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OCCURRENCE AND DIVERSITY OF MYCOFLORAL POPULATION IN SOIL OF TWO DIFFERENT LAND USE TYPES IN HARIDWAR REGION (UTTARAKHAND), INDIA

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ABSTRACT

This study was conducted to estimate the mycofloral diversity in soils of two different land use types namely forest area and the industrial area in district Haridwar. Soil samples were collected and analyzed for selected soil physico-chemical parameters using standard analytical methods and enumeration of soil fungi by serial dilution agar plating method. Results revealed that the higher Colony Forming Unit (C.F.U.) values for soil fungi was observed in the forest area significantly correlated with varying meteorological factors *viz*. atmospheric temperature (°C), relative humidity (%) and rainfall (mm). A total of 21 fungi species belonging to the Ascomycetes (01), Deuteromycetes (18) and Zygomycetes (02) were identified. The Shannon-wiener's index of species diversity was maximum (2.968) in forest area followed by industrial area (2.84), respectively. Principal component analysis showed that out of 21 fungal species 7 species were highly substantial with good PC values (70.55%), rather 14 were less occurring (13.10%) to determine the mycofloral diversity.

Keywords: Haridwar region, Land use types, Mycofloral diversity, Shannon-wiener's index, Soil fungi

1. INTRODUCTION

Soil and microbes are key components of biotic community in soil. Soil fungi play an important role as major decomposers in the soil ecosystem [1]. They also provide mankind with very useful pharmaceutical products, such as antibiotics and other valuable substances, including organic acids, enzymes, pigments and secondary metabolites used in the food industry and fermentation. On the other hand, some of them are very harmful causing food spoilage and diseases to plants, animals and humans with significant economic losses and produce mycotoxins in certain products [2]. Soil properties play an important role in determining the distribution of the various microbial groups [3].

Forest soil is a rich habitat for the growth of microorganisms unlike microbial habitats. Fungi are one of the dominant groups among the microorganisms present in soil. It was estimated that there is a minimum of 7, 12, 000 fungal species worldwide [4]. Only 5-13% of the total predictable global fungal species have been described and the definite number of fungi species are still unknown [5]. Soil fungi are microscopic cells that grow in thread like structures or hyphae that make a mass called mycelium. The colonized mycelium absorbs nutrients from the roots and surface organic matter of the soil. This developed humus complex is a natural fertilizer mixed with soil and plays a very important role in the composition of soil [6].

The removal of forest vegetation by severe anthropogenic intervention because of land-use change has led to drastic changes in plant biomass and litter inputs and has severely affected the composition, biodiversity, productivity and functionality of soil micro biota [7, 8]. Different land uses have significant effects on soil characteristics which in turn lead to the change in the nutrient status of soil [9]. Industrial and domestic wastes of the industrial area (SIDCUL and BHEL) are discharged into seasonal river Ranipur Rao, Haridwar [10] that leads to the change in nutrient status of soil in the adjoining areas. Diversity indices are the mathematical measurement of species diversity that reflects how many different types of species are there in a particular area. The Shannon-Wiener's index (H′) was chosen to measure the fungal species diversity in a land use pattern. Simpson index (D) is one of the most meaningful and robust diversity measures available. It measures the probability that two individuals randomly selected from a sample will belong to the same species. As value of Simpson index decreases, diversity

increases. Dominance index is based on the direction of aggriviness behavior between the species in each land use and evenness describes the how species close in numbers each species in environment [11].

Therefore, this study was considered to investigate the mycofloral population of soil in two different land use patterns in Haridwar region and their correlation with some important physico-chemical parameters of soil.

2. MATERIAL AND METHODS

2.1. Environmental and geographical conditions of the study sites

The study was conducted in two different land use patterns of Ranipur Rao seasonal hill river watershed in Haridwar district during April 2016 to March 2017. The total length of the Ranipur Rao seasonal stream is about 12.83 km. Because of its vicinity to Outer Himalayan hilly areas, climatic conditions of Haridwar region become moderate like plain areas of Uttarakhand. The annual mean air temperature was 22.83°C with monthly mean air temperature ranged from 3.7°C in January to 39.5°C in May during the study period.

The annual rainfall was 1128.30 mm and mean annual humidity was 72.91% recorded during the study period in Haridwar.

The monthly mean air temperature, humidity and precipitation during the study period are shown in Fig 1. However, for the study point of view the study sites and their Geo-coordinates in two different land use patterns are shown in Table 1.

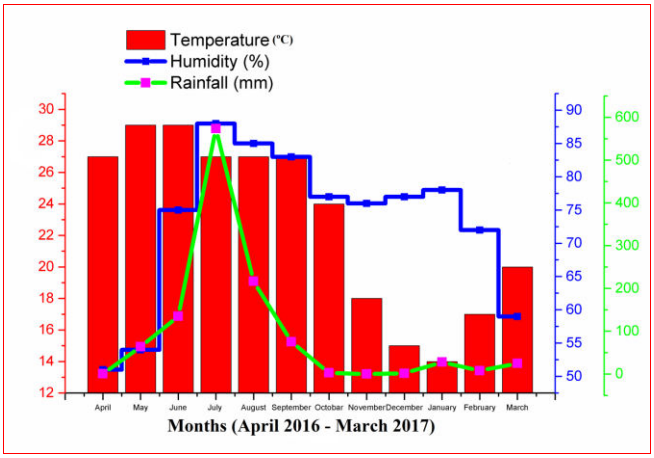

Fig. 1: Monthly variations in temperature, humidity and rainfall during the experimental period

Table.1.Showing the sampling sites at different land use areas in Ranipur Rao watershed, Haridwar

Sampling Sites	Location	Type of land use area	Geo-coordinates	Elevation
Site- A	Sureshwari temple trail sector	Forest Area	29°58'10.66"N, 78 °06'27.73" E	1057ft
Site –B	Between the SIDCUL and BHEL industrial area	Industrial Area	29°57'11.31" N, 78 °05'10.62" E	984ft

2.2. Collection of soil samples

The soil samples were collected during April 2016 to march 2017. Composite soil sampling technique was used for the collection of soil samples from two different sites. After the collection soil samples were air dried and sieved through a 2 mm sieve and brought to the laboratory for the analysis [12-14].

2.3. Analysis of physico-chemical parameters

Physico-chemical parameters of soil samples were analyzed followed by standard procedure. The average Soil Temperature was measured using soil thermometer and Moisture Content of fresh soil samples was determined by the oven dry method. Soil pH was measured using digital pH meter (TANCO Deluxe pH meter), Organic Carbon was determined by the wet digestion method [15] and Total Nitrogen was determined by Kjeldahal method [16] and Available Phosphate was determined by spectrophotometric method (Systronics Visiscan 167) by the ammonium molybdate blue method.

2.4. Enumeration and identification of soil mycoflora

Serial dilution agar plating technique and martin's agar media were used for the Enumeration of soil fungi. Dilutions were usually made in multiple of ten. The culture plates were prepared using vertical laminar air flow system for fungi analysis. The plates were incubated at 25±1°C for 4-5 days. When fungal colonies of

different size and colour grow on medium, plates were then stored at 4°C in refrigerator for further identification. The emerging fungal colonies were counted for number and indentified to the species level based on morphological characteristics.

Quantitative study: Count all the colonies on culture plates with naked eyes or with the help of electronic colony counter. Count all the Colony forming units (CFUs), take average of three plates & calculate average number of CFUs g^{-1} dry soil by using the formula [14]:

$$\text{Colony Forming Units (CFU) } g^{-1} \text{ dry soil} = \frac{\text{Averageno.ofcolonies}}{\text{Drywt.ofsoil}} \times \text{Dilutionfactor}$$

Qualitative study: Occurrence of each fungal species was measured by using the following formula:

$$\text{Occurrence \%} = \frac{\text{Averageno.ofcolony of a species}}{\text{Average no.colonies of all the fungal species}} \times 100$$

Different fungal species were identified by shape, colour, and size of their conidia, mycelium and other characteristics given in the books and literature [17, 14]. Fungi that did not understand or did not show morphological characteristics for identification were considered as 'unidentified species or sterile colony'.

Microbial Diversity Indices: Dominance Index, Simpson Index, Shannon Weiner Index and Evenness were calculating by method given in [18].

2.5. Statistical Analysis

All analyses were performed in triplicates and the data was processed in Microsoft Excel 2010 to perform all statistical analyses. Principal component analysis (PCA) was employed to evaluate the determinants of fungal diversity using Origin Lab 6.1 (9.6.0.172 (Learning Edition; Origin Lab Corporation, Northampton, MA).

3. RESULTS AND DISCUSSION

3.1. Physico-chemical properties of soil

The physico-chemical properties of soil in each land use type are summarized in Table 2. Seasonal variations were found in each parameter of soil in different seasons. The Moisture Content refers to water held by the individual particles of the sample and greatly affected by temperature [19]. It is the most important contributing factor for the growth of soil fungi. In the present study moisture content was maximum (11.50%) in rainy season due to rainfall, so that soil fungi were found to be maximum in rainy season. Soil Temperature is the most important physical property of soil system; it regulates the physical, chemical properties and biological or microbiological processes of soil organisms. In the present study, the mean values of Soil Temperature were recorded higher (23.89°C) in summer season and minimum in winter season (14.39°C) in both land use patterns.

Table 2: Seasonal variations in physico-chemical properties of soil in forest and industrial areas (Mean±S.E.)

Parameters	Depth (cm)	Forest area			Industrial area		
		Rainy	Winter	Summer	Rainy	Winter	Summer
Moisture Content (%)	0-15	11.30±0.21	9.20±0.18	9.49±0.21	11.02±0.34	8.70±0.30	8.69±0.20
	15-30	11.25±0.31	9.10±0.28	9.46±0.27	10.50±0.48	8.79±0.41	8.51±0.38
Soil temperature (°C)	0-15	20.75±0.55	16.22±0.39	23.30±0.4	20.75±0.38	15.80±0.4	25.75±0.31
	15-30	19.85±0.45	15.45±0.42	22.15±0.5	19.65±0.47	14.39±0.48	23.89±0.42
pH	0-15	6.28±0.38	6.21±0.39	6.32±0.37	6.18±0.35	6.15±0.31	6.74±0.29
	15-30	6.23±0.20	6.20±0.31	6.54±0.26	5.89±0.24	5.72±0.30	6.23±0.28
Organic matter (%)	0-15	3.2±0.13	2.25±0.14	2.39±0.12	1.25±0.16	0.89±0.15	0.92±0.13
	15-30	2.9±0.17	2.19±0.19	2.31±0.14	1.19±0.17	0.85±0.14	0.90±0.18
Total Nitrogen (%)	0-15	0.29±0.10	0.23±0.08	0.26±0.09	0.20±0.12	0.18±0.09	0.16±0.09
	15-30	0.26±0.11	0.21±0.12	0.25±0.10	0.19±0.11	0.18±0.08	0.14±0.07
Available Phosphate (%)	0-15	0.89±0.22	0.69±0.26	0.57±0.19	0.66±0.23	0.60±0.20	0.62±0.21
	15-30	0.87±0.31	0.67±0.30	0.59±0.31	0.64±0.33	0.58±0.31	0.63±0.34

Due to increasing sunshine intensity, the temperature was increasing in the month of June. Rainy season were started in second week of July and temperature was decreased, due to that the soil temperature was also decreased in surface and subsurface depth. In the present study, the pH value under industrial area was found to be

the highest (6.74) followed by forest area (6.54) respectively. This is could be due to higher values if exchangeable bases as a result of dumping site of many types of industrial waste. Soil pH influences nutrient uptake, microbial growth, tree growth and productivity. Total Nitrogen varies between 0.14 to 0.29% in the study site and higher in forest area because of high Organic Matter content in soil. Selassie et al. (2015) reported a similar observation [20]. Available Phosphate content (0.89%) was found Maximum in forest area followed by industrial area which plays a significant role in fungi growth.

3.2.Soil fungi diversity

The number of fungal species enumerated from the soils in forest area and industrial area were maximum in rainy season and summer season and minimum in winter season. Seasonal variations in mean values of colony forming units of soil fungi were found (Table 3).

Table 3: Values of colony forming unit (CFU g^{-1} dry soil) of fungal colonies in forest and industrial land use patterns

Dilution	Depth (cm)	Forest area			Industrial area		
		Rainy	Winter	Summer	Rainy	Winter	Summer
10^{-3}	0-15	$15\times10^{3}\pm0.21$	$10.03\times10^{3}\pm0.18$	$12.43\times10^{3}\pm0.21$	$12.41\times10^{3}\pm0.34$	$9.23\times10^{3}\pm0.30$	$10.37\times10^{3}\pm0.20$
	15-30	$12.82\times10^{3}\pm0.31$	$7.89\times10^{-3}\pm0.28$	$9.27\times10^{-3}\pm0.27$	$9.28\times10^{-3}\pm0.48$	$7.24\times10^{3}\pm0.41$	$8.28\times10^{-3}\pm0.38$

Table 4. Relative occurrences (%) of fungal species in forest and industrial land use pattern

Fungal species	Forest area			Industrial area		
	Summer	Rainy	Winter	Summer	Rainy	Winter
Ascomycetes						
Botrytis cinerea	-	1.29±0.77	3.08±0.48	-	-	-
Deuteromycetes						
Alternaria alternata	9.62±1.37	10.12±1.02	6.33±0.37	6.06±1.84	7.30±0.44	4.48±0.39
Alternaria tenius	5.21±0.71	6.98±0.56	6.48±0.28	1.62±1.02	5.83±0.80	2.46±0.23
Aspergillus niger	7.55±0.70	7.62±0.63	6.12±0.53	9.14±1.10	7.29±0.85	6.03±0.50
Aspergillus fumigates	6.24±0.43	4.72±1.19	5.32±0.34	8.18±0.92	5.99±1.02	6.30±0.63
Aspergillus clavity	5.98±0.90	4.50±0.73	3.69±0.50	5.33±1.85	3.59±1.33	5.34±0.56
Aspergillus flavus	4.63±0.54	3.60±0.74	2.75±0.40	2.23±1.57	3.96±1.36	4.60±1.36
Aspergillus parasiticus	5.15±1.03	4.00±1.23	3.41±0.32	4.13±2.10	2.52±0.62	2.21±0.94
Chrysosporium sp.	1.50±1.50	0.95±0.66	3.88±0.28	1.97±1.23	1.74±1.11	4.90±0.75
Cladosprium sp.	1.80±1.80	4.63±0.74	3.71±0.58	2.92±0.64	3.68±0.64	2.88±1.93
Curvularia sp.	2.53±0.75	2.26±0.49	1.78±0.16	2.66±0.52	2.80±1.23	2.18±1.01
Fusarium oxysporium	3.58±0.18	1.37±0.96	3.57±0.63	2.18±1.36	2.79±1.13	2.99±1.18
Fusarium sp	3.56±0.48	4.65±1.65	5.02±0.31	4.03±0.56	5.02±1.04	4.40±1.18
Helmenthosporium sp.	5.94±0.72	4.51±0.44	2.46±0.61	7.06±0.81	5.65±0.18	3.19±0.90
Penicillium crysogenum	1.99±1.01	2.03±0.72	4.05±0.25	5.44±1.98	2.15±0.98	4.92±1.67
Penicillium sp.	4.98±1.20	5.82±1.16	5.51±0.42	5.33±1.40	5.68±0.22	6.22±1.01
Penicillium notatum	1.91±0.47	2.28±0.41	4.68±0.36	3.20±1.45	4.09±1.15	4.71±0.87
Rhizotonia sp.	3.71±1.05	1.57±0.61	6.15±0.29	1.46±1.06	3.30±1.48	3.63±0.70
Trichoderma sp.	2.32±0.86	4.28±0.39	3.39±0.38	3.17±1.15	2.84±0.38	2.40±0.86
Zygomycotina						
Mucor sp.	9.14±0.30	7.85±0.67	5.67±0.28	8.90±1.27	7.66±0.32	8.99±0.97
Rhizopus sp.	6.89±0.98	7.15±0.72	6.65±0.46	7.48±1.65	7.21±1.17	9.19±0.75
Unidentified *sp.* A (white)	3.60±0.83	2.59±0.59	0.79±0.50	6.39±2.25	6.14±1.33	5.38±1.00
Unidentified *sp.* B (brown)	0.36±0.36	2.91±1.08	2.71±1.08	-	2.15±1.06	0.47±0.47
Unidentified *sp.*C (grey)	1.80±1.80	2.33±0.55	2.77±0.71	1.09±0.68	0.59±0.59	2.07±1.20

According to Mishra et al. (2017), significant seasonal changes occur in the diversity of soil mycoflora in Bilaspur district of Chhattisgarh state [21]. CFU's were recorded higher in rainy season followed by summer and winter season in both land use patterns. Highest value of CFU's was due to higher moisture content in rainy season because it is very important factor for fungal growth. So, there was a positive correlation between growth of soil fungi and moisture content. During the present investigation variety of fungal species were enumerated and identified (Table 4).

Deuteromycotina reported as a dominant group in comparison to other groups of fungi. On the basis of morphological characteristics total 21 soil fungi species were enumerated from two different land use patterns which belongs to three groups viz. Ascomycetes, Deuteromycetes and Zygomycetes were identified with the help of relevant literature and books and three were unidentified colony A (white), unidentified colony B (brown) and unidentified colony C (grey). Soil fungi were identified to 13 genera and 21 species, which belonged to the class Ascomycetes (1 genera and 1 species), the class zygomycetes (2 genera and 2 species) and class deuteromycetes (10 genera and 18 species). *Botrytis cinerea sp.* was found only in rainy and winter seasons in forest area. *Aspergillus sp.* was the most frequent genus followed by the *Penicillium sp.* isolated from both land use types in all seasons and these observations are similar to Manoharachary et al. (1990), Saksena et al. (1967) and Seth et al. (2016) [22-24].

3.3. Diversity indices of soil mycoflora

Mycofloral diversity index for two different land use pattern were calculated to find out the overall effect of land use pattern in soil mycofloral diversity. From Table 5 and Fig. 2, the land use pattern that had the highest diversity of soil fungi based on Shannon- Wiener index was forest area (2.96), followed by industrial area (2.84). The richness and percentage of each species was calculated by Simpson index. The forest area (0.94) had highest value of Simpson index as compared to industrial area (0.93). Direction of aggriviness behavior of species between two land uses was calculated by dominance index. The results revealed that greatest dominance in soil fungi occurred in industrial area (0.063), followed by forest area (0.056).

Table 5: Values of mycofloral diversity index between land use types.

Mycofloral Diversity index	Forest area	Industrial area
Dominance index	0.056156	0.063892
Simpson index	0.943833	0.936108
Shannon Weiner index	2.968	2.841667
Evenness	0.893275	0.890442

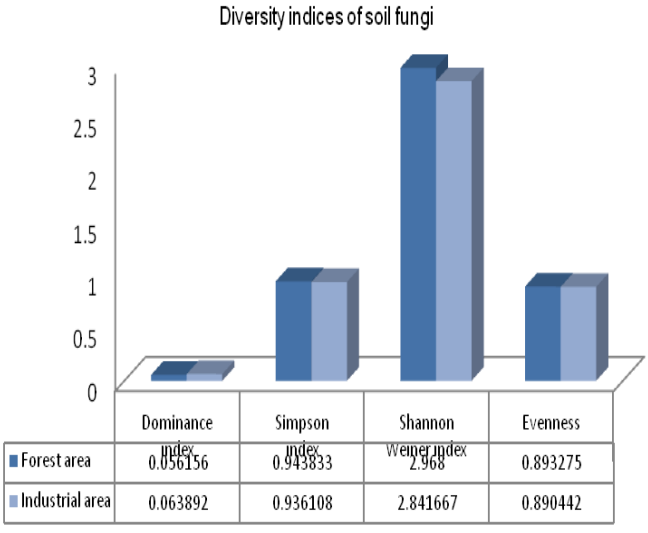

Fig. 2: Diversity indices of soil fungi in different land use patterns.

3.4. Principal component analysis

To identify the determinant fungal species of both land area of the study sites, principal component analysis (PCA) was used. In calculations of the diversity of the 21 fungal species, it was found that the selected species can be grouped into two major categories viz. first category contributing 70.55% to whole population (7 species) and second category contributing to the rest 13.10% population (14 species) as mentioned in Table 6. The data presented shows Principal component analysis results (cumulative) of fungal species in all three seasons. Beside this, scatter bi-plot for fungal species distribution in both land use patterns is provided in Fig. 3, in which labels for summer, rainy and winter are presented as B, C, and D for forest area and E, F, and G for industrial area, respectively.

Table 6: Principal component analysis results (cumulative) of fungal species in both seasons.

Fungal sp.	PCA results	
	PC1	PC2
Alternaria alternata	3.33	1.39
Alternaria tenius	0.65	2.19
Aspergillus niger	3.24	0.01
Aspergillus fumigates	1.94	-0.61
Aspergillus clavity	0.39	-0.52
Aspergillus flavus	-0.73	-0.33
Aspergillus parasiticus	-0.89	0.13
Chrysosporium sp.	-2.00	-0.26
Cladosprium sp.	-1.13	0.37
Curvularia sp.	-2.16	-0.56
Fusarium oxysporium	-1.70	0.00
Fusarium sp	0.20	0.41
Helmenthosporium sp.	0.47	-1.00
Penicillium crysogenum	-1.08	-0.71
Penicillium sp.	1.43	0.20
Penicillium notatum	-0.86	0.01
Rhizotonia sp.	-1.01	1.10
Trichoderma sp.	-1.40	0.26
Mucor sp.	4.04	-0.63
Rhizopus sp.	3.43	-0.12
Unidentified sp. A (white)	-0.22	-2.30
Unidentified sp. B (brown)	-3.11	0.73
Unidentified sp. C (grey)	-2.83	0.22
Eigen values	4.23	0.78

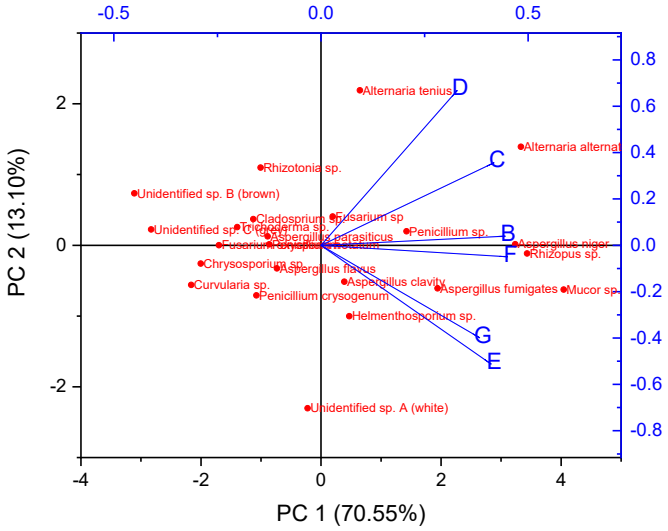

Fig. 3: Scatter plot for fungal species distribution in both land use patterns (summer rainy and winter presented as B, C, and D for forest area and E, F, and G for industrial area, respectively).

4. CONCLUSION

In conclusion, we state that the present investigation tends to understanding the soil fungi diversity with seasonal changes in different land use patterns of selected sites. Finding of the present investigation reveals that significant changes occur in the diversity of important soil fungi, therefore there is need to understand the aspects of mycodiversity for conservation and sustainability of the soil productivity on a long term basis. It was examined that mycofloral population were not equivalent whole the year they show seasonal variation. Principal component analysis showed that out of 21 fungal species 7 species were highly substantial with good PC values (70.55%); rather 14 were less occurring (13.10%) to determine the mycofloral diversity of selected soils.

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