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Influence of Environmental Parameters on Mold Sampling Results

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by

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Dedication

This thesis is dedicated to my mother, father, and brother, who believed in me and supported me through all my years away from home. Thank you all so very much.

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Abstract

Mold is a type of fungus present in nearly all environments. Mold thrives under several environmental parameters such as high humidity and an adequate food source. A professional, such as an industrial hygienist, can measure mold in indoor and outdoor environments. Industrial hygienists commonly use a cascade impactor with a culture plate to capture air within a sampling area. While collecting air samples, environmental parameters such as temperature, humidity, and carbon dioxide are recorded. A laboratory then cultures and analyzes the samples, identifying the types and amounts of viable mold found in the sampling area.

In this study, a data analysis method is used to interpret lab results and compare those results to the environmental parameters measured during collection. The study aims to show the relationship between the environmental parameters (temperature, humidity, carbon dioxide) and the types and amounts of mold that were measured in both indoor built environments and their surrounding outdoor areas.

Among all 170 different sampling locations, the outdoor areas had higher counts and concentrations of mold. In addition, both indoor and outdoor areas saw *Penicillium, Aspergillus,* and *Cladosporium* as the most prevalent molds, with *Cladosporium* having the highest counts. Lower temperatures and humidity had a very small influence on mold growth and thus, yielded the lowest counts. Furthermore, the highest concentrations of mold were found within the same temperature and humidity ranges for both indoor and outdoor environments.

Introduction and Review of Literature

Both indoor and outdoor environments are full of biological agents. These agents, which come in many shapes and sizes, include mold. In the built environment, indoor air quality may be of particular interest given the occupants may spend more time indoors on a daily basis than they do outdoors. When considering air quality in either environment, many factors come into play. Many indoor contaminants may come from outdoor air coming in through a door, window, or ventilation system. The outdoor air contains bacteria, mold, allergens, and other agents. Of particular interest, is mold (fungi), which thrives under certain conditions.

In general, fungi need sufficient temperature, moisture (water activity, relative humidity, etc.), and nutrients to survive. Light may or may not be necessary for mold growth. Temperature and water activity play a role in that they dictate different types and amounts of fungal growths. Mesophilic fungi such as As*pergillus, Cladosporium, Epicoccum,* require between 0.8 and 0.9 water activity to grow (Hung et al., 2005). These mesophilic fungi also generally have an optimal temperature range in which they grow, defined as 65 to 80 degrees Fahrenheit (°F) (Bailey, 2005). Mold growth is also dependent on humidity. It is widely accepted that although certain fungi prefer certain levels of relative humidity, most experience optimal growth at a range of 65 to 90% (Bailey, 2005). Relative humidity can coincide with water activity, which fuels fungal growth in indoor and outdoor environments, making relative humidity particularly important in the role of mold growth. Carbon dioxide levels, while often measured alongside temperature and relative humidity, are not reported to influence mold growth. Rather, carbon dioxide levels are simply used as an indicator for the acceptability of indoor spaces for human occupancy (Persily,

1997). It is important to note that carbon dioxide levels can fluctuate and are dependent on building occupancy patterns, outside sources, location, and time (Persily, 1997).

When considering fungi in indoor and outdoor environments, differences can be seen in the types and amounts of growth that occur in each environment. Generally, outdoor sampling will result in higher mold levels than indoors. For example, in a study by Meklin et al. (2007), indoor spore concentrations were 353 per cubic meter as compared to an outdoor spore concentration of 827 per cubic meter. In this study, *Cladosporium* and *Aspergillus* concentrations were similar in both environments. It is also of particular interest to study indoor and outdoor relationships of mold concentrations. One common method of characterizing indoor mold growth is by use of indoor/outdoor ratios, which compares indoor mold concentrations to those detected outdoors (Burge, 2011).

There are several methods available for sampling for mold. Cascade impaction is a very common method of quantifying the viable content of the atmosphere (BGI Inc., 2009). Specifically, a single-stage viable impactor such as an Andersen N6 can be used. Currently, there are no Occupational Safety and Health Association (OSHA) or American Conference of Governmental Industrial Hygienists (ACGIH) standards or exposure limits for biological agents and no official National Institute for Occupational Safety and Health (NIOSH) method for sampling. However, industrial hygienists commonly use the Andersen N6 device. The impactor is used for viable samples, meaning the samples are subsequently grown under specific incubation parameters. The impactor itself works by pulling fresh air onto an agar culture plate using a pump. The culture plate sits open faced on the stage and an air pump is set at 28.3 liters per minute air flow, as per the manufacturer's specifications. The air pump pulls the air into the top opening of the impactor onto the culture plate. The sampling time is subjective, depending on

manufacturer specifications, but is typically about two or more minutes. Samples are collected with the impactor set on a tripod, which has a height approaching that of the personal breathing zone of a standing adult person (Kleinheinz et al., 2006). When the process is finished, the culture plate is carefully removed from the impactor stage, sealed, labeled, and sent to a lab (Kleinheinz et al., 2006). It is important to note that the type of sample media can impact what grows. For instance, Rose Bengal Agar has selective properties that may cause some strains of fungi to grow poorly or fail to grow at all (HiMedia Labs, 2011).

Another method of air sampling for mold is the Air-O-Cell sampling cassette. This method is used for the collection of non-viable samples, which are organisms not subjected to culture. The Air-O-Cell uses inertial impaction where air flows into the slit of the cassette onto an adhesive collection media (Zefon International, 2017). The air flow into the Air-O-Cell is typically at 15 liters per minute for up to 10 minutes, depending on the indoor conditions and traffic of occupants (Zefon International, 2009). Once complete, the Air-O-Cell is shipped to a lab where biological staining processes reveal the collected particulate (Zefon International, 2017). Both the Andersen N-6 and the Air-O-Cell methods can be used for outdoor areas as well as indoors.

Once air sampling is completed and the samples are analyzed, the lab reports the results. During lab analysis by culture, the agar plate is observed and colonies are identified and counted. The count represents the number of molds that grew on the agar plate. The count is reported as colony forming units (CFU) of each particular organism. The CFU is defined as the individual colonies of mold found on the plate, expressed as a unit of a colony of a particular organism (Bailey, 2005). The concentration for viable samples collected by impaction is expressed as the

CFU in relation to the volume of air sampled (CFU/per cubic meter of air [m^3]). The concentration for non-viable samples collected by the Air-O-Cell is reported in spores/m^3.

Research Questions

This study aims to answer the following research questions:

1. How do indoor mold counts typically compare quantitatively to outdoor counts?
2. How do outdoor environmental parameters (temperature, humidity, carbon dioxide) influence detected levels of mold?
3. How do indoor environmental parameters (temperature, humidity, carbon dioxide) influence detected levels of mold?
4. Do environmental parameters (temperature, humidity, carbon dioxide) have the same influence on detected mold levels indoors and outdoors?
5. Amongst all the samples, what is the qualitative rank-ordering of detected molds?

Methods

Sample Collection Methods

The sampling method used in the area sampling for the molds was consistent for each area tested. The sampling method is in accordance with generally accepted industry guidelines. Areas sampled included indoor and outdoor locations of built structures. During sampling, ambient conditions such as dry bulb temperature, relative humidity, and carbon dioxide levels were measured and recorded. For this study, the Andersen N6 was then sanitized prior to a Rose Bengal Agar plate being inserted. With the Rose Bengal Agar plate in place, the impactor was secured, and an air pump calibrated to 28.3 liters per minute was then attached. The air pump was turned on for two minutes. After all the samples were collected, the air pump was then post-calibrated in order to record actual air flow. The agar plate was then removed from the N6 impactor, sealed with a lid, and labeled. The agar plate, along with a chain of custody containing details such as location tested and flow rate were then sent out to an independent American Industrial Hygiene Association (AIHA) accredited environmental microbiology laboratory for culture and analysis. The laboratory reported the types and counts of molds detected.

Data Analysis Methods

For this study, data from 170 sampling areas of non-industrial buildings in the state of Florida were analyzed. To do this, 21 lab reports generated between 2002 and 2009 were used. The randomly selected lab reports chosen utilized the same sampling method. The ambient conditions for each area of sampling were then listed with their corresponding lab results. After the data was sorted in a spreadsheet, a macro script was run using a Structured Query Language (SQL) program that categorized the data into tasks, which allowed quantitative analysis. This ultimately allowed each individual mold to be compared with each individual condition with the ability to choose any parameter. This included grouping each mold, listing the temperature, humidity, and carbon dioxide from greatest to least, and filtering by whether the sampling area was indoor or outdoor. First, the mean of all measured concentrations and counts was calculated for both indoor and outdoor conditions. Next, in order to find the optimal ranges of growth for indoor mold, a descending list of dry bulb temperatures from every sampling site was created. In the adjacent columns, the corresponding concentrations and counts were presented. Next, appropriate "ranges" were defined based on how much data there was for a certain temperature. For example, nothing above 80 °F was used as a range because only two sampling sites fell within that range, and therefore would not be significant in comparison to the entire data set. Below 80 °F, temperature ranges in intervals of 5 were used with the exception of temperatures in the 30s (30 – 40) in order to have enough data within a range to be significant. For each temperature range interval, the mean concentration and mean counts were calculated. Once each interval range was calculated, an observation was made as to which range had the highest mean

concentration and counts. This method of analysis was repeated similarly for relative humidity and carbon dioxide for both indoor and outdoor sample sites.

The next step of the analysis was to list the concentration and counts in descending order for indoor and outdoor sites, observing which mold was consistently at the highest marks. This was also done for indoor and outdoor sites independently. In addition, agents such as *Aspergillus*, *Penicillium*, and *Cladosporium* were individually counted throughout all 170 sampling locations in order to confirm whether any appear in all sites, as they are generally found throughout every environment.

Results of Data Analysis

The described methods were used to show the relationship between indoor and outdoor parameters and the levels of mold growth associated with these parameters. Concentrations are reported in terms of CFU/m^3, which is the count divided by the amount of air sampled in cubic meters (Burge, 2011).

Table I presents the overall comparison between the mean of the raw count and calculated concentration found in outdoor and indoor areas.

Table I - Indoor vs. Outdoor Concentration and Count

	Indoor	Outdoor
Mean Concentration (CFU/m^3)	1116	1675
Mean Count (CFU)	7.5	14

The next analysis focused on temperature of the outdoor as well as indoor environment in relation to mold count and concentration.

Tables II and III present the mean counts and concentrations found in outdoor and indoor temperature ranges, respectively

Table II – Mean Concentration/Count Compared to Dry Bulb Temperature (Outdoors)

Temperature Range (°F)	Average Concentration (CFU/m^3)	Average Count (CFU)
90 +	1429	15
85 - 90	1153	9
80 - 85	2173	22
75 - 80	2694	20
70 - 75	2445	18
60 - 70	1049	9
50 - 60	660	6

Table III – Mean Concentration/Count Compared to Dry Bulb Temperature (Indoors)

Temperature Range (°F)	Average Concentration (CFU/m^3)	Average Count (CFU)
80 - 85	1240	7
75 - 80	1143	8
70 - 75	1293	8
65 - 70	308	3
60 - 65	230	2

The next step of the analysis compared relative humidity of outdoor and indoor environments and the amount of mold found in each humidity range sampled. Tables IV and V below present these findings.

Table IV – Mean Concentration/Count Compared to Relative Humidity (Outdoors)

Relative Humidity (%)	Average Concentration (CFU/m^3)	Average Count (CFU)
75 - 80	2133	16
70 - 75	1402	11
65 - 70	2941	25
60 - 65	1166	11
55 - 60	1830	13
50 - 55	1727	19
45 - 50	845	6
40 - 45	637	7

Table V – Mean Concentration/Count Compared to Relative Humidity (Indoors)

Relative Humidity (%)	Average Concentration (CFU/m^3)	Average Count (CFU)
70 - 80	2533	22
65 - 70	2934	17
60 - 65	769	6
55 - 60	654	5
50 - 55	678	5
45 - 60	414	4
40 - 45	747	5

The next step of the data analysis involved comparing carbon dioxide concentrations measured in outdoor and indoor areas with the resulting mold concentrations and counts. Tables VI and VII present this data.

Table VI – Mean Concentration/Count Compared to Carbon Dioxide (Outdoors)

Carbon Dioxide Levels (ppm)	Average Concentration (CFU/m^3)	Average Count (CFU)
450 - 500	957	5
400 -450	1307	11
350 - 400	1279	11
300 - 350	2042	18

Table VII – Mean Concentration/Count Compared to Carbon Dioxide (Indoors)

Carbon Dioxide Levels (ppm)	Average Concentration (CFU/m^3)	Average Count (CFU)
1500 - 2000	584	4
1000 - 1500	546	4
900 - 1000	521	4
800 - 900	1884	10
750 - 800	1397	8
700 - 750	209	3
600 - 700	254	3
350 - 400	1271	10

In addition to the above tables, Tables VIII and IX below show the molds in indoor and outdoor environments that had the highest counts.

Table VIII - Prevalent Molds (Outdoors)

Mold	Count (CFU)	Dry Bulb Temperature (°F)	Relative Humidity (%)
Cladosporium	301	93.7	53
Curvularia	301	84.9	34.3
Penicillium 1	245	83.9	67.1
Cladosporium	225	85.2	56.7
Aspergillus sydowii	195	72.5	65.2
Cladosporium	173	78.7	79.7
Cladosporium	144	71.9	84.6
Cladosporium	131	76.9	68.1
Cladosporium	131	68	73.7
Cladosporium	107	81.1	71.3
	Mean	79.7	65.4

Table IX - Prevalent Molds (Indoors)

Mold	Count (CFU)	Dry Bulb Temperature (°F)	Relative Humidity (%)
Penicillium 1	152	82.1	44.9
Cladosporium	151	75.2	69.9
Cladosporium	151	75.2	69.9
Cladosporium	150	75.3	70.6
Cladosporium	150	75.3	70.6
Cladosporium	131	79.9	63.6
Cladosporium	131	79.9	63.6
Cladosporium	125	75.8	67.9
Cladosporium	125	75.8	67.9
Cladosporium	110	77.9	67.5
	Mean	77.2	65.6

Discussion

Based on the results of the 170 sampling locations and their ambient environmental parameters, the following research questions were answered:

How do indoor mold counts compare quantitatively to outdoor counts?

As seen in Table I, the average outdoor concentrations and counts were much higher than those of the indoor areas. For outdoor environments, the average overall concentration was 1675 CFU/m^3 with an average count of 14 CFU. For indoor environments, the average overall concentration was 1161 CFU/m^3 with an average count of 7.5 CFU.

How do outdoor environmental parameters (temperature, humidity, carbon dioxide) influence detected levels of mold?

At an average of 79.7 °F, the highest levels of mold were detected. Additionally, at an average of 65.4% humidity, the highest levels of mold were detected. Overall, the lowest mold counts were detected when the temperature was below 70 °F and the humidity was below 50%. Carbon dioxide levels did not have a clear influence on detected levels of mold.

How do indoor environmental parameters (temperature, humidity, carbon dioxide) influence detected levels of mold?

At an average of 77.2 °F, the highest levels of mold were detected. Additionally, at an average of 65.6% humidity, the highest levels of mold were detected. Overall, the lowest mold counts were detected when the temperature was below 70 °F and the humidity was below 52.3%. Carbon dioxide levels did not have a clear influence on detected levels of mold.

Do environmental parameters (temperature, humidity, carbon dioxide) have the same influence on detected mold levels indoors and outdoors?

Environmental parameters do in fact have the same influence on detected mold levels in both the indoor and outdoor environment. As seen from the above two research questions and their respective answers, the mean temperature and humidity from the top 10 counts of both indoor and outdoor environments show very similar numbers. For instance, as Table VIII shows, when listing the top 10 counts of outdoor environments, the mean temperature of the 10 data points is 79.7 °F. This is just 2.5 °F higher than the mean of the 10 outdoor data points from Table IX. In the same regard, the mean relative humidity of the top 10 outdoor counts is just 0.2% lower than that of the indoors.

According to Tables I and II, the highest concentration and count are seen between 70 and 85 °F. While Table I had the highest concentration at 75 to 80 °F and the highest count at 80 to 85 °F, the numbers are significant in that they are close enough together and much higher than the lower numbers found in other ranges. This methodology of statistical analysis rendered the entire range of 70 to 85 °F in Table I to be counted as the highest. The same went for Table II, as the 70 to 85 °F range had resoundingly higher numbers than the rest of the ranges in addition to the numbers being close enough together to count. As seen in Tables III and IV, the relative humidity range for optimal mold growth was between 65 to 70%. While the highest count of indoor agents actually came at the humidity range of 70 to 80%, it was taken into consideration that the concentration was much higher at the 65 to 70% range with the count being less by only a small margin. This coincides with the range found in the outdoor samples as well, which sees a significant peak of concentration at the 65 to 70% range along with a very high count. While it was mentioned earlier that carbon dioxide concentrations were not a direct or significant

indicator of mold growth, it is worth mentioning this particular parameter saw different and scattered numbers in both indoor and outdoor environments.

The optimal growth range found is consistent with the research on the subject matter. For instance, an article by Bailey (2005) stated that 65 to 90% relative humidity is a range in which mold grows the most. With mold thriving in higher temperatures and relative humidity, the numbers found through the analysis prove just that. It is worth noting that for the parameter of outdoor temperature, the concentration and count did see a drop off at temperatures above 85 °F, showing that overly high temperatures, independent of humidity, may not be ideal for mold growth. It could not be determined whether high humidity preempts higher temperatures above 85 °F, as there were not enough areas tested that had both high humidity readings (above 65%) with temperatures above 85 °F. Furthermore, humidity ranges never exceeded 80%, so no concentration or counts above 80% relative humidity were able to be determined. However, while 65 to 70% relative humidity was deemed "optimal" based on the data, the concentration and counts for both indoor and outdoor environments remain high past 70% humidity, especially in comparison to anything below 65%.

For carbon dioxide levels, the numbers are not concrete in one range. In addition, the ranges of carbon dioxide vary greatly between indoor and outdoor environments. As previously mentioned, carbon dioxide levels are a good indicator of ventilation. This is evident in the fact that in the data analyzed, the concentration of carbon dioxide in the outdoor environment peaks at 500 ppm as opposed to the 2000 ppm maximum value found in indoor environments. One reason may be that because of constant air flow in the outdoor environment, carbon dioxide concentrations remain generally low. In the indoor environment, the carbon dioxide levels can vary greatly even in a matter of minutes. This is due to the fact that the reading may have been

taken when the ventilation system was not running, or perhaps the number of building occupants influenced the number. An open window, someone walking in and out of a door, and a fan being on in a room, can also affect this.

As stated earlier, throughout all 170 sampling sites, *Cladosporium* had the highest counts in both indoor and outdoor environments. However, in the actual optimal range of 70 to 85 °F and 65 to 70% relative humidity, the indoor environment had the highest numbers in variants of *Aspergillu*s, such as *Aspergillus versicolor* and *Aspergillus fumigatus*. In the same optimal range, the outdoor environment saw the highest counts in *Penicillium 1, Aspergillus sydowii,* and *Cladosporium* respectively. As mentioned in referenced articles, *Cladosporium* and *Aspergillus* are highly common molds that are found virtually everywhere and especially thrive with higher water activity, which is commonly seen in areas of higher humidity.

Amongst all the samples, what is the qualitative rank-ordering of detected molds?

The top three detected molds were *Penicillium, Aspergillus, and Cladosporium,* respectively. Overall, variants of *Penicillium* appeared 328 times. This is due to the fact that some individual sites had as many as eight separate *Penicillium* variants. However, even with the presence of *Penicillium* in so many sites, the counts were always quite low. This is also consistent with other research that shows the abundance of *Penicillium* in the environment. It is also important to understand that although *Penicillium, Aspergillus, and Cladosporium* were seen so many times, none were detected in all 170 sampling sites. Rather, the most prevalent mold without any variants was *Cladosporium,* which showed up in 143 out of 170 sampling sites. The lack of *Cladosporium* in the other 27 sampling sites can be a result of many things such as human error in the lab, where the *Cladosporium* was either not identified or was

misinterpreted as something else. Some agar plates had an overgrowth and so it is also possible that the *Cladosporium* was hidden and unidentified in that overgrowth.

Conclusions

The results show how mold growth is influenced by environmental parameters such as temperature and humidity. In addition, the results show that carbon dioxide did not directly influence mold growth. The outdoor and indoor environments had different concentrations and counts as well as different types of molds. The optimal range in which mold grew based on the temperature and relative humidity were the same in both outdoor and indoor environments. These values are in fact consistent with literature. However, while research suggests that humidity and the moisture are the most important factors in mold growth, further research is needed to see if conditions of very low temperature but very high humidity would still yield significant mold growth. Also, while this study was unable to see if mold continues to thrive past 80% relative humidity due to the lack of this condition, it was previously mentioned that research suggests that mold thrives in humidity levels as high as 90% (Bailey, 2005). Based on the lack of such conditions in both indoor and outdoor sampling locations, such a high humidity level is likely uncommon except for a few exceptions.

Through all the sampling and data analysis, it is of particular interest and importance to keep in mind the science and current research regarding mold and other biological exposures. Because neither OSHA nor the ACGIH have set exposure limits or threshold limit values, there is no line to cross that states the numbers found in a certain area are elevated or non-elevated, acceptable or not acceptable, and safe or unsafe. Red flags may be raised if extremely higher than usual concentrations of molds are found, especially controversial molds such as

Stachybotrys chartarum, which if found, prudent practice recommends that they be removed (CDC, 2012).

Based on the data from this study, measures can be taken to try and control mold growth. The temperature range of 70 – 85 °F is very hard to control for, as an indoor temperature below 70 °F or above 85 °F are both highly uncomfortable for a building occupant. However, the relative humidity in the indoor environment should be kept at no more than 50% with proper ventilation consistently pulling in clean air and air conditioning or a dehumidifier operating during humid months (CDC, 2012).

Limitations of this study came from the lack of certain parameters. For instance, very few sample sites had temperatures above 90 °F or below 50 °F. In addition, too few sample sites had a relative humidity above 80% or below 40%.

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