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Research Paper

Enumeration of Microorganisms in Air by Plate Exposure Method: a Case Study of Mahipalpur, New Delhi

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Abstract
Human health is jeopardised by air pollution. Pollen from plants, fungus, bacteria, and viruses are the primary sources of biological air pollution. Soil, water, and organic matters are the primary sources of microorganisms in the air. The occurrence of microorganisms in the air can be seasonal or permanent, although the most common rise in their occurrence is observed in the summer and fall. According to studies, the key factors impacting the diversity and the percentage of pathogenic bacteria and fungi present in outdoor depends on the road transportation and human anthropogenic activities. The majority of microorganisms in the air are due to construction of residential buildings. Molds and fungal spores make up about 60% of the dust microbiome. The present research reports the numbers of pathogenic bacteria and fungi in indoor and outdoor air and explains the magnitude of the impact of air microorganisms on human health. The study is conducted during the COVID-19 pandemic period during the pre-lockdown and lockdown situations. The present study showed that the biological aspects of the air significantly reflect the state of the environment in which we live and function on the daily basis. Microbial load both bacterial and fungal was less in morning (10 am) than in evening (5 pm). The test results reveal that all the test results were significant at $p < 0.05$.Although the pandemic affected human life but it is evident from the present study that the prolonged lockdown has somewhere decreased the microbial load in air compared to the pre-COVID era, 2018 and 2019.

Keywords: *Air Quality, Indoor air, Outdoor air, Bacteria, Fungi, Microbiome, Human Health*

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I. Introduction

Air pollution is the most serious environmental health threat on the planet. Pollution is responsible for one out of every eight deaths worldwide. According to reports, 7 million people died in 2012 as a result of exposure to ambient and indoor air pollution. In 2012, Africa accounted for over 680,000 of the total global air pollution-related deaths (WHO).Over the years, scientific study has linked bioaerosol exposure to the incidence of undesirable health impacts such as communicable infectious diseases, acute toxic effects, allergies, and cancer.Liu et al., (2018) investigated the total numbers of harmful bacteria in the air in Hangzhou (China). Airsampling included dust of various particle sizes and quality indexes, and 16SrRNA sequencing was used to characterise the bacteria present. *Thiobacillus, Methylobacterium, Rubellimicrobium*, and *Paracoccus,* all belonging to the *Proteobactria* genus, were found to be the most numerous. *Staphylococcus, Bacillus, Clostridium, Enterobacter,* and *Klebsiella* were the most common pathogenic bacteria genera found in airborne particulate matter (PM) samples.Kubra et al. (2015) conducted microbiological air quality tests in selected kindergartens. In the morning and afternoon, air samples were obtained in classrooms. The quality of indoor air was analysed using Polish standards (PN-Z-04111-01; PN-Z-04111-02:1989; PN-Z-04111-03:1989) based on groups of indicator microorganism gathered outside the kindergartens as a control sample. The total quantity of

heterotrophic bacteria and staphylococci in the air surpassed allowed levels regardless of the research method used, while no significant contamination with mould fungi was discovered.Nabrdalik I Latala (2003) conducted study to characterise the quantitative and qualitative characteristics of fungi found in building structures. They revealed that, due to the presence of potentially dangerous fungi in the examined objects, it is required to develop testing techniques and criteria to determine the degree of air pollution in residential structures.Pastuszka et al.(2000) conducted research to evaluate the concentrations of bacterial and fungal bioaerosols in various types of buildings, as well as the risk of specific diseases caused by these bioaerosols. They discovered that there was an increased incidence of asthma symptoms in buildings with mould issues. Inhalation of fungal particles and other airborne substances most likely raised the risk. The relative concentration of the observed species, including *Penicillium* species, ranged from 3 to nearly 50% of the fungal population in the house without mould, but the highest concentration of *Penicillium* species was identified in 90% of all fungi in the indoor air.

Indoor and outdoor airs, as well as atmospheric air, are all important factors in the normal functioning of the human body. Air pollution is one of the most serious risks to people's living surroundings. Environmental contaminants are potentially dangerous airborne pollutants that substantially impact human health, according to the World Health Organization (WHO) and the European Environment Agency (EEA). All compounds in the earth's atmosphere are not natural components, greatly elevated quantities of such compounds in air signifies air pollution. Chemical compounds, acid rain, airborne ashes, dust, trace elements and biological contaminants the atmosphere.Pollen, bacteria, and viruses are examples of biological air pollutants, often known as bio aerosols. Microorganisms pollute the air, plant surfaces, rocks, buildings, and hospital environments. The dispersed phase of the bio aerosol is made up of particles with sizes ranging from 1 to 200 µm. Some may be pathogens or spread allergies, putting the population's health at risk.Microorganisms, as we all know, are one of the most important variables impacting human health. Microorganisms can be found under a wide range of conditions, including severe temperatures, pressures, salinity, and acidity. One of the remaining biological limits on Earth has been identified as the atmosphere. The microbial community's composition and biodiversity in the atmosphere are still poorly understood. Bacteria and fungi have been found in several atmospheric layers, including the boundary layer, upper troposphere, and stratosphere at altitudes of more than 20 kilometres above sea level. Airborne microorganisms have the ability to travel long distances, which has an impact on air quality.

Microorganisms present in outdoor and indoor air impacts human health through residential, hospital, and public spaces.They can be dispersed through the air by wind and precipitation, resulting in deterioration of air quality. As a result, the presence of microorganisms in both the ambient air and the indoor environment, particularly those that cause infectious diseases, can be extremely harmful. Microorganisms in the air have been shown to be responsible for immune system anomalies such as allergies, infections, airborne diseases and even cancer.The purpose of this research article is to present up-to-date facts on the air quality of Mahipalpur, New Delhi, India.Many studies by researchers established a correlated between the air quality and human cognitive performance. However, the chosen study area has not been much studied so far. During the COVID- 19 pandemic situation, the Government of India declared a complete lockdown from March 2020. A pre-lockdown, and lockdown situation was closely analysed and monitored to find out whether limited transportation and human activity outdoor plays any major role in reducing number of pathogens. The outdoor air quality also impacts indoor air quality. Hence, both outdoor and indoor air quality assessment was done considering the data on bio- aerosolised particles. The study may reveal the impact of road transportation and air pollution on altering the air quality. Changes in terms of biological air quality, was significantly studied. Air quality has an impact on the immunological development and the emergence of allergies, asthma, and allergic diseases during early childhood is evident.Bio aerosols in the air could be a source of physiological ailment. Asthma, hay fever, bronchitis, chronic lung failure, lung cancer, and other diseases are all caused by airborne germs, particularly bacteria and fungi. Aside from pathogenic microorganisms in the air, attention should also be paid on the biologically active products, such as endotoxins or mycotoxins; these airborne biological agents and their toxic components are major issues related to human health and development.The air quality of Mahipalpur, New Delhi has significantly changed due to the present situation of lockdown enforced by the Government bodies as a result of second wave of COVID- 19 pandemic in India. The present study was conducted from April 2021 to July 2021, at Institute of Public Health and Hygiene (IPHH), New Delhi for a qualitative and quantitative assay of the microbial load present in air. The main objectives of the present study are as follows:

1. To isolate bacteria, fungi from air (outdoor and indoor) using culture dependent technique and plate exposure method.
2. To enumerate the number of bacteria and fungi present in air.
3. To study the colony morphology of typical colonies obtained by plate exposure method.
4. To identify selected colonies by staining and microscopy.
5. To perform biochemical identification and type study of the selected colonies.
6. To perform statistical calculations for quantifying the number of microbial pollutants present in air.

7. Lastly, interpretation of the microbial air quality of the present study area.

This study was conducted to enumerate the numbers of living microbes or bio aerosols suspended in the air. It was intended to check the outdoor and indoor microbial quality of air. As the microorganisms are directly involved in affecting human health and the environment. The scope of work includes selection of the study area: Institute of Public Health and Hygiene (IPHH), Mahipalpur, New Delhi. IPHH hospital and laboratory was selected to check indoor air and IPHH terrace and lawn was selected for checking the outdoor air.

II. Methods and Materials

The air quality of Mahipalpur, New Delhi has significantly changed due to the present situation of lockdown enforced by the Government bodies as a result of second wave of COVID- 19 pandemic in India, in 2021. Lockdown restricted the road transport, construction and industrial activities. The air quality is expected to be better compared to the air quality index (AQI) the pre- COVID- 19 period due to the lockdowns. The present study was conducted from April 2021 to July 2021, at Institute of Public Health and Hygiene (IPHH), New Delhi for a qualitative and quantitative assay of the microbial load present in air.

2.1 Experimental Setup

Extensive microbiological culture dependent analysis was conducted for examination of the air quality. Air exposure method/ plate settling method, quantification of number of bio-pollutants, and grading the air qualitywere the main aim of the experimental study. The experimental setup included:

1. Selection of the study area: IPHH hospital and laboratory (Indoor) and IPHH terrace and lawn and the campus area (Outdoor).
2. Extensive microbiological culture dependent analysis was carried out. Air exposure method/ plate settling method were performed.
3. Statistical enumeration: Arithmetic mean and standard deviation of the parameters were calculated using Microsoft Excel 2003and student's one sample t- test was calculated using SPSS version 26 software.

Various methods were applied for enumeration and detection of microorganisms in air which are as follows: sedimentation methods, microscopic methods and culture methods.

2.1.1 Sedimentation method

The easiest method is the 'Settling Plate Technique,' which is extensively used by air microbiologists. In this experimental study bacteria-carrying particles are allowed to settle onto the medium for a length of time before being incubated at the proper temperature. The quantity of settled bacterium carrying particles can be determined by counting the colonies that has developed. Petri-dishes containing sterile nutritient agar (NA) medium and potato dextrose agar (PDA) are chosen for the study in this procedure. The medium chosen is determined by the type of microorganisms to be counted. Nutrient agar (HiMedia) was used to enumerate airborne pathogenic bacteria, and potato dextrose agar (HiMedia) was used to obtain the opportunistic fungus from air.The plates are labelled with information such as the location and time of sample, the length of exposure, and so on. The plates are then left exposed in the desired location for the specified amount of time, i.e., 30 minutes. All petri dish containing solid culture media is covered until sampling, at which point the cover is removed and the agar surfaces are exposed to air for 30 minutes outdoor at 10 am in the morning and 5 pm in the evening. After that, the petri dishes were sealed and incubated. The nutrient agar plates were kept inverted inside the bacteriological incubator at 37°C for 24 hours for obtaining aerobic bacterial counts and 3 days at 22 ˚C for saprophytic bacteria. Molds were cultured on potato dextrose agar; all the exposed plates were closed and kept straight at a temperature of 25°C for 5–7 days. On agar media, a specific number of colonies can be seen developing. Each colony symbolises a microorganism-carrying particle that has settled on the agar surface.The ideal exposure time of 30 minutes was considerable sufficient for obtaining easily countable number of well isolated colonies. It usually depends on how dusty the air being sampled is and hence the plate exposed in IPHH hospital was kept for a period of 60 minutes. It's best to keep the plates about 1 metre above the ground during sampling. The colonies on each plate are counted and recorded after incubation as the number of bacteria carrying particles settling on a specific area in a given period of time.

2.1.2 Microscopic Methods

This method allowed air to pass through a membrane filter of 0.45 micron pores size in the direction of the airflow for trapping the microorganisms, which was then examined microscopically for counting the number of cells. Acridine orange staining and observation under a fluorescence microscope was done. The total number of microorganisms in 1 m^3 of air is the end outcome. This approach has the benefit of being able to identify both live and dead microorganisms in the air, as well as those that do not thrive in culture media.As a result, the number of microorganisms detected is typically one order of magnitude more than in culture methods. Other biological agents, such as plant pollen, allergic mites, and abiotic organic dust, can also be detected and

identified. However, the methods have a fundamental flaw: where the actual determination kind of the species of microorganism is not possible.

2.1.3 Culture- dependent Methods

Microbes are transferred from the air onto the surface of the suitable selective medium such as mannitol salt agar- MSA (HiMedia) and milk agar- MA (HiMedia). The forms of colonies were counted after a 24-hour incubation period at the ideal temperature of 37°C, and the result is presented as CFU/m^3 of air. Because a colony can form from a single cell as well as a cluster of cells, the air could contain more bacteria than the colony forming unit (CFU)as the test suggests. Furthermore, the procedure enables for the detection of only live cells that are able to proliferate in the selective medium used. Microbes that have been moved to the culture medium must be revived because they have been exposed to hazardous conditions.For bacterial identification, Gram's staining was done, and lactophenol cotton blue staining was done for the fungal identification.

Pure cultures were obtained by sub-culturing distinct colonies on freshly prepared nutrient medium and streaking them sequentially to obtain pure cultures, while pure fungal cultures were obtained by spot inoculation. Colonial features, microscopy, and biochemical assays were used to characterise and identify pure bacteria cultures. In bacteriology, the Gram stain is the most extensively used staining process to distinguish between Gram-positive and Gram-negative bacteria, also known as a differential staining. This staining technique was used along with the biochemical tests for identifying the bacterial species based on differences in biochemical activities among bacterial species. Colony morphology and lactophenol cotton blue (LCB) staining was used to identify the fungal colonies.

2.2 Statistical Calculation

The results obtained were recorded as the number of colonies obtained (colony forming unit per meter cube- CFU/m^3). The statistical significance of the test results were drawn by calculating the Arithmetic Mean (AM) and Standard Deviation (SD). The Student's "one sample t- Test" was used as a statistical tool to compares the sample mean with a hypothesized population means for drawing a significance at $p < 0.05$. All the statistical calculation was done using Microsoft Excel 2003 and SPSS version 26 software.

III. Result and Discussions

3.1 Identification of microorganisms

The colony counts of bacteria and fungi obtained after a plate exposure time of 30 minutes were expressed as CFU/ m^3. At least 7 different types of bacterial species and 6 different types of fungi species were detected from the air during the study. The suspected colonies of bacteria were identified through the colony morphological study and biochemical tests (table 1). The fungal colony was detected by staining and microscopic analysis (table 2).In indoor air, the commonest genera of fungi found were*Aspergillus*, *Penicillium* and the commonest genera of bacteria found were*Staphylococci, Bacillus* and *Pseudomonas.* Microbes found in air over populated land areas below altitude of 500ft in clear weather include spores of *Bacillus* and ascospores of yeasts, fragments of mycelium and spores of molds, etc. In outdoor air, common genera of fungi such as*Cladosporium*, and the bacterial genera such as *Bacillus*, *Micrococcus, Streptococcus*, *Staphylococcus* and *Pseudomonas* were predominant.

Table 1 Morphology, Gram's stain test and Biochemical test of bacterial species

	Microorganism	**Colony morphology**	**Gram's stain reaction**	**Biochemical tests**					
				Cat-alase	**Oxidase**	**Indole**	**MR**	**Citrate**	**VP**
1	*Bacillus subtilis*	Thin long rod shape, motile,white or light brown	+ve	+ve	Variable	-ve	-ve	+ve	-ve
2	*Bacillus megaterium*	Rod-like, aerobic spore forming	+ve	+ve	-ve	-ve	-ve	+ve	-ve
3	*Streptococcus spp.*	Spherical, cocci-in chain	+ve	-ve	-ve	-ve	+ve	-ve	-ve
4	*Micrococcus spp.*	Spherical occurring in pairs, to irirregular cluster	+ve	+ve	+ve	-ve	-ve	-ve	+ve
5	*Staphylococcus aureus*	Spherical, cocci appears as grape-like cluster, non motile	+ve	+ve	+ve	-ve	+ve	+ve	+ve

6	*Pseudomonas aeruginosa*	Rod shaped, non-flagellated	-ve	+ve	+ve	-ve	-ve	+ve	-ve
7	*Aeromonasaerophilia*	Pleomorphic, rod-shaped	-ve	+ve	+ve	+ve	+ve	+ve	+ve

MR- methyl red, VP- Vogues Proskuer

Table 2 Morphology, Lactophenol Cotton blue staining for identification of fungal species

	Microorganism	Colony morphology	Lactophenol cotton blue (LCB) staining
1	*Aspergillus*	Cottony white to black	Conidial heads, stipes, colour and length vesicles shape and seriation, metula covering conidia size.
2	*Penicillium*	Greenish yellow to green mat colonies	Brush-like spore bearing, cluster of flask-shaped phialides.
3	*Cladosporium*	White cottony mass at the surface, olivaceous-brown to blackish-brown at the bottom	Septate conidia, with conidiospores and septate hyphae
4	*Mucor*	White cottony colony	The hyphae and fruiting structures with no rhizoids, non-septate hypae
5	*Fusarium*	White cottony colony, cottony aerial mycelium	Sickle shaped septate macrospores and round microspores are visible, septate hyphae
6	*Sccharomycescervesiae (Yeast)*	White to cream, smooth	Large globose to ellipsoidal budding yeast like cells

From the above tables it observed that the bacteria were characterized up to species and genera level on the basis of the Gram staining, Colony morphology and Biochemical tests (Table 1).On the other hand, the fungi were characterized up to species on the basis of their morphology and staining with Lacto-phenol cotton blue stain (Table 2). The isolated microbes include *Bacillus spp., Streptococcus spp., Staphylococcus spp., Micrococcus spp., Psuedomonas spp.,* in the case of bacteria. Whereas, *Aspergillus spp., Penicillium, Cladosporium, Mucor, Fusarium*, *Sccharomyces cervesiae* in the case of fungi.

Table 3 Colony counts of bacteria (CFU/m^3) in air at different sampling time of day at different indoor areas

Sampling site (IPHH)	Sampling time		Petridish exposure time (min)
	Day(10am)	Evening (5pm)	
Classroom	6030	8920	30
Laboratory	1028	1472	30
Library	6300	7271	30
Hospital reception	421	511	60
Hospital OPD	209	299	60
Hospital ICU	15	21	60

Table 4 Colony count of fungi (CFU/m^3) in air at different sampling time of day at different indoor areas

Sampling site (IPHH)	Sampling time		Petridish exposure time (min)
	Day(10am)	Evening(5pm)	
Classroom	93	92	30
Laboratory	52	63	30
Library	126	189	30
Hospital reception	125	172	60
Hospital OPD	19	12	60
Hospital ICU	08	13	60

Table 5 Range of microbial distribution in IPHH indoor sites

	N	Minimum	Maximum	Arithmetic Mean (AM)	Standard deviation (SD)
Bacteria CFU/m^3	12	105	8920	2708	3361.22
Fungal CFU/m^3	12	08	189	70.5	51.87

3.2 Enumeration and statistical calculations

The air microbial loads of seven different sites of IPHH were determined by taking indoor air samples (figure 1). The results of the study considering the number of colonies, concentration range, arithmetic mean, standard deviation of bacterial and fungi present in the investigated sites of IPHH are represented in the table 3, table 4 and 5.The results indicate that the highest bacterial count in CFU/m^3air of indoor samples recorded at 5 pm in Classroom at 30 min exposure, which is 8920 CFU/m^3, while the lowest bacterial CFU/m^3air were recorded at 10 a.m. in Hospital ICU at 30min exposure, which is 15CFU/m^3(table 3 and 5).The highest fungal content (CFU/m^3) in air of indoor sample has been recorded at 5 pm in the library at 30 min exposure time, which is 189CFU/m^3, while the lowest fungal CFU/m^3air were recorded at 10 am in hospital ICU at 60 min exposure time, which is 08 CFU/m^3(table 4 and 5).

Table 6 Colony count of bacteria(CFU/m^3) in air atdifferent sampling time of day at different outdoor places

Sampling site (IPHH)	Sampling time		Petri dish exposure time (min)
	Day (10 a.m.)	Evening(5p.m.)	
Main gate	2138 x 10^4	2576 x 10^4	30
Classroom Balcony	1721 x 10^4	1753 x 10^4	30
Hospital Outpatient	39	45	60

Table 7Colony count of fungi(CFU/m^3)in air at different sampling time of day at different outdoor places

Sampling site (IPHH)	Sampling time		Petri dish exposure time (min)
	Day (10a.m.)	Evening(5p.m.)	
Main gate	2242 x 10^2	2358 x 10^2	30
Classroom Balcony	1728 x 10^2	1866 x 10^2	30
Hospital Outpatient	82	190	60

Table 8Range of microbial distribution in IPHH outdoor sites

	N	Minimum	Maximum	Arithmetic Mean (AM)	Standard Deviation (SD)
Bacteria CFU/m^3	12	39	2576 x 10^4	1364.6 x 10^4	920.6 x 10^4
Fungi CFU/m^3	12	82	2358 x 10^2	1366.1 x 10^2	10.8 x 10^4

In the above result (table 6, table 7 and 8) microbial load in different outdoor sites in IPHH were presented. The highest bacterial count (CFU/m^3) inair has been recorded at 5 pm in main gate at 30 min exposure, which is 2576 x 10^4CFU/m^3, while the lowest bacterial load in air (CFU/m^3) was recorded at 10 am in hospital outpatient at 60 min exposure, which is 39 CFU/m^3 (table 6 and 8).On the other hand, the highest fungal CFU/m^3 air has been recorded at 5pm in main gate at 30 min exposure, which is 2242 x 10^2 CFU/m^3, while the lowest fungal CFU/m^3 air were recorded at 10 am in hospital outpatient at 60 min exposure, which is 82 CFU/m^3 (table 7 and 8).

Figure 1. Colony of bacteria and fungi isolated from air through plate exposure method, library, IPHH

The experimental investigation carried out in different indoor and outdoor areas of Institute of Public Health and Hygiene (IPHH), Mahipalpur, in New Delhi shows presence of 7 species of bacteria and 6 species of fungi. Among which most predominant species were isolated was*Staphylococcus, Bacillus* and *Aspergillus* indoor and outdoor sample.

The study showed that fungal spores *Aspergillus* and *Penicillium* were present indoorwhich indicates that the indoor environment provides a favorable condition for the survival of fungi. The highest indoor and outdoor culturability of fungi was observing in the libraby of IPHH.

Bacteria occur in most environments; particularly in dusty, dirty places that may not be harmful to human or other animals. In this study many species of bacteria isolated from the laboratory and hospitals were found to be*Bacillus, Pseudomonas* and *Staphylococcus;* where the latter two were associated with causing opportunistic and nosocomial infection.

Concentration of fungi are usually higher outdoors than indoors in spring, summer, and fall, although in spring, the difference was significant as concentration of bacteria was lower indoors than outdoors, and indoor levels directly correlates with outdoor levels for either exposure.Microbial flora of indoor air depend on several factors, including the number and hygienic standard of people present, the quality of household system and mechanical movement within the enclose space. For both indoor and outdoor air samples, the concentration of total bacteria was higher. Theconcentration of total fungi was even higher for all the areas. The microbial load of the indoor air depends on seasonal variation, environmental conditions, and personal hygiene of the people and dampness of the rooms. Bad indoor air quality imposes health risks due to presence of these biological contaminants. In the present study among bacteria; *Bacillus spp, Staphylococcus aureus, Micrococcus spp., Streptococcus, Psuedomonas,* was predominant in air; most of which were Gram-positive cocci belongs to saprophytic microflora, generally associated to human skin and mucosal infection. The result suggests that the main bacterial contaminats suspended in the air derives from human presence. The fungalisolates include*Aspergillus spp., Penicillums pp., Cladosporium spp,* recognized as opportunistic pathogens of human.

The outdoor air was much contaminated with presence of fungi and bacteria. But during the complete lockdown, the number and counts of the pathogenic bacteria and fungi were found to be eventually decreasing. It is evident from the present study that the biological aspects of the air significantly reflect the state of the environment in which we live and function on our daily basis. Limited transportation and limited anthropogenic activity reduced the load of pathogenic fungi in air. Microbial load both bacterial and fungal was less in morning (10 am) than in evening (5 pm). Outdoor air quality was much better in the morning as the sunlight may play major role in reducing the number of bacterial and fungal cells present in air. The study revealed that all the test results were significant at $p < 0.05$.

IV. Conclusion

An assessment of bacteria and fungi in the indoor and outdoor environment were experimentally investigated. It is understood from the present environmental study that the air quality is better in early morning as less anthropogenic activity occurs during morning. The road transport affects the air quality. The road transport systems deteriorate air quality. Seems transportation occurs throughout the day, thus air quality is affected. Microbial populations in the air may be seasonal or all-year in character, although their occurrences are generally most intense in summer and autumn. Also in the daytime the sunlight reduces the number of

pathogens from air. There is essential to monitor air quality in both indoor and outdoor environments for microorganisms, in particular with regard to their effects on human health. Increased awareness of the threats posed by biological air pollution will enable greater care of the conditions in which people live every day. In particular, the proper ventilation of buildings, ventilations of all classrooms library and laboratory and prevention of excessive air humidity are all essential. In this pandemic of COVID 19, we need physical distancing, wearing a mask, keeping rooms well ventilated, avoid crowds and cleaning in our environment.

4.1 Microorganisms in the outdoor air: sources, transit, and variables determining their existence

Viruses, bacterial cells or fragments, mycelium pieces, fungal spores, and protozoa are generally found in atmospheric air. Natural sources such as soil, water, and plant surface contamination, as well as microorganisms transferred by gusts of wind, dust in raindrops, or insects, are the principal causes of microbiological air pollution. Anthropogenic sources, on the other hand, include different types of landfills, composting plants, and transportation. It is worth noting that the microbiological composition of the atmosphere is controlled not only by the source, but also by the volume of emissions, the distance from regions where emissions are higher, the kind and survival of microorganisms, their dispersal, and the weather. It is also worth noting that human activity, notably transportation, is the primary source of air pollution. Humans also contribute to the generation of biological aerosols by introducing considerable amounts of microbes into the atmosphere through sneezing, coughing, or physical exertion. Air movement is responsible for the long-distance transportation of dust particles, bacteria, and other pollutants. The makeup of airborne microorganisms is crucial in understanding how they spread and interact. Natural physical phenomena such as mutual attraction remove pollen, microorganisms, and mineral dust particles from the atmosphere. Bacterial cells dispersed by dust are vulnerable to a variety of environmental conditions, including temperature, humidity, atmospheric pressure, wind speed and direction, and the intensity of solar radiation. Changes in the weather may also have an impact. It is worth noting that the presence of a living bacterial cell in the air can be disseminated over large distances.

4.2 Microorganisms found in indoor air: sources, transit, and variables determining their existence

Biological contaminants in direct interaction with humans in residential structures may have an adverse effect on human health. Indoor air quality has a greater impact on people's well-being and health than outdoor air quality because of the amount of time spent in residential buildings. Increased bio-pollution is a result of 21st century construction approaches such as the installation of airtight windows and inadequate ventilation systems.

Microorganisms in the air, in residences are most often detected in dust, which has become a significant research topic for evaluating indoor air quality. Dust is primarily distinguished by the presence of chemical molecules, trace elements, and mite metabolites, and is the source of many hypersensitivity reactions and allergies. The presence of microbe; their numbers depend on the humidity of the air. The presence of potentially health-hazardous metabolites such as PAHs is detrimental to human health. PAHs along with a wide variety of chemical and biological contaminants pollute the indoor air. These pollutants of air are confined in spaces indoor. It is very much necessary to detect these chemical and biological pollutants of air. As understanding and controlling these pollutants of indoor air helps to reduce the risk associated with indoor health concerns.

The number of microorganisms is significantly greater in indoor spaces with a relatively dense population than in open areas; microbes, especially harmful microorganisms, are more abundant in such places. Talking, coughing, and sneezing all release bacteria from the human respiratory system into the airways. They may enter the air via scaling and clothing, therefore the amount of bacteria with in air is dependent on the number of people in the room and the ventilation system's efficiency.

Although the pandemic affected human life but it is evident from the present study that the prolonged lockdown has somewhere decreased the microbial load in air compared to the pre-COVID era, 2018 and 2019.

V. Limitation Of The Present Research

In this method only the rate of deposition of the total number of bacteria and fungi carrying particles per volume, was measured. Growth of bacteria in the settled particles may be affected by the medium used since not all microorganisms are growing well on all media. Moreover since air currents and any temporary disturbances in the sampling area can affect the count.

Since only particles of certain dimensions tend to settled on to the agar surface and, also, the volume of air entering inside the petri dish is not known, this technique gives only a rough estimate and can be used only to isolate airborne microorganisms.However, one can gather information about the kind of airborne microbes occurring in a particular area by repeated used of settle plate technique for a fixed period of time.The use of settle plates is not recommended when sampling air for fungal spores, because single spores can remain suspended in air indefinitely. Settle plates have been used mainly to sample for particulates and bacteria either in research studies or during epidemiology investigations.

Results of sedimentation sampling are typically expressed as numbers of viable particles or viable bacteria per unit area per the duration of sampling time; this method cannot quantify the volume of air sampled. Because the survival of microorganisms during air sampling is inversely proportional to the velocity at which the air is taken into the sampler, one advantage of using a settle plate is its reliance on gravity to bring organisms and particles into the contact with its surface, thus enhancing the potential for optimal survival of collected organisms. Thus process, however, takes several hours to complete and may be unfeasible for some situations.

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