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AIR QUALITY ASSESSMENT IN SENGAMALA THAYAAR EDUCATIONAL TRUST WOMEN'S COLLEGE (AUTONOMOUS) SUNDARAKKOTAI, MANNARGUDI

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ABSTRACT;

Airborne transmission occurs when pathogenic microorganisms are transferred from an infected source to a susceptible individual through the air. The present investigation was conducted to study the microorganisms present in the air surrounding the four building blocks of S.T.E.T Women's College (A), Sundarakkottai, Mannargudi, Thiruvarur District, Tamil Nadu, India.

The collected air samples were processed for the isolation of microorganisms. The bacterial colonies obtained were subjected to motility tests, Gram staining, and various biochemical tests for identification. A total of 11 bacterial isolates and 5 fungal isolates were identified. The bacterial species isolated included *Escherichia coli (E. coli), Streptococcus* spp., *Staphylococcus* spp., and *Bacillus* spp., which were confirmed using Bergey's Manual of Systematic Bacteriology. The fungal isolates were identified based on their morphological characteristics and confirmed using Gillman's Fungi Manual. The fungal species included *Candida* spp., *Aspergillus* spp., and *Saccharomyces* spp.

Bacterial growth was observed to be higher compared to the relatively slower growth of fungi. Among the different sampling locations, Block 1 showed a higher bacterial population than the other blocks. This may be attributed to laboratory exposure and a crowded environment.
Additionally, *Aspergillus* species showed higher growth in outdoor environments. This suggests that areas with high human activity and visitor movement are more prone to increased microbial contamination and associated health risks.

KEYWORDS; Air,*E.oil,Streptococcus,Aspergillus,* Bergey's manual,Gillman Fungi manual.

INTRODUCTION

AIR is the earth's atmosphere .Air is a mixture of many gasses of many gasses and tiny dust particle.it is the clean gas in which living things live and breathe .it has an indefinite shape and volume .it has mass and

weight,because it is matter .The weight of air creates atmosphere pressure.There is no air in outer space.(Hwang *et.al.,2017)*Atmosphere is a mixture of about 78% Nitrogen,21% Oxygen,and 1% Other gasses,such as carbon dioxide.(Martin *et.al*..,2016) Air can be polluted by some gasses (such as carbon dioxide,hydrocarbon and nitrogen oxide),smoke and ash.

This air pollution causes various problem including smog,acid rain and global warming.it can damage people's health and the environmentAir is invisible it cannot be seen by the eye.(Zahnle;Schaefer;Ferry *et.al*..,2010)

OUT DOOR

Outdoor air pollution is the contamination of air due to the presence of substancesin the atmosphere that are harmful to the health of humans and other living beings or cause damage to the climate or to material.outdoor air pollution is contamination occurring outside a building.(Hinton .,*et.al 1983*)Exposure To outdoor air pollutant ccurs virtuallycontinuously.Although most of the more abundant pollutants are known and can be measured, many trace species have not been identified,much less quantified routinely.(Haas ,*et.al*..,2014.

INDOOR

Indoor air pollution means contamination of air quality within and around buildings and structures.Some of the most common examples of indoor air pollutants are solvents, moulds,smoke,pesticides,gases and pet dander.(Afzali.;*et.al..,2015)*

THE BIOAEROSOLS

Bioaerosols are the atmospheric particles,mists or dust of um range,associated metabolically active and/or inactive viable particles.(Dimmic.,*et.al.,1969)*Bioaerosols vary considerably . in size and composition depends on a variety of factors including the type of microorganism or toxic,the type of particles they are associated with such a mist or dust and the gasses in which the bioaerosol is suspended.The smaller particles <0.1um in diameter are consider to be in the nuclei mode ,those ranging from 0.1 to 2um are in the accumulation mode and large particles are considered to be in the coarse mode.(Brooks *et.al..,2004)*

DISEASE CAUSED BY POLLUTION

Air pollution leads to environmental damage, but it can also harm the health of people.some of the most common ailments in the world are diseases caused by air pollution .The air pollutants that cause disease include what the EPA has categorized " Criteria Air Pollutants".(Hugh-Jones *et.al.,1967)*These are pollutants that are most abundant in the atmosphere,and consequently,they do the damage to human health.Hence the present study was planned to assessment of air quality in STET Women's College (A),Sundarakottai,Mannargudi.

MATERIALS AND METHODS

SAMPLE COLLECTION

The air samples were collected from 4 different Blocks of our college Sengamala Thayaar Educational Trust Women's college (Autonomous) Sundarakottai, Mannargudi, Thiruvarur district,Tamilnadu,India.The sampling sites are named as,
BLOCK 1 ,BLOCK 2,
BLOCK 3,BLOCK 4:

Block 1

Block-1: has 3 floors (i.e) Ground, First, Second, Third floor and each floor has 3 wings (A,B,C). Air samples were collected from this block. The first air sample was collected on the Ground floor followed by placing 3 plates in 3 wings (A,B,C) of the First floor to gather microbes from the air. The same procedure was followed for the Second and Third floor which has 3 wings of A,B,C.

Block-2:

It has 3 floors such as Ground, First and Second floor. The next 3 samples were collected from this block. These three plates with Nutrient agar and Potato dextrose agar were exposed for air sampling over 2-3 mins at each floor.

Block-3:

It has 4 floors viz., Ground, First, Second and Third floor. Totally 4 samples were collected from this block. These 4 samples were collected from each floor by exposing the plates with the suitable media like Nutrient agar and Potato dextrose agar.

Block-4:

3 site samples were collected from the 3 floors (i.e) Ground, First, Second floor of the T.V.S block by using Open plate method. Air samples were taken twice a day in the morning and afternoon. These 22 samples were collected in pre-labelled Petri dishes containing culture media which is suitable for the growth of Bacteria and Fungi. These Petri dishes were incubated for the Microbial Analysis.

MEDIA USED:

Nutrient agar(NA),MacConkey agar and Blood agar medium was used for Isolation of Bacteria and Potato dextrose agar(PDA)medium was used for Isolation of Fungi.Both the Medium were prepared and sterilized.Those medium were poured onto a sterilized and pre labeled petri plates and allowed to solidify.The petri plates were maintained in an aseptic conditions.

ISOLATION AND IDENTIFICATION OF BACTERIA:

Air samples were collected by using open plate method,exposed for 2-3 mins and incubated for 24-48hrs. After incubation period,the isolated colonies were obtained and identified up to species level.All Bacterial isolates were identified based on their colony characteristics,morphological characteristics and biochemical reactions as per Bergy'sManual of systematic Bacteriology.

ISOLATION AND IDENTIFICATION OF FUNGI AND USING ANDERSON AIR SAMPLER:

Air samples were collected by using open plate method,exposed for 2-3 mins and incubated for 3-5 days. After incubation period,all fungal isolates were identified based on their Macroscopic and Microscopic appearance.

MORPHOLOGICAL CHARACTERISTICS:

Gram Staining Technique: (Gillman, 1957)

A thin smear of bacterial isolates were separately made on a clean glass slide and heat fixed. Then the smear was stained by crystal violet for one minute and then washed with water and flooded with Gram's iodine. After one minute, the slide was washed again with tap water and decolorized with alcohol. After decolorization the smear was counter stained with safranin for one minute.

The slide was washed and dried. Finally it was observed under a microscope.

Motility test: (Bailey and Scott, 1966)

A ring of petroleum jelly was applied around the concavity of the depression slide. A loopful of isolates was placed separately in the center of a clean coverslip by using sterile technique. The Depression slide was placed with the concave surface facing down over the cover slip and pressed gently to form a seal between the slide and cover slip.

Then the slide was quickly turned upside down. So, the drop continues to adhere to the inner surface of

the cover slips. Then the slide was observed through the oil immersion microscope.

Lactophenol cotton blue (Lpcb) Staining Technique: (Smith, 1970) A drop of lactophenol cotton blue was placed on a glass slide. A small tuft of the fungus properly with spores and bearing structure was transferred into the drop using an inoculation needle. The material was tested using the two inoculation needles. The stain was mixed with the fungi. A cover slip was placed over the preparation, taking care to avoid trapping air bubbles in the stain. The slide was observed under the Microscope to examine the individual Fungal species and were identified.

BIOCHEMICAL CHARACTERISTICS: (Norris and Ribbons, 1972) Indole test Tryptone broth was prepared by mixing 10g of peptone in 100 ml of distilled water. The pH of the test medium was adjusted to 7.3 and sterilized. The test tubes containing tryptone broth were inoculated with isolates separately and uninoculated broth was maintained as control. Inoculated and uninoculated tubes were maintained at 35°C for 48 hrs. 1ml of Kovac's reagent was added to each tube, including control. The tubes were gently shaken at an interval of 10-15 minutes and allowed to stand until the reagent reached the top. Cherry red ring was formed that indicates indole positivity. Absence of red color ring formation is considered as a negative result.

Methyl red test

MR-VP broth was prepared by mixing peptone-7.0g dextrose-5.0g and potassium phosphate-5.0g in 1000 ml distilled water. Then PH of the medium was adjusted to 6.95 ml of the broth and poured into each tube and sterilized MR-VP tubes were inoculated with the isolates separately and control was maintained. All the tubes were inoculated at 28±2°Cfor 48 hrs. After 48 hrs, a few drops of methyl red indicator were added to each tube, red color was observed which indicates positive result. If it remains yellow, it is considered a negative result.

Voges - Proskauer test

5ml of MR-VP broth was poured into different tubes and sterilized. The uninoculated tubes were maintained as control. All the tubes were incubated at 28±2°C for 48hrs. A few drops of Barritt's reagent was added to each tube and observed for the appearance of deep rose color. If the tube remains yellow, it is considered a negative result.

Citrate utilization test

Simmon's citrate agar slants were prepared by mixing ammonium dihydrogen phosphate-1.0g, Sodium chloride-5.0g, magnesium sulfate-2.0g. bromothymol blue-0.08g and agar-15.0g in 1000 ml of distilled water. The slants were inoculated with the isolates and incubated at 28±2°C for 48hrs and the color change was observed from green to blue. Otherwise it is considered a negative result.

Oxidase test

Trypticase soy agar plates were prepared, and single line streak inoculated bacterial isolates were made separately on the agar surface and incubated at 28±2°C for 24 hrs. Then 2 or 3 drops of para amino dimethyl aniline oxalate were added to the surface of the inoculated plates and observed for the color change. Otherwise it is considered a negative result.

Triple Sugar Iron Test

Triple sugar iron test was performed to identify the ability of organisms to utilize sugar like glucose, lactose and sucrose with the production of acid or alkali end products with or without production. TSI agar medium was dispersed in a test tube and sterilized. After sterilization, the slants were prepared in a test tube. The slants were inoculated by means of stab and streak. The test tubes were incubated for 24 to 48 hours at 37°C.After incubation, the tubes were observed for the color change in the slant and butt and for the production of gas. The yellow color of the medium indicates acid only. The pink color of the medium indicates the alkaline end products. The pink

colouration of butt indicates the production of a sugar amount of acid, which gets oxidized in later hours. The H_2S gas production was identified by black precipitation in the medium.

Urease test

Urea agar medium was prepared by mixing peptone - 10g, sodium chloride - 5.0g, and Potassium - 2.0g. The pH of the medium was adjusted to 6.8 before adding agar, and sterilized. The medium was poured into the sterile Petri plates and allowed to solidify. The Petri plates were then inoculated with the isolates and incubated at 28+2°C for 28 hrs and a deep pink color was observed. No color formation indicates negative results.

Catalase test

Bacterial isolates were picked up aseptically from the SLA t and a smear was made on a clean glass slide. Then a drop of hydrogen peroxide was placed on the slide with bacterial culture and observed for the production of a gas bubble. If there is no bubble formation, that indicates a negative result.

STATISTICAL ANALYSIS:

1.Mean was calculated to facilitate the comparison of the data of various growth parameters in All samples (Salil Bose, 1982)

2.Limits of mean to the probability of deviation of the sample mean to the probability of Deviation from the population of mean.

3. In other words, the sample mean is to be related to the population mean of a confidence Interval.

4.In most biological studies 95 % significant is the acceptable level of accuracy. While Showing limits of a mean one should attach the level of significance to the result analyzed.

5. The formula for calculating standard deviation is $SD = \Sigma(X-X)2/N-1$

RESULTS AND DISCUSSION:

Air pollution caused by airborne microorganisms poses a significant threat to human health. Hence, the present investigation was undertaken to assess microbial air contamination in four blocks of Sengamala Thayaar Educational Trust Women's College,(A) Sundarakkottai, Mannargudi, Thiruvarur District, Tamil Nadu, India.

Air samples were collected twice daily, during the morning and afternoon sessions. Both bacterial and fungal groups were isolated and identified using standard microbiological techniques. It was observed that environmental microorganisms generally predominated, with significantly higher microbial counts detected in areas where hygiene levels and housing standards were relatively poor.

A total of four blocks were screened. The bacterial species isolated included *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli (E. coli)*, *Proteus* spp., *Pseudomonas* spp., *Klebsiella* spp., *Acinetobacter* spp., and *Citrobacter* spp. The fungal species identified were *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., *Mucor* spp., *Fusarium* spp., *Saccharomyces* spp., and *Candida* spp.

Assessment of outdoor airborne microorganisms was carried out using the open plate (settle plate) method.

Isolation and Identification of Microorganisms

The colony counts of bacteria and fungi obtained after a plate exposure time of 2–3 minutes were expressed as CFU/m^3. Various types of bacterial and fungal species were detected from the air samples collected during the study period. The suspected bacterial colonies were identified based on colony morphology and confirmed through biochemical tests. Fungal colonies were identified using staining techniques and microscopic analysis.

Block 1

Block 1 consisted of four floors, namely the ground, first, second, and third floors, each having three wings (A, B, and C). On the ground floor, three plates containing Nutrient Agar, Blood Agar, MacConkey Agar, Potato Dextrose Agar, along with control plates, were exposed. A total of 2 bacterial and 3 fungal species were isolated, including *Streptococcus* spp., *Staphylococcus* spp., *Aspergillus* spp., and *Candida* spp.

In the upper three floors (first, second, and third), nine plates were exposed across the A, B, and C wings. The results showed:

- First floor: 4 bacterial species
- Second floor: 3 bacterial species and 1 fungal species
- Third floor: 4 bacterial species and 4 fungal species

Block 2

Block 2 consisted of three floors: ground, first, and second floors. Air samples were collected from each floor using three plates exposed for 2–3 minutes with suitable media. A total of 3 bacterial and 3 fungal species were isolated and identified.

Block 3

In Block 3, air samples were collected from all four floors (ground, first, second, and third floors). Plates containing Nutrient Agar and Potato Dextrose Agar were used. The findings were:

- Ground floor: 2 bacterial species and 1 fungal species
- First floor: 1 bacterial species and 1 fungal species
- Second floor: 1 bacterial species and 2 fungal species

Block 4

Block 4 consisted of three floors: ground, first, and second floors. Three air samples were collected from each floor using exposed plates. A total of 3 bacterial and 3 fungal species were isolated and identified.

Overall Observation

The results indicated that Block 1 had the highest bacterial and fungal population compared to Blocks 2, 3, and 4. This increased microbial load may be attributed to laboratory activities and a crowded environment within the block.

CONCLUSION

Further studies are required to analyze indoor air contamination and to statistically compare the results with outdoor air samples. The concentration of airborne microorganisms varies not only across different seasons but also throughout the course of a single day.

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TABLE;1 CHARACTERISTICS OF SAMPLING SITES

BLOCKS	NUMBER OF PETRI DISHES EXPLORED (BACTERIA)	NUMBER OF PETRI DISHES EXPLORED (FUNGI)
Vivekananda block	12	12
V.V.block	9	9
G.N.block	12	12
T.V.S block	9	9

TABLE 2: DETAILS OF ASSESSMENT OF AIR QUALITY

Block	Time schedules	Total number bacterial (cfu/m²)	Total of filamentous fungi (cfu/m²)
Vivekananda block	MORNING	6.5×10^2	7.8×10^2
	AFTERNOON	5.2×10^2	8.0×10^2
V.V block	MORNING	3.2×10^2	3.9×10^2

	AFTERNOON	2.6×10^{2}	2.6×10^{2}
G.N block	MORNING	1.0×10^{3}	1.3×10^{2}
	AFTERNOON	1.4×10^{3}	1.1×10^{2}
T.V.S block	MORNING	2.3×10^{3}	1.0×10^{3}
	AFTERNOON	2.6×10^{3}	1.6×10^{2}

TABLE 3:STATISTICAL ANALYSIS OF THE SAMPLING SITES

SAMPLES	**BACTERIA**
BLOCK-1	78.86±12.12
BLOCK-2	44±13.11
BLOCK-3	60.62±16.37
BLOCK-4	54±10.58

Values are represented as Mean Standard Deviation