ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

Evaluation of the Presence of Some Pathogenic Microorganism in Air and the Surface of the Facilities of a Gymnastic Club in Puerto Rico

Waleska Díaz-Muñoz[1] and Karlo Malavé-Llamas[2]*

[1]*Department of Science and Technology, Universidad Ana G. Méndez-Cupey Campus, Puerto Rico*
[2]*Department of Science and Technology, Universidad Ana G. Méndez-Carolina Campus, Puerto Rico*

***Corresponding Author**: Karlo Malavé-Llamas, Associate Professor and PI-NSF-HIS-STEM at the Department of Science and Technology, Universidad Ana G. Méndez-Carolina Campus, Puerto Rico.

Received: February 27, 2023; **Published:** March 17, 2023

Abstract

Background: The Environmental Protection Agency (EPA) identifies poor indoor air quality as one of the top five environmental health hazards affecting public health. The average person spends more than 90% of their time in enclosed environments, which is why; pollutant free air in those environments should be a priority. Exposure to indoor allergens can result in allergies, asthma, bronchial hyper-reactivity, respiratory tract inflammation, dermatitis and sinusitis.

Method: We measured environmental factors in six different points inside an athletic facility including temperature, humidity, CO, CO_2, dust characterization and airflow. We collected 98 samples in total; from which 36 were surface samples in areas were athletes are in contact with gymnastic equipment; 32 random air samples and 30 random samples for dermatophytes. We incubated all the samples and individual colonies were isolated for their identification.

Result: Results showed the presence of fungi such as *Acremonium strictum, A. curvulum, Cladosporium cladosporioides, Curvularia brachyspora, C. clavata, C. senegalensis, Penicillium chrysogenum, P. citrinum, Aspergillus niger, A. clavatus.* Among the most prevalent pathogenic dermatophytes were *Trichophyton soudanense, T. verrucosum, and Epidermophyton floccosum.* Bacteria were also accounted for; among the identified species were *Sphingomonas paucimobilis, Klebsiella pneumoniae, Bacillus megaterium, Serratia odorifera, Micrococcus luteus, Staphylococcus hominis and S. saprophyticus*, among others.

Conclusion: This study analyzed the microbial diversity of the site and potential health risk for users, athletes and visitors of the gymnasium. Many of the microorganisms isolated during this investigation could present a health hazard for the public in the facility specially the children that practice there. Therefore, a mitigation process was performed, minimizing the probability if infection by those using the facilities.

Keywords: *Pathogenic Microorganism; Air; Surface; Gymnastic Club*

Background

During the last decades, we have seen progress in the area of science and technology brought about by the industrial revolution. As a result, global population has increased through the years [1]. This has caused a change in the way people visualize their place of residence and labor, thus, the need for more houses and work places lead to the excessive construction of buildings and homes. Due to lack of land, an urban vertical growth has been the rational option, allowing a greater amount of people to live in the limited space we have, so vertical has been the way to go [2]. These structures, in combination with a tropical climate are prone to be inhabited by microorganisms.

All our technological advances have been increasing throughout the years producing an adverse effect in the quality of our atmosphere [3]. In view of that, air pollution can be defined in general terms as; the introduction of biological, chemicals materials or particles into the atmosphere changing the natural characteristics of it, exacerbating the probabilities for harmful effects to the immediate population of human, animals and other living things. Consequently damaging our environment and the quality of natural resources [4]. Among other things, it is important to recognize that the quality of our outdoor environment will directly affect, positively or negatively our indoor air.

During the1970's, it was acknowledged that the quality of the air inside non-industrial buildings, under certain circumstances like poor maintenance, is harmful to human health [5]. Exposure to poor indoor air quality poses a public health threat due to the fact that the public spends 90% of their time in closed environments such as buildings [6]. The EPA recognizes poor indoor air quality as one of the top five environmental health hazards affecting public health. According to the US Center for Disease Control (CDC), Puerto Rico has a very high prevalence of lifetime (19.6%) and current (11.6%) asthma in comparison with any other state or territory of the United States [7]. Incidence among children's eighteen years and younger is particularly high in the eastern part of the island [8].

Indoor air quality is defined by the air parameters found inside buildings, businesses, schools, and homes [9]. The sources of contamination can be anthropogenic or natural; examples are automobiles, paint, photocopier machines, electric generators, numerous particles, fibers, dust, bacteria, fungus, or gases [10]. Some environmental factors such as high temperatures and humidity, if not under adequate parameters, foster the proliferation of biological contaminants that can cause long and short term health problems [11]. Bacteria, mold, fungi, viruses, mite, cockroaches, pollen, and animal particles contributes to indoor air quality and are known as biological indicators of air quality [12]. These contaminants greatly contribute to the symptoms of irritation or reactivity presented in people exposed to them [13].

Although biological allergens are very important and have priority in the health area, they have certain special properties or characteristics that make them difficult to evaluate and identify [14]. This difficulty rests on the great amount and complexity of their surface antigens and other protein molecules of these agents, being responsible not only for their pathogenic capacity but also for their difficult evaluation. Asthma is caused by the combination of genetic and environmental factor [15]. Hakonarson and Halapi (2002) attributed the condition to the interaction among many genes and how these genes react with the environment. Different genes have been reported to show linkage of asthma and bronchial hyper-sensitivity [16]. In the last decade significant progress has been made in the field of asthma but the clinical implication due the genetic variation remains indeterminate [16].

Asthma can be broken down into two groups based on the causes of an attack: extrinsic and intrinsic. Extrinsic asthma has a known cause, such as allergies dust mites, pollen, grass, weed, or pet dandruff [17]. Intrinsic asthma has a known cause, but the association between the cause and the symptoms is not clear because antibody hypersensitivity is not necessarily present [18].

When talking about air quality, especially indoor air quality it is important to remember that asthma is not the only health hazard that should preoccupies us, with this said, dermatophytes come into the equation. These fungi are very important because they are the most common infectious agent of humans [19,20]. Dermatophytes are filamentous fungi that are able to digest and obtain nutrient from keratin, the primary component of skin, hair, and nails [20]. Dermatophytes are the only fungi that have evolved a dependency on human or animal infection for the survival of the species [19,20]. *Trichophyton soudanense*, *Epidermophyton floccosum* and *Trichophyton verrucosum* are some of the most common Dermatophytes identify in different buildings around the world. One of those examples is an outbreak experience by a Judo team in Europe during 2005.

Chemical and biological pollutants have caught our attention because of the various health effects they generate due to their mechanism to produce inflammation as well as for the variety of environmental stressors they are responsible for [21]. Some chemicals include Carbon monoxide (CO), Ozone (O_3), Particulate matter, Tobacco smoke, Volatile Organic Compounds (VOC's), Radon, Pesticides, Asbestos,

Lead, and Arsenic among others. The physiological effects of these contaminants are numerous; they can trigger asthma, and irritate eyes, nose, throat, respiratory illnesses and lung cancer [22].

Knowing this, and to maintain an indoor environment under adequate levels of quality that will keep us healthy, we need to study the environmental characteristics that promote the growth and proliferation of the organisms mentioned above. Environmental characteristics include temperature, humidity, CO, Carbon dioxide (CO_2), dust characterization and air flow, among others. One of the most important factors to consider if we intend to have good indoor air quality is the temperature; it should be kept at 70ºF to 76ºF. Higher temperatures promote the growth of bacteria, fungi, and dust mites. Therefore, temperature controls are important since if appropriate conditions are provided for the proliferation of these pathogenic organisms, the probabilities of severely affecting the health of the people exposed to them will increase [23]. Another important factor which is influenced by temperature is relative humidity. Relative humidity should be kept at 30 - 60%; higher levels could be critical for people sensitive to these organisms and could result in possible asthma episodes on chronic sufferers of this disease since the growth of these microorganisms could propagate [24].

Temperature and humidity controls are very significative for the public health. If we don't meet with these parameters inside buildings our health could be compromised, skin and eyes irritations start to appear and if these symptoms are related with the working environment then it is known as Sick Building Syndrome (SBS). One important characteristic of this syndrome is that it is always present in susceptible individuals while inside the building and absent or more moderate when they leave or are not present on the premises. The main complaints among the personnel that work in the premises include ear, nose, and throat problems; dermatitis, concentration problems, headaches, and fatigue, shortness of breathe, and smell sensitivity. The term building related illnesses is used when the symptoms of the disease are identified, diagnosed, and directly attributed to the air contaminants of the building. The signs and symptoms include cough, chest pain, respiratory problems, edema, palpitations, cancer, alveolitis, pneumonia, occupational asthma among others [25]. Many people know the health hazards of atmospheric pollutants, but others ignored how the contaminants inside a building can significantly affect their health. Some studies on the exposure to air pollutants indicate that indoor levels of contamination can be 2 to 5 times and sometimes up to 100 times higher than the outside air [26].

The majority of biological contaminants that are found inside a building originate on the natural environment and are classified as outdoor allergens [27]. These penetrate the indoor environment through windows, doors, and ventilation systems. The aeroallergens vary with the seasons, weather conditions, geographic location, and the indoor environment [28]. Researchers have established the following hypothesis: the higher the flow of air from the exterior to the interior of the building, the lower is the concentration of contaminants and the probability of getting sick due to air contamination [29]. Therefore, contrary to popular belief closing our doors and windows to limit the flow of air goes against our desire to protect our health [30].

Understanding the symptoms and acknowledging the cause of airborne contamination will empower individuals, therefore illness because of SBS will minimize. The excess of humidity in structures contributes to the growth of fungi and provides a favorable environment for dust, roaches, rats, and other plagues [31]. Structural problems, plumbing deficiencies and poor maintenance in buildings provide mechanisms for those plagues to enter the buildings. On the other hand, the fungi found in the interior come from two sources, from the outside through doors and windows and from fungus colonization in the building [31]. Once fungi obtain specific nutrients and sufficient humidity to grow, they will appear on walls, insulation material, carpets, mattresses, and other surfaces [31]. The above-mentioned biological agents sensitize the immunological system producing antibodies after the first exposure. After repeated exposure, the immune responses are faster and more intense and can result in allergic asthma, bronchial hyper-reactivity and respiratory tract inflammation [32].

In the mid- 1990's, the University of Michigan or UMHS began to diagnose serious infections caused by *Staphylococcus aureus* and *Streptococcus sp.* among athletes, healthy children, military recruiters, and groups of professional football players. The CDC recommends

that communities and athletic centers work to develop prevention strategies since this bacterium are opportunistic especially when it comes to open skin wounds [33].

Infections with pathogenic organisms are due to direct or indirect contact with those organisms. With the direct contact, the pathogenic microorganism goes directly from the infectious source to the healthy host. The indirect contact is produced through an intermediary that can be a vector. It is important to remember that an exposure will result in an adverse health reaction if the pathogenic microorganisms along with the abiotic conditions needed by that microorganism to survive are present. Among those abiotic factors are temperature, humidity, pressure fluctuations, and other microorganisms; all of them influencing for the contagion of diseases [34].

Children and the elderly are more sensitive to pets and birds allergens when in enclosed spaces. Inhalation, skin contact, and ingestion are the most frequent ways of exposure. Bacteria, dust, mites, animal epithelium cells, pollen, fungi, and animal excrement particles when exposed to a susceptible individual result in detrimental health effects like allergies, dermatitis, sinuses, and asthma among others [35].

The importance of this evaluation relies on the fact that the majority of the present individuals, using the facility, are children. Due to their physiological characteristics and behavior, children are one of the groups more susceptible to infections by the endogenous pathogens. During the year 2008, one athlete was hospitalized because of *Streptococcus sp* infection, though the origin of the bacterium was not identified. Other factors that worsen the situation are the presence of pigeons, dogs, cats and mice, elevated temperatures, and humidity. Is important to remember that diseases caused by *Streptococcus sp.* are considered sporadic, but can cause epidemics and are common in places where there is overcrowding.

Method

In this study, our objective was to evaluate the presence of pathogenic microorganisms in the air and the surface, and the indoor air quality at the Gymnastic Club in Caguas Puerto Rico. We conducted this investigation following a preliminary visual assessment and the identification of some pathogenic microorganisms that may be a potential risk to the health of the children and coaches that use those facilities.

The assessment included an inspection of the relevant areas for visual microorganism growth, air and surface sampling. The visual inspection was important to us because it allowed the identification of possible factors that affect the quality of indoor air. Beside the visual inspections environmental factors likes temperature, humidity, Carbon monoxide (CO), Carbon dioxide (CO_2), Particulate matter were analyzed with direct reading instrumentation and Spore Trap Air Filter was collected utilizing a Air-O-Cell cassette (manufactured by Zefon International).

For the identification of the sampling points, we created a quadrangle following the asbestos quadrangle. We divided the quadrangle in 134 equal squares; each one has length of $10ft^2$. Using the statistical program RANDOM.ORG, we selected 30 sample points for the local identification of the air sampling using the SAS 100 (manufactured by Bioscience Inc.) (Figure 1). This program will be used to select random samples from large data sets with a uniform distribution. We used the same grid for the identification of the physical and chemical parameters but divided in six equal parts (Figure 2). The physical parameters evaluated in those points were temperature, humidity, CO, CO_2, Particle matter and Spore Trap Air Filter. We also collected two samples as background for the microbiological and physical parameter to compare with the sample inside the athlete training area.

We conducted this research in three phases, starting on June 9, 2009 and finishing on October 10, 2009. We sampled during the first phase sterile carpet test, exposure plates and measure of direct reading instrumentation. The sampling of air and surfaces was performed to identify microbial presence. We measured physical and chemical parameters like Temperatures, Relative humidity, CO, and CO_2 utilizing an IAQ Calc. manufactured by TSI. We measured particulate matter with direct reading instrument and Spore Trap Air Filter.

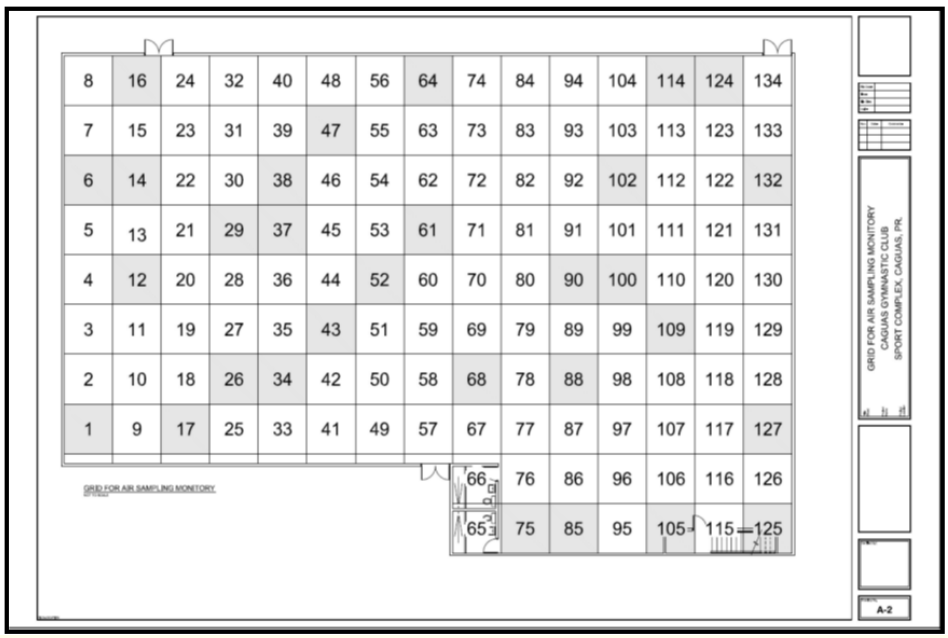

Figure 1: *Grid for the air sampling monitory.*

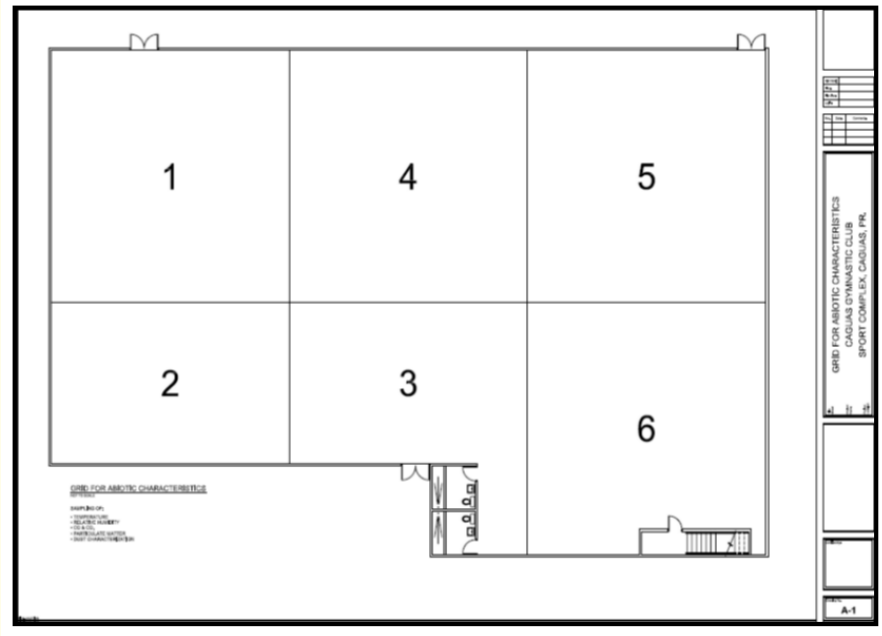

Figure 2: *Grid for the physical-chemical sampling monitory.*

We conducted a second phase with the purpose of perform the mitigation process for fungi and bacteria in Caguas Gymnastic Club. During this phase, we performed a cleaning and sanitization process using Microban QGC Disinfectant Cleaner manufactured by Sylvane. Microban is a product approved by EPA for the use as disinfectant, fungicide, virucide, sanitizer, mildewstat, deodorizer and heavy duty cleaner. Microban has been used in microbial remediation, pest control and odor removal. We performed a second sampling of sterile carpet test, air and surfaces to compare the effectivity of the cleaning procedure. Temperature, Relative humidity, CO, $CO_{2,}$ Particulate matter and Spore Trap Air Filter was taken.

The third phase was conducted with the interest of the application of the Anti-microbial product (Trimethoxysilyl Quaternary Ammonium Chloride) H.E.L.P Technologies that can prevent the presence of a broad spectrum of microorganisms during 90 days.

Physical parameter procedure

Temperature and relative humidity (%RH)

We analyzed these parameters utilizing a direct reading instrument model TSI-8760, IAQ-Calc™, manufactured by TSI. The field calibration was performed before the sample collection as recommended by manufacturer. The samples were collected in each of six different points identifies in the quadrangle. Two different samples as background were also collected, one in the office and other in the exterior of the building.

Carbon monoxide (CO) and carbon dioxide (CO_2)

We analyzed these parameters utilizing a direct reading instrument model TSI-8760, IAQ-Calc™. The field calibration was performs before the sample collection as recommended by manufacturer. We collected the samples in each of six different points identified in the quadrangle. Two different samples as background were also collected, one in the office and other in the exterior of the building.

Particulate matter (PM)

These parameters were analyzed utilizing a direct reading instrument IAQ model 316 manufactured by Lighthouse World Wide Solution. We collected six samples of non-viable particles in the training area using the above-mentioned quadrangle. Two different samples as background were also collected, one in the office and other outside of the building. The instrument read the concentration of the most common particulate matter of air in ft^3; 0.3 microns, 0.5 micron, 1.0 micron, 2.5 micron, 5.0 micron, 10.0 micron and Total Suspended Particle (TSP).

Spore trap air filter

Spore sampling was performed using the Air-O-Cell cassette. This sampling technique collected viable and nonviable particulates in the training area. We collected six 15 Liter air samples of viable and non-viable particle in the training area using the quadrangle and two different samples as background, one in the office and other in the exterior of the building.

Sampling materials

- Air-O-cell cassette
- Rotameter
- Flexible tubing
- High volume air pump.

Sampling procedures

Removed and retained the tape seal covering the Air-O-Cell inlet and outlet. We connected the pump tubing into the outlet. The sampling pump flow rate was set of 15 liter during five minutes. We removed the Air-O-Cell Cassette from the tubing and resealed with the

original tape. We placed all the samples in a plastic bag and send to RAMS Environmental Laboratory, Inc in Miami, Florida for the Dust Characterization by Optical Microscopy techniques.

Sampling analysis

We removed the glass slide from the Cassette. We placed the glass slide into a microscope slide with one drop of Lacto Phenol Cotton Blue. We covered the microscope slide with the cover slip. We conducted the counting and quantification by counting cross-sections of the deposited trace. The particle deposit area is approximately 1.1 mm wide by 14.5 mm an approximate area of 15.95 mm^2.

Biological parameter procedure

Exposure plate

The purpose of this sampling method was to estimate the contamination level in the study area. We conducted this sampling technique following the SOP 300-021 of Clendo Industrial laboratories Inc. and APHA 4th Edition (2001). We selected Tripticase Soy Agar (TSA) media to identify bacteria, and Rose Bengal Agar (RBA) was choosing for the collection of fungi. The RBA is a selective medium since the antibiotic inhibits the growth of bacteria, consequently, avoiding the contamination of the samples. We placed a totally of 30 TSA plate and 30 RBA plate in different point selecting the random samples given by the program RANDOM.ORG and the quadrangle. Two different samples as background were also collected, one in the office and other in the conference room. We collected one plate of TSA and RBA as negative control for sterility test purpose.

Sampling materials

- TSA plates
- RBA plates
- Personal protective equipment
- Biological waste disposal autoclave bags
- Incubator 30º - 35ºC
- Incubator 20º - 25ºC.

Sampling procedure

We placed the exposure plates immediately after the athletes had left the training area. We placed all samples at 9:00 pm and removed the next day at 8:00 am in the morning. We packed and transported all samples in a cooler to the laboratory for analysis. We incubated all TSA plates in inverted position at 30º - 35ºC for 48 hours. We incubated also the RBA plates in inverted position at 20º - 25ºC for 48 hours.

Sampling analysis

We removed all the samples from the incubator at the 48 hours due an overgrowth in all TSA and RBA plates. We evaluated all the samples only for macroscopic identification and counted. All the samples were disposed as biohazard material.

Sterile carpet test

Dermatophytes are one of the most pathogenic fungi that have been identifying in human skin, hair or nails infections. Usually is transmitted by contact, particularly in common showers and gym facilities. This testing is a modification of the technique follow by Calcanti, 2002 and Bentubo 2006. We collected 30 samples in equipment were athletes are in direct contact. We also collected one sample for sterility check purpose.

Sampling materials

- 2 x 2 sterile carpet
- Mycosel agar petri dishes.

Sampling procedure

We removed the sterilized carpet from the bag and rubbed in the area of interest. We pushed the piece of carpet into the Mycosel Agar plate and then removed from the agar plate. After packing, we transported all the samples in a cooler to the laboratory for analysis. We incubated all the Mycosel agar plates in inverted position at 25ºC during four week.

Sampling analysis

We removed all the samples from the incubator when presenting sufficient growth for identification. We evaluated all samples by their macroscopic and microscopic morphological characteristics. For the microscopic identification, we used a slide and cover slip mounting with Lactophenol Aniline Blue. The analysis techniques was conducted following the Clendo Industrial Lab specification and different taxonomic guide.

Surface monitoring using swabs

We collected the surface samples with Tecra Enviroswabs in areas where athletes have greater contact. This type of sample is not destructive method in evaluating the presence of microorganisms on surface. We collected 35 samples, 33 inside the training area and two as background, one in the office and other in the Conference Room. We collected one swab as negative control for sterility test purpose. We conducted this sampling technique following the SOP 300-021 of Clendo Industrial laboratories Inc. and APHA 4th Edition (2001). The sampling technique, sampling analysis, incubation period were also followed the procedure that have been previously validated and approved by the laboratory. For the specific steps in the process of isolating and identifying bacteria, we used the Vitec 2 Compact and followed the SOP 300-008. We evaluated all the samples for fungi identification by their macroscopic and microscopic morphological characteristics.

Sampling materials

- Trypticase soy agar (TSA) plates
- Trypticase soy broth (TSB) - 10 ml
- Eosin methylene blue (EMB) agar plates
- Sterile tecra enviroswabs
- Personal protection equipment
- 20º - 25ºC incubator
- 30º - 35ºC incubator
- Rose Bengal agar (RBA)
- Lactophenol aniline blue.

Sampling procedure

We labeled each swab with the date, the spot of the sample and the control number of the laboratory. We removed each swab from the tube and pushed the tip of the swab to the side of the tube to remove excess diluents. We rubbed the sterile swab over the surface of a diameter that measures 2 x 2 centimeters. We placed the swab again in its packing and sealed. We transported all Enviroswabs to the laboratory for analysis.

Sampling analysis

We worked aseptically all Enviroswabs samples in the Biological Safety Cabinet to avoid contamination. In each Enviroswabs, we added 20 ml of Trypticase Soy Broth (TSB). We closed each tube and swirled in the vortex during one minutes. For each Enviroswabs sample, we used TSA plates in duplicate. In each plate, we added 1.0 ml in duplicate and 0.1 ml in duplicate of the sample. After added the samples in each Petri dish we added TSA using pour plate technique. We incubated all plates at 30º - 35ºC for 48 hour. We counted all colonies and calculated the number of colonies recovered from 50 cm^2 (equivalent to 1 ml of poured media). We re-incubated the plates at 20º - 25ºC for another 120 hours. All TSA plates were counted and then calculate the number of colonies recovered from 50 cm^2 (Colonies/50 cm^2) and reported in CFU. Use appropriate selective and differential media like EMB, Nutrient Agar, TSA and Sabouraud Dextrose Agar and incubated as required.

Air sampling

The objective of this study is to capture and quantify the different cultivable fungal and bacteria present in the air to determinate if the level present indicate a problem in the indoor environment. The samples was taken with the instrument SAS SUPER 100, a portable instrument that use the impaction of a medium of solid culture using Petri dishes of 100 mm. We selected TSA media to identify bacteria and RBA was choosing for the collection of fungi. We conducted this sampling technique following the SOP 100-023 of Clendo Industrial laboratories Inc and the EMLab P & K IAQ Pocket Reference Guide 2008. We sampled a totally of 30 TSA plate and 30 RBA plate using SAS 100 in different point selecting the random samples given by the program RANDOM.ORG and the quadrangle. We also collected two different samples as background, one in the Office and other in the exterior of the building. We collected one plate of TSA and RBA as negative control for sterility test purpose.

Sampling materials

- SAS SUPER 100 air sampler
- 70% ethanol
- TSA plates
- RBA plates
- 20º-25ºC incubator
- 30º-35ºC incubator
- Personal protective equipment.

Sampling procedure

We removed the coverlid from the Air Sampler. We inserted the contact plate into the Air Sampler. As manufacture recommendation, the air aspiration cycle was three minutes for each TSA and RBA plates. We removed the contact plate from the instrument and replaced with the lid of the SAS. After finished each sample we identified each contact plate with the sample ID point and the control number of the Clendo laboratory. After finished all the samples, packed it and sent to the laboratory in a cooler for analysis. We incubated all the samples for the specific time at the appropriate temperature: TSA at 30º to 35ºC for 48 hours and RBA at 20º to 25ºC for 5 days.

Sampling analysis

We counted all cultivable microorganisms at the end of the incubation period and related this number to the volume of air sampled. We made subculture of representative isolates for identification using an appropriate selective and differential media like EMB, Nutrient Agar, and Sabouraud Dextrose Agar. We incubated all the subcultures as required. We identified all the bacteria with gram stain. For the specific

steps in the process of isolating and identifying bacteria, we used the Vitec 2 Compact and followed the SOP 300-008. We evaluated for fungi identification, all the samples by their macroscopic and microscopic morphological characteristics.

Result calculation

We applied a correction factor to each sample prior to calculation of concentration of fungi and bacteria in each sample, expressed in CFU per cubic meter of air. We used the Most Probable Number (MPN) given by the manufacturer for the correction factor because more bacteria could be aspirate from the same hole and land on top of another bacterium on the surface media.

Example of calculation results: $X = \frac{Pr \times 100}{V}$

Where:

V = Volume of sampled air = 200 liters of air

R = Colony Forming Units counted on "55mm Contact Plates" = 67

Pr = Probable count obtained by positive hole correction = 80

X = Colony Forming Units per 1000 liters = $1m^3$ of air

$X = \frac{80 \times 100}{200}$

To express the result in CFU/ft^3, multiply the CFU/m^3 value by 0.02832 (Note conversion formula = 1 cubic foot = 20.32 liters.

Results

Temperature and relative humidity (% RH)

During the first phase, the average temperature in the training area was 91.1ºF and the relative humidity was 52.7%. The temperature in the office collected as background inside the building was 89.1ºF and the relative humidity was 56.7%. The temperature in the exterior of the building collected, as background was 89.6ºF and the relative humidity was 55.8% (Table 1).

During the second monitoring in the first phase, the average temperature in the training area was 93.0ºF and the relative humidity was 52.3%. The temperature in the office collected as background inside the building was 90.0ºF and the relative humidity was 59.3%. The temperature in the exterior of the building collected, as background was 82.2ºF and the relative humidity was 55.3% (Table 1).

During the second phase, the average temperature in the training area was 82.5ºF and the relative humidity was 72.6%. The temperature in the office collected as background inside the building was 81.5ºF and the relative humidity was 74.0%. The temperature in the exterior of the building collected, as background was 80.8ºF and the relative humidity was 74.6% (Table 1).

Carbon monoxide (CO) and carbon dioxide (CO_2)

During the first phase the average Carbon monoxide measured in the training area was 1.6 ppm and the CO_2 measured was 407 ppm. The CO measured in the office collected as background inside the building was 1.7 ppm and the CO_2 measured was 382 ppm. The CO measured in the exterior of the building collected as background was 1.4 ppm and the CO_2 measured was 384ppm (Table 1).

During the second monitoring in the first phase performed the average CO measured in the training area was 2.5 ppm and the CO_2 measured was 542 ppm. The CO measured in the office collected as background inside the building was 2.3 ppm and the Carbon Dioxide

measured was 618 ppm. The Carbon Monoxide measured in the exterior of the building collected as background was 2.4 ppm and the Carbon Dioxide measured was 562 ppm (Table 1).

During the second phase, performed on, the average carbon monoxide measured in the training area was 1.4 ppm and the carbon dioxide measured was 389 ppm. The carbon monoxide measured in the office collected as background inside the building was 1.5 ppm and the carbon dioxide measured was 388 ppm. The carbon monoxide measured in the exterior of the building collected as background was 1.5 ppm and the carbon dioxide measured was 496 ppm (Table 1).

Id point	Date			Temp (ºF)			RH%			CO			CO_2		
1	Jun 09	Jun 15	Oct 06	92.0	82.8	93.8	51.8	74.5	49.9	1.7	2.9	1.3	398	460	382
2	Jun 09	Jun 15	Oct 06	92.0	82.6	94.1	51.5	72.5	49.5	1.8	2.6	1.1	390	546	378
3	Jun 09	Jun 15	Oct 06	92.7	82.4	93.1	51.5	73.6	51.7	1.8	2.3	1.3	497	690	376
4	Jun 09	Jun 15	Oct 06	90.7	82.2	93.2	52.6	72.4	53.1	1.5	2.6	1.7	380	503	393
5	Jun 09	Jun 15	Oct 06	89.8	82.4	92.3	52.7	72.3	54.9	1.5	2.6	1.2	385	570	392
6	Jun 09	Jun 15	Oct 06	88.9	82.8	91.8	56.1	70.2	55.0	1.6	2.0	1.7	395	484	411
Bkg 1	Jun 09	Jun 15	Oct 06	89.1	81.5	90.0	56.7	74.0	59.3	1.7	2.3	1.5	382	618	388
Bkg 2	Jun 09	Jun 15	Oct 06	89.6	80.8	82.2	55.8	74.6	55.3	1.4	2.4	1.5	384	562	496

Table 1: *Physical characteristics of the facilities during the first, second and third monitory.*

Particulate matter (PM)

During the first phase performed, the average of PM measured in the training area was PM0.5 = 2.22 ug/m^3, PM1.0 = 7.93 ug/m^3, PM2.5 = 37.30 ug/m^3, PM5.0 = 192.40 ug/m^3, PM10 = 252.60 ug/m^3 and TPM = 298.50 ug/m^3. The average of PM measured in the office collected as background inside the building was PM0.5 = 1.72ug/m^3, PM1.0 = 6.91 ug/m^3, PM2.5 = 33.03 ug/m^3, PM5.0 = 161.18 ug/m^3, PM10 = 188.87 ug/m^3 and TPM = 201.35 ug/m^3. The PM measured in the exterior of the building collected as background was PM0.5 = 1.72ug/m^3, PM1.0 = 6.88 ug/m^3, PM2.5 = 32.90 ug/m^3, PM5.0 = 153.56 ug/m^3, PM10 = 180.28 ug/m^3 and TPM = 189.53 ug/m^3 (Table 2).

During the second monitoring in the first phase performed, the average of PM measured in the training area was PM0.5 = 1.35 ug/m^3, PM1.0 = 2.98 ug/m^3, PM2.5 = 12.32 ug/m^3, PM5.0 = 62.91 ug/m^3, PM10 = 87.84 ug/m^3 and TPM = 115.36 ug/m^3. The average of PM measured in the office collected as background inside the building was PM0.5 = 1.24 ug/m^3, PM1.0 = 2.92 ug/m^3, PM2.5 = 12.34 ug/m^3, PM5.0 = 63.75 ug/m^3, PM10 = 87.93 ug/m^3 and TPM = 117.05 ug/m^3. The PM measured in the exterior of the building collected as background was PM0.5 = 1.17 ug/m^3, PM1.0 = 2.65 ug/m^3, PM2.5 = 10.51 ug/m^3, PM5.0 = 51.42 ug/m^3, PM10 = 60.39 ug/m^3 and TPM = 71.48 ug/m^3 (Table 2).

During the second phase performed, the average of PM measured in the training area was PM0.5 = 2.07 ug/m^3, PM1.0 = 8.51 ug/m^3, PM2.5 = 39.51 ug/m^3, PM5.0 = 196.82 ug/m^3, PM10 = 241.64 ug/m^3 and TPM = 260.21 ug/m^3. The average of PM measured in the office collected as background inside the building was PM0.5 = 1.95 ug/m^3, PM1.0 = 8.02 ug/m^3, PM2.5 = 35.82 ug/m^3, PM5.0 = 167.06 ug/m^3, PM10 = 191.83 ug/m^3 and TPM = 205.70 ug/m^3. The PM measured in the exterior of the building collected as background was PM0.5 = 3.64 ug/m^3, PM1.0 = 9.44 ug/m^3, PM2.5 = 24.82 ug/m^3, PM5.0 = 64.48 ug/m^3, PM10 = 68.38 ug/m^3 and TPM = 76.70 ug/m^3 (Table 2).

Id point	PM 0.5			PM 1.0			PM 2.5			PM 5.0			PM 10			TPM		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	2.70	1.43	2.26	9.55	2.98	9.22	46.63	11.99	44.97	241.95	57.35	236.09	332.83	76.07	314.48	434.06	91.79	378.28
2	2.67	1.42	2.09	8.38	3.10	8.48	37.95	13.21	39.28	201.04	69.76	195.86	270.08	98.43	237.98	321.85	136.86	246.77
3	2.31	1.34	2.03	8.23	2.93	8.39	38.64	11.42	38.73	201.63	55.18	189.94	273.20	66.29	226.80	326.82	79.24	235.12
4	2.02	1.33	2.09	7.35	3.00	8.53	33.99	12.55	39.38	172.24	66.42	190.11	215.14	103.09	230.87	239.18	150.24	242.42
5	1.78	1.31	2.02	7.06	3.05	8.31	33.67	13.22	37.95	169.45	71.33	186.04	219.38	106.43	221.53	245.26	145.73	229.39
6	1.85	1.25	1.95	7.02	2.84	8.11	33.21	11.54	36.79	168.23	57.45	182.88	205.29	76.75	218.18	223.78	88.31	229.27
Bkg 1	1.72	1.24	1.95	6.91	2.92	8.02	33.03	12.34	35.82	161.18	63.75	167.06	188.87	87.93	191.83	201.35	117.05	205.70
Bkg 2	1.72	1.17	3.64	6.88	2.65	9.44	32.90	10.51	24.82	153.56	51.42	64.48	180.28	60.39	68.38	189.53	71.48	76.70

Table 2: *Particulate matter (PM) result during the first, second and third monitory.*

Spore trap air filter

We collected air samples using the Air-O-Cell Cassette for particle dust characterization. During the first phase performed on, ere collected six samples inside the training area. We collected two samples as background, one in the offices and other outside the building. The air samples in the training area indicated the presence of carbonaceous materials, dust, skin cells and the presence of several fungal spores being the predominant the *Penicillium/Aspergillus* and *Cladosporium* spores. The sample collected as background in the office indicated the presence of dust and skin cells and the sample in the exterior of the building indicated the presence of dust.

During the second phase performed on, were collected six samples inside the training area. We collected two samples as background, one in the office and other outside the building. The air samples in the training area indicated the presence of dust. All samples collected as background indicated the presence of dust.

Exposure plate

During the first phase performed, samples of TSA and RBA were carefully collected after has been exposure during 10 hours. The results indicated the presence of fungi and several bacteria. Due an overloaded growing in all the TSA and RBA plates all samples were observed for macroscopic identification. The result indicated Too Numerous to Count for all TSA and RBA plates (TNTC) (Table 3).

Sterile carpet test

During the first phase performed on we carefully collected samples with a sterile carpet. The result indicated the presence of various species of Dermatophytes likes *Microsporum ferrugineum*, *Microsporum cookei*, *Microsporum audouinii*, *Trichophyton verrucosum* and *Epidermophyton floccosum*. Other species of fungus identified was *Blastomyces dermatitidis, Fonsecaea pedrosoi, Aspergillus avenaceus, Aspergillus hollandicus, Scopulariopsis asperula, Penicillium citrinum, Paecilomyces viridis, Phialophora reptans, Phialophora richardsiae, Phialophora verrucosa, Cladosporium cladosporioides, Scytalidium infestans, Polypaecilum insolitum, Candida albicans* and *Histoplasma capsulatum* (Table 4).

During the second phase performed were collected the sterile carpet test followed by the cleaning and disinfestations of the CGC. The result indicated no growth of fungi in all Mycosel Agar plates after 21 days of incubation period.

Sample Id	TSA plate (#CFU)	RBA plates (#CFU)	Sample Id	TSA plates (#CFU)	RBA plates (#CFU)
109	TNTC	TNTC	64	TNTC	TNTC
34	TNTC	271	88	TNTC	TNTC
100	TNTC	TNTC	14	TNTC	TNTC
85	TNTC	TNTC	47	TNTC	TNTC
124	TNTC	TNTC	125	TNTC	TNTC
114	TNTC	TNTC	37	TNTC	TNTC
127	TNTC	TNTC	132	90	TNTC
29	TNTC	TNTC	68	TNTC	TNTC
102	TNTC	TNTC	17	TNTC	TNTC
43	TNTC	TNTC	6	TNTC	TNTC
105	TNTC	TNTC	75	TNTC	TNTC
26	TNTC	TNTC	38	TNTC	TNTC
16	96	TNTC	61	TNTC	81
52	TNTC	TNTC	1	TNTC	TNTC
90	TNTC	TNTC	Bkg 1	60	171
12	TNTC	TNTC	Bkg 2	TNTC	TNTC

Table 3: *Exposure plate result.*

Sample	First Testing	Second Testing	Health effect
Back rug 1B	*Blastomyces dermatitidis*	No growth	Cutaneous infections
Back rug 1E	*Fonsecaea pedrosoi*	No growth	Chromoblastomycosis
Foam pit mat	*Microsporum ferrugineum*	No growth	Tinea capitis
Bar	*Epidermophyton floccosum*	No growth	Infect skin and nail
Bar mat	*Aspergillus avenaceus* *Scopulariopsis asperula* *Penicillium citrinum*	No growth	Aspergillosis, cutaneous infections, corneal infections
Yellow cheese	*Aspergillus avenaceus* *Polypaecilum insolitum* *Penicillium citrinum*	No growth	Aspergillosis, corneal infections
Beam A	*Paecilomyces viridis*	No growth	Endocarditis
Pommel horse A	*Phialophora richardsiae*	No growth	Keratitis, cutaneous infections
Pail for legs	*Trichophyton verrucosum*	No growth	Infect scalp, nails, skin
Mat (baby gym)	*Aspergillus avenaceus*	No growth	Aspergillosis

Pommel horse B	*Aspergillus avenaceus* *Cladosporium cladosporioides* *Phialophora richardsiae*	No growth	Aspergillosis, pulmonary infections, cutaneous infections
Mat (pommel horse area)	*Microsporum cookei* *Aspergillus avenaceus* *Phialophora reptans*	No growth	Hair, cutaneous and pulmonary infections
Entrance floor	*Aspergillus hollandicus* *Scopulariopsis asperula* *Scytalidium infestans*	No growth	Pulmonary infections, Keratitis and cutaneous infections
Wooden cubicle	*Histoplasma capsulatum* *Cladosporium cladosporioides* *Phialophora verrucosa*	No growth	Pulmonary infections, hair, nail and cutaneous infections
Front rug 1A	*Candida albicans*	No growth	Infect skin, mucosal tract
Front rug 1B	*Candida albicans*	No growth	Infect skin, mucosal tract
Front rug 1C	*Microsporum audouinii*	No growth	Epidemic ringworm

Table 4: *Sterile carpet test result.*

Surface monitoring using swab

During the first phase performed on, were carefully collected samples with Tecra Enviroswabs. The result indicated the presence of various species bacterias. The most common pathogenic bacteria found were *Micrococcus lylae*, *Sphingomonas paucimobilis*, *Brevibacillus choshinensis*, *Staphylococcus epidermidis* and *Kocuria kristinae* (Table 5).

Microorganisms	Gram stain	Human health effect
Micrococcus lylae	Gram-positive	Meningitis, Endocarditis
Sphingomonas paucimobilis	Gram-negative	Bacterial infection of the bloodstream
Brevibacillus choshinensis	Gram-positive	Keratitis, urinary tract infections
Staphylococcus epidermidis	Gram-positive	Endocarditis
Kocuria kristinae	Gram-positive	Bacterial infection of the bloodstream

Table 5: *Most common pathogenic bacteria found with the Enviroswabs.*

During the second phase performed on, were collected Enviroswabs samples followed by the cleaning and disinfestations of the CGC. The result demonstrated that the process of cleaning and disinfection was effective with a cleaning effectivity of 97.44%.

Air sampling

During the first phase performed on, were collected air samples using SAS instrument. The result indicated the presence of various species of microorganisms. The most common pathogenic fungi founded in Air sampling was *Aspergillus niger*, *Aspergillus avenaceus*, *Aspergillus clavatus*, *Acremonium curvulum*, *Curvularia clavata* and *Penicillium chrysogenum* (Table 6). The most common bacteria were *Staphylococcus haemolyticus, Staphylococcus saprophyticus, Pantoea spp., Klebsiella pneumoniae, Bacillus megaterium* and *Staphylococcus epidermidis* (Table 7).

Microorganisms	Human health effect
Aspergillus niger	Aspergillosis, human carcinogenicity
Penicillium chrysogenum	Potential hazard for human
Aspergillus avenaceus	Opportunistic invaders that cause Aspergillosis
Aspergillus clavatus	Opportunistic invaders that cause Aspergillosis
Acremonium curvulum	Corneal infection, and nail infection
Curvularia clavata	Chronic allergic sinusitis with cerebral involvement

Table 6: *Most common pathogenic fungi found in air sampling.*

Microorganisms	Gram stain	Human health effect
Staphylococcus haemolyticus	Gram-positive	Conjunctivitis, infection in urinary tract
Staphylococcus saprophyticus	Gram-positive	Acute urinary tract infections
Pantoea spp.	Gram-negative	Opportunistic pathogen
Klebsiella pneumoniae	Gram-negative	Respiratory tract infections
Bacillus megaterium	Gram-positive	Involved in opportunistic infections
Staphylococcus epidermidis	Gram-positive	Opportunistic pathogen

Table 7: *Most common pathogenic bacteria found in air sampling.*

During the second phase performed on, were collected air samples followed by the cleaning and disinfestations of the CGC. The result demonstrated that the process of cleaning and disinfestations was effective with a cleaning effectivity of 80% (Figure 3 and 4).

Discussion

At this moment, there are no state of federal regulation for fungi, bacteria and indoor air quality standards. The industrial hygiene profession and the American Society of Heating, Refrigeration, and Air-Conditioning Engineers (ASHRAE) have recommended airborne concentrations of one-tenth the ACGIH Threshold Limit Value (TLV). This limit of concentrations will not produce compliance in non-industrial populations such schools, offices and others public buildings.

Temperature and relative humidity (%RH)

Air temperature and relative humidity are measured to assess thermal comfort and the possibility of mold growth. According to ASHRAE Standard 55, indoor air humidity levels should be maintain between 30 and 65 percent for optimum comfort and the temperature should be kept at 70º to 76ºF.

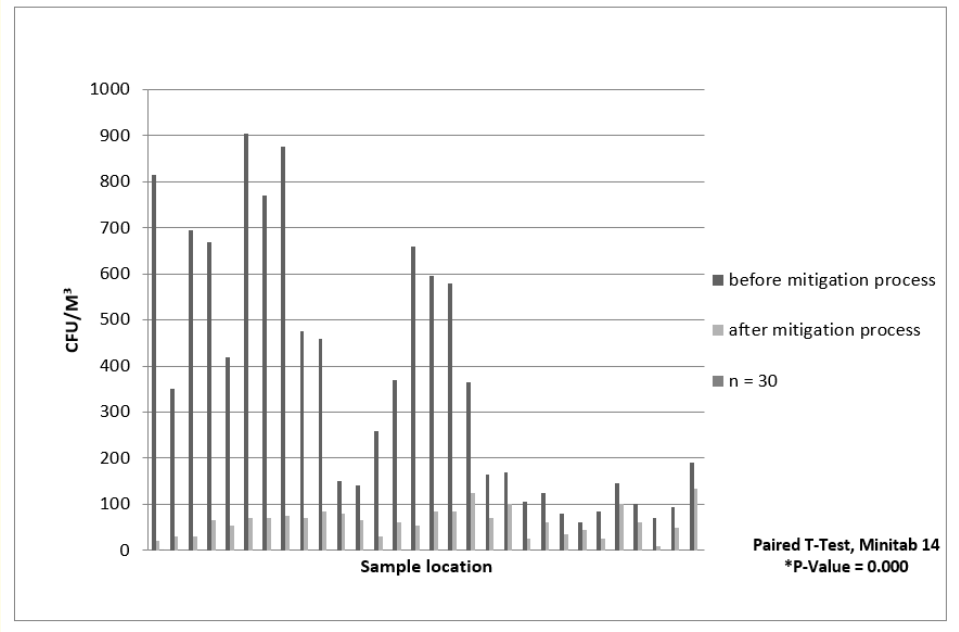

Figure 3: *Bacteria air sampling result before and after mitigation process.*

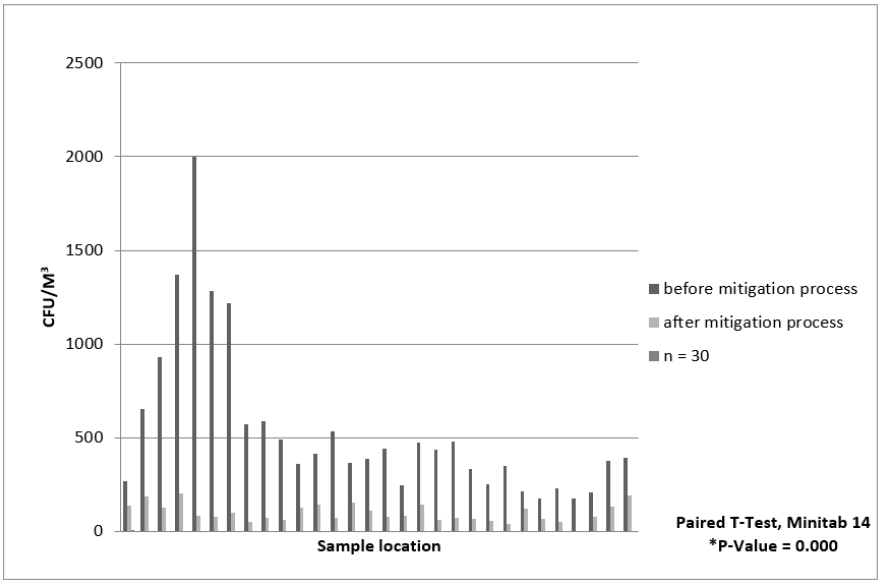

Figure 4: *Yeast and mold air sampling result before and after mitigation process.*

The average temperature measured was 88.8ºF and the relative humidity 59.2% in the training area. These results are above the recommended limit and the results were consistent with the background measurements obtained from the exterior of the building. The relative humidity in the training area is between the recommending limits and was consistent with the background.

Carbon monoxide (CO) and carbon dioxide (CO_2)

Carbon Monoxide is a colorless, odorless, and tasteless gas. It results from incomplete oxidation in combustion. Auto, truck or bus exhaust from attached garages, nearby roads, or parking areas can also be a source. No standards for CO have been agreed for indoor air. The US National Ambient Air Quality Standards for outdoor air are 9ppm for eight hours, and 35ppm for one hour. The Carbon monoxide measurements were below the permissible exposure limit during our study.

Carbon dioxide (CO_2) is a colorless, odorless product of carbon combustion. Human metabolic processes and all combustion processes of carbon fuels are sources of CO_2. Exhaled air is usually the largest source of CO_2. ASHRAE Standard 62 recommends an indoor level not to exceed about 700 ppm above outdoor ambient air, which is typically between 300 to 400 ppm. The Carbon dioxide measurements result were below the permissible exposure limits during our study.

Particulate matter (PM)

There are currently no federal government standards for PM2.5 in indoor air environments. The annual limit in National Ambient Air Quality Standards list is 15 ug/m^3 and 65 ug/m^3 is known as the 24-hours limit for PM2.5 in indoor air. The particulate matter in homes are related to carpet and clothing fibers, dust and dirt tracked into the home by its occupants, particles from food preparation, insect parts, plants, etc. These particles can cause symptoms such asthma, cardiac function and allergies in people, especially young children.

The result of particulate matter indicated an increase in all size range compared with the background samples from the exterior of the building. This size range of abnormal particles can be an indicator of potential risk for athletes and coaches inside the CGC.

Spore trap air filter

Spore trap samplers are capable of capture viable and non-viable fungal spores present in air. This sampler technique also captures particulate matter; quantify pollen, fiberglass, hair, skin cells, and hyphae fragments among others. If use this technique alone may miss a potential indoor air quality problem. That is why in our study we use cultivable samples and non-cultivable samples with the purpose to compare results.

The analytical result obtained from RAMS Environmental Laboratory, Inc in Miami, Florida for dust characterization by Optical Microscopy techniques indicated the presence of several fungal spores were the most predominant are *Aspergillus, Penicillium* and *Cladosporium* spp. These results are consistent with the cultivable samples using SAS instrument. During the visual inspection, we observed dust accumulation throughout the training area especially around the foam pit and the back rug. These finding are consistent with the dust characterization result. The results indicated the presence of dust, carbonaceous material and skin cell. After the cleaning and disinfestations process, the result obtained from the spore trap sampler indicated that the quantity of carbonaceous material, skin cell and fungal spore was decreasing significantly. Good housekeeping practices can lower the levels of the skin cell in indoor environment. However, at the same time, the quantity of dust are both >800. We observed during the cleaning process all the windows were close and actually are still close. The problem of bad ventilation in addition to maintain close window in the training area does not allow that the particulate going out the building.

Exposure plate

The purpose of this sampling technique was to estimate the contamination in the study area. The results obtained in a short incubation period allow us to create strategy for obtain accurately results and avoid technical mistake in the laboratory.

Sterile carpet test

This kind of testing is very uncommon for the indoor air quality specialist. Dermatophytes are the only fungus that has evolved in a dependency of human body that is why is very common to produce cutaneous infections in people. This group is composed of three genera (*Microsporum, Trichophyton* and *Epidermophyton*). During our study, we identified different species for each genus. We performed after the cleaning and disinfestations process in CGC a second monitoring of sterile carpet test. The sterile carpet test results indicate that no evidence of Dermatophytes after 21 days of incubation period.

Surface monitoring using swabs

There are no governmental or federal regulations concerning permissible level of fungi and bacteria. The result obtained from this testing indicated that 98% of the total microorganisms founded in CGC was bacteria and 2% fungi (Figure 5). For the bacteria identification, we used the Vitec Senior Model 120. Before the identification, we needed to have confirmed Gram stain from isolated colonies in purity plates. The result indicated that the most common pathogenic bacteria found with the Enviroswabs are Gram-positive bacteria (Table 5). *Micrococcus lylae, Brevibacillus choshinensis, Staphylococcus epidermidis* and *Kocuria kristinae* are Gram-positive bacteria. Most pathogenic bacteria in humans are Gram-positive microorganisms. Two of these groups are *Streptococcus* and *Staphylococcus*. *Sphingomonas paucimobilis* is Gram-negative bacteria. Gram-negative bacteria are associated with nosocomial infections.

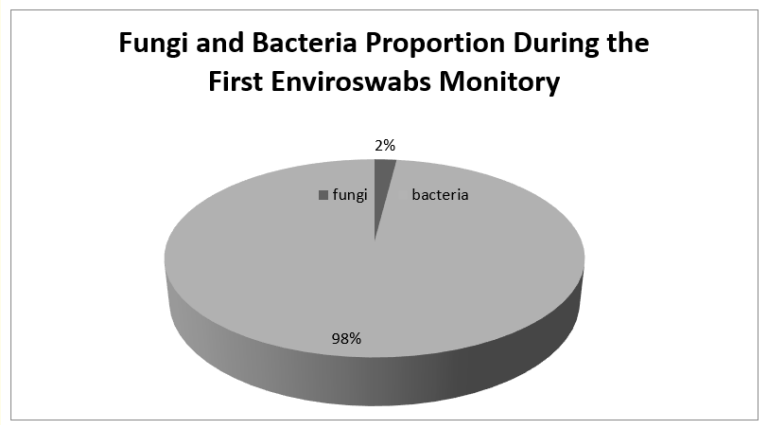

Figure 5: *Proportion of fungi and bacteria after the first Enviroswabs monitory.*

Air sampling

Actually there are none federal and governmental regulation concerning permissible levels of fungi and bacteria in indoor air. All the resent standards and guidelines range from 200 cfu/m^3 is an acceptable level for indoor environments. The ACGIH have guidelines that less than 500 cfu/m^3 is acceptable for certain species except for pathogenic species.

During our study, the 60.4% of the microorganisms identified were fungi and 39.6% bacteria (Figure 6). We identified 13 different fungi and 12 different bacteria. The most common pathogenic fungi are *Aspergillus spp.* These results are consistent with the spore trap technique. *Aspergillus* and *Penicillium* spp was the most common fungi identified in spore trap sampler. *Aspergillus spp.* has been associate with aspergillosis, human carcinogenicity due to aflatoxins produce by that genus of fungi and be involved in respiratory cancers among food and grain workers. The result indicated that the most common pathogenic bacteria found in air samples are Gram-positive bacteria,

for example *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis* and *Bacillus megaterium*. The Gram-negative bacteria are *Pantoea spp*. and *Klebsiella pneumonia*. *Staphylococcus epidermidis* is a human commensal bacterium. An increase number of human commensal bacteria in indoor environment may indicate high occupant density and poor ventilation; this situation may suggest an environment where airborne pathogens can be more easily spreader from person to person.

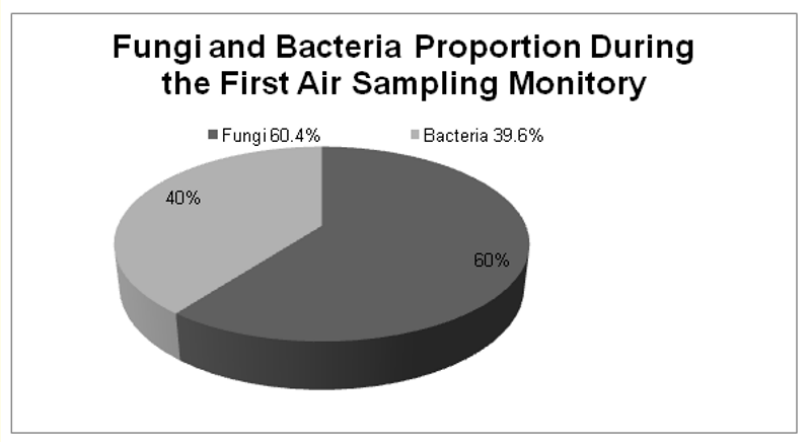

Figure 6: *Proportion of fungi and bacteria after the first air sampling.*

Conclusion

Gymnastics is a high-risk sport. Cuts and abrasions are inevitable for gymnastics athletes creating an elevated risk of infectious disease spread by skin-to-skin contact and contaminated equipment shared by athletes. Gymnastic mats, bars, beams, pommel horse and carpet are constant sweated increasing the environment for the growth of pathogenic microorganisms. The Caguas Gymnastic Club was built in 1972. At the beginning the facilities was used as a volleyball court. For this purpose the airflow, needed to be controlled for the practice of this sport. During our study, we identified the lack of airflow using the Drager Rohrchen air tube. The Caguas Gymnastic Club is a facility with high traffic of children during six day a week. During the visual assessment we can observed a lack of cleaning inside the training area.

Our results demonstrate a lot of dust, skin cell, particulate matter, fungi and bacteria inside the training area. The temperature levels in the facilities exceed the recommended by ASHRAE standards 55-2204. The Particulate matter result could be an indicator of potential risk health effect. The sampling before mitigation process revealed the presence of dermatophytes and pathogenic microorganisms in the air and the surface that could pose a health hazard to those in the facilities. The monitory after mitigation process demonstrated that the microbial remediation was effective. With a cleaning effectivity of Enviroswabs test = 97.44%, SAS air sampling = 80% and Sterile carpet test = 100%. After the mitigation process was performed a statistically significant difference (P-value = 0.000) between the number of cultivable bacteria and fungi before and after the process was detected (Figure 3 and 4). Indicating that the process is effective in dismissing bellow the public health concerns the number of potential pathogenic microorganism in the Caguas Gymnastic Club.

With an effective method of cleaning and decontamination of the facility we can made the microbiology remediation for Caguas Gymnastic Club. The use of an antimicrobial product (Trimethoxysilyl Quaternary Ammonium Chloride) we can prevent the presence of a

broad spectrum of microorganisms during approximately 90 days. With a good cleaning monitoring and good housekeeping practice, we can prevent the risk to the health of the children and coaches that use the facility.

We know that the microorganisms found during our study have a potential risk of infection for athletes and coaches in Caguas Gymnastic Club. The lacks of Standards and Guidelines for indoor environments do not give us the opportunity to evaluate the dose/responds and the exposition to humans. It is very important that the local and state agency and the Department of Sport create a cleaning and disinfection plan for all sports facilities to avoid bacterial and fungal infections acquired in athletic settings, including ringworm, athlete's foot, and community acquired Methicillin-resistant *Staphylococcus* infection (MRSA), herpes and impetigo.

Bibliography

1. Bartlett A. "Reflections…On sustainability, population growth, and the environment-revisited". *The Social Contract* (2005): 35-54.
2. Ali MM and Aksamija A. "Toward a better urban life: Integration of cities and tall buildings". The 4th Architectural Conference on High Rise Buildings, Amman-Jordan (2008): 9-11.
3. Krishna BK. "Atmospheric carbon and food security in Nepal". *Journal of Agriculture and Environment* 9 (2008): 54-61.
4. Simon H., *et al*. "Fine particulate Matter emissions inventories: Comparisons of emissions estimates with observations from recent field programs". *Journal of the Air and Waste Management Association* 583 (2008): 20-343.
5. Rentas A., *et al*. "Isolation and identification of airborne fungi that can cause asthma: a case study from Eastern Puerto Rico". *International Journal Environmental Technology and Management* 10 (2009): 243-259.
6. Spengler JD and Chen Q. "Indoor air quality factors in designing a healthy building". *Annual Review of Energy and the Environment* 25 (2000): 567-600.
7. US Environmental Protection Agency (EPA). Buildings and the Environment: A Statistical Summary (2009).
8. Schenck P., *et al*. "Climate change, indoor air quality and health". (Review Article) University of Connecticut Health Center Section of Occupational and Environmental Medicine Center for Indoor Environments and Health (2010).
9. Wu F., *et al*. "Improving indoor environmental quality for public health: impediments and policy recommendations". *Environmental Health Perspectives* 115.6 (2007): 953-964.
10. Sexton K and Dyer R. "Indoor air and human health". (2nd Edition). USA: CRC Press (2004).
11. Ye X., *et al*. "Environmental exposure and children's health in China". *Journal of Environmental and Occupational Health* 62.2 (2007): 61-73.
12. Chan T., *et al*. "Spatiotemporal analysis of air pollution and asthma patient visits in Taipei, Taiwan". *International Journal of Health Geographics* 8.26 (2009): 1-26.
13. Steinke J. "Genetics of hypersensitivity". Allergy Frontiers: Epigenetics, Allergens and Risk Factors 1.11 (2009): 227-238.
14. Carrer P., *et al*. "Allergens in indoor air: environmental assessment and health effects". *The Science of The Total Environment* 270.1-3 (2001): 33-42.

15. Charpin D., *et al*. "Triggers in intrinsic asthma in the EGEA study". *Journal of Asthma* 40.1 (2003): 87-91.

16. Mbata T and Nwajagu C. "Dermatophytes and other fungi associated with hair-scalp of nursery and primary school children in Awka, Nigeria". *The Internet Journal of Dermatology* 5.2 (2007): 1-9.

17. Garg J., *et al*. "Rapid detection of dermatophytes from skin and hair". *BMC Research Notes* 2.60 (2009).

18. Gomez-Mejaba S., *et al*. "Inhalation of environmental stressors and chronic inflammation: autoimmunity and neurodegeneration". *Mutation Research* 674.1-2 (2009): 62-72.

19. Clifford M., *et al*. "Current state of the science: health effects and indoor environmental quality". *Environmental Health Perspectives* 115.6 (2007): 1-8.

20. Boquete M., *et al*. "House Dust mite species and allergen level in Galicia, Spain: a cross-sectional, multicenter, comparative study". *Journal of Investigational Allergology Clinical Immunology* 16.3 (2006): 169-176.

21. Agency for Toxic Substance and Disease Registry. Case Studies in Environmental Medicine (CSEM) Environmental Triggers of Asthma Treatment, Management and Prevention (2009).

22. Sumedha J. "The sick building syndrome. (Review Article) (Clinical Report)". *Indian Journal of Occupational and Environmental Medicine* 12.2 (2008): 61-64.

23. US Environmental Protection Agency. Una breve guía para el moho y la humedad en su hogar (1997).

24. Morawska L. "Droplet fate in Indoor environments, or can we prevent the spread of infection". *Indoor Air* 16.5 (2006): 335-347.

25. Gots R., *et al*. "Indoor health: background level of fungi". *American Industrial Hygiene Association Journal* 64 (2003): 427-438.

26. Menzies D., *et al*. "Aeroallergens and work –related respiratory symptoms among office worker". *Allergy Clinic Immunology* 94.4 (2003): 38-44.

27. O'Connor G., *et al*. "Airborne fungi in the homes of children with asthma in low-income urban communities: the inner city asthma study". *Journal of Allergy and Clinical Immunology* 114.3 (2004): 599-606.

28. TorvinenE., *et al*. "Mycobacteria and fungi in moisture-damage building materials". *Applied and Environmental Microbiology* 72.10 (2006): 6822-6824.

29. Jacobs D., *et al*. "A systematic review of housing interventions and health". *Journal of Public Health Management and Practice* 16.5 (2010): S5-S10.

30. University of Michigan Health System. Potentially dangerous staph infections spreading (2004).

31. Koren H and Bisesi M. "Environmental Problems and Human Health". Handbook of Environmental Heath, Biological, Chemical and Physical Agent on Environmentally Related Disease 1 (2008): 79-85.

32. US Environmental Protection Agency. A guide to indoor air quality. (EPA 402-K-93-007). Washington, DC: Indoor Environments Division, Indoor Air Quality Tools for Schools (1995).

Volume 19 Issue 4 April 2023