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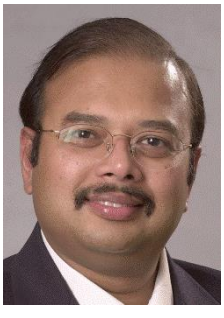

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An Assessment of the AFL Mini Sanifier II® in Terms of Reducing the Incidence of Canine Allergy

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With the current unprecedented situation of COVID-19, indoor air-quality has become a major concern all over the world. Air quality has become a greatly challenging issue and millions of people are dying due to polluted, unacceptable air quality in different countries of the world. There is a great demand for an energy efficient air purifier that cleans up the air efficiently. With revolutionary, energy-efficient technology, that is filterless, the AFL Mini II® fights impurities by cleaning and sanitising the air and surfaces in rooms up to 330 square feet. This includes nurseries, bedrooms, living rooms, kitchens, bathrooms, hotel rooms, aeroplanes, motor homes and more.
The long-life UVC lamp fitted in the AFL Mini Sanifier II® destroys germs as they pass through UV light. The light rays react with the nano metal AFLPCO® catalyst to produce an abundance of negative ions, which find and destroy carbon based molecules including bacteria, viruses and VOCs. Hydrocarbon contaminants are broken down into water and carbon dioxide. The AFL PLASMA® ionisers reduce aero-allergenic particulate matter such as dust, pollen, PM2.5 and pet dander.
We have used domesticated canines like dogs as the animal model to study the efficiency of the AFL Mini Sanifier II®. The objective of our study was to determine if the pollen concentration in the air has any relationship with the incidence of inhalant allergies in dogs. Dogs suffer from the same type of inhalant allergies as people such as pollen, mold, and other allergens. The data on dogs admitted to an animal hospital in Amarillo for allergy treatment were collected and compared to the aeroallergen indices of respective years. We analyzed the data to determine if there is any correlation between the increase of aeroallergen concentration and patients receiving treatment at the animal hospital. We also analyzed the effect of the AFL Mini Sanifier II® on aeroallergen in

the indoor air of the clinic by setting slides with double sticky tapes and observing with a BX-40 Olympus microscope with a digital camera. Analyzed data indicate that there exists a significant correlation between the aeroallergen indices with the incidence of allergy in dogs.

Key Words: AFL Mini Sanifier II®, Canine Atopic Dermatitis, air purification system, PM2.5, Aerosol, VOC, AFLPCO®

Introduction

The indoor air quality is a factor that can affect both people and animals like dogs. Studies show aeroallergens and volatile organic compounds (VOCs) may have many negative health impacts when present in high concentrations in the indoor air. We have tested to assess the efficiency of the AFL Mini Sanifier II® built by the Air For Life (AFL, UK) that uses AFLPCO air purification technology used in reducing indoor aeroallergens and VOCs. The effect of the AFL Mini Sanifier II® air purifiers with Air For Life Photo-Catalytic Oxidation (AFLPCO) technology were tested at the Coulter Animal Hospital in Amarillo, Texas for the concentration of debris in the air, such as aeroallergens and VOCs. The AFLPCO purifier can help control allergy related aliments as well as prevent new disorders from accumulating by cleansing the air. The current unprecedented situation with the COVID-19 poses a great challenge to the whole world in terms of technologies to fight with and prevent transmission of pathogens. Air For Life (UK) has successfully developed a plethora of gadgets to fight against infection by improving the air quality. AFL has developed the AFL Mini Sanifier II® with revolutionary dual innovative technology that eliminate viruses, bacteria, germs and other contaminants by sanitizing and sterilizing the air and surrounding surfaces. More than twenty-five research students have been analyzing the AFL Mini Sanifier II® at West Texas A&M University to evaluate its effect on the indoor airborne allergens including the airborne pollen, bacterial and fungal populations, dusts, and burnt residues. We have also assessed and analyzed the capacity of the AFL Mini Sanifier II® in terms of the reduction of the suspended PM2.5. We have used dogs as the animal model, which showed a gradual reduction of symptoms of allergy while kept in a room with AFL Mini Sanifier II® on for a duration of 72 hours.

Photo Catalytic Oxidation Nanotechnology

Photocatalytic oxidation (PCO) is a very powerful air purification technology and has the ability to destroy particles as small as 0.001 microns (nanometer) such as carbon based impurities in air like bad odor, volatile organic compounds (VOC), allergens like household dust mites and their droppings, mold, pollen and fungal spores. Harmful pathogenic organisms like fungi, bacteria and viruses, as the one responsible for causing contagious diseases like the ongoing pandemic of COVID 19 can also be successfully destroyed and neutralized[6].

Materials and Methods

We used a fiberglass chamber to assess the AFL Mini Sanifier II® air purifier in terms of reduction of airborne pathogens and Particulate Matter PM2.5. We followed the following steps: We used the Clorox wipes to clean up the surfaces and allowed them to dry up for 24 hours. We spreaded the ISO 12103-1 Ultrafine Dust Particle with an average size of 2.75 micron (PTI Powder Tech., Minnesota) with the help of 4 small blower fans placed in the 4 corners of the chamber. We

allowed the aerosol to get saturated for 24 hours and allowed them to settle down. We recorded the initial reading of the PM2.5 concentration using a *Garosa Air Quality Monitor Formaldehyde Detector* and Accurate PM2.5 Micron Particulate Matter Dust Pollution Multi Tester.

We ran the AFL Mini Sanifier II® for 24h, 48h, 72h and 120h and 1 week intervals and recorded the PM2.5 concentration.

Collection of Aeroallergen data from the Burkard volumetric Spore Trap: Aeroallergens cause serious allergic and asthmatic reactions. Characterizing the aeroallergen provides information regarding the onset, duration, and severity of the pollen season that clinicians use to guide allergen selection for skin testing and treatment. Digital Microscopy has useful approaches to understand the structure and function of the microscopic objects. The analysis of air was performed through the collection of pollen and spores through the use of the Burkard 7-day Volumetric Spore Trap (Burkard Agronomics Division of Burkard Scientific Sales Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, England). In general, volumetric spore traps generate superior data, as a fixed volume of air is sucked into the trap, which permits the calculation of pollen and spore concentrations. We standardized the Burkard 7-day Volumetric Spore Trap by using a flow meter provided by the manufacturer. We mounted the spore trap on the flat roof of the third floor of the Agriculture and Natural Sciences building of West Texas A&M University in Canyon, Texas and standardized it by using a flow meter provided by the manufacturer. The sampling equipment was strategically placed in wind-protected locations on a flat surface away from walls and other obstacles. The spore trap was mounted on the flat roof of the Agriculture and Natural Sciences building of West Texas A&M University in Canyon, Texas. This area has adequate exposure to the prevailing winds of West Texas, and is above the trees of the surrounding community. This location is beneficial because it allows adequate sampling of the wind-blown pollen and spores carried to the sampling apparatus on the air currents, while preventing unwanted surface contamination such as excess dirt or sand. In addition, there are no overhanging trees or vegetation to compromise the collection of data. The spore trap operates on 25 watts of electricity at 240 volts, or 50 watts at 110 volts. Air is suctioned through the trap at a rate of 10 liters per minute. A fan on the ventral portion of the trap rotates at a rate of 2900 rpm to draw air into the trap[1,2] . The drum is driven by a precision 7-day jewel clock located on the lid inside the canister, which rotates the drum at a constant speed. This clock is designed to allow one complete rotation of the drum over a 7-day period. Hourly observations can be made because the drum will rotate at a standard rate of 2 mm/hr. The Melinex tape (a clear plastic tape, Burkard Agronomics, UK) was attached to the drum through the application of double-sided tape at the orifice start position. Once attached, the Melinex tape was coated with a thin layer of Beckman's Silicone vacuum grease (Fisher Scientific cat # 004 S80119WX) to adhere the aerial particles that include fungal spores, pollens, plant debris, and carbonated materials from burnt elements. The distance from the beginning of the tape designates spore collection time. A trimming block is segmented to allow for the division the tape into 24-hr periods. A wet mount is used for the preparation of the slide. Two to three drops of distilled water are put on a clean glass slide. To get the sample, the Melinex tape is taken off of the drum with fine forceps. The tape is laid flat on the trimming block and aligned with the segment marks. The tape is cut on the segment lines to get the sample for each day. Each strip is then put with the impregnated surface sample side up, using the forceps, on a slide. The tape is then manipulated so that the long edges of the tape are parallel with the long edges of the slide. Therefore, the transverses of the microscope will match up with the tape on the slide. After positioning, any excess water is pulled off with a paper towel to keep strip in place. The aeroallergens are stained with 1% Safranin O (Sigma Cat no. 84120, Fluka, Microscopy Grade) and Gelvatol (Burkard Agronomics Division of Burkard Scientific Sales Ltd., UK) a permanent mountant mix. Gelvatol is composed of 35g Gelvatol powder (Burkard Manufacturing Co Ltd., UK), 50 ml Glycerol, 100 ml distilled water, and 2g phenol. Gelvatol is

prepared by mixing the Gelvatol powder and phenol in water allowing it to sit overnight. Glycerol and distilled water were added to the mixture while heating over a water bath (65^{o} C) and continuous stirring produced the proper emulsion. The addition of few drops of 1% Safranin O to the Gelvatol enhances the visibility of the pollen, including their detailed morphology like cell wall patterns, orientation of the pores and colpi. Gelvatol-Safranin-O emulsion is administered evenly to the cover slip using a glass rod, which is essential for a good slide. The cover slip is placed at a 45° angle on one side of the tape and is slowly laid down across the reminder of the tape with a bent needle to allow for proper placement of the coverslip. The slide is then ready to be viewed with the compound microscope[1,2].

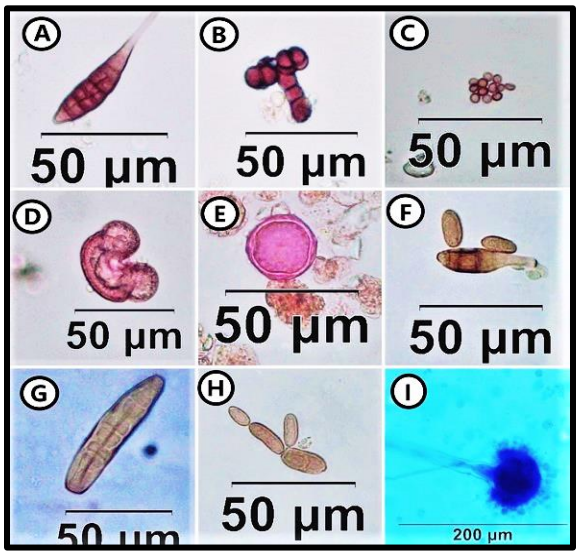
Comparing the incidence of allergy among dogs with the aeroallergen data: The analysis begins with descriptive statistics, which include time plots for the examination of possible trends in the data e.g. aeroallergen concentrations based on time (season, daily, and hourly details). The daily weather will be recorded including temperature, wind speed, precipitation, humidity, average soil temperatures, etc. The characteristics of the pollen season will be studied via Principal Pollen Period (PPP) which is defined as the first day, on which at least 1 pollen grain/m^3 was reached, with subsequent days containing 1 or more pollen grains/m^3. The end of a season is considered as the first of 5 consecutive days without pollen grains. The correlation between pollen concentrations during the PPP and the main meteorological parameters are studied by means of a Pearson's correlation coefficient and Spearman's correlation test with significance levels of 1% and 5%. This data helped us in identifying the most prevalent pollen and spores. Atmospheric pollen counts are indexed based on a contemporary application of Thommen's postulates. This allowed the estimation of the clinical significance of the various pollen types by combining data concerning *in vivo* allergenicity and terminal velocity as a means to judge the clinical significance. Collection of pollen and spores is done for at least 3 years from the area specified earlier. At the end of 3rd year, the pollen and spores are charted to see a trend of pollen peaks throughout the year. Daily recording of the pollen and spore counts and meteorological data are recorded that reflects the weather patterns. From the data pollen peaks are determined to find the basic patterns for the pollination periods of the local vegetation.[1,2,3]

Result and Discussion

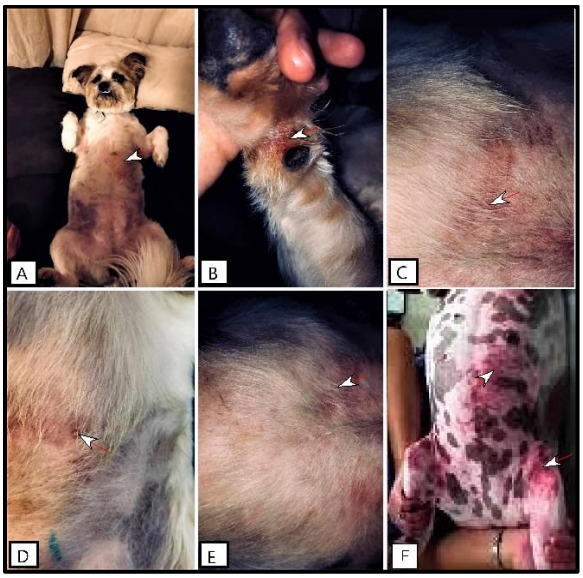
The analysis with dog's allergy as compared in the control (no air purifier) with AFL Mini Sanifier II® running, the treated set, showed a gradual reduction of the allergy cases with the treatment with the air purifier. The geographical location of the Texas Panhandle allows this area to have varied composition and concentration of aeroallergen (Fig. 1A-I). The pollen grains are composed of: *Pinus sylvestris*, *Solanum elaeagnifolium, Ambrosia artemisiifolia,* and grass pollen. Fungal spores are mainly composed of *Alternaria alternata, Ustilago, Cladosporium, Stachybotrys,* and *Drechslera* spp.

Dogs exhibit varied types of hypersensitive reactions starting from redness on the skin and paws, continuous scratching and respiratory discomfort (Figs. 2A-F) [4,5]. There was a gradual reduction of the microbial colonies on using the AFL Mini Sanifier II® (Fig. 3B). The analysis of three years' data reveal that there was a constatnt variation in the presence of different aeroallergen. We used a SZ-40 Sterescope attached to two Gooseneck Optical fiber light sources to differentiate the microbial colonies as bacteria or fungi. Later, we have stained the colonies with Gram stain, Lactophenol Cotton Blue and Safranin Gelvatol staining to identify the bacteria and fungi (Fig. 4A). As tested in the AFL fiberglass chamber, there was a gradual reduction of the mold spore count and PM2.5 concentration with the increased interval (Graph-2). The Total VOC and Formaldehyde readings went down significantly on running the AFL Mini Sanifier II® for one week. After one week the Formaldehyde count went down from 563 to 15, TVOC count went down from 2134 to 185, and the PM2.5 count went down from 2416 to almost 0. (Graph-2, Fig. 3D). At the same time the colony count went down to 0, after

running the air purifier for one week (Fig. 3C), proving thereby the high efficacy of the AFL Mini Sanifier II®. In the Texas Panhandle area, the outdoor and indoor aeroallergens vary in combinations as shown in the Figs. 1A to I.



Figs. 1A-I showing most frequent aeroallergens of Texas Panhandle: Fungal spores; A. *Alternaria alternata*, B. *Ustilago*, C. *Cladosporium*, F. *Alternaria*, G. *Drechslera*, H. *Stachybotrys*, I. *Aspergillus* spp. Pollens: D. *Pinus sylvestris*, E. *Solanum elaeagnifolium*. These aeroallergens were trapped using a Burkard Volumetric Spore Trap, stained and mounted with Safranin-Gelvatol. The images were captured using a BX-40 Olympus Microscope with a DP-74 Digital camera.



Figs. 2A-F. Hypersensitive skin reactions and scratch wounds on the body, paws of the subjects, pointing with arrows.

Fig. 3A. Examining the Petriplates. B. AFL Mini Sanifier II®. C. Petriplates showing gradual reduction of the microbial colonies on running the AFL Mini air purifier. D. Final counts of Formaldehyde, Total VOC and PM2.5 using a Garosa Air Quality Monitor Formaldehyde Detector and Accurate PM2.5 Micron Particulate Matter Dust Pollution Multi Tester.

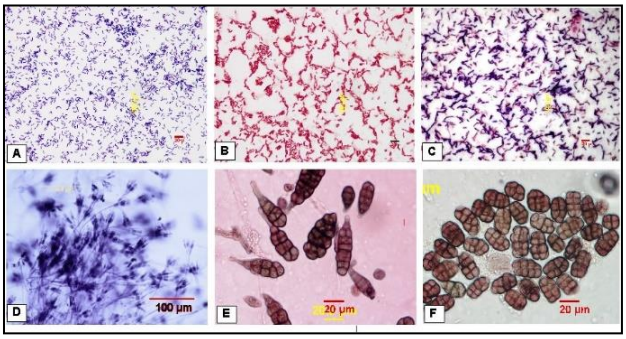
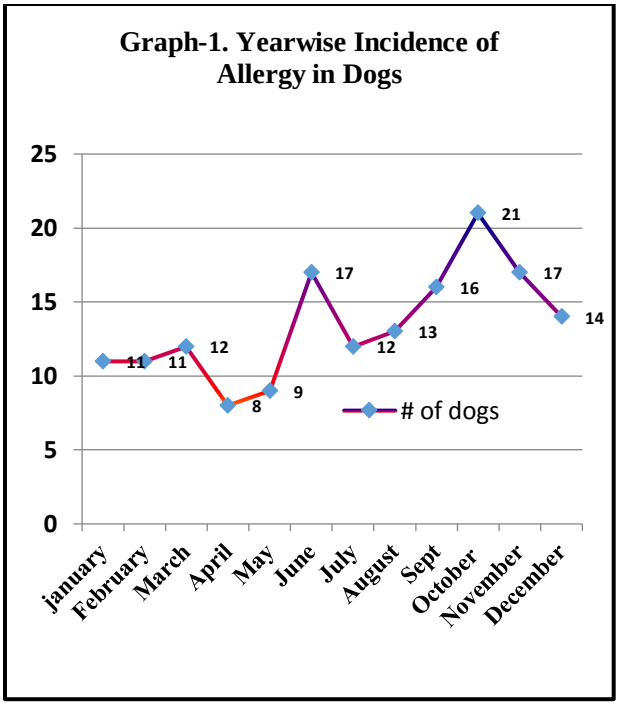


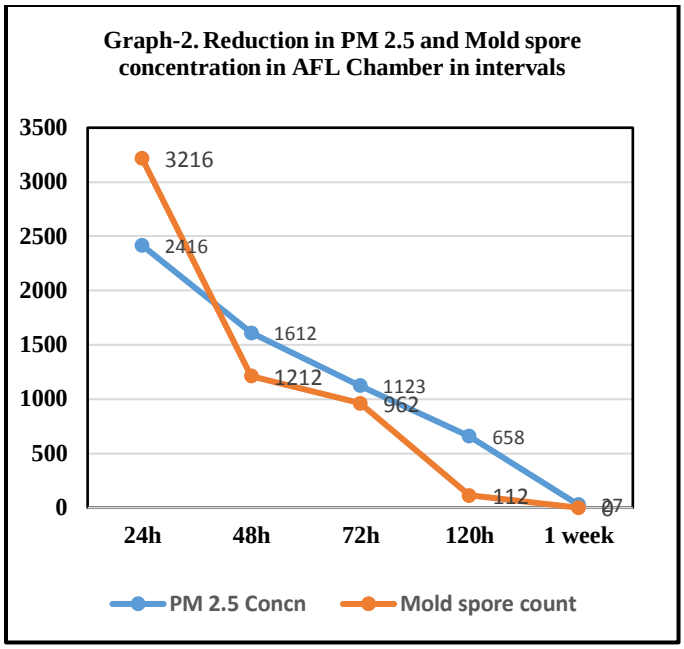
Fig. 4A. showing the Gram-positive *Bacilli* from the colonies from the Petri plates (no air purifier). B. Gram-negative *Bacilli*. C. spore-forming *Bacilli* D. *Penicillium* sp. E. *Alternaria alternata* conidia and F. *Pithomyces* sp. spores.

Conclusion: Dogs suffer from the same type of inhalant allergies as people, caused by pollen, mold, and other allergens[3,4,5]. We have collected and analyzed the data on dogs' allergy from the dogs admitted to an animal hospital in Amarillo. AFLPCO® or Air For Life Photocatalytic oxidation is a very powerful and advanced air purification technology that has

the ability to destroy particles as small as 0.001 microns (nanometer) such as carbon based impurities in air like bad odor, volatile organic compounds (VOC), allergens like household dust mites and their droppings, mold, pollen and fungal spores. This AFLPCO® nanotechnology[6] was applied to build the AFL Mini Sanifier II®. When AFL Mini Sanifier II® was used, it showed a gradual reduction of aeroallergen including PM2.5 and mold spore concentrations, total VOC (TVOC) and Formaldehyde concentration in the room air. This, in turn reduced the symptoms of allergy among the sick dogs. Use of AFL Mini Sanifier II® can improve the indoor air quality (IAQ) and help people fighting against infection, allergic rhinitis and other respiratory ailments.



Graph-1. Incidence of allergy in dogs, an average of three years' data. The graph shows two peaks in June and in Sept.-Oct. when the aeroallergen counts also reached the peaks in a year[1].



Graph-2. Reduction in PM2.5 concentration and Mold spore count in AFL Chamber in intervals.

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