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Investigation on Abundance of Microbial Communities in Ambient Air over Urban Site in Semi Arid Region

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Abstract—*The present research work was conducted to investigate the presence of the microbial component (bacteria and fungi) in the ambient environment of the urban sites in Agra over a semiarid region. The presence of microbial components and high pollution load in air of city of Taj may pose threats to the people as Agra is a tourist place and attract attention from all over the world due to Taj Mahal. An assessment of the airborne bacteria and fungi were experimentally determined. Enumeration and Identification of air borne fungi and bacteria have been studied using Nutrient Agar Medium (NAM) and Sabouraud Dextrose Agar (SDA) medium respectively. The total microbial concentration in Respirable particles (PM_{10}) was 270.5 cfu/m^3 while bacterial and fungal concentration was 236.4 cfu/m^3 and 34.1 cfu/m^3 respectively. The bacterial isolates having highest prevalence i.e., 100% throughout the year were selected and identified on the basis of morphological, physiological and biochemical characteristics. The bacteria were identified as Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, Chromobacterium violaceum from BD-BBL crystal autoreader. Eight fungal isolates were identified comprising of Fusarium, Aspergillus, Cladosporium, Trichoderma. Alternaria, Mucor, Rhizopus, Helminthosporium. Some of the isolated bacteria and fungi are pathogenic in nature and may pose threat to the survival of human beings.*

Keywords*: Biochemical, Microbial load, Morphological, Physiological, Respirable particles*

1. INTRODUCTION

Exposure to bio-aerosols, containing airborne microorganisms and their by-products, can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions [1, 2]. Bioaerosols has been associated with human health effects and symptoms usually manifest inflammation of the respiratory system, coughs and fever [3] and inhalation of bioaerosol may cause or exacerbate respiratory diseases. They have been also known to cause gastrointestinal illness, eye irritation and dermatitis. Among various bioaerosols, fungal and bacterial spores are of major concern because of their abundant sources and ubiquitous presence in environments [4]. Globally, the presence of bioaerosols has been reported from plants and soils vegetables, water bodies, sewage sludge, animal feeding, fermentation process, and agricultural activities, swine breeding farms, feedstuff-manufacturing factories, human [5, 6]. Agra is best known for the Taj Mahal and as an important tourist destination, transport hub and commercial center. Agra, the city of Taj, a landlocked city is geographically located between 78°2' E and 27°11' N at 169 m above sea level (asl) with a tropical steppe climate influenced by the Thar Desert of Rajasthan in its South East, West and North West peripheries and is therefore, a semiarid area. The climate of Agra is divisible into three distinct seasons; summer, monsoon and winter [7]. The major industries in Agra are two thermal power stations, foundries, two railway marshalling yards (work on coal) industries like rubber, chemical, engineering, and brick. In spite of the above industries, Mathura Refinery and Firozabad Glass Industry are also very close to Agra, contributing to air pollution. Total area of Agra district is about 4 041 square kilometers. Agra has 580 396 motor vehicles registered, out of which 27 462 were transport vehicles. The aim of the study was to assess the degree of bioaerosols and the examination and characterization of isolated microorganisms (bacteria and fungi) in outdoor environment.

2. METHODOLOGY

2.1 Sampling and isolation methods

The sampling was carried out in Sanjay Place (Mahatma Gandhi Road), Agra**,** an urban site, is densely populated with residential and office buildings. PM_{10} aerosol samples were collected by Envirotech APM 460 (BL) Respirable Dust Sampler using GFA (Glass Fiber Filter) flow rate were noted before and after each sampling. Each sampling ran up to 24 hours with flow data acquisition every 5 minutes with ± 2% accuracy. The air samples of PM_{10} were taken at a flow rate of 1.2 m^3/min, respectively After the sampling filter paper were withdrawn and kept in dessicators and in Laminar Air Flow for analysis of bioaerosols. The sampling was carried out for different fractions of airborne microorganisms i.e., bacteria and fungi.

Culturable concentrations of fungi collected on impactors were determined or quantified using Sabouraud Dextrose Agar (SDA) media (Peptone -10 g, Dextrose- 40 g, Agar- 20 g, Distilled water-1000 ml and pH-5.6). 0.1 ml of the samples was inoculated in the petri dishes using Pour-Plating technique and petri dishes were incubated in BOD incubator at 28^0C for 3-5 days. The number of fungi colonies present were assessed on fifth days of incubation, and the number of colonies were determined in terms of colony forming per cubic meter (CFU m^{-3}) of air. Bacteria were isolated using Nutrient Agar Medium (Peptone-5g, Beef Extract-3g, NaCl- 5g, Agar- 20 g, Distilled water-1000 ml and pH-7). 0.1 ml of the aliquot of the sample was inoculated in the petri dishes using Pour-Plating technique and petridishes were incubated at 37^0C in BOD incubator for 24 h. The number of bacterial colonies was assessed after the 24 h of incubation, and the number concentration were reported as CFU m^{-3}.

2.2 Estimation of Protein

Total protein was estimated as a surrogate measure of biological mass concentration in the PM_{10} and $PM_{2.5}$ samples [9, 10, 11]. Protein was measured using the standard methods of Lowery [12]. Standard curve was developed using nine dilutions of Bovine Serine Albumin (BSA) between 0.25 and 100 µg ml^{-1} in sterile, deionized water.

2.3 Identification of Isolates

Identification of Bacteria

The bacterial cultures were identified on the macroscopic (shape, size, color, margin, elevation, opacity consistency, appearance of colony and hemolytic reactions) and microscopic (grams staining and endospore staining) examinations. Biochemical characterization of recovered isolates were performed according to Bergey's Manual of Determinative Bacteriology [13]. Further final identification was done using BD-BBL crystal autoreader. [14]

2.4 Identification of Fungi

The fungal cultures were identified on the basis of macroscopic (colonial morphology, color, texture, shape, diameter appearance of colony and basis of lacto phenol staining) and microscopic (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia, and presence of sterile mycelium) characteristics [15].

Relative microbial distribution was conducted according to Smith [16] where,

$$\text{Relative distribution} = \frac{\text{No. of colonies of the genus or species}}{\text{Total no. of colonies of all genera or species}} * 100$$

3. RESULTS AND DISCUSSION

The microbiological air quality (bacteria and fungi) and frequency distribution were assessed in the present research work. The concentration of RSPM in ambient air was 339.4 µg/m^3 and the mean concentration of bacteria and fungi was 236.4 cfu/m^3 and 34.1 cfu/m^3 respectively and the total protein concentration was 0.164 µg/m^3 (Table 1). The biological component can be identified specifically by species or collectively accounted for in the measurement of protein concentrations that reflect the levels of all PM that is biological in origin [17]. Menetrez [18] during research work revealed that the coarse PM fraction contained a higher percent of biological mass as was demonstrated by the protein measurement.

Table 1: Average mass concentration, microbial concentration and protein content in ambient air

	Av.	S.D	Min.	Max.
Mass Concentration (µg/m^3)	337.2	262.4	49	660.1
Bacterial Count (cfu/m^3)	236.4	241.2	16.2	1355.3
Fungal Count (cfu/m^3)	34.1	33.05	6.94	201.3
Total Microbial Count (cfu/m^3)	270.5	255.8	37.03	1399.5
Protein (µg/m^3)	0.164	0.017	0.135	0.207

*Av.-Average, S.D-Standard Deviation, Min.-Minimum, Max.-Maximum

In case of bacteria, twenty different bacterial strains were isolated from the samples collected. The bacterial isolates having highest prevalence i.e. 100% throughout the year were selected identified on the basis of their morphological, physiological and biochemical characteristics, a new technique of BD-BBL Crystal Mind Auto reader was also used for rapid identification of bacteria. It showed results between 95 to 99% purity of identified bacteria. The bacteria were identified as: *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Chromobacterium violaceum.*(Table 2, Fig. 2). Mancinelli [19] and Shaffer [20] reported that gram positive bacteria, particularly *Micrococcus* and *Bacillus* genera, often dominate the culturable fraction of airborne bacteria. Naddafi [21] isolated 14 bacterial species and genera, among them the dominant species were *Staphylococcus epidermidis*, *Micrococcus luteus* and *Bacillus* spp. *Bacillus* spp. is spore-forming soil bacteria and the most persistent in the atmosphere. Our results are in concordance with the findings of Gangamma [22] which stated that, the genera such as *Bacillus*, *Escherichia*, *Micrococcus* were predominant in airborne bacterial species distribution. Badri [23] during their research work isolated six bacteria pathogens comprising of *Staphylococcus* spp., *Bacillus* spp., *E Coli*, *Pseudomonas* spp., *Salmonella* spp., and *Shigella* from the samples of urban area.

Table 2: Summary of the Biochemical Tests for the identification of bacterial isolate

Biochemical tests	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Gram's Staining	+	+	+	-
Shape	Cocci	Rod	Cocci	Rod
Catalase	+	+	+	+
Oxidase	-	+	+	+
Urease	-	-	-	-
Indole	-	-	-	-

MR	+	-	-	+
VP	+	+	+	-
Glucose	+	-	+	+
Sucrose	-	-	+	+
Fructose	-	+	+	+

*****1- *Staphylococcus aureus*, 2- *Bacillus subtilis*, 3- *Micrococcus luteus*, 4- *Chromobacterium violaceum*

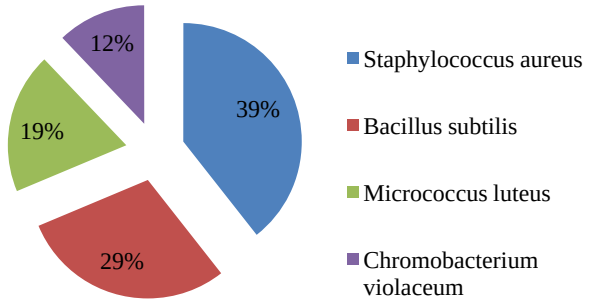

Fig. 1: Relative Frequency of Bacterial Isolates

In case of fungi, fungal isolates were identified on the basis of the micro- and macro-morphological features, coloration of colonies, morphological characteristics of the vegetative mycelium, reproductive structures and arrangement of conidia. From the samples, a total of fifteen fungal isolates were identified comprising of *Fusarium, Aspergillus, Cladosporium, Trichoderma. Alternaria, Mucor, Rhizopus, Helminthosporium.*

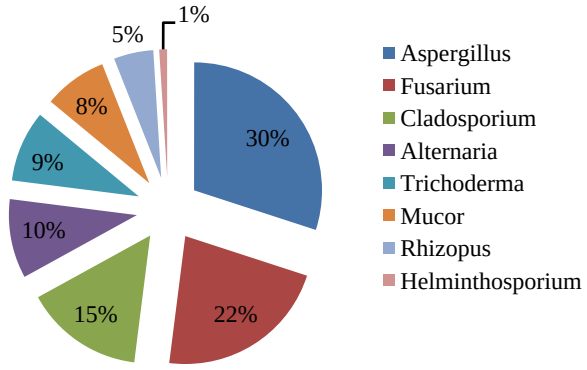

Fig. 2: Relative Frequency of Fungal Genera

(Fig.2) The most common fungi isolated from air environment belong to the Deutoromycota division (imperfect fungi) and the most frequently genera found are *Penicillium, Aspergillus, Eurotium, Wallemia, Cladosporium* and *Alternaria* [24]. Pereira [25] in their study reported *Aspergillus* spp. and *Penicillium* spp. as the predominant genera of organisms isolated from the air. Our results are similar with the report of Thirumala [26] the fungal species were *Aspergillus, Pencillium, Curvilaria, Cladosporium, Fusarium Rhizopus, Alternaria* species were identified. *Aspergillus* species (47.2%) showing maximum contribution is observed where as *Rhizopus* shows minimum contribution.

4. CONCLUSION

Airborne microbial flora (bacteria and fungi) were isolated enumerated and identified in outdoor environment of city of Taj, Agra, India. From the samples collected total 04 bacterial strains and 03 fungal strains were characterized and identified. Bacteria show higher growth in comparison to slow growing fungi. BD-BBL Crystal Mind Auto reader showed results between 95 to 99% purity of identified bacteria. The bacteria were identified as: *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus*, and *Chromobacterium violaceum.* The most common fungal genera were identified as *Fusarium, Aspergillus, Cladosporium, Trichoderma. Alternaria, Mucor, Rhizopus, Helminthosporium.*

5. ACKNOWLEDGEMENT

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