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# Sampling And Identifying Of Mould In The Library Building

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**Abstract.** Despite the growing concern over mould and fungi infestations on library building, little has been reported in the literature on the development of an objective tool and criteria for measuring and characterising the mould and fungi. In this paper, an objective based approach to mould and fungi growth assessment using various sampling techniques and its identification using microscopic observation are proposed. This study involved three library buildings of Higher Institution Educational in Malaysia for data collection purpose and study of mould growth. The mould sampling of three libraries was collected using Coriolis air sampler, settling plate air sampling using Malt Extract Agar (MEA), IAQ MOLD Alexeter IAQ-Pro Asp/Pen® Test and swab sampling techniques. The IAQ MOLD Alexeter IAQ-Pro Asp/Pen® Test and traditional method technique identified various mould species immediately on the site, and the microscopic observation identifies common types of the mould such as Aspergillus, Penicillium and Stachybotrys's. The sample size and particular characteristics of each library will result in the mould growth pattern and finding.

## 1 Introduction

As stated by Storey et al., (2004), most of mould favour a temperature of 15°C-30°C (59°F-86°F), but there are varieties that will grow below or above these temperatures. Mould be potential to grow by influenced by moisture or high humidity, nutrition, light, oxygen, and temperature. Mould will grow anywhere indoors and outdoors over a broad temperature range where there are sufficient moisture and a nutrient source. It results in visible wetting of walls, ceilings boards and floors, blistering paint, bulging plaster, mould on the surfaces and fabrics, rotting timber and sulphate attack on brickwork. It can also lead to less obvious problems. In example, thermal insulation is reduced in the effectiveness of brickwork because metal components imbedded in it have been corroded. Among the effects that had attracted the most attention is biological growth such as moulds with various species of Cladosporium, Aspergillus and other fungi types. This mould species can be found in both indoor and outdoor environments of buildings. Hollis (2005) stressed that it is completely linked to most building deterioration.. Findings by Jan (2006) on microenvironments exposure where isolated areas of books stacks or storage and other buildings may not applicable to tropical countries such as Malaysia. Malaysia experienced little variation in temperature and humidity and rainfall throughout the year. Interrelated the above issue, according to Zuraimi & Tham (2008) significant difference between temperature and relative humidity in tropical Singapore compared to cold countries were reported in IAQ of Child Care Centres(CCC). Hussin, Sann, Shamsudin, & Hashim (2011) conducted fungi research on selected primary schools in Malaysia. Their findings revealed that most frequently mould genera found were Aspergillus, Penicillium, Fusarium, Rhizopus and Zygomycetes.

There are many types of moulds exist. As stated by Burton & Gibbins (2011) certain types of moulds are toxigenic, whereby they can produce toxins. However the mould themselves are not toxic or poisonous. One of the moulds that are commonly found inside a building is Stachybotrys Chartarum. It is black in colour and produce its conidia in slime heads. Another common mould is Cladosporium, which produces itself in olive green to brown or black colonies. Besides that, it is quite often to find green colour mould which is called as Penicillium and also Aspergillus which is common been seen in either red or gold colour.

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## 2 Methodology

Visual inspection was conducted to investigate the visible mould growth, measure indoor condition and find the possible moisture sources. It is the most important initial step in identifying a possible contamination problem.

### 2.1 Buildings and moisture investigation

This study included a total of three library buildings of Higher Institution Educational in Malaysia for data collection purpose and study of mould growth. The Information Resource Centre (IRC), UTP was choosing for the controller purpose. Besides, it decides according to the modern design and technology. Perpustakaan Tun Abdul Razak 1 (PTAR1), UiTM Shah Alam and Perpustakaan Tuanku Zanariah; UTM Skudai Malaysia were selected due to its age, features and location. An inspection included an interview with a chief librarian, building maintenance and management personnel and workers. Visual Inspection being conducting to identify the signs of moisture and mould and any defects that found in the buildings. NYC Department of Health and Mental Hygiene highlight the extent of any water damage and mould should be visually assessed. A visual inspection is the most important initial step in identifying a possible contamination problem. Remediation of visually identified fungal contamination should proceed without further evaluation. According to Palaty (2010) outcomes of visual inspections can lead to three possible outcomes as Table 1 below.

**Table 1**: Outcome of visual inspections

| No | Possible | Interpretation |
|---|---|---|
| 1 | No evidence of mould growth or moisture damage | No remediation action and further inspection required |
| 2 | Evidence of mould growth or moisture damage | Remediation actions need to be initiated to remove the mould |
| 3 | Unclear if mould or moisture is present | Proceed to take mould sampling and further analysis |

The equipment such as humidity data logger, moisture metre and infrared thermography were used to measure and identify the moisture or dampness on the structures or surfaces of the materials. The inspection had identified some of the books on the book rack contained dust, especially on the book cover. The books were also looking swelling and shrinking. The researcher also found dirt, dust and microbial growth on the book that been displayed at the bookshelves as shown in **Figure 1**. The moisture staining and mould also found on the building elements such as floor, wall and ceiling.

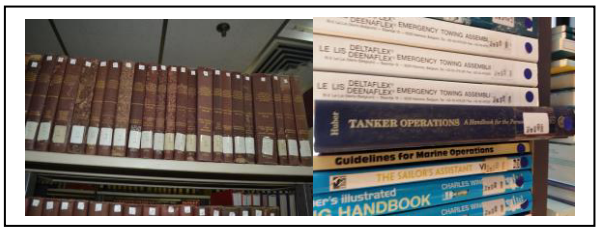


**Figure 1**: Possible mould and microbial growth on the book surfaces

The inspection revealed dampness and visible growth of the mould on a ceiling board, timber column, floor or carpet and wall. Infrared thermography and moisture metre were used to confirm that moisture on the building structures occurs.

Infrared thermography is one of the useful equipment that helps the researcher in identify the causes of moisture occur in building. It is easy to use as a camcorder or a digital camera and can give a full image of the situation. It also allows us to perform inspections when systems are under load and could identify and locate the problem. In addition, it could measure temperatures and store information. In this research, it will detect and help eliminate mould infestations. A mould problem is a moistur problem, so when it is used to find moisture and it finds moisture, it becomes possible to prevent mould and rot from taking hold or to remove the mould that grows. Figure2, Figure 3 and Figure 4 shows the visible mould and humidity on building finishes using infrared thermography.

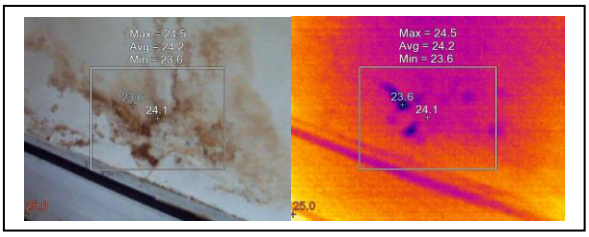


**Figure 2:** Infrared photo showing moisture intrusion into interior wall cavities

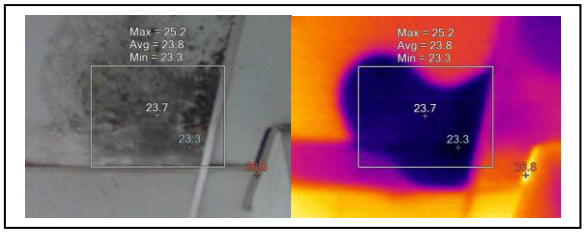


**Figure 3:** Infrared photo of ceiling showing moisture location

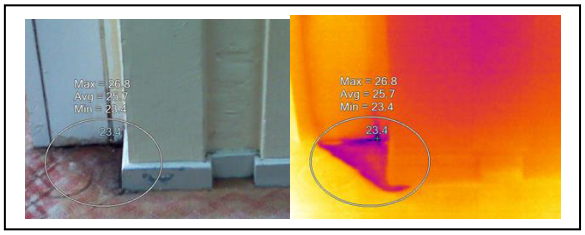


**Figure 4:** Infrared photo of carpet showing moisture intrution from rain water down pipe

## 2.2 Mould Sampling Method

The sampling method chosen depends upon resource availability, method, availability and information required and it is mention by AIHA (2008). In general, mould sampling method used to collect mould from air, surfaces and settles dust. The sampling needed in this research is to find the types of mould in the selected building. Swab samples were obtain from all visible mould found, and indoor air samples using Coriolis and malt extract agar were collected from reading area in three selected libraries. The sampling also focuses on the books and bookshelf which appearance of mould or stain. Also, the sampling is also done to get the total count of mould and yeast in the building. With the amount of total number using the specific formula, the researcher can get the colony forming unit (cfu) for the particular area or surface. The summarised of Mould Sampling Method that used for three selected libraries building shown in Table 2 below:

**Table 2:** The summarised of sampling perform in library buildings

| Method | IRC | PTAR1 | PSZ | Purposed |
|---|---|---|---|---|
| Coriolis Air Sampler | √ | √ | √ | Identification |
| Settling Plates Air Sampling | √ | √ | √ | Identification |
| Swab Test (IAQ Mold) | √ | √ | √ | Identification |
| Swab Test | √ | √ | √ | Identification |

## 2.3 Coriolis Air Sampler

The Coriolis µ was developed to go beyond traditional techniques performances. Traditional techniques indeed rely on the impaction of particles on a solid agar medium and are thus limited by low flow rates, unreliable impaction and a longer analysis time. The Coriolisµ air sampler manufactured by Bertin Technologies (France) is a continuous air sampler, dedicated to indoor monitoring of airborne spores and pollen grains. Bertin Technologies has developed a liquid cyclone high-volume air sampler for collection and identification of airborne bio-particles including micro-organisms such as bacteria, fungi, and pollen grains. This technology is based on a cyclone types device that selects and captures the airborne particles and instantaneous transfer them into a liquid sample. This sample is directly compatible with standard culture methods Carvalho et al., (2008). The sampler operates for 4 min by pulling air at a flow rate of 250 per l minute into a cyclonic separator containing 15 ml of swirling collection fluid. After the sampling period complete, the remaining fluid was collected; the volume measured (evaporation loses some volume) and it was assayed for the presence of microorganisms. The process of sampling using Coriolis air sampler is shown in Figure 5 and below.

The Coriolis air sampler located in the reading area of each level in the libraries building selected as shown in Figure 5. Repeat twice to obtain triplicate before sampling to the next site. Consecutive samples using a new sterile cone and collection liquid dose at each sampling. For the growth culture analysis, a portion of the liquid sample in Coriolis cone about 0.5mL using the sterile pipette will spread onto Malt Extract Agar (MEA) as a culture medium. Before transferred the liquid sample need to vortex about five to ten second. The culture medium finally put into the incubator at room temperature. Wait until the mould growth.

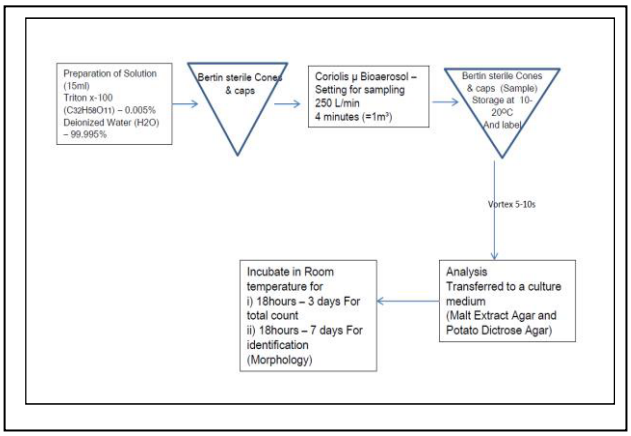


**Figure 5:** Sampling process using Coriolis machine and laboratory preparation

## 2.4 Settling Plate using Malt Extract Agar (MEA)

The visual inspection result that recorded on the checklist of the area in libraries selected identified for sampling. It is also focusing the area that the students or the user most spent their time in an individual area. In this research, reading area, being choose for sampling places because the student spent their time more than 1hour. However, the place that visible mould found also targeted for sampling. The books collection also one of the places that the researcher take for sampling since huge volume for each library with a different type of papers and books covering.

**Figure 6**: Air Sampling Mould Isolate at Reading Area

## 2.5 Surface Sampling

### *2.5.1 Swab Sampling*

Specimen collection depends on the site affected. Surface sampling can determine whether a stain has resulted from fungal growth or some other problem. For example, discolorations associated with leaks might be due to dissolved materials in the water accumulating on the surface as the water evaporates. Dirt might cause the dark material that accumulates near air vents. An environmental consultant who is familiar with the appearance of these types of discolorations should be able to discern areas of fungal contamination on a surface, minimising the number of surface samples needed.

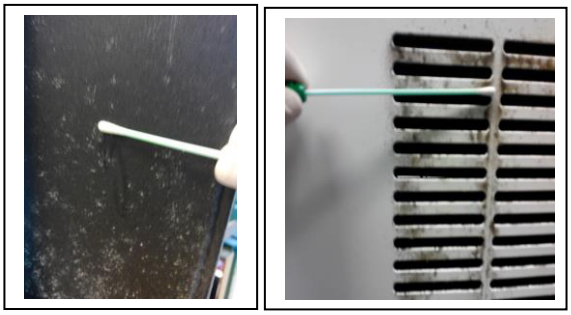

**Figure 7:** Swab Test Sampling

### *2.5.2 Mould Identification Using IAQ MOLD Alexeter IAQ-Pro Asp/Pen® Test*

The Alexeter IAQ-Pro Asp/Pen test strip is a lateral flow immunochromatographic device that uses two antibodies in combination to correctly detect the antigen in solution. One of the specific antibodies is labelled with a colloidal gold derivative. When the sufficient antigen is present, the colloidal gold label provides a reddish-brown colour line that is visualised after accumulating in the test sample region on the device. When a sample is added to the Alexeter IAQ-Pro Asp/Pen test strip, the sample begins to mix with the colloidal gold labelled antibody and simultaneously moves along the strip membrane by capillary action. In the sample region of the test strip, if the antigen is present, the second specific antibody captures the colloidal gold labelled antibody and bound antigen, forming a colour line or band in the sample (left side) window of the test strip. As an internal control, a second band visualised in the control (right side) window of the test strip is an indication that the test strip functioned properly. Two bands or colour lines are required for a positive result determination.

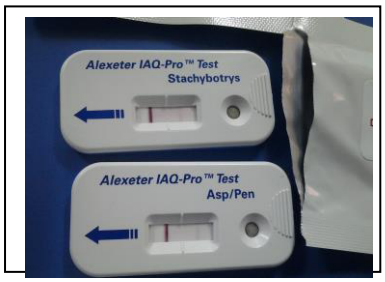


**Figure 8:** Result on IAQ MOLD Alexeter IAQ-Pro Asp/Pen® Test

## 2.6 Measurement of Mould Growth

### *2.6.1 Microbiological assays*

Microscopy is used to observe clinical specimens for the presence of fungal elements or to identify the fungus following culture. Culturing methods cannot and should not be excluded from any fungal analysis. Merck provides Media Melt Extract Agar (MEA) and Potato Ditrose Agar (PDA) plates (90 mm) for use in all the physical efficiency testing.

All plates mould isolates were incubated at room temperature 30$^{O}$C for up to 18 hours before counting the colonies. Before analysis, accurately measure the final volume of collection liquid in the cone in a clean area and record it. Vortex 5-10s second the liquid sample in the Coriolis cone (or in the transferred container) and remove 0.5mL with a sterile pipette. Spread this volume onto repeat once to obtain a duplicate by each culture medium. Captions should be typed in 9-point Times. They should be centred above the tables and flush left beneath the figures.

### *2.6.2 Mould Identification and quantification*

Traditional mould quantification was based on the enumeration of cells captured on a sticky surface and counted under a microscope or by culturing moulds from the sample on various media. Most moulds cannot be identified directly by looking at the spores. Because of this limitation, most commercial mould analyses only describe the moulds to the genus level. Moreover, in the cases of Aspergillus and Penicillium cells, these two genera cannot be distinguished by microscopic observation alone. For that reason, most commercial labs only combine these genera together as the mega-category "Asp/Pen."[9].

All of these inoculated media (original and duplicate) were placed in an incubator set to 30$^{O}$C, and they remained there for a week (MEA). The identification of mould on the microscopic visualisation of morphological characteristics using lactophenol cotton blue dye and demonstrated manuals [10]. In this research, microbiologist expertise was a help to confirm the morphology data found as shown below.

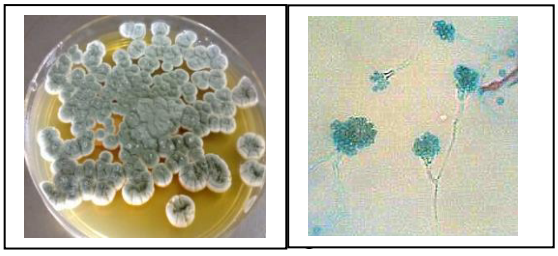

**Figure 9:** Petri dish on which colonies of Stachybotrys have been cultured and identified.

## 3 Result Obtained

### *3.1 Mould Identification Using IAQ MOLD Alexeter IAQ-Pro Asp/Pen® Test.*

The result obtained by the application of IAQ Mould Alexeter IAQ-Pro is as shown in Table 3. The identifications of mould confirmed active for Aspergillus and Penicillium type of mould presence. Whereas only 5 sample show positive on Stachybotrys and 11 of 12 samples shows the positive result of Aspergillus.

**Table 3**: IAQ Mold Alexeter IAQ-PRO Asp/Pen® test expanded data result

| Client ID | Date Collected | Sample Location | Item/ Material | Result | | Relative Humidity % (AVERAGE) | Temperature °Celcius (AVERAGE) |
|---|---|---|---|---|---|---|---|
| | | | | Asp/ Pen | Stachy botrys | | |
| IRC | 30/10/14 | Ground Level | Book | +ve | -ve | 68% | 22.5 |
| IRC | 30/10/14 | Level 3 | A/c outlet | +ve | -ve | 69% | 23.2 |
| PTAR | 15/11/14 | Level 3 | Book | +ve | -ve | 69% | 23.9 |
| PTAR | 15/11/14 | Level 3 | Window frame | +ve | +ve | 69% | 23.9 |
| PTAR | 15/11/14 | Level 3 | A/c | +ve | +ve | 69% | 23.9 |
| PTAR | 15/11/14 | Level 4 | Carpet | +ve | +ve | 64% | 26.3 |
| PTAR | 15/11/14 | Level 4 | Ceiling | +ve | +ve | 64% | 26.3 |
| PSZ | 6/12/14 | Level 2 | Book | +ve | -ve | 74.6% | 20.4 |
| PSZ | 6/12/14 | Level 2 | A/c | +ve | +ve | 74.6% | 20.4 |
| PSZ | 6/12/14 | Level 3 | A/c | +ve | -ve | 70% | 21.2 |
| PSZ | 6/12/14 | Level 4 | Book dust | -ve | -ve | 70% | 22.4 |
| PSZ | 6/12/14 | Level 4 | A/c | +ve | -ve | 70% | 22.4 |

### *3.2 Traditional Identification of Isolates*

All samples collected from library demonstrated that the sites of sampling were contaminated with microorganisms and fungi. A total of 22 fungal were isolated from all sampling sites involved as shown in Table 4. From morphological study through microscopic observation, most of the mould isolated from the selected study areas were known to be the common airborne fungi or common contaminants as shown in Figure 9. Out of 22 isolates from all sampling sites involved, the most abundant mould was found to be *Aspergillus sp.* with 27.27% of total isolates, followed by *Penicillium sp.*, *Stachybotrys sp.* and *Rhizoctonia Solani* with 18.18%, 18.18% and 13.63% of total isolates, respectively. Five isolates are unable to identify at genus level but had been confirmed to be *Fungal sp.* as they possess the characteristics of filamentous fungi. Most fungi, through most of their life cycle, consist of hyphae the cylindrical cells that increase in length by growth at one end. Their life cycle start with a spore, germinates into the suitable substratum, grows and branches forming a radiating system of hyphae called mycelium. Some fungi are not hyphae, but hypha and mycelium are associated with the growth and form of fungi to a greater extent.

**Table 4:** Mould identification from isolates

| Location | Isolates |
|---|---|
| IRC Ground Floor at Reading area | *Stachybotrys sp.* |
| PSZ Level 2 /Book | *Fungal sp.* |
| PSZ Level 2/ Air sampling(1) | *Stachybotrys sp.* |
| PTAR1 /Level 3/ Aircond swab | *Aspergillus sp.* |
| PSZ/Level 3/ Air sampling | *Fungal sp.* |
| PTAR1/Level 3/ Window frame (1) | *Stachybotrys sp.* |
| PTAR1/Level 3/ Window frame (2) | *Stachybotrys sp.* |
| PTAR1/Level 4/ Air sampling (R) 1 | *Aspergillus sp.* |
| PTAR1/Level 4/ Air sampling (R) 2 | *Aspergillus sp.* |
| PSZ/Level 4/ Air sampling | *Fungal sp.* |
| PTAR1/Level 4/ Carpet swab | *Fungal sp.* |
| PTAR1/Level 4/ Ceiling swab (1) | *Rhizoctonia solani* |
| PTAR1/Level 4/Ceiling swab (2) | *Rhizoctonia solani* |
| PTAR1/Level 4/ Timber column swab | *Rhizoctonia solani* |
| PSZ/Level 5/ Air sampling(1) | *Penicillium sp.* |
| PSZ/Level 5/Air sampling (2) | *Penicillium sp.* |
| PSZ/Level 5/Air sampling (3) | *Fungal sp.* |
| PSZ/Level 5/Aircond | *Aspergillus sp.* |
| PSZ/Level 5/Aircond(2) | *Penicillium sp.* |
| PSZ/Level 5/Aircond(3) | *Penicillium sp.* |
| PSZ/Level 5/Ceiling swab (1) | *Aspergillus sp.* |
| PSZ/Level 5/Ceiling swab (2) | *Aspergillus sp.* |

## 4 Conclusion

In this paper, various methods of mould sampling and identification using morphological study through microscopic observation had been conducted. The area of contribution of this research work is the identification of mould growth and the acquired image. The most abundant mould was found to be *Aspergillus sp.* *Penicillium sp.*, *Stachybotrys sp.* and *Rhizoctonia Solani*. Future work of this research will focus on the determined total count of mould base on the sampling area with the rate contamination of the library environment. The sample size and particular characteristics of each library will result in the mould growth pattern and finding. The research expected to identify the best solution to protect a collection that will depend on various factors such as type and number of visitors, characteristics of the library building and level of maintenance work provide.

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