples were made in triplicates [16,17,18].

CFU visible with the naked eye were counted as per the following formula:

$$CFU / mL = CFU\ X\ dilution\ factor\ X\ 1/aliquot \text{ [19]} (1)$$

2.3.2. Standard Plate Count (SPC)

Microbial growth was determined in terms of SPC in each soil sample, using the pour plate method. 10^{-4}, 10^{-5}, and 10^{-6} series of soil sample suspensions in distilled water were prepared [14,15]. 1ml of these stock solutions was transferred to the labeled sterile Petri dishes. A molten agar medium (40-45°C) was then transferred to the plate containing soil suspension and blended well by slightly rotating the plate. Later the agar has cooled and hardened, the plates were incubated overnight in an incubator at 37°C. Pour plates for all samples are made in triplicate [16,17,18].

2.3.3. Gram Staining Method

Bacterial growth was determined using Gram staining method. Smear of cells was taken on an inoculated glass slide with the help of an inoculating loop. Then it was air dried and heat fixed with the help of flame. Crystal Violet stain was applied on the slide for 1 min then washed off by distilled water. The same procedure was followed by Gram iodine staining. After this, slide was rinsed with decolorizer - 95% ethanol followed by distilled water wash. Finally, safranin stain was applied on the slide for 1 min and washed off with distilled water. After drying the slide was ready to observe under a microscope [20]. LABOPHOT-2 (Nikon, Japan) was used for bacterial identification.

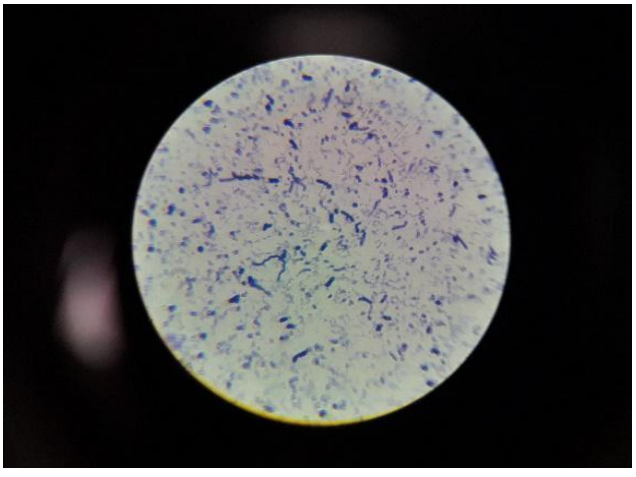

Figure 3. Selected Gram-Negative Bacteria

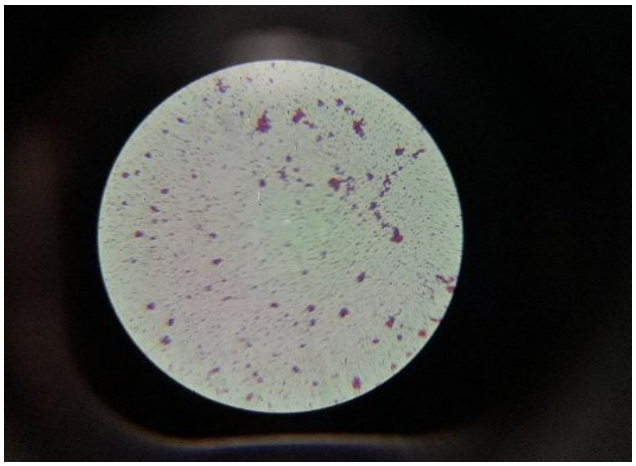

Figure 4. Selected Gram-Positive Bacteria

2.4. Statistical Analysis

Pearson Correlation test, two-way ANOVA test, and Tukey's test were utilized to analyze the results of Microbial counts seasonally. The correlation test was conducted between three seasons (Rainy, winter, and summer). R Studio software was used for qualitative and statistical data analysis.

3. Result and Discussion

The study attempted to cover the microbial diversity of the whole Gautala forest through covering maximum sampling spots. The average TMC in the rainy season for plate 1 and plate 2 was 36.73 and 35.46 respectively, and the final count was 36.4 X 10^6. The average TMC in the winter season for plate 1 and plate 2 was 32.4 and 31.93 respectively, and the final count was 32.46 X 10^5. The average TMC in the summer season for plate 1 and plate 2 was 37.13 and 36.4 respectively, and the final count was 37.6 X 10^4 (Table 1). (Figure 5 - Figure 8)

The bacterial colony at sampling points 1, 4, and 7 showed presence of Gram-Negative Bacteria; whereas the rest of the sampling points showed presence of Gram - Positive Bacteria. Further details about the bacterial colony characters, microscopic observation of isolates, and shape are depicted in Table 2. There were three types of Gram-negative and 12 were found to be Gram-positive bacteria with three genera i.e. Pseudomonas spp., Bacillus spp., and micrococcus spp. (Table 2).

Microbial growth rates were thoroughly interconnected to temperature and carbon content. The high growth rates mostly occur at 20°C. In general, under deciduous forest higher carbon value occurs during summer months temperature always more than above 20°C. This may be responsible for the highest count in the summer season. It depends strongly on temperature [21], therefore, in particular spots i.e. S1, S3, S4, S6, and S8 highest specific growth rate in summer is observed as compared to rainy and winter seasons (Table 1). The work which is carried out by Kavitha *et al.*, [22] population of microbes was found to be affected in contaminated soil which could be observed by increase in the bacterial count in the forest ecosystem especially in Kallar region (78.5 x 10^8 CFU g-1)

and Ooty (74.75 x 10^8 CFU g-1) soil on compared to the Agro-ecosystem eastern block (51.0 x 10^8 CFU g-1) and contaminated soil (37.0 x 10^8 CFU g-1) in nearby town area. Significantly higher bacterial count was noticed in soil near forested areas.

The most important nutrient supply to the forest is derived from litter decomposition by the action of organisms under conditions of high air temperature and soil moisture. The chemical factors present inside the litter are mobilized and reabsorbed through plant roots, restarting a new plant nutrient cycling and ensuring perennial situations to the system even in instances of low fertile soils. The greater accumulation of litter in the rainy season can be attributed to the greater precipitation, which leads to an increase in plant biomass. The existence of a resilient culturable bacterial population that is not affected by season [23]. Decreased microbial diversity in winter seasons agrees to the propositions that microbial populaces were lower in winter and maximum in the summer [24]. Soil bacterial communities within the soil large macro and micro aggregates are shaped in part by various resources and Physico-chemical ambient conditions [25,26,27,28].

The Finding of present research work similar to the work carried out by Martinez [24]. Seasonal variation in diversity and richness of microbes in a dry deciduous forest may be due to seasonal fluctuations in ecological factors [29]. Consideration of soil combination microbial communities additionally led us to detect potential soil microbial diversity than the entire soil sampling approaches. Previous work shows that approaches of easily available substrates in an ecosystem supports biodiversity across various levels of biological organization [30,31], including the potential within soil aggregates [32]. As a beneficial microbe, Bacillus, a part of the Firmicutes family, can enhance plant growth and reduce soil-borne disorders [33]. Bacillus, for instance, prevents Ralstonia solanacearum-induced bacterial loss [34,35,36,37]. Furthermore, Bacillus-inoculated fertilizer was shown to enhance bacterial diversity in the soil [38,39].

Table 1. Total Microbial Counts (2017-18)

Sample No.	Rainy season (Dilution 10^{-6})			Winter season (Dilution 10^{-4})			Summer season (Dilution 10^{-6})		
	Plate 1	Plate 2	Final Count CFU/g Soil	Plate 1	Plate 2	Final Count CFU/g Soil	Plate 1	Plate 2	Final Count (CFU/g Soil)
S1	25	18	22 X 10^6	7	5	6 X 10^5	88	74	81 X 10^4
S2	14	21	18 X 10^6	55	62	59 X 10^5	15	24	20 X 10^4
S3	27	16	22 X 10^6	14	12	13 X 10^5	57	49	53 X 10^4
S4	55	49	52 X 10^6	17	18	18 X 10^5	41	37	39 X 10^4
S5	31	37	34 X 10^6	15	14	15 X 10^5	7	11	9 X 10^4
S6	25	31	28 X 10^6	9	8	9 X 10^5	37	29	33 X 10^4
S7	47	52	50 X 10^6	68	73	71 X 10^5	55	49	52 X 10^4
S8	48	67	58 X 10^6	24	19	22 X 10^5	37	26	32 X 10^4
S9	51	49	50 X 10^6	58	64	61 X 10^5	45	68	57 X 10^4
S10	27	29	28 X 10^6	43	37	40 X 10^5	33	27	40 X 10^4
S11	48	39	44 X 10^6	49	54	52 X 10^5	23	18	21 X 10^4
S12	10	8	9 X 10^6	36	39	38 X 10^5	77	83	80 X 10^4
S13	61	50	56 X 10^6	45	38	42 X 10^5	5	9	7 X 10^4
S14	44	39	42 X 10^6	15	11	13 X 10^5	21	27	24 X 10^4
S15	38	27	33 X 10^6	31	25	28 X 10^5	16	15	16 X 10^4
Average	36.73	35.46	36.4 X 10^6	32.4	31.93	32.46 X 10^5	37.13	36.4	37.6 X 10^4
Max	61	67	58 X 10^6	68	73	71 X 10^5	88	83	81 X 10^4
Min	10	8	9 X 10^6	7	5	6 X 10^5	5	9	7 X 10^4

Table 2. Colony Characteristics and Microscopic Observation of Isolates

Isolates	Colony Characteristics					Microscopic Characteristics		
	Size (mm)	Colony Shape	Margin	Elevation	Colony Color	Surface	Grams Reaction	Shape
S1	2	Irregular	Uneven	Umbonate	Yellow	Rough	Gram Negative	Cocci
S2	1	Circular	Entire	Flat	Orange	Smooth	Gram Positive	Cocci
S3	4	Circular	Entire	Flat	Yellow	Smooth	Gram Positive	Cocci In Cluster
S4	3	Irregular	Uneven	Umbonate	White	Smooth	Gram Negative	Cocci
S5	5	Irregular	Uneven	Umbonate	Orange	Smooth	Gram Positive	Cocci In Cluster
S6	3	Circular	Uneven	Flat	White	Smooth	Gram Positive	Cocci
S7	4	Circular	Entire	Convex	Orange	Smooth	Gram Negative	Cocci
S8	3	Irregular	Entire	Convex	Yellow & White	Smooth	Gram Positive	Cocci In Chain
S9	3	Circular	Entire	Convex	Yellow	Smooth	Gram Positive	Coccobacillary Rods
S10	1	Circular	Entire	Flat	White	Rough	Gram Positive	Rods
S11	3	Irregular	Uneven	Convex	Brown	Rough	Gram Positive	Long Rods
S12	4	Circular	Entire	Convex	Off -White	Smooth	Gram Positive	Bacilli In Chain
S13	6	Irregular	Uneven	Convex	Off -White	Smooth	Gram Positive	Rods
S14	2	Circular	Entire	Convex	Yellow	Smooth	Gram Positive	Rods
S15	3	Circular	Entire	Concave	White	Rough	Gram Positive	Coccobacillary Rods
				Pseudomonas Sp.			Gram Negative	
				Micrococcus Sp.			Gram Positive	
				Bacillus Sp.			Gram Positive	

Table 2 describes the detailed analysis of primary soil characteristics i.e. Colony Characteristics and Microscopic Observation of Isolates. The microbial groups existing (e.g., Gram + and Gram -) [40]. With reference to the colony characteristics, all the isolates except at S1, S4, and S7 were Gram-positive in nature.

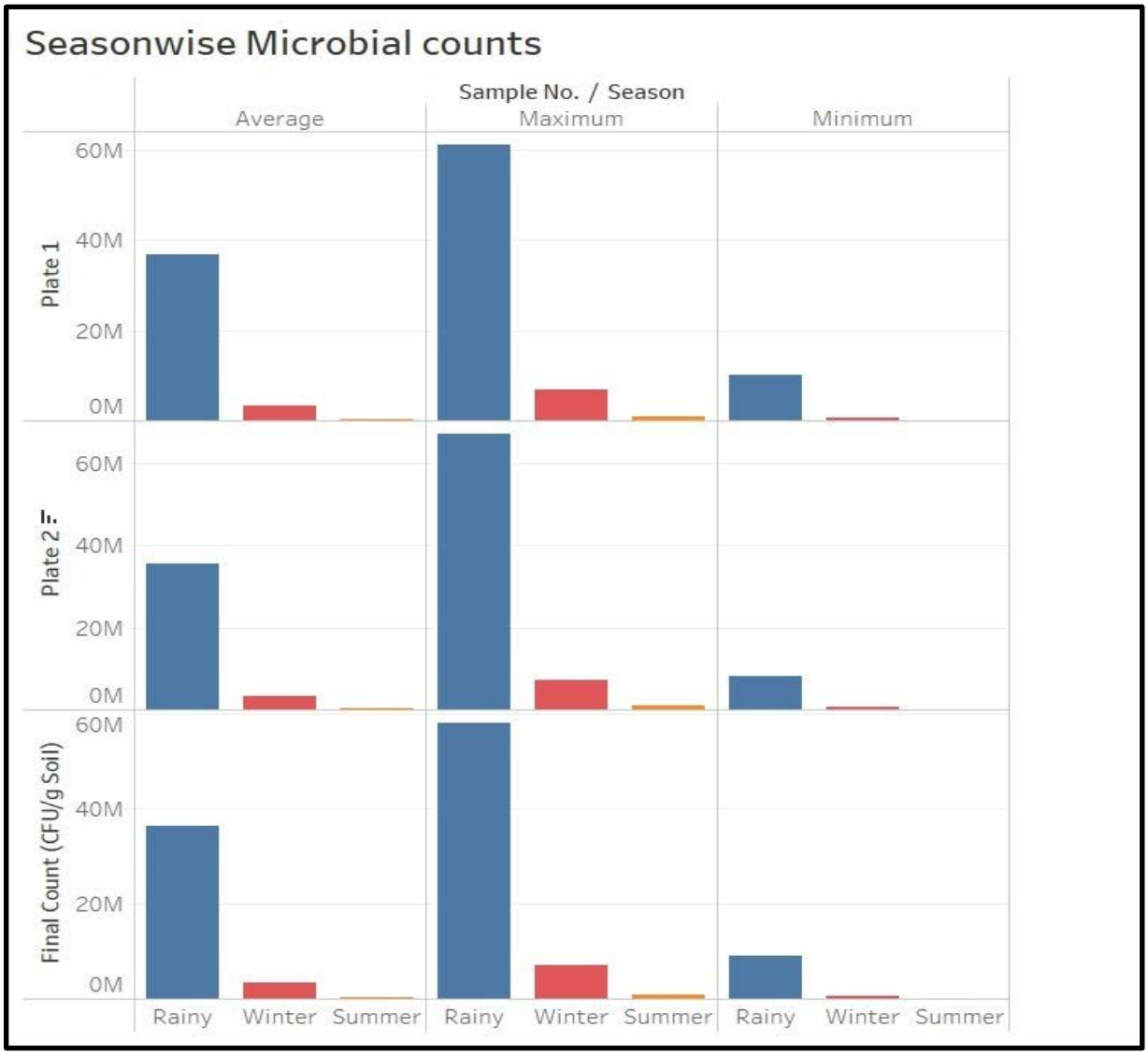

Figure 5. Seasonal Microbial Counts

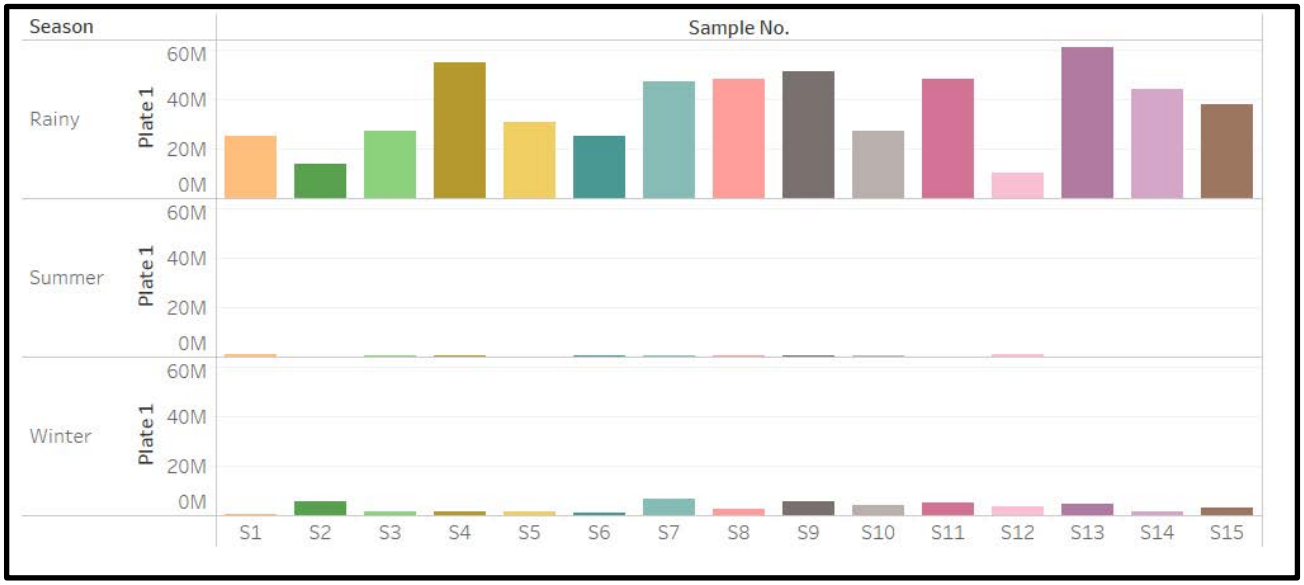

Figure 6. Seasonal and Spot wise microbial counts in Plate 1

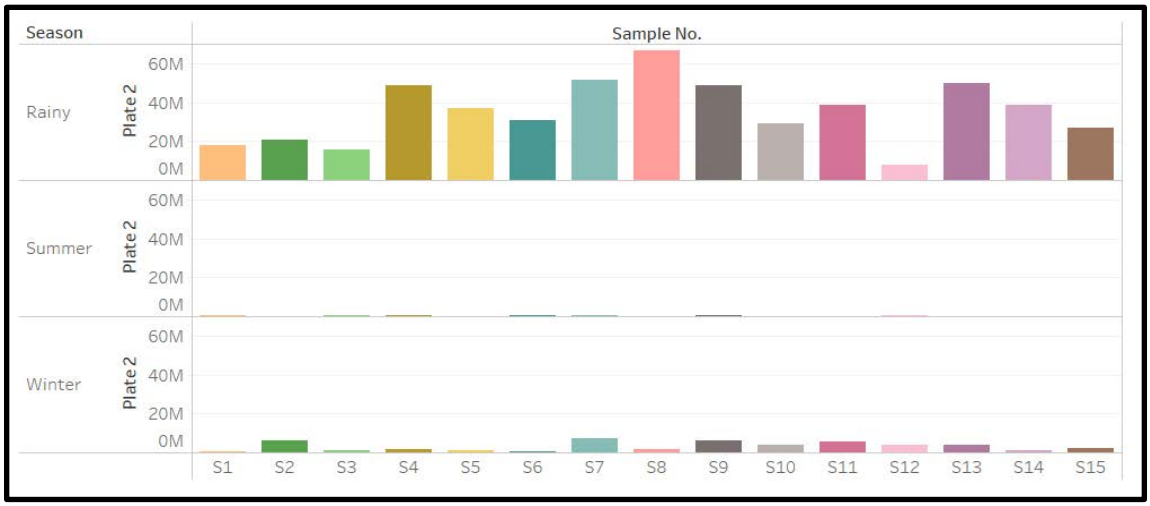

Figure 7. Seasonal and Spot wise microbial counts in Plate 2

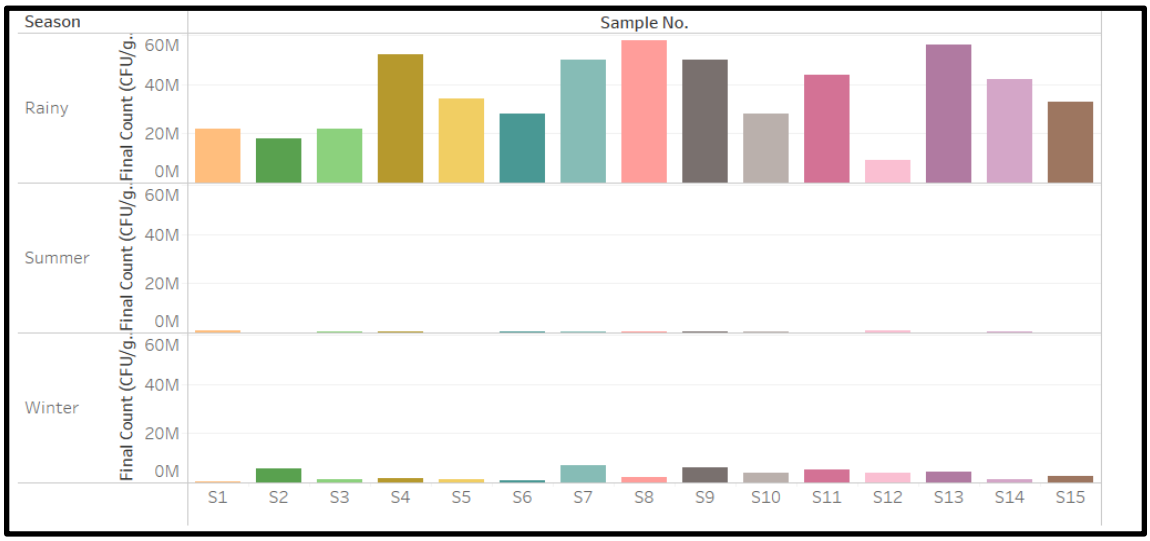

Figure 8. Seasonal and Spot wise microbial counts of Final Counts

3.1. Statistical Relationships

Microbial count in the rainy season and summer season are significantly negatively (-0.4) associated, according to Pearson correlation test results. The microbial counts of the rainy, winter, and summer seasons have no significant correlation. (Figure 9), shows the comparison of microbial counts during the rainy, winter, and summer seasons. Spots has a P-value of 0.426, which is greater than 0.05. (Level of significance). As a result, we accept the null hypothesis at a significance level of 5%. In other words, the average microbial count of different places does not differ significantly. Season has a P-value of 3.21*10 (-12), which is less than 0.05. (Level of significance). As a result, at a 5% level of significance, we reject the null hypothesis. In other words, there is a considerable variance in one of the seasons' average microbial counts (Table 3). Tukey's test is used to determine which pair of seasons has the most significant difference. We can conclude from Tukey's test that there is a substantial variation in average microbial counts across all seasons (Table 4).

Table 3. ANOVA test results

Source	Df	Sum of Square	Mean Sum of Square	F	p-value
Spot	14	1.14E+15	8.14E+13	1.066	0.426
Season	2	1.20E+16	6.01E+15	78.711	3.21E-12
Residuals	28	2.14E+15	7.64E+13		
Total	44	1.5309E+16	6.17079E+15		

Table 4. Tukey's test results

Season	Diff	lwr	upr	p adj	Conclusion
Summer-Rainy	-36024000	-43920797	-28127203	1.85E-11	Significant Difference
Winter-Rainy	-33153333	-41050130	-25256537	1.23E-10	Significant Difference
Winter-Summer	2870667	-5026130	10767463	6.45E-01	Significant Difference

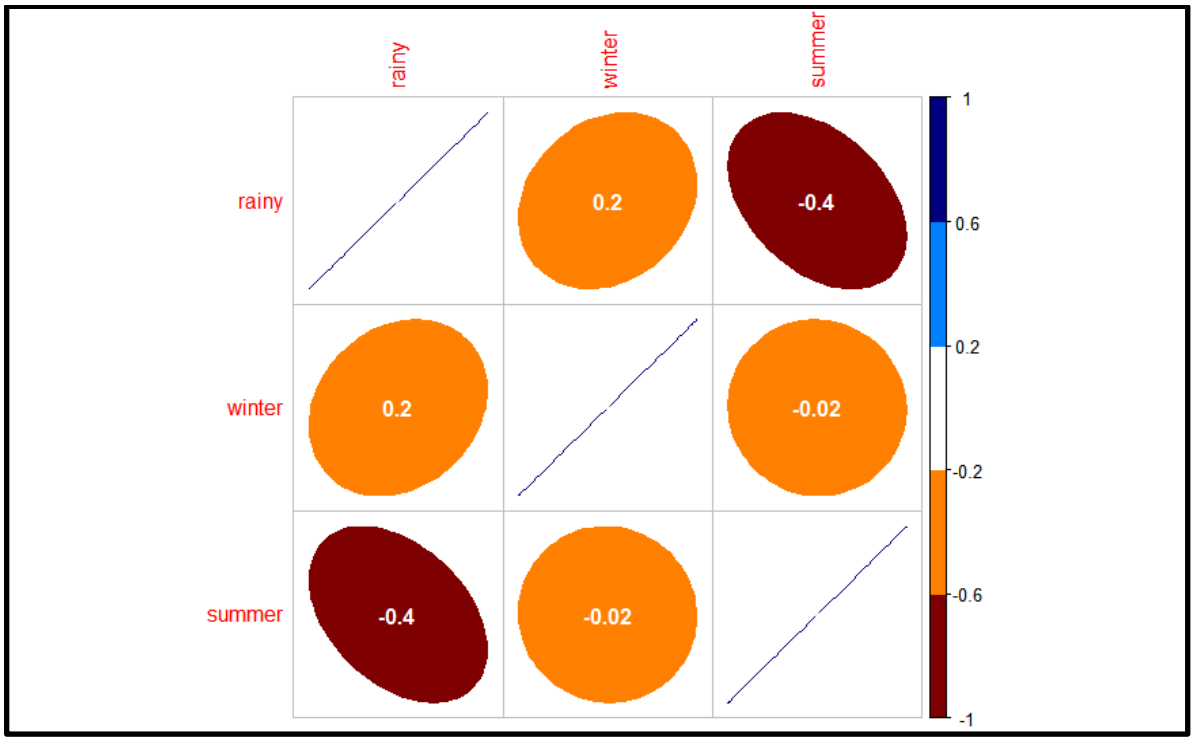

Figure 9. Seasonal Correlation Analysis. *(*Hypothesis: i) Ho: There is no significant difference between average microbial counts of different spots. Ha: At least one spot has a significantly different average microbial count than other seasons. ii) Ho: There is no significant difference between average microbial counts of Rainy, winter, and summer seasons. Ha: At least one season has a significantly different average microbial count than other seasons.)

4. Conclusion

The study was carried out for mapping the microbial diversity and seasonal changes of microbial counts in the soil of selected sites of Gautala Reserve Forest. The population of soil bacteria varies rapidly depending on geographical, ecological and climatic conditions etc. Soil microorganisms are a potential indicator of soil superiority, because plants rely on soil microorganisms to mineralize organic nutrients and promote their growth. Prolonged rains may create anaerobic conditions in the soil thus reducing microbial activities. As per the present results Bacterial process rates were commonly lower in winter than in summer. In statistical analysis, the Pearson correlation tests used for microbial populations are correlated. The microbial counts of rainy and summer seasons are slightly negative (-0.4). There is no significant correlation between microbial counts of rainy, winter, and summer seasons, but the two-way ANOVA test, and Tukey's test showed a significant difference between the average microbial counts at all sites seasonally. The seasonal variations in microbial counts in different seasons are increased or decreased (Impact on counts).

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