Detection and Identification of *Stachybotrys chartarum* Microbial Volatile Organic Compounds (MVOCs) On-Site Using a Person-Portable Gas Chromatograph/Mass Spectrometer (GC/MS) with a Thermal Desorption Accessory

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Background

- Exposure to molds and fungi can cause health issues that can become severe
- Water damage in residential and commercial buildings provides an optimal environment for mold and fungal growth
- The ability to quickly analyze and identify toxigenic molds and fungi will facilitate an efficient remediation process

Background

- **Sick Building Syndrome and Damp-Building Related Illnesses contribute to not only allergies but also more serious conditions for individuals with compromised immune systems**
  - **Sick Building Syndrome**
    - **Symptoms associated with acute discomfort, such as headache, eye, nose, or throat irritation, dry cough, dry or itchy skin, dizziness and nausea, difficulty in concentrating, fatigue, and sensitivity to odors**
    - **The cause of the symptoms are unknown**
    - **Relief occurs soon after leaving the building**

Background

- **Damp-Building Related Illness**
  - **Symptoms such as cough, chest tightness, fever, chills and muscle aches**
  - **Symptoms can be clinically defined and have clearly identifiable causes**
  - **Complainants may require prolonged recovery times after leaving the building**

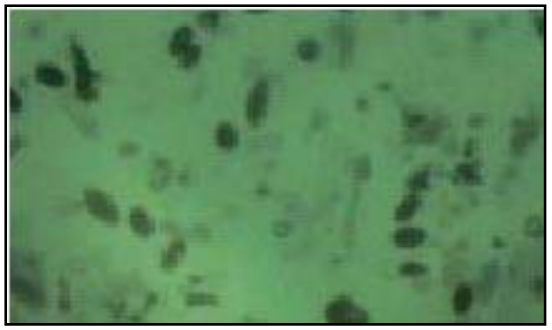
Background

- *Stachybotrys chartarum*
  - Saprophytic fungus grows rapidly on water-damaged, cellulose-rich materials
  - Two main chemotypes[1]
    - One chemotype produces highly toxic trichothecene satratoxins G and H, and other proteinases
      - Satratoxins G and H are protein synthesis inhibitors; together with the proteinases produced by black mold allows for the breakdown of vascular and cellular walls
      - Exposure to this chemotype can cause severe illnesses such as Sick Building Syndrome and Damp Building-Related Illnesses
    - The second chemotype produces less hazardous mycotoxins

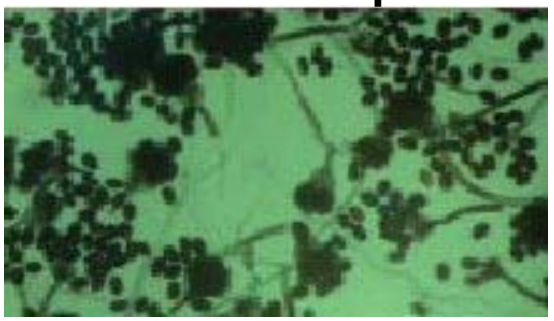
Current Mold Sampling Techniques

- **Airborne samples from the sampling environment are collected then sent to laboratories for analysis of spore types**
  - **Spore Trap Analysis**
    - **Collected samples are analyzed under a microscope and the individual spores are analyzed**
    - **Mold is considered to be present when these spores are found**
  - **Mold cultures**
    - **Samples are collected, transferred to growth media, and incubated**
    - **Colonies that form after incubation are viewed under a microscope and analyzed**

Spore trap sample



Culture sample



Current Mold Sampling Techniques

- **Concerns**
  - **The accuracy and precision of the results of both of these methods are questionable due to frequent (day-to-day or hour-to-hour) fluctuations of spore concentration**
  - **Not all toxigenic molds have airborne spores that can be collected and analyzed**
  - **Even “rush” analysis can take 1-2 days**

Current Mold GC/MS Sampling Techniques

- For *S. chartarum*, a fungus that does not have airborne spores, analysis of unique MVOCs is more accurate for identification
  - Samples are collected on-site via a thermal desorption tube, then sent to a remote site for GC/MS analysis
    - More accurate than individual spore analysis, however potential for sample contamination is constantly present
    - Samples still take 1-2 days even for rush analysis

On-site GC/MS Sample Analysis

- Utilizing a person-portable GC/MS, samples can be collected, desorbed onto the GC/MS, and analyzed on-site
  - Eliminates the possibility of sample contamination in transportation
  - Samples can be collected and analyzed within minutes

Sampling Equipment

- **HAPSITE ER person-portable GC/MS equipped with the Thermal Desorber Sampling System**

Experimental

- Studies by Gao et al[2] introduced the concept of detecting unique MVOCs as a method of confirming the presence of microorganism growth in buildings

- Studies by Betancourt et al[3] and Mason et al[4] list unique MVOCs produced by three toxigenic strains of *S. chartarum* grown on gypsum wall board and sugar plates

Experimental

- **Based on information found in mold characterization literature, the following MVOCs were obtained for sampling**
  - Standard 1
    - Propanoic acid methyl ester
    - Acetoin
    - 3-Furanmethanol
    - Styrene
    - Anisole
    - 3-Octanone
    - 3- and 4-methylanisole
    - Napthalene
    - 3,5-dimethoxytoluene
  - Standard 2
    - Geosmin
    - 2-Methylisoborneol
    - α-Terpineol

Experimental

- For initial characterization of the MVOC mixture, 0.2 µL of a 1000 µg/mL standard was injected into a 1 L polytetrafluoroethylene (Teflon) bag containing Ultra High-Purity nitrogen
- 250 mL of analytes were collected at a rate of 150 mL/min onto a carbon-based thermal desorption tube
  - Supleco Carboxen thermal desorption tube

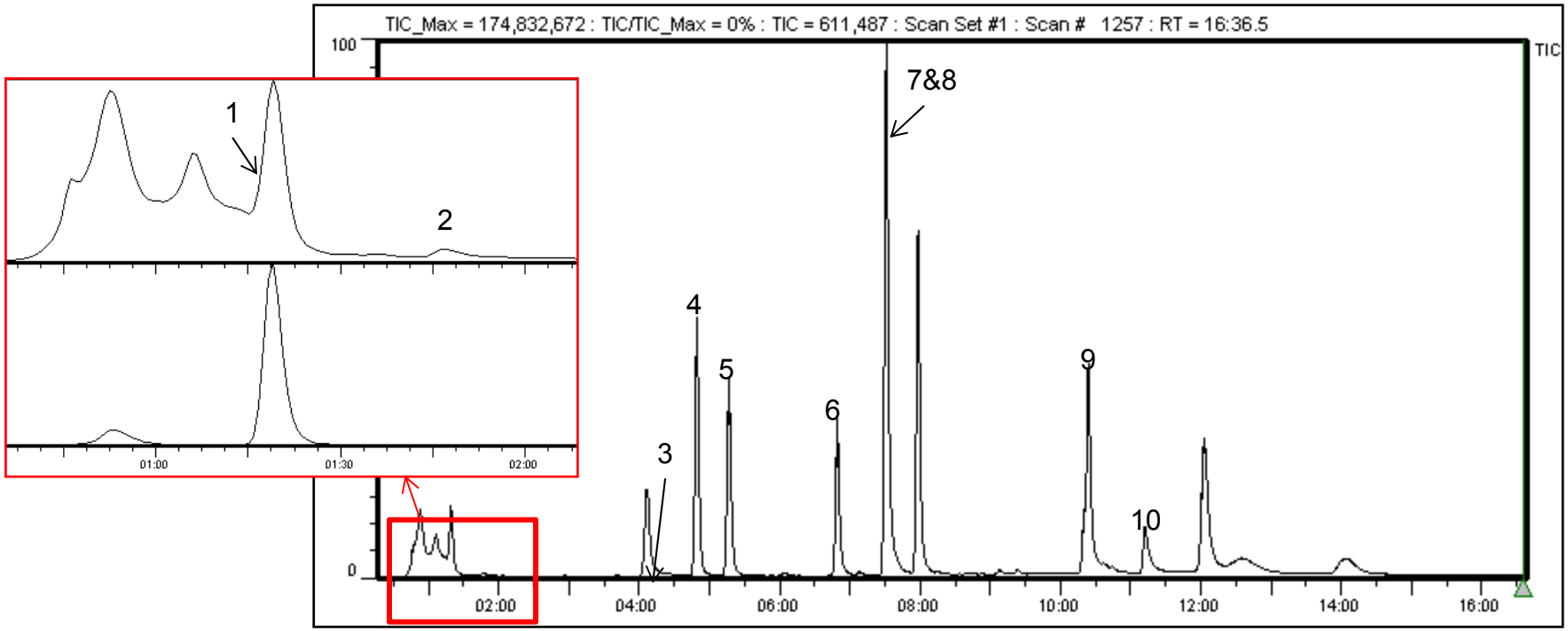
Experimental

- Samples were desorbed from the thermal desorber tube at 300 ºC for 5 minutes
- The sample was then transferred to an on-board concentrator within the HAPSITE ER via an external desorption accessory attached to the HAPSITE ER

Experimental

- Samples then were desorbed from the on-board concentrator onto the column where they were separated using a ~16-minute analysis
  - Column- HP-1MS, 15 m, 0.32 i.d., 1 µm
  - Hold 60 °C for 2 minutes, 6 °C/min to 80 °C, 10 °C/min to 120 °C, 26 °C/min to 200 °C, hold for 5 minutes

MVOC mixture in Nitrogen

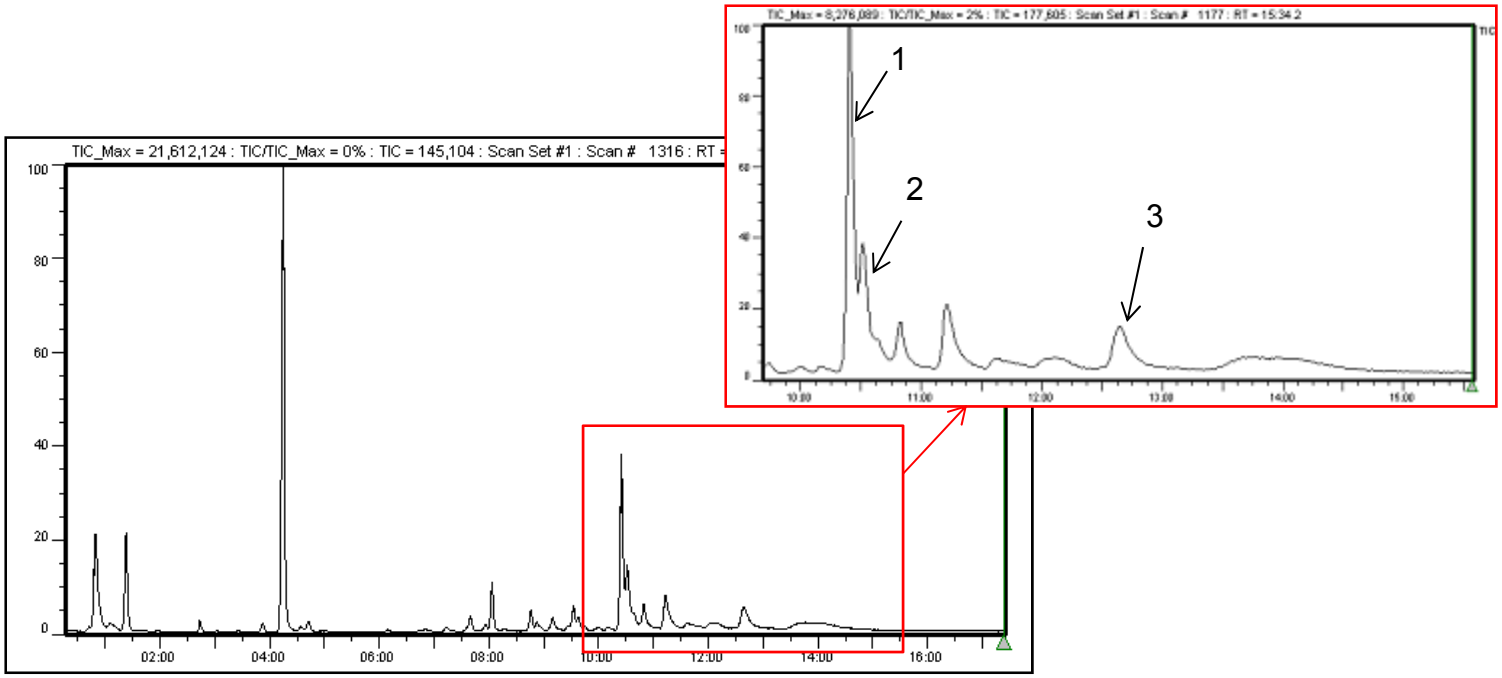


No.	Compound	Conc. (ppbv)	Conc. (µg/m^3)	RT
1	Propanoic Acid Methyl Ester	61	200	1:20
2	Acetoin	61	200	1:48
3	3-Furanmethanol	55	200	4:12
4	Styrene	51	200	4:57
5	Anisole	49	200	5:23
6	3-Octanone	42	200	6:54
7	3-Methylanisole	44	200	7:35
8	4-Methylanisole	44	200	7:35
9	Naphthalene	42	200	10:24
10	3,5-Dimethoxytoluene	35	200	11:12

Experimental

- A second standard containing the additional MVOCs of interest (geosmin, 2-methylisoborneol, and α-terpineol) in methanol was diluted into a 1 L Teflon bag of nitrogen at a final concentration of 20 µg/m^3 per compound
  - Samples were desorbed from the carbon TD tube at 300 °C for 5 minutes and transferred to an on-board carbon-based concentrator in the HAPSITE ER
- Analytes were then desorbed from the on-board concentrator to the column and separated using a 16-minute analysis
  - Column used: HP-1MS, 15 m, 0.32 i.d., 1.0 µm
  - Hold 60 °C for 2 minutes, 6 °C/min to 80 °C, 10 °C/min to 120 °C, 26 °C/min to 200 °C, hold for 5 minutes

Geosmin, α-Terpineol, 2-Methylisoborneol in Nitrogen

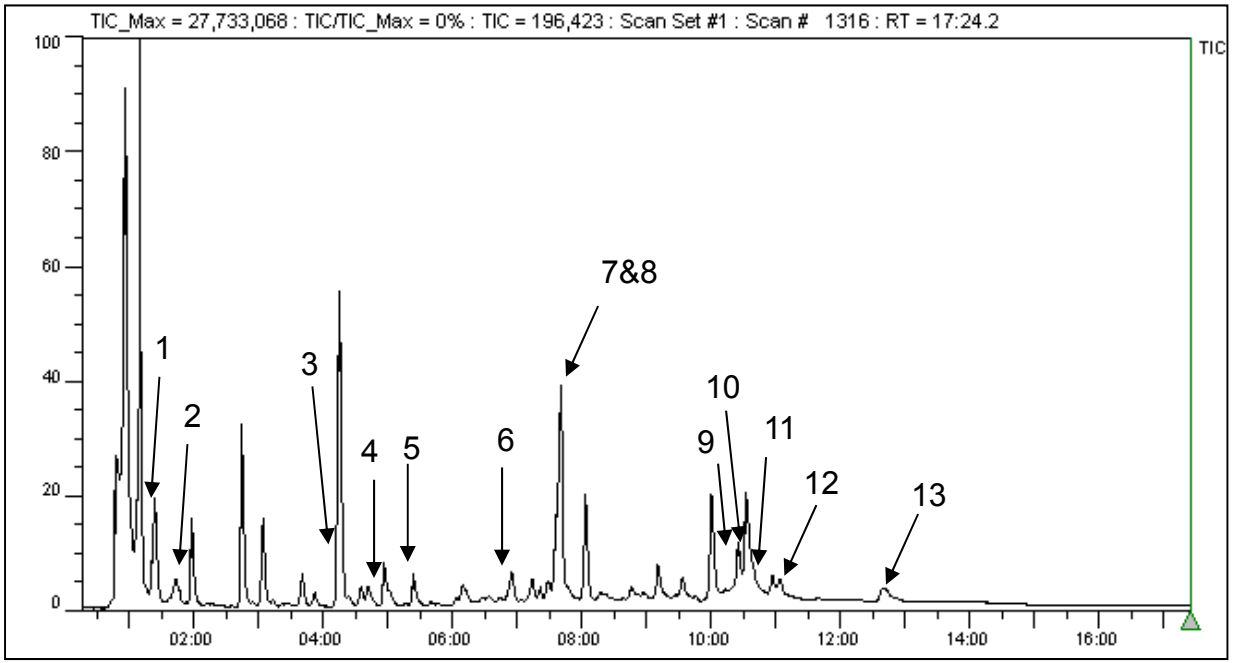


No.	Compound	Conc. (ppbv)	Conc. (µg/m^3)	RT
1	2-Methylisoborneol	2.86	20	10:31
2	α-Terpineol	3.12	20	10:33
3	Geosmin	2.64	20	12:38

Experimental

- The MVOCs from both standards were injected at 20 µg/m^3 into 1 L Teflon bag containing room air
  - A 700 mL sample was collected onto the carbon TD tube
    - Sampling rate of 150 mL/min
- Analysis was performed using the same parameters as the previous analyses
  - Samples were desorbed from the carbon TD tube at 300 °C for 5 minutes and transferred to an on-board carbon-based concentrator in the HAPSITE ER
  - Analytes were then desorbed from the on-board concentrator to the column and separated using a 16-minute analysis

Experimental



No.	Compound	Conc. (ppbv)	Conc. (µg/m^3)	RT
1	Propanoic Acid Methyl Ester	5.47	20	1:20
2	Acetoin	5.47	20	1:48
3	3-Furanmethanol	4.91	20	4:12
4	Styrene	4.63	20	4:57
5	Anisole	4.46	20	5:23
6	3-Octanone	3.76	20	6:54
7	3-Methylanisole	3.94	20	7:35
8	4-Methylanisole	3.94	20	7:35
9	Naphthalene	3.76	20	10:24
10	2-Methylisoborneol	2.86	20	10:31
11	α-Terpineol	3.12	20	10:33
12	3,5-Dimethoxytoluene	3.17	20	11:12
13	Geosmin	2.64	20	12:38

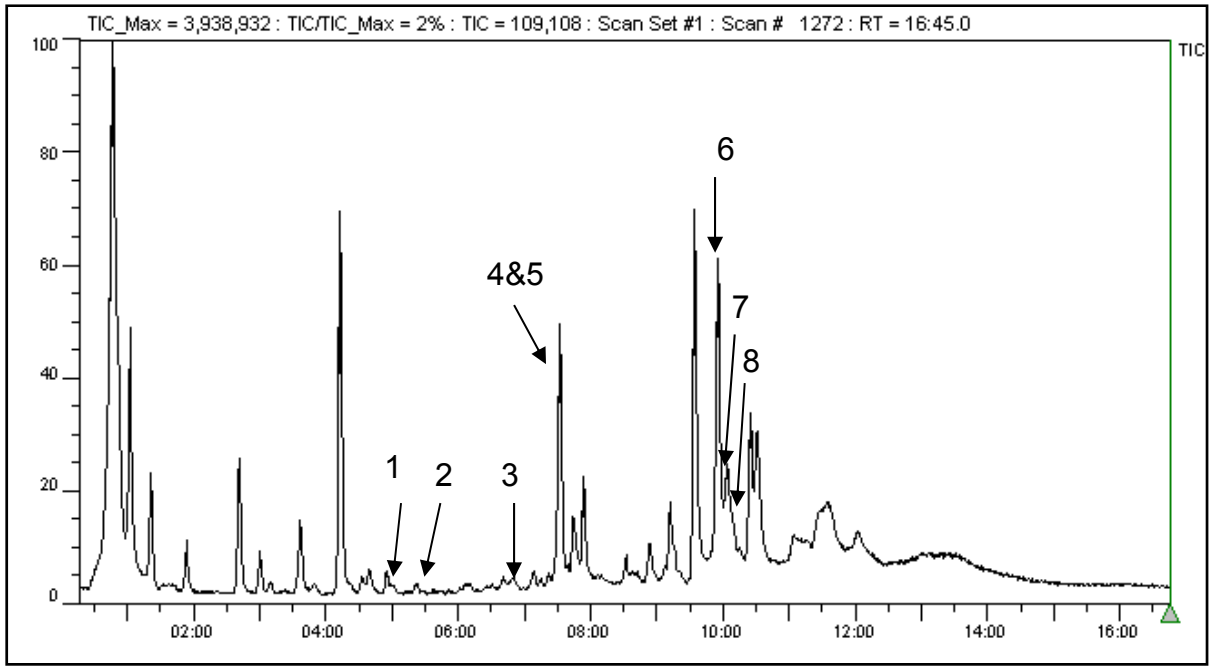
Experimental- Sampling with the Air Probe

- A similar study was performed using the HAPSITE ER and sampling directly with the Air Probe
  - Eliminates the need for collecting samples and bringing to the GC/MS
  - Analysis can be performed while in motion
  - Air probe comes standard with the HAPSITE ER

Sampling with the Air Probe

- A mix of the two standards of MVOCs was prepared in a 1 L bag filled with room air for a final component concentration of approximately 20 µg/m^3
- This mixture was sampled using the air probe and collected on the on-board concentrator for 1 minute
- Analytes were then desorbed from the on-board concentrator to the column and separated using a 16-minute analysis
  - Column- HP-1MS, 15 m, 0.32 i.d., 1.0 µm
  - Hold 60 °C for 2 minutes, 6 °C/min to 80 °C, 10 °C/min to 120 °C, 26 °C/min to 200 °C, hold for 5 minutes

Sampling with Air Probe



No.	Compound	Conc. (ppbv)	Conc. (µg/m^3)	RT
1	Styrene	4.63	20	4:54
2	Anisole	4.46	20	5:23
3	3-Octanone	3.76	20	6:50
4	3-Methylanisole	3.94	20	7:28
5	4-Methylanisole	3.94	20	7:28
6	Naphthalene	3.76	20	9:57
7	2-Methylisoborneol	2.86	20	10:03
8	α-Terpineol	3.12	20	10:04

Conclusion

- **The person-portable HAPSITE ER allows for sampling and analysis of *S. chartarum* MVOCs to be performed on-site for faster results**
  - **Eliminates questionable sample integrity due to sampling containers and delay in analysis due to transport time**
  - **Focuses on unique signifiers of *S. chartarum***
  - **Two sampling modes:**
    - **Air probe allows for a direct sampling of MVOCs without the need for additional accessories, but the amount of MVOCs detected are limited**
    - **Thermal desorber accessory allows for a broader range of detectable compounds and higher sensitivity**

References

[1] Pestka, J. J., I. Yike, D. G. Dearborn. M. D. W. Ward, J. R. Harkema. 2009. *Stachybotrys chartarum*, Trichothecene Mycotoxins, and Damp Building-Related Illness: New Insights into a Public Health Enigma. *Toxicological Sciences* 104(1): 4-26

[2] Gao, P., F. Korley, J. Martin, B. T. Chen. 2002. Determination of Unique Microbial Volatile Organic Compounds Produced by Five Aspergillus Species Commonly Found in Problem Buildings. *AIHA Journal* 63:135-140

[3] Betancourt, D.A., Dean, T.R., Menetrez, M.Y., Moore, S.A. 2006. Characterization of Microbial Volatile Organic Compounds (MVOC) Emitted by *Stachybotrys chartarum*. Proceedings for the AWMA/EPA Indoor Environmental Quality: Problems, Research and Solutions Conference, Research Triangle Park, NC.

[4]Mason, S., D. Cortes, W. E. Horner. 2009. Detection of Gaseous Effluents and By-Products of Fungal Growth that Affect Environments. *HVAC&R Research* 16(3):109-121

QUESTIONS???