

Original Research Article

<https://doi.org/10.20546/ijcmas2017.604.191>

Soil Microbial Population in Rasomati Forest of Pundibari Range, Cooch Behar, West Bengal, India

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A B S T R A C T

Keywords

Soil,
Microbial
population,
Forest.

Article Info

Accepted:
15 March 2017
Available Online:
10 April 2017

An experiment was carried out in Rasomati forest of Pundibari range of West Bengal, India in respect to the variation in bacterial and fungal populations. The mean colony forming unit (cfu) of bacteria was 6.02 whereas average fungi colony forming unit (cfu) was 5.31. It was observed that Gram -ve bacteria was found higher as compared to Gram +ve. The genus *Aspergillus* and *Penicillium* was recorded highest percentage as per the counting of colonies. Over all soil were sandy loam to clay loam and slightly acid in all sites with mean pH 6.24 and the percentage of organic carbon was 1.96%.

Introduction

Soil microorganisms are vital for the continuous cycling of nutrients and for driving above-ground ecosystems (Van der Heijden *et al.*, 1998; Cairney, 2000; Klironomos *et al.*, 2000; Ovreas, 2000; Kirk *et al.*, 2004). The soil microbe decompose the plant and animal residues entering the soil and convert them into soil organic matter, which influence the physico-chemical and biological properties and creates a complimentary medium for biological reactions and life support in the soil environment (Olson *et al.*, 2000; Das *et al.*, 2013). Soil microorganisms are not only the driving force to carry and transfer the nutrient substrate through

biochemical process (Lima *et al.*, 1996; Jorden *et al.*, 1999; Garau *et al.*, 2007) but also play a crucial role in the functional process of the entire ecosystem since they can exert essential effect on the dynamics of multi-directional microbiological processes. Soil microbial enzymatic activity is central to the soil biological processes as it is associated with organic matter breakdown and nutrient cycling which is being mediated by soil microorganism (Karam *et al.*, 2012). That's why the study on microbiological diversity are essential to understand the microbial ecology and other ecosystems (Atlas, 1984).

A number of works on microbial population and diversity has been carried out in agricultural field but few works have been conducted in forest ecosystem. So, this study deals with the soil bacterial and fungal population and soil physico-chemical properties of the Rasomati forest. Rasomati forest is situated near to river Toorsa basin which comes under Pundibari range of Cooch Behar forest Division of West Bengal. The overall forest is consisting of mixed deciduous tree species under tropical moist deciduous forest (Champion *et al.*, 1968). However, this forest is the home of many important flora and fauna, which are highly vulnerable due to the anthropogenic activities as it is surrounded by many villages. Therefore, the scientific conservation majors need to be taken to conserve this rich biodiversity.

Materials and Methods

The study was conducted in Rasomati forest located between 26° 27' 44.0'' N latitude and 88° 19' 57.8'' E longitude with an elevation of 66 m above mean sea level. Rasomati forest comes under Pundibari forest range of Cooch Behar forest Division of West Bengal, at northern fringe of the state in the foothills of sub-Himalayan mountain belts. The detail of the geographical map of the study area is shown in figure 1.

The climate is moist tropical (Anonymous, 2001). The average minimum and maximum temperature varied from 23.08⁰C during winter (January) to 33.42⁰ C during summer (July). On an average the annual rainfall varies from 2000 mm to 3500 mm, bulk of which is being received during pre-monsoon and monsoon period i.e. May to September. The quantum of precipitation is very low during winter. The relative humidity of the area varies from 55% to 90 %. Consequently, the area is warm and humid except a short

spell of winter extending from December to February.

Soil samples were collected from 45 random plots at (0-10 cm) depth by covering the total area and 9 composite samples were prepared for the further studied during the month of February to March 2016. The sample put in a sterile poly bags and immediately brought into the laboratory. The pH of the soil was determined by making a soil solution with the help of digital pH meter and organic carbon was measured by Walkley and Black, 1934. Serial dilution plate method (Parkinson *et al.*, 1971) was followed for the isolation of fungal and bacterial populations. One gram of soil was taken in a 250 mL conical flask containing 100mL of sterilized distilled water to give a 1:100dilutions. It was then diluted to 10⁻⁴ to 10⁻⁶ respectively for fungal and bacterial counts. Bacteria were enumerated using nutrient agar (Difco manual, 1953) and fungi on rose Bengal agar (Martin, 1950). In each case 0.5 ml of the soil suspension was spread onto plates containing 20 ml of the solidified medium and incubated at 28 ± 1⁰C for 5 days for fungi and at 28± 1⁰ C for 48 hours for bacteria. Three replicate were maintained for each set. The Colony Forming Unit (CFU) per gram soil was calculated on the dry weight basis.

CFU/gD_w

$$= \frac{\text{Number of colony} * \text{dilution factor} * \text{inocollum}}{\text{Dry weight of soil (g)}}$$

Where, D_w = Dry weight of the soil (g)

Discrete fungal colonies appeared and were identified under the microscope with the help of standard manuals (Gilman, 1995; Barnett and Hunter, 1972). The bacterial isolation was done by morphological character by observing under microscope using gram-staining techniques (Gram, 1884).

Results and Discussion

The soil pH and organic carbon was varied among the sample. The pH of the soil varied from 5.97 to 6.50 with a mean value of 6.24 whereas the organic carbon contain varied from 1.51 to 2.29 % with an average value of 1.93%. Table 1 revealed that highest colony forming unit (cfu) of bacteria was observed in G₆ (6.54) and lowest was in G₃ (5.3). The CFU of bacteria was in G₄ (6.38) which were statistically at par with G₅ (6.29) and G₇ (6.29) respectively. The bacterial population in petri plate's indicated that maximum colonies were found as Gram -ve bacteria than Gram +ve. Table 2 and figure 2 described and illustrated the cultural variations in bacterial populations. Similarly, in case of fungi highest and lowest cfu was recorded in G₇ (5.88) and G₃ (3.77)

respectively. The fungal population was significantly at par among the other sample groups.

Among fungi population, genus *Aspergillus* and *Penicillium* was identified and recorded highest population whereas other colonies need to be identified. The cultural variations in fungal populations were depicted in table 3 and figure 3. This result indicated that the study area was highly diverse because of the vegetation cover with maximum nutrients. Maximum population or diversity of the genus *Penicillium* and *Aspergillus* may be due to their greater rate of spore production and dispersal and partly due to their resistance over extreme environmental conditions. This finding is close agreements with the findings of Schimel (1995); Kayang (2006) and Das *et al.*, (2013).

Table.1 Variations of bacterial cfu ($\times 10^5$ g⁻¹ soil) and fungal cfu ($\times 10^5$ g⁻¹ soil)

Group	Bacteria	Fungi
G ₁	(5.69) 489778.8	*(5.63) 426579.5
G ₂	(5.77) 588843.7	*(5.2) 158489
G ₃	(5.3) 199526.2	(3.77) 5888.437
G ₄	*(6.38) 2398833	*(5.36) 229086.8
G ₅	*(6.32) 2089296	*(5.46) 288403.2
G ₆	(6.54) 3467369	*(5.2) 158489.3
G ₇	*(6.29) 1949845	*(5.88) 758577.6
G ₈	(5.94) 870963.6	*(5.69) 489778.8
G ₉	(6.03) 1071519	*(5.65) 44668.3
C.V (%)	1.5	20.23
CD (P=0.05)	0.16	1.86

Parentheses indicate the log transformed value. Symbol * do not differ significantly.

Table.2 Cultural variations in bacterial population

Group	Variability in cultural characteristics of bacterial population
G ₁	Moderate single colonies, circular smooth white translucent with raised elevation as Gram ve- (70.59%) Minute whitish single dry circular colony raised elevation gram as Gram ve+ (29.41%).
G ₂	Small convex, entire colony yellow pigmentation at edge of petri plate as Gram ve- (53.85%) Filamentous irregular opaque white colonies as Gram ve- (15.38%) Moderate round opaque, spreading edge colonies as Gram ve- (30.77%)
G ₃	Moderate opaque irregular undulated, lobate colonies as gram ve- (58.82%) Tiny circular yellow colony as Gram ve- (41.18%)
G ₄	Dull irregular smooth convex whitish cottony growth and single small opaque filamentous flat white colony as Gram ve+ (44.55%) Tiny white pigment round irregular and entire opaque colonies, covering the total surface of petri plate as Gram ve- (47.27%) Small irregular and round rhizoids form opaque with yellow pigmentation as Gram ve- (8.18%)
G ₅	Pinpoint round small colony and moderate convex translucent colony at the edge of petri plate as Gram ve- (70%) Punctiform irregular and round rhizoids form opaque with yellow and black pigmentation small colonies as Gram ve- (30%)
G ₆	Small irregular and round rhizoids form opaque with yellow pigmentation as Gram ve- (42.45%) Small round irregular form, entire opaque colonies covering the total area of petri plate as Gram ve- (57.55%)
G ₇	Moderate round opaque, spreading edge colonies as Gram ve- (39.30%) Tiny white pigment round irregular and entire opaque colonies as Gram ve- (60.70%)
G ₈	Very minute, single circular whitish colonies as Gram ve- (34.88%) Moderate round opaque, spreading edge colonies as Gram ve- (46.51%) Tiny white pigment round irregular and entire opaque colonies, covering the total surface of petri plate as Gram ve- (18.60%)
G ₉	Small convex, entire colony yellow pigmentation at edge of petri plate as Gram ve- (76.81%) Dry whitish yellow slimy colonies with raised elevation as Gram ve- (23.19%)

Fig.1 Google earth map of the study area



Fig.2 Isolated bacterial culture



Fig.3 Isolated fungi culture



In conclusion, the present study concluded that bacterial and fungi populations in Rasomati forest are influenced by the diverse tree composition and physico-chemical properties of soil. Even, the climatic behaviour and soil nutrients status cannot be completely ignored in this aspect. The preliminary work has been carried out to understand the microbial population for a one season variation. Thus, further more scientific studies are needed to conduct in this area for the species identification and to understand their diversity on seasonal basis.

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How to cite this article:

Biplov Ch. Sarkar, Amarendra N. Dey, Ayon Roy and Barun Rai. 2017. Soil Microbial Population in Rasomati Forest of Pundibari Range, Cooch Behar, West Bengal, India. *Int.J.Curr.Microbiol.App.Sci.* 6(4): 1554-1560. doi: <https://doi.org/10.20546/ijcmas.2017.604.191>