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Study of microbial population under these land uses and water

Bharti Pathak[1], S B Sharma[2]
[1] Research Scholar, Department of Microbiology, Faculty of Science, Motherhood University, Roorkee, Uttarakhand, India
[2] Professor and Dean, Faculty of Science, Motherhood University, Roorkee, Uttarakhand, India

Abstract
Haridwar district, covering an area of about 2360 sq. km. is in the western part of Uttarakhand state of India. Its latitude and longitude are 29.58°N and 78.13 °E respectively. The height from the sea level is 249.7 meters. The experiments were conducted in Uttarakhand i.e. Haridwar and Roorkee for two years (2018-2020) from three land use site, Industrial area Agricultural area, Natural Forest area. Mucor species showed an increasing trend with the decreasing temperatures in Haridwar sites. It was most abundant in agricultural fields. Mucor species was minimum in rainy season, medium abundance in summers and most abundant in winter season. Aspergillus *candidus* was found in most of the sites of Haridwar (Agricultural, Industrial as well natural forest sites). It was found most in summer season and least in rainy season.

Keywords: mucor *Sp*., apergilus *Candidus*, Haridwar, Roorkee, Uttarakhand

Introduction
Haridwar district, covering an area of about 2360 sq. km. is in the western part of Uttarakhand state of India. Its latitude and longitude are 29.58°N and 78.13 °E respectively. The height from the sea level is 249.7 meters. The district came into existence on 28th Dec. 1988. Prior to its inclusion in the newly created state of Uttarakhand, this district was a part of Saharanpur Divisional Commissionaire. The district is ringed by Saharanpur in the west, Dehradun in the north and east, Pauri Garhwal in the east, Muzzaffar Nagar and Bijnor in the south. The district is administratively subdivided into three tehsils i.e. Haridwar, Roorkee and Laksar and six development blocks i.e. Bhagwanpur, Roorkee, Narsan, Bahadrabad, Laksar and Khanpur. Haridwar is one of the first towns where Ganga emerges from the mountains to touch the planes. As per the 2001 census, the population of the district is 14,44,213. Due to Haridwar's location on the bank of river Ganga, it has plenty of water resources and almost all kind of food grains are produced here in abundance.

The Ganga is a holy river in India. It traverses a distance of 507 km in the state of West Bengal and falls into Bay of Bengal. The river Ganga flows through more than 700 cities and about 120 million liters of waste water added daily by treated and partially treated effluents discharged into river Ganga directly and indirectly from different industries like pesticides, tanneries, paper and pulp mills, petrochemicals and fertilizer complexes, rubber factories and host of others use rivers. Also a large number of municipal wastes are also being discharged and polluted the water of river Ganga. With ever growing population and increasing demand of water for food production, industrial and domestic activities increases every year. No doubt, water is essential for life but 99% of the river in the world has been polluted by man-made resources mainly due to rapid urbanization, industrialization and increasing population (Saha, *et al*., 2012). The resulting degradation of quality of water in the water body creates a condition so that water cannot be used for intended beneficial uses including bathing, recreation and as a source of raw water supply. Haridwar is one of the important cities in India as a center of cultural, religious and industrial activities.

Geography and Climatic Conditions
Roorkee has a largely monsoon climate with high humidity levels. Annual average rainfall around 70-100 inches (1170mm), concentrated in the months of May to September. Temperature ranges from 21ºC to 45ºC. In winter temperature drops below 4ºC, but frost is common at high elevation. The temperature during the summer season remains between 21ºC to 45ºC. Strong Southeast winds blow across the state during the months of February and March.

Experimental
The area is situated in Uttarakhand, North India. The first sampling site is from Roorkee which is located at 29.87ºN latitude and 77.88ºE longitude and 268 meters above the sea level. And the second sampling site is from Haridwar which is located 29.94ºN latitude and 78.16ºE longitude and 314 meters above the sea level.

The experiments were conducted in Uttarakhand i.e. Haridwar and Roorkee for two years (2018-2020) from three land use sites

Table 1: 1. Industrial area 2. Agricultural area 3. Natural Forest area

Sl. No	Research area name
1	Industrial area
2	Agricultural area
3	Natural Forest area

Table 2: Location details of Haridwar sampling sites with GPS location

Land use area	Site name	Site notation	Latitude & longitude
1. Industrial area	Bhagwanpur	D1	N 25°54'35.98'' & E 93°41'32.25''
	Roorkee	D2	N 25°55'35.98'' & E 93°46'35.37''
2. Agricultural Area	Landhaura	D3	N 25°50' 31.19'' & E 93°45' 46.35''
	Laksar	D4	N 25°48' 25.12'' & E 93°48'0.65''
3.Natural Forest Area	Chilla Wild Life Sanctuary	D5	N 25°46' 22.76'' & E 93°48' 58.13''
	Roshanabad	D6	N 25° 45' 51.34'' & E93°52' 49.05''

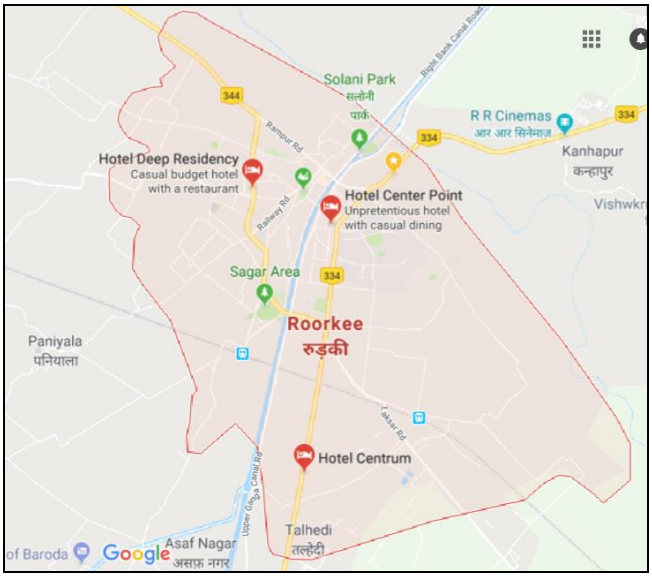

Fig 1

Fig 2

Sampling period

Samples will be collected at different time intervals from different land use areas of Roorkee and Haridwar for consecutive years 2017-2018 & 2018-19.

Sampling frequency

First sample of soil and groundwater will be collected in the month of February, second sample of soil will be collected in the month of June, third sample of the soil will be collected in the month of October and the fourth sample in the month of February from different sites of Roorkee and Haridwar for one year (2018-2019).

Collection of soil samples

The soil samples will be collected at depth 0-15cm, using screw-auger. 21 points subsamples will be taken randomly from each site and mixed thoroughly to form composite samples (approx. 3kg soil) each. The soils will be put into plastic bags, labeled and immediately transported to the laboratory for analysis (Subra, 2001; Okonkwo, 2010) [15]. Each soil sample was divided into two parts; the first part was sent to be analyzed for soil physical and chemical properties. The second part of the soil sample was isolated to obtain pure soil bacterial and fungal cultures at the Environmental laboratory of College of Forestry, SHUATS, Prayagraj.

Results

Colony Forming Unit

Soil samples will be taken from different land use areas, were sieved through a 2mm sieve. One gram of each sample was mixed with 10ml of deionized water in a screw-capped bottle. The soil solutions were then serially diluted down to five steps. One ml of each sample was pipetted into a petri dish in 03 replications, and was then mixed with nutrient agar (Subra, 2001; Jackie, 2013) [15]. The mixtures in the petri dishes were allowed to solidify and were incubated at 28˚C for 48 hours. The petri dishes were then removed from the incubator and the colonies enumerated using the plate count method (Julia *et al*., 2005; Jackie, 2013).

Microbial analysis

The isolates of bacteria will be identified using morphological and biochemical characteristic studies as suggested by Bergey and Holt (Bergey, D.H.; Holt, J.G). The fungal isolates will be stained with lactophenol cotton blue and observed under the microscope for identification of mycelial and spore structures. The isolated bacterial species will be classified into many groups based on their morphology and biochemical characteristics studies (Bergey, D.H.; Holt, J.G and Prescott, L.M.; Harley, J.P.; Klein, D.A) and the fungal species will be classified based on cultural and microscopic spore characteristics (Benson, H.J and Aneja, K.R).

Isolation and Identification of Soil Fungi

The soil dilution plate method (Manoch, 1998) was used to isolate fungi from the soil samples. Each soil sample was diluted to 1×10^{-4} concentration suspension. Then, 1 mL of the soil suspension (containing 0.0001 gm wet weight soil) was drawn by pipette into a Petri dish 90 mm in diameter.

A mixture of 25 mL of warm, melted glucose-ammonium nitrate agar (GAN) added with Rose Bengal and streptomycin

was poured over the soil suspension and the Petri dish was rotated gently to let the soil suspension mix well with the agar medium.

Five replications were completed for each soil sample (0.0005 g wet weight soil/sample). Since nine soil samples/land use type were collected, there were 45 Petri dishes/ land use type (0.0045 g wet weight soil/land use type). All the Petri dishes were incubated at room temperature (26-28°C) in darkness for 3-5 d or longer. After incubation, the emerging fungal colonies were examined. Hyphal tips of the different colonies were transferred to potato dextrose agar (PDA) slants using a transfer needle. The tube slants were incubated in indirect light at room temperature for two week, then grouped into presumed entities on the basis of the morphological characters of the colonies.

The total number of colonies of each entity was recorded. Identification of fungal species was based on morphological characteristics in plate cultures on suitable media and observation under compound and dissecting microscopes.

Statistical analysis

The number of colonies of a soil fungus/0.0045 g wet weight soil/land use type was converted to the number of colony forming units (CFU)/g wet weight soil/land use type or the abundance of a soil fungus. By summing up all the individual abundance records of a soil fungus in a land use type, the total abundance or total CFU/g wet weight soil for a land use type was obtained.

Discussion

The present study was carried out in Uttarakhand i.e. Haridwar and Roorkee for two years (2018-2020) from three land use sites 1. Industrial area, 2. Agricultural area, 3. Natural Forest area. The natural forests, agricultural areas and agricultural areas were taken for the study. A total number of 02 fungal species *Mucor sp.*, *Aspergillus candidus.* (Table 1).

Table 3: Mucor sp. and Aspergillus candidus

	Haridwar			Roorkee		
	Industrial Area	**Agricultural Area**	**Natural Forest**	**Industrial Area**	**Agricultural Area**	**Natural Forest**
Mucor sp.	+	+	+	+	+	+
Aspergillus candidus	-	+	-	+	+	+

Seasonal Abundance of Fungal and bacterial species

Mucor *sp.*: Mucor species showed an increasing trend with the decreasing temperatures in Haridwar sites. It was most abundant in agricultural fields. Mucor species was minimum in rainy season, medium abundance in summers and most abundant in winter season. On the other hand, it was not detected in agricultural fields of Roorkee site in rainy season. But it was detected in Industrial area sites of Roorkee, still most abundant in winter season.

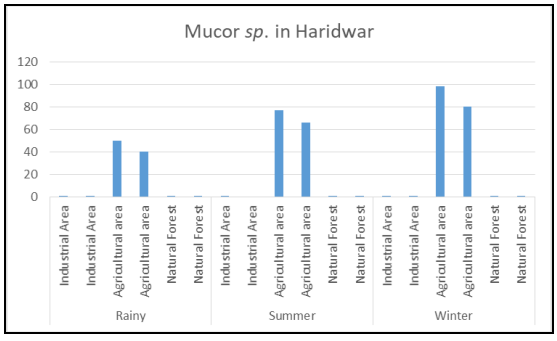

Fig 3

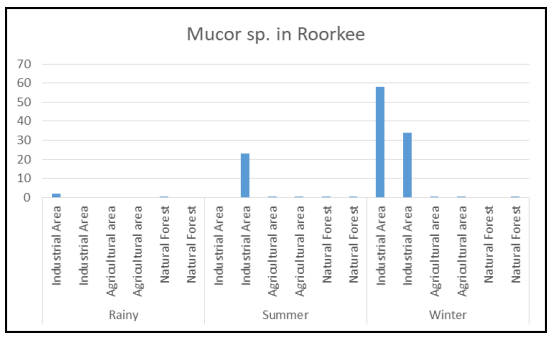

Fig 4

Aspergillus *candidus*

Aspergillus *candidus* was found in most of the sites of Haridwar (Agricultural, Industrial as well natural forest sites). It was found most in summer season and least in rainy season. On the other hand, in Roorkee sites, it was found mostly in Industrial area sites during rainy and winter seasons.

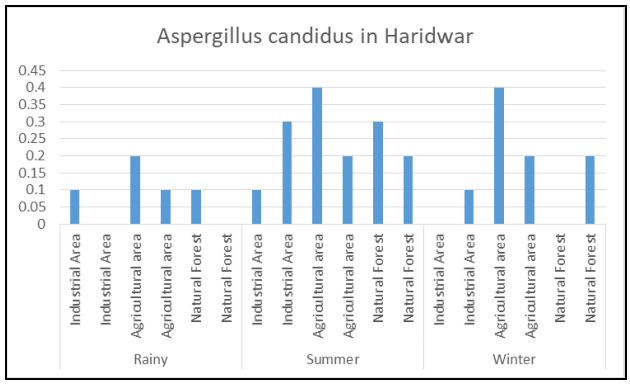

Fig 5

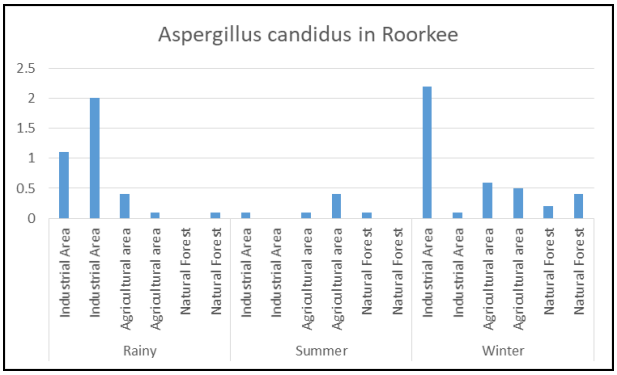

Fig 6

The diversity of Mucor *sp*. was observed and the maximum value was found. Mucor *sp* found 0.4 in Haridwar natural forest area. Aspergillus *candidus* found mainly in Haridwar in every season where as in Roorkee it is found more in winter as well as rainy season.

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