

Qualitative Assay of Indoor Air Using an Air Oasis Air Purifier with Special Reference to the Reduction of Aero Allergens and Pollutants



Nabarun Ghosh Ph.D.

Associate Professor of Biology

Department of Life, Earth and Environmental Sciences

West Texas A&M University, Canyon, Texas 79016

Amanda Whiteside

Research Associate

Rupa M. Patel

Research Associate

Lisa Dyer

Research Associate

Introduction

Aeroallergens are often the cause of serious allergic and asthmatic reactions, affecting millions of people each year. To aid to the diagnosis and treatment and reduce the ailments of these individuals suffering from various indoor aeroallergens, a thorough understanding and control of the indoor aeroallergens and other airborne particles are necessary. Weather conditions and diurnal cycles play an integral role in the passive and active discharge of spore and pollen. Warm dry weather conditions promote passive dispersal of dry air spora, including *Alternaria*, *Cladosporium*, *Curvularia*, *Pithomyces* and many smut teliospores. Diurnal levels of these spores usually have peaks during the afternoon hours under conditions of low humidity and maximum wind speeds. Moist weather conditions promote the active dispersal of moist air spora, such as the explosive release of ascospores from Ascomycetes, and the expulsion of basidiospores from the gills of the Basidiomycetes.

Pollen grains are released by the flowering plants following the particular season for pollen production specific for each plant species. As we recorded for the last five years the most significant aeroallergens of the Texas Panhandle were fungal spores from *Alternaria*, ascospores from Pezizales, *Dreschlera*, *Cladosporium*, *Curvularia*, and pollen from short ragweed (*Ambrosia artemisiifolia*), grass pollen (Poaceae) common sunflower (*Helianthus annuus*), hairy sunflower (*Helianthus hirsutus*), buffalo bur (*Solanum rostratum*), purple nightshade (*Solanum elaeagnifolium*) and lamb's quarters (*Chenopodium album*). Offices, working places and schools face the challenge of an increasing number of workers and children with pre-existing health conditions which are affected by the Indoor Air Quality and other environmental factors. Indoor Air Quality (IAQ) in offices is an important aspect. Indoor levels of air pollutants can be 2-5 times higher, and occasionally 100 times higher, than outdoor levels. Surprisingly, nearly 55 million people, 20 percent of the U.S. population, spend their days inside offices and schools. An estimated 50 percent of the nation's schools have problems linked to Indoor Air Quality (Ref. 1). The neglect of IAQ can cause or contribute to short and long-term health problems including asthma, respiratory tract infection and disease, allergic reactions, headaches, nasal congestion, eye and skin irritations, coughing, sneezing, fatigue, dizziness and nausea. Not only does poor indoor air quality contribute to an unhealthy environment; it also hastens building deterioration. One study on an elementary school showed that if \$8,140 had been spent over 22 years on preventative maintenance, \$1.5 million in repairs could have been avoided by the use of a proper monitoring of aeroallergens and air purifying system (Ref. 2). In order to prevent a risky working environment the employers must be appropriately educated about indoor air quality.

Implementation of the Strategies and Discussion:

The quality of the environment within buildings is a topic of major importance for public health. (Ref. 3). Presently Indoor Air Quality (IAQ) is a major concern at various work places. I am quoting an important quote on air quality from the journal: “With all the publicity, more and more people are realizing that pollutants in the indoor air could make them sick. The worst thing that has happened to the indoor air quality marketplace in the last year or so is also mold. This is because much of the media coverage is designed to sensationalize the topic and frighten the public - so much so, that the word ‘mold’ always seems to be preceded by the adjective ‘toxic.’ Thus, homeowners and building managers are scared to death of any minor infestation that might possibly be toxic mold, and they often ignore other health issues, such as combustion byproducts, VOCs, second-hand tobacco smoke and poor ventilation.” (Ref. 4)

When a mold can be seen or smelled, it poses a concern. A building with leakage or moisture problems contributes to flourishing live mold spores. Once a mold has established itself, it may increase quickly and colonies can spread if enough moisture is available. Therefore moisture control is an important aspect to preventing and controlling mold problems. However, if an office space or residential buildings encounter a mold problem on the premises it should be able to demonstrate that the moisture problem is being removed using effective methods to restore the sight to a clean condition. Although all buildings contain some mold, as do our homes, offices, and the outdoor air, becoming indoor air quality conscious will help in the prevention (Ref. 5).

Qualitative Assay of the Fungal Aero Allergens (Molds) after using an Air Oasis air purifier

While the fungal exposure assessment was based on the determination of fungal propagules for a long time, recent progress has led to the development of methodology for other fungal agents, e.g. the fungal cell wall components, metabolites, and allergens that may be responsible for health effects caused by fungal exposure. This proposal includes a summary of the sampling techniques and analytical methods that are currently used or are in progress for the fungal exposure assessment. (Ref. 6). The first phase of this study is aimed at analyzing the effect of an Air Oasis Air Purifier the spore and pollen counts of allergenic taxa in the indoor air randomly sampled from different office facilities and residential buildings. Sampling will be done using a standard spore trap (Burkard Corporation). Petroleum jelly emulsion was applied to the tapes to capture the allergens. This study will cover observations on the effect of an ionizer/air purifier in reduction of indoor aero allergens including mold spores, pollen, dust mites, cat and dog dander, dust mites and microbial flora.

We shall use digital and fluorescence microscopy to quantify the aeroallergens present in a particular location. We shall also assess the air quality by screening microbial colonies and analyzing the gaseous composition of a room by the using of a Gas Chromatography (GC) Mass Spec.

We shall focus on a target aeroallergen and compare the composition and concentration of each of them before and after the usage of the Air Oasis Air Purifier at 2hours, 8 hours, 24 hours and 72 hours intervals at a particular location.

We shall compare the composition and concentration of each of the following target aeroallergens before and after the usage of the Air Oasis Air Purifier:

1. Mold spores, mycelia, hyphae or other fungal bodies present in the indoor air.
2. Pollen from various species of flowering plants present in the indoor air.
3. Microbial composition in the indoor air focusing on particular specie: *Staphylococcus aureus*, *E. coli* and *Streptococcus*.
4. Burnt residues, gums, resinous droplets and plant fibers in the indoor air.
5. Dust mites, insect droppings, other insect residues and animal dander (cats and dogs namely).

This project will be carried out in 4 phases.

Phase I: In the first phase we will standardize the procedure to analyze and compare the mold spores, mycelia, hyphae or other fungal bodies present in the indoor air. We will also cover the comparative analysis of the other aeroallergen like pollen.

Phase II: In the second phase we will compare the concentration of bacteria described above (#3) before and after using the Air Oasis air purifier.

Phase III: In the third phase we will cover the comparative observations on burnt residues, gums, resinous droplets and plant fibers in the indoor air. We will also cover the study on dust mites, insect droppings, other insect residues and animal dander.

Phase IV: We shall compare the gaseous composition of the indoor air before and after the exposure with the Air Oasis air purifier with the special focus on volatile organic compounds, Carbon disulfide, d-Limonene Toulene, Hydrogen Sulfide, Benzene, Formaldehyde and Ammonia. Bioaerosol sampling will be done following standard techniques (Ref. 7) and by the using of the Gas Chromatography facility at Dr. David Parker's Lab. All the data will be analyzed and statistical analysis will be done after comparing the data from every phase. Graphs will be erected and data will be used for possible publication. Data from every phase will be provided to Air Oasis time to time after the completion of every phase.

Report on Phase I: Fall 2006

We have divided this research projects in four different phases. The first phase of research was aimed at evaluating the Air Oasis Air Purifier and assesses its efficiency in reducing the indoor aeroallergens and air-microflora, especially the bacteria and mold spore concentration in the indoor air. The first phase research was divided into two groups: 1.Observation on the effect of the air purifier in reducing the concentration of bacteria and mold in the air,

2. Observation on the effect of the air purifier in reducing the concentration of aeroallergens like pollen, spores and other particulate matters concentration in the air.

The first part was carried out by Rupa M. Patel under my supervision (Dr. N. Ghosh).

Report by Rupa M. Patel: Air Oasis Air Purifier on Microbial colonies

To evaluate the Air Oasis Air Purifier we set up the following criteria and variables.

Criteria: Evaluation of the Air Oasis Air Purifier using petri-plates and coated slides to collect the microbial spores, propagules (like fungal hyphae) and aeroallergens (like pollen, spores and other particulate matters) with a standard distance of 1foot, 2 feet, 4 feet, 6 feet and 12 feet away from the air purifier. The petri-plates and slides were previously made before setting up the experiment. The slides were coated and placed in clean slide boxes and the boxes were sealed with parafilm to avoid any contamination. The petri-plates were made following standard aseptic procedure by autoclaving the media at 15lb steam pressure /sq Inch at 121 degree Celsius. After pouring the media the petri-plates were stored on the table top to cool down and then stored in the refrigerator after sealing the plates with parafilm.

Variables:

Distance: A number of sets of the petri plates and coated slides were placed with those distances of 1foot, 2 feet, 4 feet, 6 feet and 12 feet away from the air purifier with various time intervals.

Time Period:

Control (exposure 0 hours): Assay done with the petri plates and coated slides keeping the air purifier off.

24 hours: Assay done with the petri plates and coated slides after running the air purifier for 24 hours in the room.

48 hours: Assay done with the petri plates and coated slides after running the air purifier for 48 hours in the room.

72 hours: Assay done with the petri plates and coated slides after running the air purifier for 72 hours in the room.

Space:

Size of the room: 15 ft x 25 ft (Room 317)

8 ft x 15 ft (Room 336)

Air purifier setting

At Low

At High

The Air Oasis Air Purifier was evaluated keeping it on a table top and placing the petri-plates prepared from Brain Heart Infusion agar. The Air Oasis Air Purifier was placed on a table in a large laboratory room (15 ft x 25 ft), bacterial and mold samples were obtained from Brain Heart Infusion media plates. The petri- plates were set surrounding the air purifier and assayed after no exposure (Control), after 24 hours and 48 hour exposure of the room air to the air purifier. All of the plates were set at the distances of 1 foot, 2 feet, 4 feet, 6 feet and 12 feet away from the base of the air purifier and assayed after various time intervals of 24 hours and 48 hours. The air purifier was also tested at High and low settings. The control plates and the plates exposed to the high setting of the air purifier were analyzed using a SZ-40 Olympus Stereo Microscope. The bacterial and mold specimens were further identified by Gram staining and Lacto-Phenol-Cotton-Blue Staining techniques for size, shape, and morphology. Samples were examined, counted and photographed every 24 and 48 hours using a BX-40 Olympus microscope attached to a DP-70 Olympus Digital Camera devised with Image Pro-6.0 software. Data were correlated with the distance, time of exposure to find the differences in bacteria and mold population between room air treated with and without the air purifier. We found significant differences in microbial spore population in the room air before and after the treatment with Air Purifier 3000 at different intervals.

Report from Mandy Whiteside: Air Oasis Air Purifier on Aeroallergen

Evaluation of an Air Oasis Air Purifier by Mandy Whiteside

For this project we had to first standardize our technique. We did this by setting guidelines on what we wanted to accomplish with this project. We first wanted to setup slides at specific distances. So far we have evaluated in close proximity to the Air Oasis air purifier. We setup slides with distances of 1 foot, 2 feet, 4 feet, 6 feet and 12 feet away from the air purifier in 4 directions. Our ultimate goal is to get samples all the way up to 12 ft.

For slide preparation we use standard microscope slides 75x25. We used scotch brand double sided sticky tape cut to a length of 2 inches which is the length of the cover slip. The tape is taken off the roll using forceps and a needle to avoid the contamination from the oil from our hands. To stain aeroallergens, we applied safranin gelatin to the cover slip and then placed the cover slip on top of the tape.

On our first trial was for our control group, these slides were setup at the predetermined distances from the air purifier. This first trial was setup for 24 hours. The results were very discouraging because there were very little aeroallergens on some slides to nothing on others. We decided that we needed to add some extra adhesion glue to the sticky tape. We thought that there was probably not enough glue for the aeroallergens to get stuck to. There are two types of glue that we tried the first was an acid free Ross Stick washable glue, and the other was Beckman's Silicone Vacuum Grease. We set up another trial for 24 hours to test which of these two glues was better. Half of the slides were setup with the Ross Stick glue and the other half were setup with the Beckman's Silicone Vacuum Grease. We then compared the results of the two glues. Our conclusion was that the Beckman's Grease trapped more aeroallergens than the Ross glue.

We then set up our control groups for 24 hours, 48 hours, and 72 hours. We compared the results of each. We established that the most aeroallergens were on the 72 hour slides, followed by the 48 hour slides, and the slide with the least amount of aeroallergens were the 24hour slides.

After evaluation of our control group we setup for our trail groups. We first wanted to evaluate the low setting on the air purifier. Again we set the slides up at the predetermined distances for 24, 48, and 72 hours. The results were after 24 hours there was little change in the aeroallergen count. There was more improvement after 48 hours; we found that there were less of the aeroallergens on these slides than in the control group. The best results were found on the 72 hour slides, we found that there was the most improvement on these slides. There was least concentration of aeroallergens on these slides than on the 72 hour control group.

We next wanted to evaluate the Air Oasis air purifier at the high setting. As before, we setup the slides at the predetermined distances for 24, 48 and 72 hours. The 24 hour trial we found less aeroallergen than both the control group and the low setting group. The 48 hour trail at the high setting, the results were also that there were less aeroallergen than the control and the low setting. Finally, we found that there were very little aeroallergens at all.

Our conclusion from the results that we found is that the Air Oasis air purifier works best over a longer period of time and at the high setting.

Procedure for the preparation of microscopic slides:

A clean microscopic slide is coated with a thin layer of water. The segment of tape is then laid upon the microscopic slide with the impregnated surface using forceps so that the long edges of the tape are parallel with the long edges of the slide. Positioning should be such that viewing of the entire slide may be accomplished from subsequent traverses of the slide. It is essential that the sides of the tape be mounted parallel to the sides of the slide so that traverses are to be truly traversed with the microscope. The use of Gelvatol is required to secure the cover slip in place. This compound is applied with a glass rod and is a permanent mountant. Gelvatol is composed of 35 g Gelvatol powder (Burkard Manufacturing Co Ltd., UK), 50 ml Glycerol, 100 ml distilled water, and 2 g phenol. We prepared Gelvatol by mixing the Gelvatol powder and phenol in water allowing sitting overnight. The mixture of phenol and Gelvatol was placed on a water bath (65°C) and glycerol and distilled water was added to it slowly while stirring produced the proper emulsion. Upon distribution of the Gelvatol on the cover slip, a drop of Safranin O (Sigma Cat no. 84120, Fluka, Microscopy Grade) was placed upon the cover-slip and stirred evenly, when set on the slide it stained the pollen and spores facilitating the observation with a microscope. Safranin was prepared beforehand and stored in a glass bottle. We found 1% Safranin was very effective stain in viewing pollen, including their cell walls and colpi (pores). The stain was comprised of 1.0 grams of Safranin O powder dissolved in a 50:50 mixture of 95% ethanol and distilled water. These components were dissolved in a round flask and were allowed to boil for 15-20 minutes. After cooling to the room temperature, this solution was filtered through Whatman's filter paper (Grade No. 43 Cat. no. 28481-302, 12.5 cm in diameter, VWR) into a second round flask. After filtering, the solution was transferred to glass vessels for long-term storage at room temperature.

Microscopic analysis of collected allergens:

Sample tapes from the spore trap will be analyzed regularly after the collection and transfer of the pollen

pollen sampling tape take place at suitable intervals. Slides will be analyzed with five latitudinal traverses corresponding to the mean concentration will be assessed. The mean concentration will be determined mathematically by taking a sum total of all the prepared slides and multiplying this sum by a correction factor. Correction factors are microscope-objective specific and are determined prior to counting. It can be expressed as the total area sampled divided by the graticule width (8).

The prepared slides will be examined, counted, and photographed using a BX-40 Olympus microscope attached to a DP-70 Digital Camera attached to a Dell Computer equipped with Image Pro 6.0 Image Analysis software. This assessment will involve the optical counting of pollen grains, fungal spores and other particulate matters through a microscope and the use of a micrometer scale and graticule (100 square microns). The graticule is a scale that measures distances to the 10^{-2} division of a millimeter. The graticule was calibrated using a stage micrometer. The pollen, fungal spores and insect residues will be identified using standard keys from literature and the websites (Ref. 9, 10, 11, 12 and 13).

Result and Discussions

Figure 1 Control ((No treatment with air purifier)

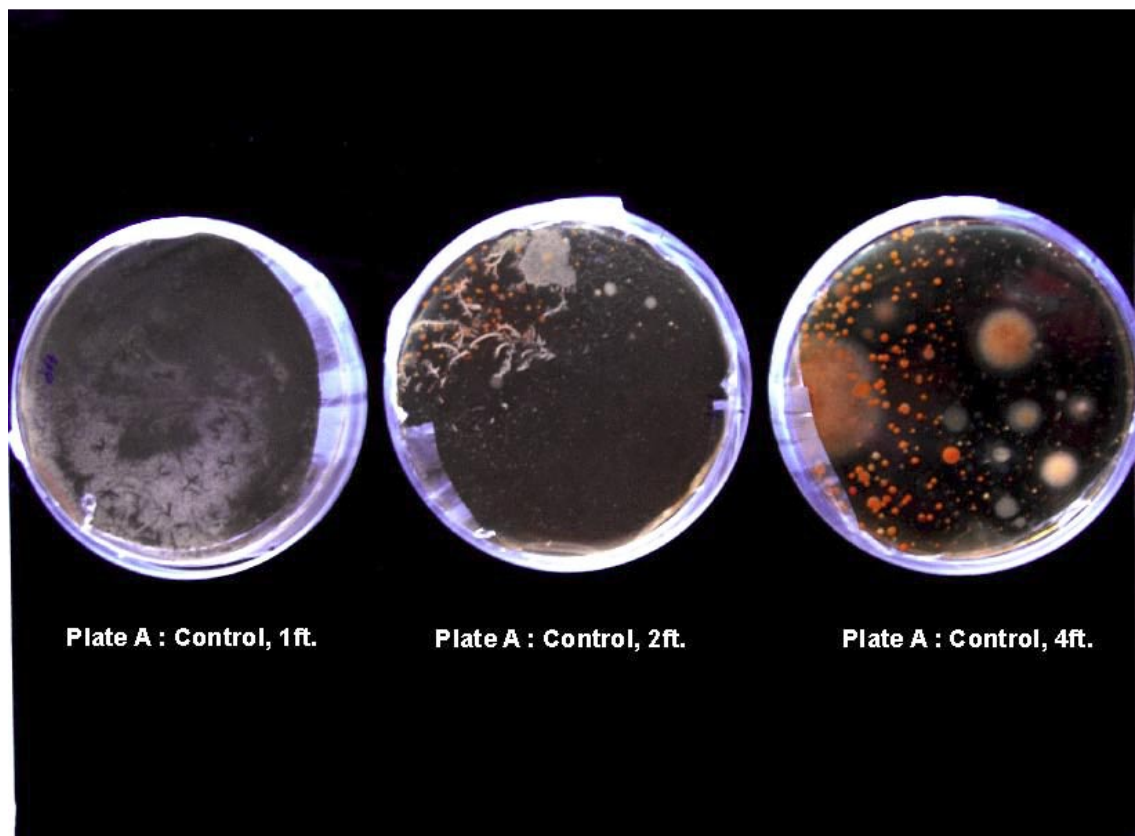


Figure-1 shows the number of microbial colonies produced in the Control set, before any treatment of indoor air with the air purifier.

Figure - 1A

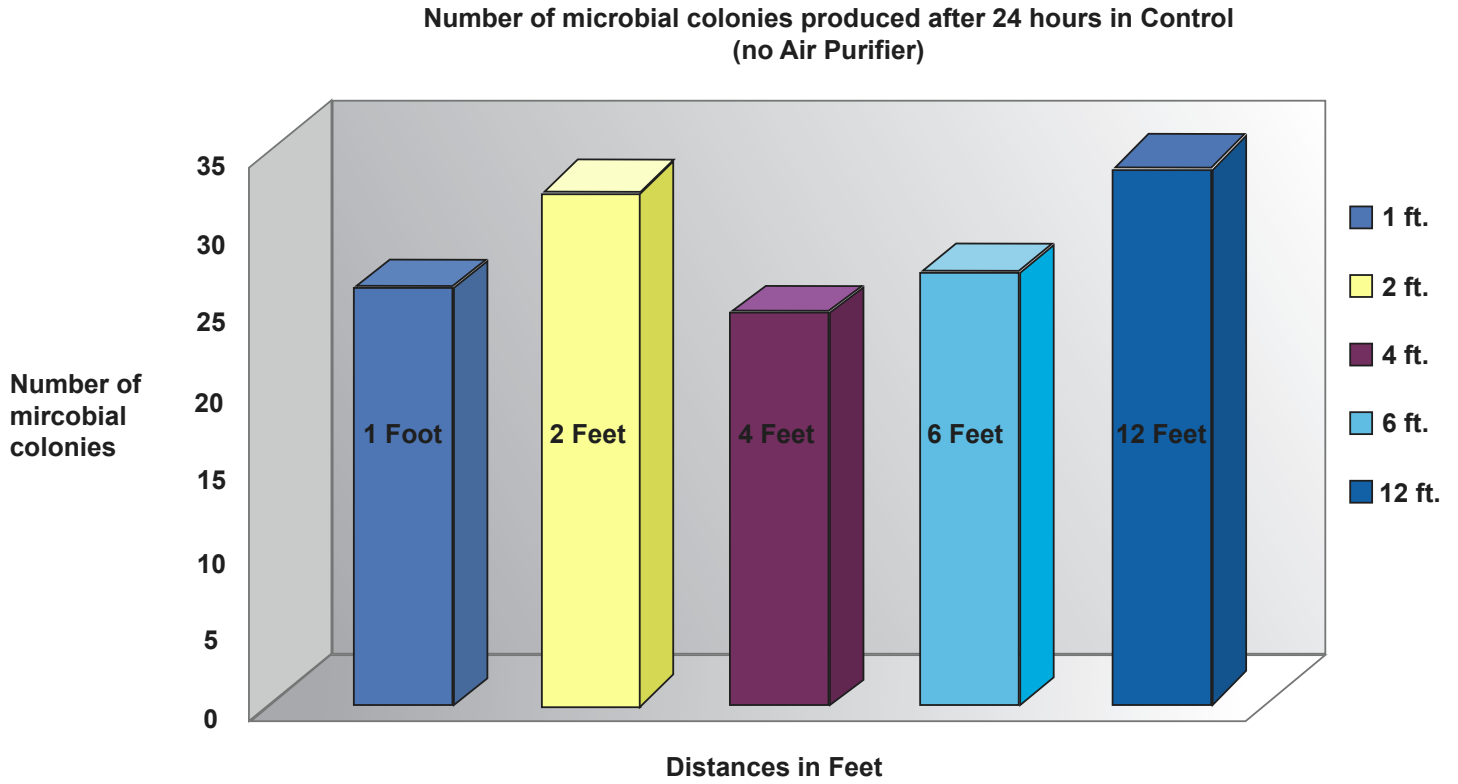
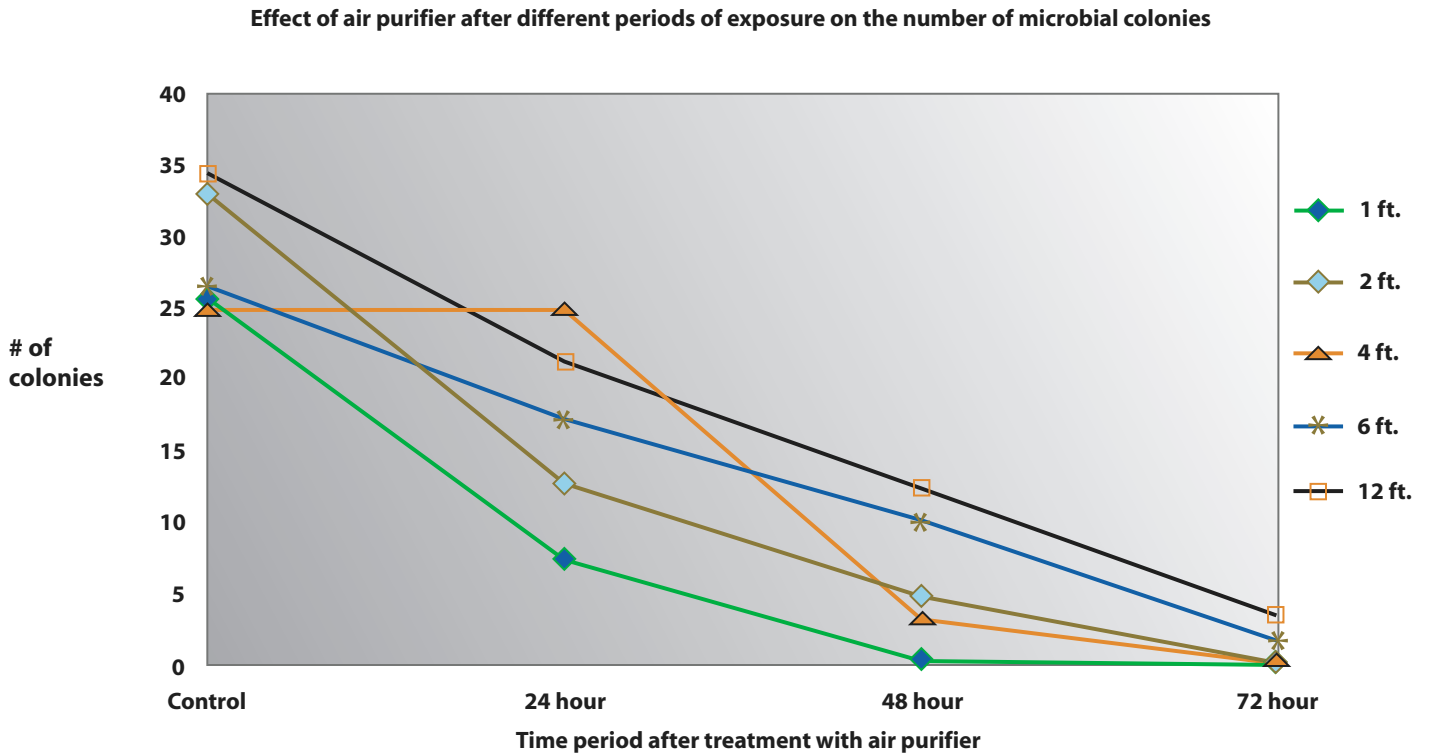


Figure 1A shows the distribution of microbial colonies in the Control set (No treatment with air purifier).

Figure - 2



From the figure-2 is a graph on the distribution of the number of microbial colonies before and after the treatment of indoor air with the air purifier, it is very clear that there was a gradual reduction in the number of microbial (bacterial and fungal) colonies with greater interval of exposure with the air purifier.

Figure 3

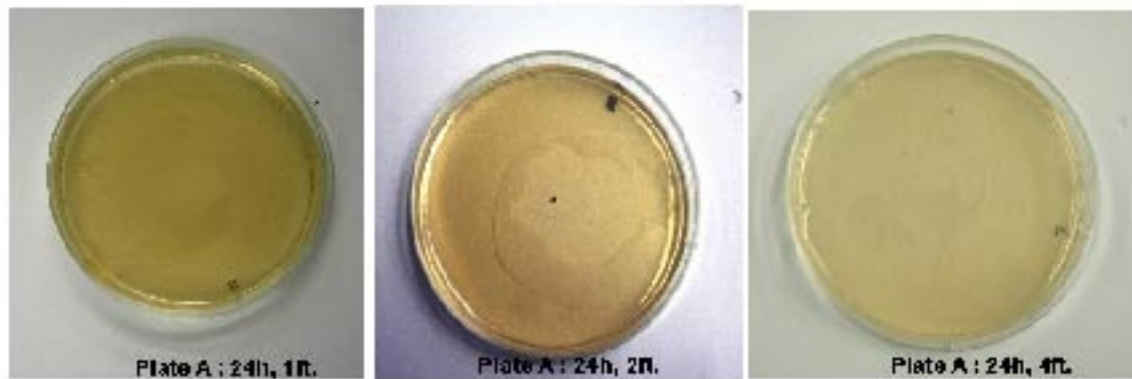
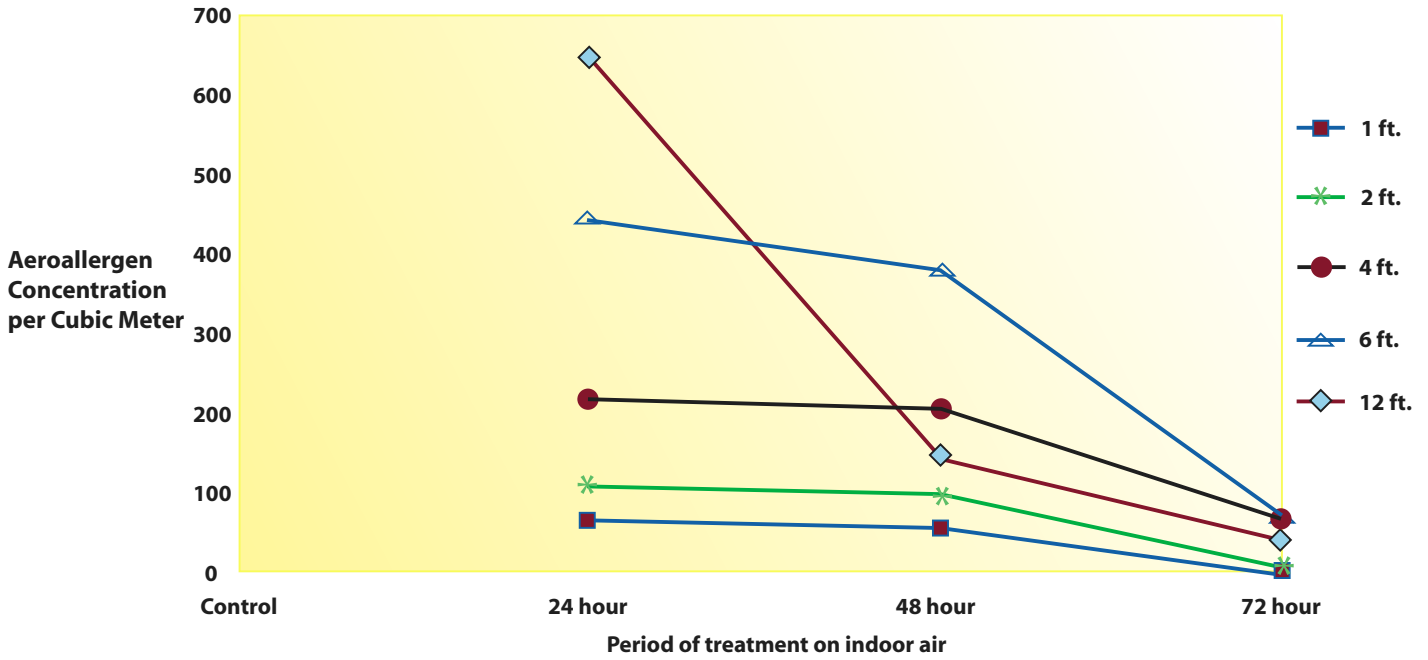


Figure 3 shows the petri-plates after a 24 hour treatment with the air purifier. Note the reduction in number of colonies compared to figure-4 (Control, No treatment with air purifier).

It was also evidenced that the petri-plates placed closer to the distance to the air purifier (1 ft. and 2 ft.) produced least number of colonies after 24h., 48h. and 72 hours of treatment of the indoor air with the air purifier. Figure-3 shows the level of infection after 24 hours with distances 1 ft, 2ft. and 4 ft. away from the air purifier. There was very minor trace of inoculums from the petri-plates from 1 ft. or 2 ft. and 4 ft. distances after 24 hours of treatment of the room air indicating thereby definite reduction of the microbial aeroallergen in the room air. After 72 hours of treatment of the indoor air with the air purifier there was almost no microflora or propagules left in the indoor air since there was no microbial colony produced on the petri-plates. All the experiments were carried out with setting at High in the air purifier. Figure 1 shows the Control plates without any treatment. All the 3 petri plates from the set up of 1ft, 2ft and 4 ft show vigorous growth of the microbial colonies after incubation in an incubator at 37°C.

Figure - 4

Effect of treatment of air purifier on the concentration of indoor aeroallergen concentration.



The petri plates from the set up of 24 hour treatment with air purifier from distances 1ft, 2ft and 4 ft from the air purifier showed no significant microbial growth. At the distance of 1 ft no microbial colony was recorded on the petri-plate while the petri plates from 2 and 4ft showed very minor trace of inoculums. Figure 4 shows the distribution of the aeroallergen count after the treatment of the room air after 24hour, 48 hour and 72 hours. After 72 hours of treatment there was almost no aeroallergen left closure to the air purifier.

Future Research

We will carry out the research in Phase-II for

1. Assay for the rest of the time intervals and distances away from the air purifier in a different room.
2. Identification of specific aeroallergen the concentrations of which were reduced with the treatment of the indoor air with the air purifier.
3. We will also use the variable of “Space” using a different size of room to assess the efficacy of the air purifier.
4. We will use digital and Fluorescent Microscopy to characterize the aeroallergens the concentration of which is reduced by the air purifier.
5. We will correlate our findings on reduction of bacterial population and identifying the specific bacteria in relation to their pathological importance.

Reference:

1. Indoor Air Quality Program: Web site: <http://www.tdh.state.tx.us/beh/IAQ/default.htm>
2. School Health: www.health.state.mn.us/divs/eh/schools/index.htm
3. Journal Indoor Air: at <http://alcor.concordia.ca/~raojw/crd/essay/essay000897.html>.
4. John Bower, owner, The Healthy House Institute, Bloomington, Ind.: In Mold Reporter at: <http://www.moldreporter.org/vol2no6/2002Air>
5. Investigating Mold in Minnesota Public Schools at: www.health.state.mn.us/divs/eh/indoorair/schools/mold.html
6. Journal Indoor Air at <http://alcor.concordia.ca/~raojw/crd/reference/reference001468.html>
7. BIOAEROSOL SAMPLING (Indoor Air) NIOSH Manual of Analytical Methods (NMAM), Fourth Edition Method 0800 January 15, 1998 at: <http://www.cdc.gov/niosh/nmam/pdfs/0800.pdf#search='BIOAEROSOL%20SAMPLING'>
8. Horner, E., Levetin, E., Shane J.D., Solomon, W. 2002. Advanced Aeroallergen. 58th AAAAI Annual Meeting. 1-68
9. 9. Horner, E., Levetin, E., Shane J.D., Solomon, W. 2002. Advanced Aeroallergen. 58th AAAAI Annual Meeting. 1-68.
10. Moore, P.D. 1991. Pollen Analysis, Second Ed. Blackwell Scientific Publications. Oxford. 62-166.
11. Website for American Academy of Allergy Asthma and Immunology: <http://www.aaaai.org/>
12. Website: University of Arizona: <http://www.geo.arizona.edu/palynology/polonweb.html>
13. Jelks, M. L. 2002. Allergy Pollen Keys with Images. Sarasota, FL. 1-19.
14. [Sessions](#), S. K. 1993. **Review: The Art and Science of Fluorescence Microscopy.** *Systematic Biology*. Vol. 42, No. 2 (Jun., 1993), pp. 224-225
15. Stephens, D. J. and V. J. Allan (2003). Light Microscopy Techniques for Live Cell Imaging (2003). *Science*. Vol. 300 Issue 5616 p-82-84.
16. Ghosh N., B. Patten, Lewellen, G. T., C. Saadeh, M. Gaylor. (2003). Aeroallergen survey of the Texas Panhandle using a Burkard Volumetric Spore Trap. *The Journal of Allergy and Clinical Immunology*. Vol. 111, No. 2: S91
17. Ghosh, N., R. Camacho, E. Schniederjan, C. Saadeh, M. Gaylor. (2003). Correlation between the meteorological conditions with the aeroallergen concentration in the Texas Panhandle. *Texas Journal of Microscopy*. 34:1:12-13
18. Ghosh N., C. Saadeh and M. Gaylor. "Quantification and characterizing the Aeroallergen by scanning and analyzing the tapes from the Burkard Spore-trap". *Journal of Scanning Microscopies*, Vol. 28, No. 2 (March/ April 2006). p. 127-128.
19. Ghosh N. and M. Whiteside. (2006). Fluorescent Microscopy in Characterizing Some Biological Specimens". *Journal of Scanning Microscopies*, Vol. 28, No. 2 (March/ April 2006). p. 129-130.