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EFFECT OF PRESCRIBE FIRE ON BACTERIAL ABUNDANCE AND THEIR ENZYMATIC ACTIVITY IN BURNT AND UNBURNT SOIL OF CHILLA FOREST, RAJA JI NATIONAL PARK, UTTARAKHAND, INDIA

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Abstract

To minimize the incidences of wildfire and their effect on forest ecosystem prescribe burning is being used .It is used to burn the excess of litter fall, flammable material and to prepare seed beds. This study was designed to understand the effect of fire on bacterial population and their enzymatic activity. Soil samples of burnt and unburnt site were compared for their individual bacterial abundance and were screened for their cellulolytic and proteases activity. The study indicated *Bacillus* is dominant genera in both the samples but their abundance was higher 41% in burnt soil than unburnt soil i.e. 26%. The enzymatic study revealed that bacteria showed 94% cellulase and 65% protease activity in burnt were as 86% cellulase and 43% protease activity in unburnt soil. It is concluded that fire affect not only the bacterial abundance but also their enzymatic properties.

Key words: Fire, Bacteria, Enzymatic activity, Soil microbiology

Introduction

Forest fire is the most hazardous phenomenon especially in chir pine dominated forest strand of Himalaya region every year. Its reoccurrence become a matter of attention now. Fire produce several unwanted alteration in the forest ecosystem. Fire induces major changes on the biogeoch-mical cycle of ecosystem. Soil physical, chemical and biological properties were significantly affected. Fire is one of the important factor which affect the forest ecosystem, the positive and negative response depends on the severity and reoccurrence of the fire (Chandler *et al*.,1983). After fire treatment in forest ecosystem, soil microbiology and biochemical attributes such as biological activity of fertility layer of soil and nutrient availability (Grady and Hart, 2006) or the enzyme activities may affected (wic-Baena *et al.,* 2013). As we know microorganism are the basic functional unit of biogeochemical cycle and they serves as the indicator for soil quality and ecosystem health. They act as the serving source of nutrients and catalyst to accelerate the rate of nutrient transformation reactions. Microorganism like Bacteria, Fungi, Viruses act as the maintainers of soil structure by performing mutualism relationship with the root of plants (Hannanm *et al*., 2006).

The activity of soil microorganism is an another important factor to assess after fire. Saravanan *et al*. (2013) reported the effect of fire by quantitative analysis of the soil microorganism, the microbial population was found to be higher in the burnt sample as compare to the unburnt. The quantitative measure of soil respiration include overall microbial activity and organic carbon decomposition with respect to biological activity. It is interesting to analyze the enzymatic characteristics of microorganism after fire because every organism have its specific enzymatic activities have in catalyzing biological reactions, which results to the production of essential elements in biogeochemical cycles. However, it must be indicated that some of the enzymes are not exclusive to microorganism. The impact of forest fire on the soil microbial properties has not been carried out so far. Keeping the above points in view, the present investigation was undertaken in the Chilla forest range, Raja ji National park Uttarakhand India with the following

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objective: to assess the effect of forest fire on the enzymatic properties of the bacterial population which were isolated after fire. Although soil organisms include Bacteria, Fungi, Actinobacteria Viruses, Protozoa and other microflora, here in our study we have focused on the post fire effect of Bacterial population and their enzymatic activity.

Material and Methods

Sampling : For sampling, two sites (Burnt and Unburnt) were selected in Chilla Forest Range, Rajaji National Park, Uttarakhand. After fire soil samples were randomly collected from 5 different point from both the site (burnt and unburnt), sample were taken from 0-10cm depth in a sterile poly bag and brought them to the laboratory for further analysis.

Isolation and Identification : Composite of soil sample were made separately and than Samples were serially diluted (10^{-2}-10^{-6}) for the isolation of bacterial colonies using pour and spread plate method on Nutrient agar media .The plates were incubated for the observation of bacterial colonies at $35^0C \pm 2$ for 24-48 hrs. The pure isolates were maintained in 50% glycerol stock at -20^0C (Anheja, 2003). Biochemical characterization was performed by following tests Viz. Indole-Methyl red-Voges Proskauer IMViC) , Triple sugar Iron (TSI) ,Urease utilization and citrate utilization tests (Cappucino and Sherman, 2007)

Enzymatic activity : Enzymatic activity or characterization of isolates were studied for Cellulase (Teather and Wood, 1982) Protease and Amylase (Cappucino and Sherman, 2007). The Enzymatic activity index for Protease, Cellulase, Protease and Amylase were calculated. (Mangunwardoyo *et al*., 2011).

$$\text{Index of relative enzymatic activity} = \frac{\text{Diameter of clear zone (mm)} - \text{Diameter of bacterial colony (mm)}}{\text{Diameter of bacterial colony (mm)}}$$

Results and Discussion

The population of bacteria were found to be significantly different in burnt as compared to the unburnt site. *Bacillus* was found to be dominant in both the sites followed by *Paenibacillus* and *Pseudomonas* but in comparison with the individual genera *Bacillus* is more (41%) in burnt site (fig. 1) than that of unburnt (26%) (fig. 2). This is because of the endospore forming ability of *Bacillus*, this special feature provide this genera to better adapt in the adverse conditions. Fire creates major changes in soil property by the increase or decrease in N,P,K, pH, moisture content etc which directly or indirectly affect the soil physico-chemical parameters. Soil microorganism use the macro-micro nutrient from soil to perform their metabolic activities and regulate the functioning of ecosystem cycle.

The bacterial isolates were screened for their enzyme production activity for cellulase and Protease. Out of the total isolates from Burnt site 94% isolates were reported for cellulase production and 64% for protease production. The highest enzymatic index for cellulase was found as 9.50 and for protease, it was 0.07 (table 1). Where as in Unburnt site the isolates shown 86% for cellulase and 43% for protease The highest enzymatic index of the isolates for cellulase was 5.50 and for protease it was 0.60 (table 2). Enzymatic index for cellulase in burnt flora Bn-12-S is high and 5.50 in Un-10-S of unburnt flora. Whereas Protease enzymatic index of Bn-2-S (burnt flora) was observed 0.31 and 0.23 in Un-1-S and Un-7-S (unburnt flora).

After comparing the functional enzymatic activity of burnt and unburnt isolates it was found that the burnt site flora was more functionally active than unburnt. The enzyme production percentage is higher in burnt site flora than unburnt site. The study is suggested that their over all percentage of dominant genera varied in both the site but their individual properties does not affected significantly. After burnt the number of fire resistant bacteria increases and the other genera could not adapt their self for this condition. That would be one of the reason for more enzymatic activity in burnt than unburnt. Balagobalan *et al*. (2002) also reported that quantitatively the population of soil bacteria was more in burnt soil than in unburnt soil. Sharma *et al*. (2015) reported that *Bacillus* is most adapted to the high temperature as compare to the other genera. Saravanan *et al.* (2013) observed that *Bacillus* is the most dominant genera found in the burnt soil as compare to the others. The microbial population modified by fire treatment in several ways as a result of heat sensitivity, survival strategies, colonization and sensitivity to soil and change in microclimate. Yeager *et al*. (2005) reported that in different ecosystem some bacterial group like *Bacillus* spp. and *Clostridium* spp. can create heat resistant (temperature $100\text{-}120^0C$) forms after fire.

Some researcher reviewed in their study effect of fire on soil nitrogen transformation (Raison, 1979), Soil water repellency was done by De Bano (1981, 2000) and Doerr *et al*. (2000), Neary *et al.* (1999) and Certini (2005) provide the general review on soil physico-chemical and biological properties. Soil microbiology of fire has received less attention than physical chemical property

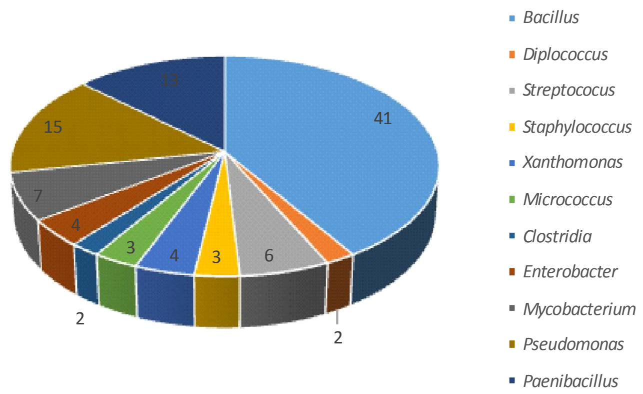

Fig. 1 : Percentage distribution of bacterial population burnt site.

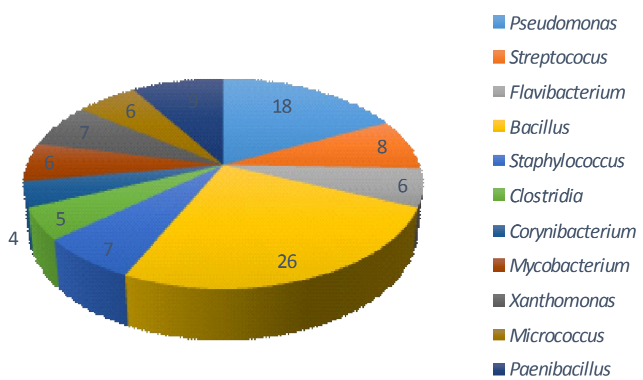

Fig. 2 : Percentage distribution of bacterial population in unburnt site.

Table 1 : Indexes for cellulose and protease activity of burnt site bacterial isolates.

Name of isolates	Cellulase activity index	Protease activity index
Bn-1-S	1.50	-
Bn-2-S	4.60	0.31
Bn-3-S	0.80	0.25
Bn-4-S	3.25	0.12
Bn-5-S	3.40	-
Bn-6-S	4.40	0.11
Bn-7-S	3.80	0.19
Bn-8-S	5.00	-
Bn-9-S	4.00	-
Bn-10-S	0.80	0.07
Bn-11-S	-	-
Bn-12-S	9.50	0.10
Bn-13-S	0.60	0.25
Bn-14-S	4.40	0.25
Bn-15-S	3.00	0.19
Bn-16-S	3.00	0.12
Bn-17-S	4.60	-

(-) show no enzymatic activity.

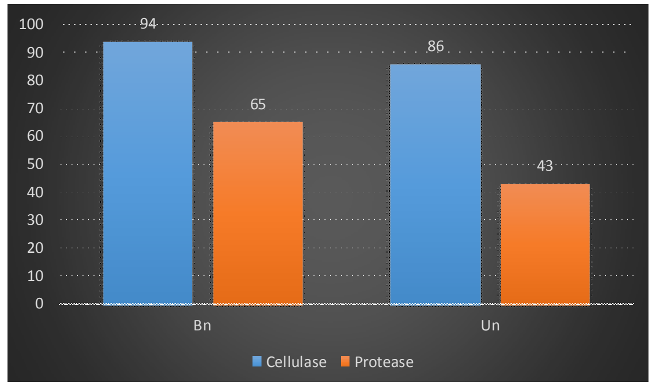

Fig. 3 : Comparative percentage distribution of enzymatic potential of isolates from burnt and unburnt site.

Table 2 : Indexs for cellulose and protease activity of unburnt site bacterial isolates.

Name of isolates	Cellulase activity index	Protease activity index
Un-1-S	4.40	0.25
Un-2-S	3.00	0.19
Un-3-S	3.00	0.12
Un-4-S	4.00	-
Un-5-S	4.00	-
Un-6-S	-	-
Un-7-S	5.40	0.25
Un-8-S	4.00	-
Un-9-S	3.83	0.20
Un-10-S	5.50	0.06
Un-11-S	2.13	-
Un-12-S	4.33	-
Un-13-S	4.33	-
Un-14-S	2.00	-

(-) show no enzymatic activity.

of soil. Although, there are a number of way to study the change after fire in soil microbiology .the estimation of overall microbial population in soil after fire is complicated . for this reason most of studies have been carried out on microbial biomass and respiration rather than their individual enumeration and functional properties (Jorge Mataix-Solera *et al*., 2010).

Conclusion

In the present study, the bacterial isolates obtained from burnt and unburnt site were screened for their enzymatic property which shows that the burnt flora was highly active than unburnt flora. A no of scientific investigation were previously performed to understand the effect of fire on microbial community quantitatively but less of concern pay to the effect of fire on the property of microorganism. It is essential to know that if we are going to do fire treatment what would be the effect of fire on microbial population and their properties. Bacterial enzyme have play an important role in biogeochemical cycle too. This study suggested that the prescribe fire should be more in practice because it give positive results in soil microbes in there number, their community and individual properties. Microbes regulates the soil by their metabolic activites and after fire their activities are not altered negatively. Their property contribute more to the vegetation profile and soil physico-chemical constituents.

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