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A Simple, Cost Effective and Rapid Air Borne Mold-Monitoring Model Developed in St. Kitts for Ensuring Global Public Health Safety and Food Security

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Abstract

The morbidity and mortality caused by pathogenic and opportunistic air borne molds (ABM) has been on the rise. ABM are not confined by national boundaries but can travel distances over time and can cause respiratory dysfunction, crop damage, food spoilage and serious meningitis outbreaks due to contamination of pharmaceuticals. Presence of mold spores in abnormally high numbers can present hazardous risks to human population world-wide causing mycosis, hypersensitivity reactions or poisoning by mycotoxins. Tropical places and places receiving heavy rains in the monsoon season can end up with a massively higher mold count and the aftermath of hurricanes and floods can result in dangerously high levels of mold counts in the environment. ABM does not uniformly affect the population but is more dependent on individual hypersensitivity, age, nutritional status, vegetation, ventilation etc. While the world has been acutely aware of particulate and minute organic and inorganic pollutants from automobile emissions and industrial release of gases in the air, the presence of molds in the environment has received little attention until the recent outbreak of Meningitis, which resulted in over 60+ deaths and 100s of hospitalizations in the USA. There is an urgency now to develop the tools that can be universally adopted to monitor the mold count in the air around the landmass of the world and then to characterize the type of mold to be followed by ways of cleaning the environment of harmful molds. Here we propose a simple, cost effective and rapid model for monitoring molds that does not require sophisticated infrastructure to gain initial insight of what is in the air in terms of molds we breathe locally and assess the impact it could have. Developed countries could certainly go steps ahead in terms of sophistication in identifying the molds such as by use of PCR but for now to establish a baseline of what is the mold count and what are the general types of molds in surrounding air, a simple low cost model could be a start. Our results from assessment of molds on an island nation have revealed that mold count varies within certain regions and settings. The diversity of the molds is proportional to the mold count. Most molds found can cause little direct harm to individuals but can contribute to crop damage and food spoilage. Overall the benefit to the inhabitants in getting to know the types of molds in the surrounding air they breathe is going to be of considerable benefit.

Introduction

Molds are found both indoors and outdoors. Estimates of species of air borne molds range from tens of thousands to perhaps three hundred thousand. Molds grow best in warm, damp, and humid conditions, and spread and reproduce by spores [1,2]. Mold spores can survive harsh environmental conditions, such as dry conditions, that do not support normal mold growth. Some of the more common air molds include *Cladosporium, Penicillium, Alternaria,* and *Aspergillus*. Several studies have suggested a potential link of early mold exposure to development of asthma in some children, particularly among children who may be genetically susceptible to asthma development [1,2]. There is enhanced awareness of the importance of mold contamination in residential environments. Individual immune responses to these fungi may range from no symptoms to severe, depending on each person's susceptibility to the allergen [3]. Several investigators have reported on the prevalence of outdoor and indoor air molds in different countries [4-7].

A wide variety of microorganisms including fungi are dispersed into the Caribbean region by the Saharan dust aerosols during the summer months. These microorganisms can cause diseases in plants and animals, and might be responsible for an increase in incidence of asthma and respiratory diseases in this region [8,9]. Information on the prevalence of air molds in the Caribbean region has been very much lacking. In a study from Cuba, the principal air-borne molds detected were *Cladosporium, Aspergillus,* and yeasts, with many other genera being encountered occasionally. In another study in Cuba, *Aspergillus, Cladopsorium* and *Penicillium* spores were detected abundantly at different times of the day [10,11]. A Dominican study identified six genera of air born fungi including *Aspergillus, Penicillium, Cladosporium, Fusarium, Curvularia,* and *Nigrospora* [12].

The rationale for the present 2-part study is to propose a simple, cost effective and rapid model to monitor air borne molds around the world by first obtaining a mold count within the air of one of the smallest independent nations in the world, using Google maps to catalog the mold counts by location, and further characterizing these mold species [13]. We hope this 4-step model will provide the know-how to identify regions of high mold count, followed by characterization of the diversity of both pathogenic and non-pathogenic molds distributed world-wide. Here, we report the latter part and together, the model could be adopted to cover the air over the landmass of the rest of the world. This information would be vital for ensuring the public health safety and food security.

Materials and Methods

The methodology to trap the molds is essentially the same as

described earlier [13]. Duplicate Petri plates of Difco Sabaroud's Dextrose agar (SDA (40 g/L dextrose, 10 g/L peptone, 20 g/L agar, pH 5. 6)) were exposed for one hour at various pre-determined locations followed by a 48 hour incubation period at room temperature inside a bio-safety hood. Final mold count was obtained by repeating the entire process once again in duplicate with an exposure for periods shorter than 1 hour for those that had too many to count (TMTC) quantities of molds. The mold counts for those with shorter exposure were then multiplied by a factor equal to the fraction. If there was a 15-minute exposure in the second round then the mold count was corrected for 1 hour by multiplying by a factor of 4. The average count/hour of exposure was obtained by averaging the mold count from the 2 separate exposures for the same location. Exposure locations included apartments and places of business because even these locations are susceptible to pathogenic fungal colonization. Places using electrical appliances to artificially modify the environment were included in the experiment as they have been shown to potentially carry the same risk for mold contamination [14]. Identification of the molds was attempted by a detailed study of colonial morphology and microscopic features as observed in lactophenol cotton blue mounts, and comparing the observed characteristics with descriptions given in standard books and manuals [15-17]. The distribution of molds identified was then, using Google maps, organized into pockets of air sampled providing a map of the location with the corresponding molds in the air of that location (Google map 1 and 2).

Results

Twelve different locations on the island of St. Kitts were examined for fungi present in the air. More than 20 different species of fungi and
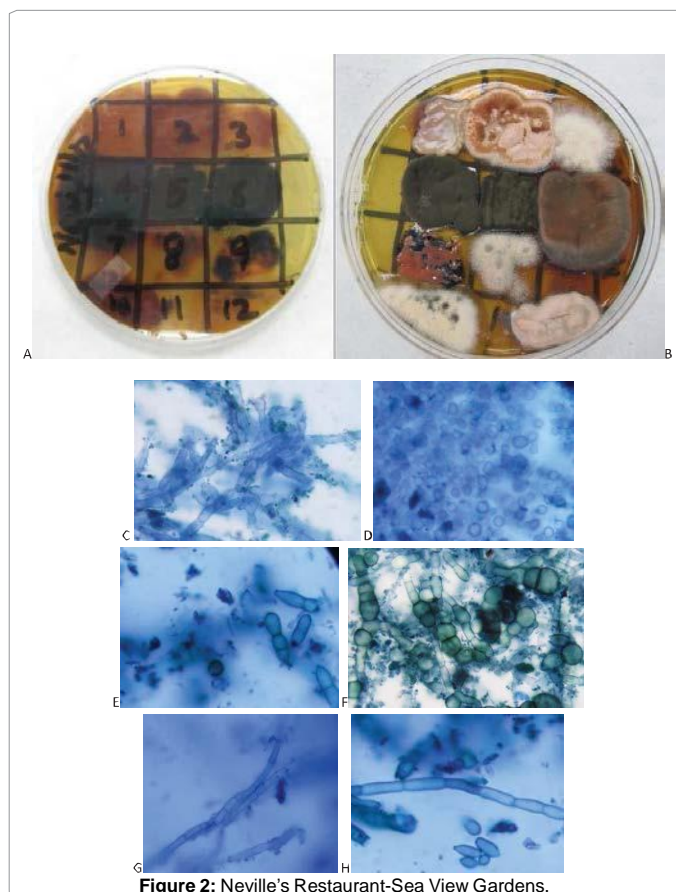

Figure 1: Mattingly Heights.

Figure 2: Neville's Restaurant-Sea View Gardens.

yeast were identified. *Cladosporium cladosporioides*, *Mucor* sp and *Aspergillus sp.* were all found in three or more locations throughout the island. Other infrequently recovered molds included *Curvularia, Alternaria, Fusarium solani, Metarrizum, Stachybotry, Trichosporon, Geotrichum, and Sporobolomyces*. The brief detail of the isolations of molds from different locations are mentioned below

Mattingly Heights-Residential Housing

A kitchen area in a house in Mattingly Heights was examined. This was one of the sites that showed mold overgrowth and the plates of SDA were re-exposed to air in this location for a shorter duration of 15 minutes and incubated until mold growth was manageable enough to isolate colonies. Based on microscopic analysis of fungal growth in Mattingly Heights, it was noted that predominant fungi prevalent in the air in the Camps Area were *Mucor* sp and *Cladosporium cladosporioides* (Figure 1).

Sea View Gardens- Neville's restaurant

A restaurant by the main road in greater Camps area called Neville's was examined. This site also showed mold overgrowth and the plates of Sabouraud dextrose agar were re-exposed to air in this location, and incubated until appreciable mold growth occurred to recover cultures. *Cladosporium cladosporioides* was the predominant fungal agent in the air; a few colonies of *Geotrichum candidum* were also observed (Figure 2).

Half Moon Bay- Horizon's villa

Residential housing area in the Half Moon Bay was examined. This site showed significant growth of *Aspergillus fumigatus* and *Mucor* sp,
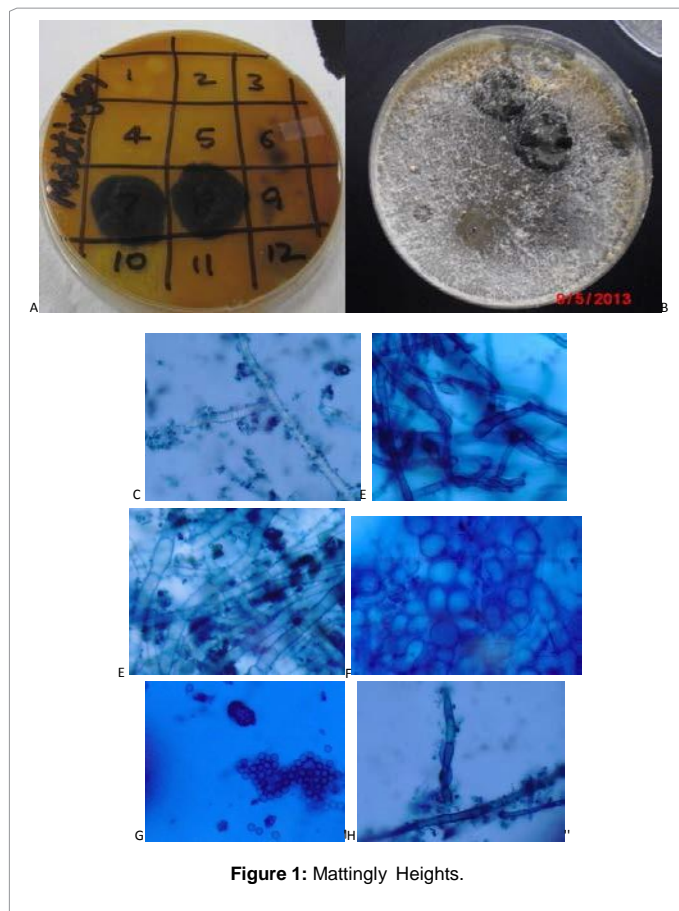
Figure 3: Half Moon Bay-Horizons Villa.

Figure 4: Rituals Coffee House- Frigate Bay.

as evidenced by a large number of colonies of both species appearing on the plates (Figure 3).

Frigate bay

Rituals Coffee House, a restaurant and coffee house in Frigate Bay area was examined. This location showed growth of several colonies of fungi, mainly *Cladosporium sp*, *Alternaria* (*A. alternata*) and the yeast, and *Trichosporon* sp. (Figure 4).

Rituals Sushi Restaurant in the Frigate Bay area was examined. Location showed multiple colonies of *Cladosporium* sp and *Candida* sp (Figure 5).

Camps

A residential housing in the Camps area was examined. Significant overgrowth was seen on test plates as well as master plate. Growth included several colonies of species of *Aspergillus* (mainly *A.niger*, *A. fumigatus*, and *A. flavus*), *Mucor, Cladosporium* sp, and *Trichosporon* sp. (Figure 6).

The Best Buy Grocery Store located in Camps was examined. Large amount of mold growth was seen, including that of *Rhodotorula sp.* and *Cladosporium sp.* (Figure 9).

UMHS campus

The lunch tent on the UMHS campus where most students eat meals while on campus was examined. Significant growth was seen and fungal species included *Mucor* sp, *Aspergillus* sp (Figure 7).
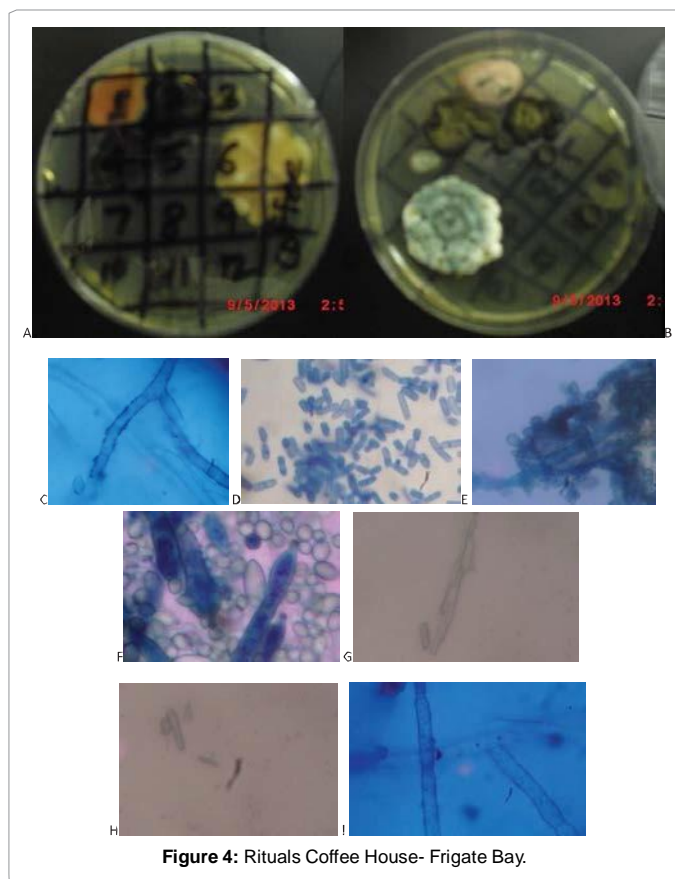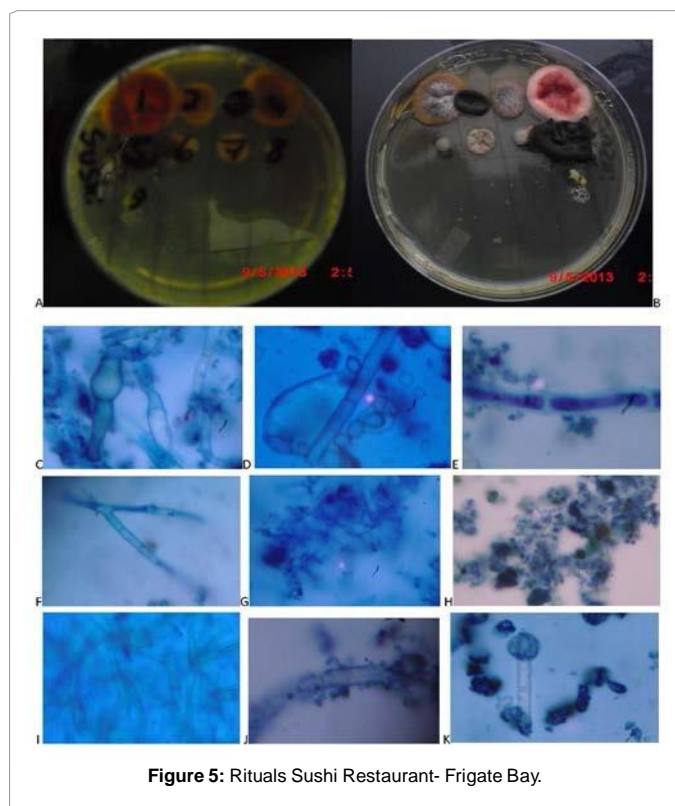

Figure 5: Rituals Sushi Restaurant- Frigate Bay.

The Student Lounge on the East side of the Administration building was examined. Large colonies of growth were seen, including fungi and yeasts. The predominant species was *Aspergillus fumigatus*, followed by *Curvularia* species. A few colonies of *Bipolaris* sp were also observed (Figure 8).

Figure 6: Camps Housing.

Figure 7: UMHS Lunch Tent-Camps.

The results are summarized in Table 1 and Table 2. In Table 1, all the molds are identified against locations around the Island. This gives a clear picture of which molds are found in several locations and Table 2 summarizes all the molds found within each location. It may be mentioned here that it was not possible to identify all the mold species year round to reflect seasonal differences and the identification of yeasts to specific level was not done due to lack of reagents and yeast identification kits. Therefore, while adopting this model for global surveillance in the future, it is advised to repeat mold counts during all seasons experienced in the given geographical area.

Discussion

The present investigation constitutes the first study of its kind dealing with the types of airborne molds in an island nation of St. Kiits. It is noteworthy that a variety of species were recovered in the agar plates exposed to air in different types of locations. The predominant fungi were *Cladosporium* (*C. cladosporioides*) and Aspergillus (mainly *A. fumigatus*, *A. Niger* and *A. flavus*). The presence of Aspergillus fumigatus in quantity in air has important health implications for humans and animals [18]. *A. fumigatus* is quite common in house dust, indoor and outdoor, in different types of soil, on decaying plant material, compost, and also on hay and crops. It is also an important etiological agent of systemic mycosis in domestic animals and in humans, especially the immunosuppressed and immunocompromised
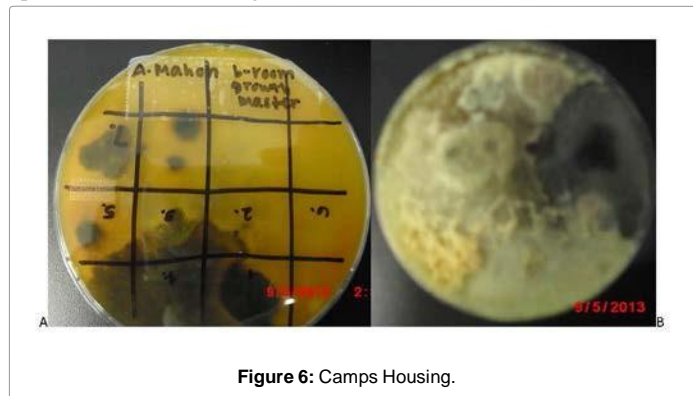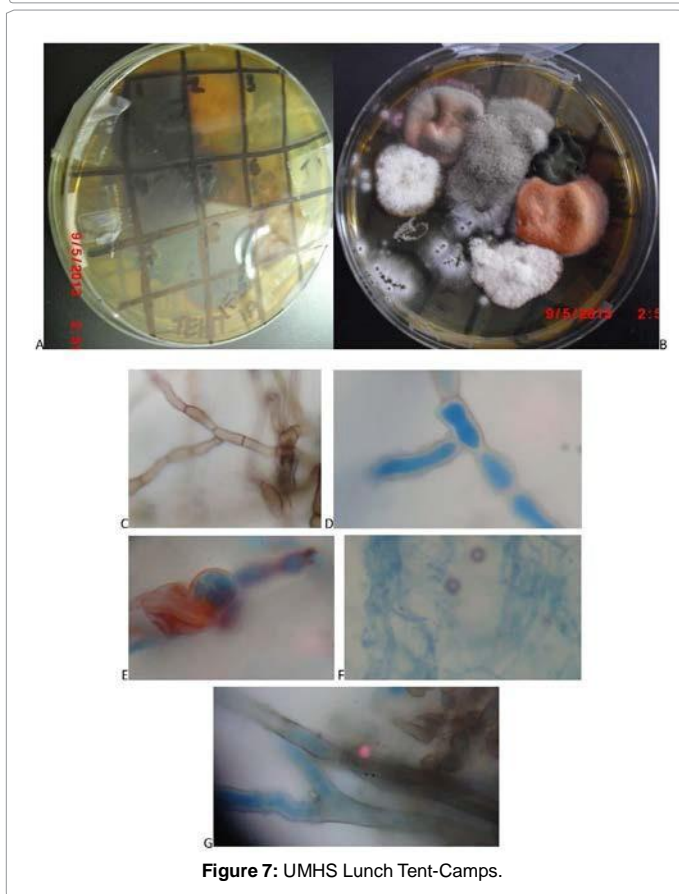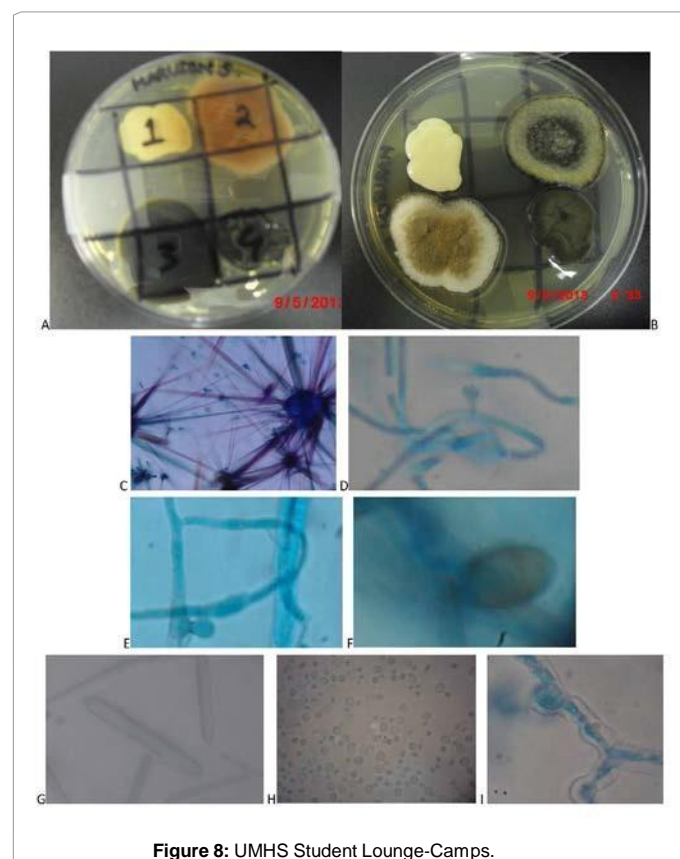

Figure 8: UMHS Student Lounge-Camps.

Figure 9: Best Buy.

	Alternaria alternata	Aspergillus flavus	Aspergillus fumigatus	Aspergillus niger	Aspergillus sp.	Bipolaris sp	Candida sp.	Cladosporium cladosporioides	Cladisporiodies sp.	Curvularia sp.	Fusarium solani	Geotrichum candidum	Metarhizum sp.	Mucor sp.	Rhodoturola sp.	Sporbolomyces	S. chartarum	Stachybotrys	Trichosporon sp.
Camps- Best Buy Grocery Store								✓						✓					
Camps- Residential housing		✓	✓	✓								✓		✓					✓
Camps- UMHS Campus			✓			✓				✓									
Camps- UMHS Lunch Tent					✓									✓					
Frigate Bay- Coffee House	✓							✓											✓
Frigate Bay- Sushi Restaurant							✓	✓											
Half Moon Bay- Horizon's Villa		✓												✓					
Mattingly Heights- Residential housing								✓						✓					
Sea View Gardens- Neville's Restaurant								✓				✓							
Other											✓		✓			✓	✓	✓	

Table 1: Mold II Sheet 1.

Location	Mold Species
Residential Housing- Mattingly Heights	Cladosporium cladosporioides Mucor sp.
Neville's Restaurant- Sea View Gardens	Cladosporium cladosporioides Geotrichum candidum
Horizon's Villa- Half Moon Bay	Mucor sp. Aspergillus fumigatus
Ritual's Coffee House- Frigate Bay	Cladosporium sp. Trichosporon sp. Alternaria alternata
Ritual's Sushi Restaurant- Frigate Bay	Cladosporium sp. Candida sp.
Residential Housing- Camps	Aspergillus niger Aspergillus fumigatus Aspergillus flavus Mucor sp. Trichosporon sp. Cladosporium sp.
UMHS Lunch Tent-	Mucor Aspergillus sp.
UMHS Campus- Camps	Aspergillus fumigatus Curvularia sp. Bipolaris sp.
Camps- Best Buy Grocery Store	Rhodoturola sp. Cladosporium sp.
Species infrequently found on St. Kitts	Metarhizum sp. Fusarium solani Stachybotrys S. chartarum Sporobolomyces

Table 2: Location of mold species.

[19,20]. *Aspergillus fumigatus*, *A. Niger* and *A. flavus* have also been reported to cause allergies, asthma, and rhinitis in children and adults [20]. *C. cladosporioides*, a dematiaceous fungus is frequently encountered in indoor and outdoor environment occasionally linked to health problems [14]. *Fusarium solani*, a species infrequently recovered in this study is known to cause mycotic keratitis and skin infections in humans [20, 21]. *A. alternate*, another dematiaceous mold occasionally encountered in this study has been recognized as risk factor for asthma [22]. *Stachybotrys*, occasionally encountered in our study is known to cause human disease through direct irritation, type 1 hypersensitivity, or production of toxins. A variety of respiratory, dermatological, eye, and constitutional symptoms have been associated with heavy and prolonged exposure to *S chartarum* [23]. The design and methodology detailed in this novel study can be adopted globally to generate a Google map of the total count and types of air borne molds prevalent worldwide. This information can also be used to ensure public health by using appropriate masks, and ensure food security by its proper handling and preservation. Currently such a map exists for a few pathogenic molds like *Histoplasma capsulatum* present in the Ohio valley in USA, *Coccoidiosis immitis* present in the Southwest region of USA etc. The diversity of fungi that could directly or indirectly cause harm has not been sufficiently studied in the past making this one of the earliest studies and attempts to obtain a handle at what else is in the air surrounding human inhabitants, plant and animal life. Capturing the molds on culture plates, followed by their morphological identification and comparison with molds from the immediate vicinity can serve as a simplified and cost-effective model for recognizing and monitoring airborne molds in much of the atmosphere. We envision that by ultimately expanding our model of organizing the information gathered and emphasizing the unique molds that have been identified, studies similar to ours can be conducted in other geographical areas.

References

1. Centers for Disease Control and Prevention (2009, May 21). Air Pollution and Respiratory Health. Retrieved from http://www.cdc.gov/nceh/airpollution/indoor_air.htmhttp://www.cdc.gov/nceh/airpollution/indoor_air.htm.

2. World Health Organization (2009). WHO guidelines for indoor air quality: dampness and mould. Retrieved from http://www.who.int/indoorair/publications/7989289041683/en/

3. Cooley JD, Wong WC, Jumper CA, Strauss DC (1988) Correlation between the prevalence of certain fungi and sick building syndrome. Occup Environ Med 55: 579-584.

4. Abdel-Hafez SII, Shoreit AAM (1985) Mycotoxins producing fungi and mycoflora of air-dust from Taif, Saudi Arabia. Mycopathologia 92: 65-71.

5. Abdel-Hafez SII, Shoreit AAM, Maghraby MOE (1986) Mycoflora and mycotoxin-producing fungi of air-dust particles. Mycopathologia 93: 25-32.

6. Vital RPR, Rasool SK (1995) Enumeration of airborne molds in some indoor environments of Madras city (India) by cultural and non-cultural volumetric samplers. Aerobiologia 11: 201-204.

7. Shelton BG, Kirkland KH, Flanders WD, Morris GK (2002) Profiles of airborne fungi in buildings and outdoor environments in the United States. Appl Environ Microbiol 68: 1743-1753.

8. Griffin DW, Kellogg CA, Garrison VH, Lisle JT, Borden TC, et al. (2003) African dust in the Caribbean atmosphere. Aerobiologia 19:143-157.

9. Griffin DW (2007) Atmospheric Movement of Microorganisms in Clouds of Desert Dust and Implications for Human Health. Clin Microbiol Rev 20: 459–477

10. Alvarez JC, Castro JF (1955) Numbers and kinds of air-borne, culturable fungus spores in Havana. Cuba. J Allergy 26: 152-162.

11. Almaguer M, Aira MJ, Rodríguez-Rajo FJ, Rojas TI (2013) Temporal dynamics of airborne fungi in Havana (Cuba) during dry and rainy seasons: influence of meteorological parameters. Int J Biometeorol 58: 1459-1470.

12. Rivera-Denizard O, Betancourt C, Armstrong RA, Detres Y (2003) Airborne fungi in Sahara dust aerosols reaching the Eastern Caribbean: I. Taxonomic characterization by morphological features. American Geophysical Union, Fall Meeting 2003, abstract #B21F-0775.

13. Prasla I, Duman K, Ciochetto Z, Burman A, Mahon A, et al. (2014) Significant Heterogeneity in Airborne Mold Quantities on the Caribbean Island of St Kitts: Health Implications and Impact on Food Preservation. Virol Mycol 3: 123.

14. Joe WK, Lee JH (2008) Airborne fungi and bacteria associated with automobile airconditioners, heaters, and humidifiers. Arch Environ Occup Health 63: 101-107.

15. Kwon-Chung KJ, Bennett JE (1992) Medical Mycology. Philadelphia, LeaFebiger.

16. DeHoog GS, Guarro J, Gene J, Figueras MJ (2000) Atlas of Clinical Fungi. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands/Universitat Rovirai Virgili. Spain.

17. Ellis D, Davis S, Alexiou H, Handke R, Bartley R (2007) Descriptions of Medical Fungi, Mycology Unit, Women's and Children's Hospital, and School of Molecular and Biomedical Science, University of Adelaide. University of Adelaide: Adlelaide: 198.

18. Pattron DD, Aspergillus (2006) Health implication & recommendations for public health food safety. Internet J Food Safety 8: 19-23.

19. Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM (2006) The link between fungi and severe asthma: a summary of the evidence. Eur Respir J 27: 615–626.

20. Gugnani HC, Gupta SC, Talwar RS (1978) Role of opportunistic fungi in ocular infections in Nigeria. Mycopathologia 65: 155-166.

21. Oyeka CA, Gugnani HC (1992) Skin infections due to Hendersonula toruloidea, Scytalidium hyalinum, Fusarium solani and dermatophytes in cement factory workers. Journal de Mycologle Medicale 2: 197-201.

22. Salo PM, Arbes Jr SJ, Sever M, Jaramillo R, Cohn RD, et al. (2006) Exposure to Alternaria alternata in US homes is associated with asthma symptoms. J Allergy Clin Immunol 118: 892-896.

23. Bitnun A, Nosal RM (1999) *Stachybotrys chartarum* (atra) contamination of the indoor environment: Health implications. Paediatr Child Health 4: 125–129.