Chemosphere xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Dominant microbial volatile organic compounds in 23 US homes

Timothy J. Ryan [a,*], Catherine Beaucham [b,1]

[a] W 357 Grover Center, Ohio University, Athens, OH 45701, United States
[b] National Institute for Occupational Safety and Health, 4676 Columbia Pkwy., MS C-14, Cincinnati, OH 45226, United States[2]

HIGHLIGHTS

- ▶ Only low microgram m^{-3} concentrations of MVOCs prevail in basements generally.
- ▶ "C8" MVOCs were generally elevated in homes with detectable MVOCs.
- ▶ 2-Octen-1-ol was significantly elevated in non-problematic basements.
- ▶ C8 alcohols and ketones were found with molds known to predominate in wet locations.
- ▶ A semi-quantitative predictor of mold termed "MOW" can aid in applied mold studies.

ARTICLE INFO

Article history:
Received 18 January 2012
Received in revised form 29 May 2012
Accepted 23 June 2012
Available online xxxx

Keywords:
Bioaerosol
IAQ
Indicators
Moisture
Mold
MVOCs

ABSTRACT

Associating Microbial Volatile Organic Compounds (MVOCs) with the species producing them may open the path to more rapid and reliable chemical methods to detect mold problems, especially for mold hidden in wall cavities or small enclosed spaces. This study associated the dominant MVOCs in a convenience sample of 23 homes with the mold species present. Three semi-quantitative predictors of mold growth ("MOW scores") were assessed in the homes through a comparison of basement to main floor areas. MVOC samples were collected and analyzed by GC/MS. Aerotek N-6 samplers were co-located with the MVOC samplers to collect bioaerosols. Concentration and prevalence data for 19 definitive MVOCs were compared with the bioaerosol data. Mold predictor scores were elevated in basement locations as compared with main floor areas. Of the 23 mold genera identified, the predominant genera (ranked occurrences) were *Cladosporium*, *Penicillium*, *Basidiomycetes*, and *Aspergilli*. The MVOCs 2-octen-1-ol, 3-octenone, 2-heptanone, 1-octen-3-ol, and 1-butanol showed the highest average concentrations (11–37 μg m^{-3}), but no single MVOC was significantly elevated in basement locations as compared with main floor living areas in these non-problematic homes. Using a less conservative one-tail test of significance, average 2-octen-1-ol concentrations in basements were higher ($p < 0.040$), and both 3-octenone and 1-octen-3-ol were elevated ($p < 0.095$). Differences in MVOC occurrence were greatest between homes, with MVOCs found in basement locations typically detected in living areas at similar concentrations and frequencies. Based on these findings, the C_8 MVOCs show promise as gross indicators of fungal growth related to the most frequently found mold genera.

1. Introduction

Available evidence has demonstrated that a multitude of factors are associated with poor indoor air quality, including temperature, humidity, lighting and noise; social/psychological stressors; and microbiological causes such as fungal allergens or other growth byproducts like Microbial Volatile Organic Compounds (MVOCs) (Dillon et al., 1996). The Institute of Medicine has concluded that there is "sufficient evidence of an association" between exacerbation of asthma and exposure to mold allergens (Pope et al., 1993).

Multiple federal funding priorities demonstrate an interest in the root causes of poor indoor air quality and in the techniques for their early detection and elimination (U.S. Department of Health and Human Services, 2001; U.S. Department of Housing and Urban Development (HUD) and Office of Healthy Homes and Lead Hazard Control, 2004). The use of MVOCs as a rapid and reliable method to detect mold problems holds promise in this regard, since MVOCs can serve as important indicators for the presence of molds in indoor air pollution (Fiedler et al., 2001). At present, however, the relevance of fungal metabolites in indoor air remains insufficiently studied (Fischer and Dott, 2003).

* Corresponding author. Tel.: +1 740 593 2134 (O); fax: +1 740 593 0555.
E-mail addresses: ryant@ohio.edu (T.J. Ryan), cbeaucham@cdc.gov (C. Beaucham).
[1] Tel.: +1 513 533 8524 (O); fax: +1 513 533 8230.
[2] Present address.

0045-6535/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.chemosphere.2012.06.066

Despite the availability of highly sophisticated laboratory-based methods for trace chemical detection, most routine assessment of indoor fungal contamination problems is presently performed by visualizing large-scale fungal growth (e.g., the ''New York City Guidelines'') and by environmental wipe or air sampling (New York City Department of Health and Mental Hygiene, 2002). In addition, comparative measures of moisture, such as the output of a properly used moisture meter, can be taken to predict locations at which hidden mold growth is probable. However, these relative moisture methods cannot detect excessive mold growth or the causative species, and the intuition and experience of the investigator must be relied upon to identify and sample suspect growth locations. Services of dubious value have emerged offering qualitative inspection services that capitalize on fears of ''black mold'' or ''toxic mold'' contamination where resources directed toward such analyses are unlikely to produce scientifically meaningful results (Blackmold.awardspace.com, 2011; Home Air Check, 2011). For these reasons, advances toward more objectively characterizing indoor mold contamination are of real interest to practicing professionals and the IH profession as a whole.

Competently performed field studies of suspected microbially affected indoor environments rely heavily on sometimes subjective, semi-quantitative visual inspections, spore trapping, dust cultures, or bioaerosol collection. Well recognized deficiencies of such viable airborne mold sampling techniques include high seasonal, geographic, temporal, and analytical variability, as well as comparatively long turnaround times. For this reason, the documentation of one or more validated correlations between MVOCs and the molds producing them may open the path to more economical and precise chemically-based quantification methods. Given the extensive commercial volatile organic compound analysis infrastructure in the US today, mold assessments based on MVOCs would be significantly quicker than culture-based analyses.

Delays in obtaining sampling results can greatly inconvenience building residents and clients, and often add significantly to the construction-related costs of mold-issue resolution at affected buildings. Although existing methods are passable for some needs, all mold assessment surveys could benefit greatly from a method capable of providing a more immediate turnaround, with results requiring less interpretation by the professional. Recent work with portable ion-mobility spectrometry has resulted in a promising field application of real-time MVOC detection with the lower limit of detection necessary for most MVOCs (Tiebe et al., 2009). Interpretation of conventional culture results, whether bioaerosol or solid surface in nature, is often of questionable scientific merit. In fact there is presently no definitive, widely accepted statement as to the meaning of most cultured fungal concentration data. Correlating MVOCs to known mold genera and colonization extent, moisture content, and related environmental factors, will serve to create an investigative tool that is commercially feasible now, as well as highly cost-effective.

A number of studies have been conducted on the utility of MVOCs as indicators of fungal growth. Possible advantages to the use of MVOCs for fungal contamination quantification include (a) rapid analysis as no culture is needed, and (b) indication of not only visible but also hidden microbial growth, since MVOCs are known to permeate building structures (Gao and Martin, 2002; Matysik et al., 2008). All culture-based methods suffer from an inability to non-destructively detect mold hidden in wall cavities or small enclosed spaces. A key characteristic of MVOCS—their ability to diffuse from enclosed spaces, from behind vinyl wallpaper or vapor barriers, or off heating, ventilation, air conditioning (HVAC) filters free of visible contamination—has been of interest to investigators seeking to employ MVOCs as indicators of latent mold growth (Strom et al., 1994; Ahearn et al., 1997; Elke et al., 1999; Hachem et al., 2009). It is this property, in part, that makes MVOCs so attractive as a mold assessment tool. However, as noted by the US Environmental Protection Agency, a definitive determination on how to use MVOC data remains lacking and ''Research on MVOCs is still in the early phase.'' (USEPA, 2011).

Finally, information about the prevalence of specific MVOCs is significant to basic research for several reasons. MVOCs in indoor air present a group of compounds worthy of additional study both because of their innate toxicological properties, or as precursors for more toxic mycotoxins. For almost two decades now there have been suggestions that such volatiles, and the mycotoxins for which they may act as precursors (Borjesson et al., 1992; Zeringue et al., 1993; Jelen et al., 1995; Pasanen et al., 1996) could be partially to blame for indoor air quality (IAQ) problems caused by fungal growth (Land et al., 1987; Tobin et al., 1987; Burge, 1989; Sorenson, 1989; Flannigan et al., 1991; Miller, 1992; Samson, 1992). Thus the prevalence of MVOCs is a basic public health question of potential merit.

In one of the most frequently cited early works on MVOCs a total of 23 compounds (grouped into Classes A and B) were labeled by Wessen and Schoeps (1996) as arising uniquely from microbial sources. According to them, Class A MVOC occurrence is usually of higher frequency than Class B but Class B compounds are necessary to correlate microbial impact with the indoor environment. With the publication of additional studies since Wessen and Schoeps, it is possible to compile a more definitive list of presumptive MVOCs. A single grouping of fourteen (14) empirically derived MVOCs has been compiled by the authors in Table 1 (Ryan, 2011). The definitive criterion for this listing is the independent quantification of an MVOC in three or more separate field-based investigations (Hung et al., 2005).

Compared to other areas of IAQ research, there exist comparatively few field studies of MVOC occurrence patterns. The often cited work done in Swedish houses is based on only three houses (one of which was a control dwelling) (Stom et al., 1994). The significance of those findings must be guarded given the lack of a statistically valid number of observations. With respect to other field studies of MVOCs there are relatively few reports ($n \sim 7$) in the peer-reviewed literature (Miller et al., 1988; McJilton et al., 1990; Bayer and Crow, 1992; Wessen and Schoeps, 1996; Schleibinger et al., 1997; Elke et al., 1999; Matysik et al., 2009) and no recent surveys of US housing stock. All studies which attempted to correlate mold species with predominant MVOCs have yet to be repeated. The great majority of published studies to date have in fact investigated mold genera and specific MVOCs only under laboratory conditions (not discussed here), where a great diversity of compounds is reported. Perhaps because of the complexity presented by this diversity there exists in the literature at present a dearth of studies that examine the nature of the mold-MVOC relationship under field conditions. Recent work with artificial neural

Table 1
Fourteen MVOCs as suggested by consistent isolation in ⩾3 field surveys (AIHA, 2005).

3-Methyl furan
1-Butanol
3-Methyl-1-butanol
3-Methyl-2-butanol
2-Pentanol
2-Hexanone
2-Heptanone
3-Octanone
3-Octanol
1-Octen-3-ol
2-Octen-1-ol
2-Nonanone
Borneol
Geosmin

networks (ANNs) holds promise for distilling down this complexity but it has thus far been reported only as preliminary findings (LeBouf et al., 2010).

In this study, 23 non-problematic homes were convenience sampled for definitive MVOC prevalence. Both drier main floor living areas and presumably wetter basements were sampled. Basement locations were a key component because of earlier findings which showed significantly higher airborne fungal spore counts of commonly encountered molds (i.e., *Aspergillus*, *Penicillium*, *Cladosporium*, and unidentified basidiospores) in damp residences (Li and Kendrick, 1995). As none of the homes sampled were identified by their owners as problematic, basement areas were included as surrogates for damp residences. A total of 19 MVOCs could be determined with acceptable analytical precision (i.e., standards were obtainable). Airborne molds present at each location were also determined. Thus, in this work the MVOCs present were examined in relation to mold species presumably generating them.

2. Methods

Study homes were convenience sampled from a pool of approximately 45 community households, which had been previously identified by participation in an ongoing baseline IAQ study of non-problematic homes. Key selection criteria were the presence of a basement and resident availability during the 3 month summer sampling campaign. Sampling logistics allowed a total of 25 homes to be studied during this period, although complete data could only be obtained for 23.

2.1. Environmental data

Environmental data at each location was collected on each sampling day. Temperature and relative humidity were continuously recorded in basement locations using a Quest AQ-5001 (Quest, Manitowoc, WI) and were grab sampled in main floor living spaces using an Omega HHM25 meter with a calibrated probe (Omega, Stamford, CT). Outdoor temperature and relative humidity were also grab sampled with the Omega meter.

2.2. Qualitative assessments

Three semi-quantitative measures of mold growth associated factors were defined and employed in this study. The association of microbial growth with visible water or elevated moisture content is well known (Hung et al., 2005). Furthermore, well-accepted criteria have been developed for the visual determination of the extent of mold presence (New York City Department of Health and Mental Hygiene, 2002). Therefore, these two predictors of mold (‘‘Water Extent’’ and ‘‘Mold Extent’’) were assessed in this study for comparison to MVOC levels detected. A novel third predictor (‘‘Organic Volume’’), designated for assessing the amount of organic substrate material potentially colonized or otherwise available for mold amplification and MVOC production, was also employed. Using these three measures, an attempt was made to semi-quantitatively assess the degree of moisture, mold, and mold substrate, respectively, present in sampling locations. Prior to the initiation of the study, definitions of the extent of each of these three parameters were developed (Table 2). The descriptors' assessment criteria were written such that they could be objectively measured, without exceptional reliance on the experience of the field sampling personnel. In practice all three predictors were determined by the same individual {Beaucham} thus eliminating inter-assessor variability related error (but raising the possibility of assessor bias). As a group, these three predictors are referred to as ‘‘MOW’’ measures, for Mold, Organics, and Water extent. During each sampling event, upstairs and basement locations were visually inspected and the MOW scores recorded. Photographs of all basement areas were obtained to blindly verify the assignment of the Organics present. (It should be noted that none of the homes were described as problematic by their owners, an observation reflected in an average Mold score of 1.7 (see Results). No home received a Mold score above 3, confirming there was little to no visible mold detected in the homes sampled.)

2.3. MVOC sampling

Two (2) MVOC samples were collected in each house; one in the main floor living area (excluding kitchens) and one in a centrally located basement location. MVOC area samples were collected by active sampling onto stainless steel siliconized tubes containing 300–400 mg Tenax TA and Carbopack B (Markes International, Pontyclun, UK). Field sampling employed a battery operated diaphragm pump (SKC Aircheck Sampler Model 224-PCXR4; Eighty Four, PA) with a flow rate of 45–55 mL min m^{-1} operated for 3–5 h to give a typical sample volume of 0.017–0.029 m^3. Calibration of the sampling train was accomplished through the use of a graphite piston calibrator (DryCal DC-Lite, BIOS International Pompton Plains, NJ) before and after sample collection.

Table 2
Mold, organic volume, and water (MOW) measures and assigned values used in scoring sampling locations.

Extent of mold present	
1	No visible mold
2	Slight odor and no visible mold or <2 s.f. in diffuse colonies
3	Distinct odor and no visible mold or <5 s.f. in diffuse colonies
4	Distinct odor and visible patches of mold (2–5 s.f.)
5	Distinct odor and visible colonies covering a large area (>10 s.f.)
Extent of organic volume present	
1	0–10% of floor space used for organic materials, essentially empty basement.
2	25% of floor space utilized, mainly inorganic materials; limited wood shelves, or cardboard boxes present.
3	50% of floor space used. Half organic material, half inorganics. Multiple stored cardboard boxes, hanging clothes, etc.
4	75% of floor space used for organic materials. Floor covered in old rugs/carpet, stacks of old books/magazines.
5	90–100% of floor space used. Leather, food debris, old clothes, wood, etc. covering the majority of available floor space.
Extent of water present	
1	None
2	Intermittent or only limited presence of water (e.g. washer floor drain), or past damage visible
3	Currently moist areas or extensive past water damage, no currently pooled water
4	Current pooled water in one area
5	Current pooled water covering a large majority of basement.

Please cite this article in press as: Ryan, T.J., Beaucham, C. Dominant microbial volatile organic compounds in 23 US homes. Chemosphere (2012), http://dx.doi.org/10.1016/j.chemosphere.2012.06.066

Table 3
Average MVOC Concentrations and descriptive statistics ordered by decreasing concentration in basements.

	Basement				Main floor			
	Average concentration (μg m^{-3})[a]	Range (μg m^{-3})	Standard deviation	Variance	Average concentration (μg m^{-3}) [a]	Range (μg m^{-3})	Standard deviation	Variance
3-Octanone	37.3 (12)	4.0–180.2	50.0	2496.4	11.6 (10)	4.1–45.9	12.7	161.5
1-Octen-3-ol	31.3 (8)	7.1–120.9	39.9	1595.1	11.8 (8)	8.5–16.7	3.5	11.9
2-Octen-1-ol	28.9 (18)	12.4–79.6	16.2	261.6	21.5 (17)	14.4–33.6	5.3	28.0
2-Heptanone	16.8 (23)	10.0–26.5	4.1	16.8	16.9 (22)	10.3–25.4	3.7	14.0
1-Butanol	14.3 (16)	6.1–57.2	13.6	186.4	11.0 (16)	5.4–30.5	7.5	55.7
2-Methyl-1-propanol	13.7 (4)	9.3–20.1	4.8	22.7	10.4 (2)	8.5–12.2	2.6	6.7
Carveol	11.5 (3)	8.8–13.6	2.5	6.0	11.9 (2)	8.0–15.7	5.5	30.1
3-Octanol	9.3 (8)	5.3–16.3	3.3	11.2	8.8 (9)	4.4–16.3	3.7	13.6
Gamma Terpineol	7.9 (17)	4.8–11.7	1.9	3.6	8.7 (18)	4.9–17.6	3.9	15.1
Alpha Terpineol	7.6 (19)	4.6–13.5	2.3	5.5	9.0 (15)	5.2–27.4	5.7	32.8
Borneol	6.8 (20)	4.4–12.7	1.9	3.9	6.9 (21)	4.4–13.6	2.4	5.8
2-Nonanone	5.6 (15)	3.8–8.1	1.3	1.8	5.8 (12)	4.2–11.8	2.1	4.3
2-Pentylfuran	5.4 (17)	3.1–11.3	1.9	3.7	5.1 (17)	2.9–12.6	2.6	6.8
2-Methylfuran	5.4 (10)	2.6–10.9	2.2	4.9	6.3 (8)	2.8–9.9	2.3	5.4
Thujopsene	5.3 (9)	2.9–8.8	1.9	3.9	5.9 (8)	3.2–9.8	2.4	5.6
Fenchone	4.9 (15)	2.8–6.8	1.3	1.6	5.2 (14)	3.3–9.8	1.7	2.8
Geosmin	4.3 (3)	3.3–5.8	1.3	1.7	6.0 (3)	2.8–9.5	3.4	11.6
Ethyl Butyrate	4.2 (20)	2.3–14.2	2.5	6.4	4.8 (19)	2.3–15.5	3.8	14.3
2-Pentanol	3.62 (1)	–	–	–	4.8 (3)	3.4–5.9	1.3	1.6

[a] Number in parentheses refers to the number of samples on which the average concentration was calculated.

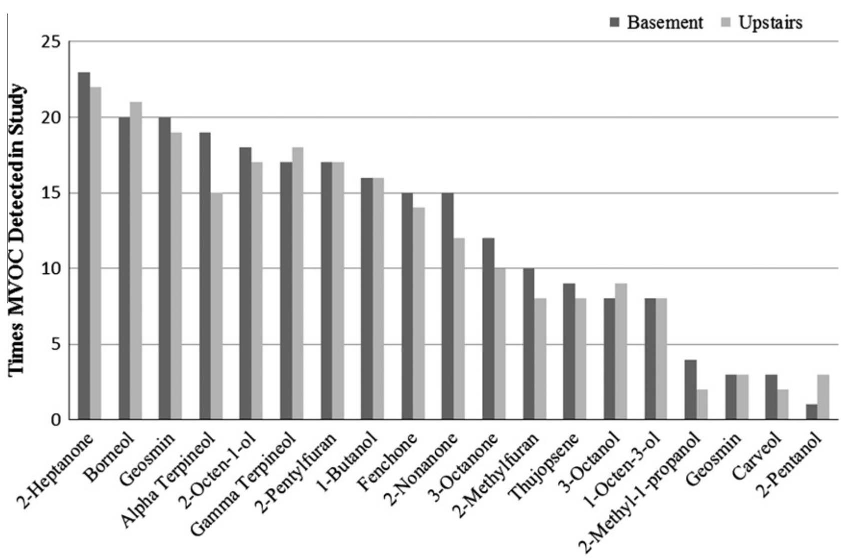

Fig. 1. Occurrence of MVOC species included in study.

2.4. MVOC analysis

Thermal-desorption is generally the preferred method when sampling for MVOCs, as levels as low as 250 pg L^{-1} are possible (Hung et al., 2005). Sample processing and analysis was performed by placing the adsorbent tube in a thermal desorber (Markes International, Pontyclun, UK) and flushing with ultra high purity Helium gas (>99.999%) at a rate of 50.0 mL for 1 min in order to remove the air and water present. The initial purge of the sample tube was followed by a desorption purge at 300 °C for 10 min, in which the compounds present were carried onto a cryotrap (−10 °C) to focus all organic volatiles present. The trap was then heated ballistically to 300 °C and held at temperature for 5 min to load the organic volatiles onto the gas chromatograph column.

A Hewlett Packard (HP) 6890 gas chromatograph (GC) with an HP Ultra 2 column (50.0 m × 320 μm × 0.52 μm) coupled to an HP5973 mass selective (MS) detector was used for the separation, identification, and quantification of the volatile compounds collected. The GC/MS conditions were based on the MVOC elucidation work of Gao and Martin (2002). After thermal-desorption, the oven temperature program initiated at 35 °C for 5 min followed by an increase of 10 °C min^{-} until a temperature of 220 °C was reached (followed by a final increase to 280 °C for 15 min). To maximize sensitivity, the MS was operated in scan mode from 40 to 260 atomic mass units.

Table 4
Mold genera and species detected.

Abisidia	*Curvularia geniculata*
Acrodontium crateriforme	*Epicoccum nigrum*[a]
Alternaria alternata[a]	*Fusarium graminearum*[a]
Aphanociadium	*Fusarium solani*[a]
Arthrinium	*Gliocladium*
Aspergillus fumigatus[a]	*Paecilomyces marquandii*[a]
Aspergillus ochraceus[a]	*Paecilomyces variotii*[a]
Aspergillus versicolor[a]	*Penicillium*[a]
Aspergillus niger[a]	*Phoma*[a]
Aspergillus ustus[a]	*Pithomyces chartarum*
Basidiomycetes[a]	*Rhinocladiella*
Beauveria bassiana	*Rhizopus stolonifer*[a]
Botrytis cinerea	*Rhodotorula glutinis*[a]
Choanephora	*Trichoderma Harzarum*[a]
Cladosporium[a]	*Sterile fungi and yeasts*

[a] Indicates designation as "Moisture Indicator Fungi", per Yang et al.

Table 5
Genera occurrence and average concentrations ranked by times detected (46 maximum).

Genera	Times detected	Average CFU m^{-3}	Average CFU m^{-3} by sampling location	
			Downstairs	Upstairs
Cladosporium	44	227	213	241
Penicillium	42	165	203	128
Basidiomycetes spp.	40	150	171	129
Aspergillus spp.	30	97	142	51
All other genera, spp.	82	35	33	36

Compound identification was accomplished through the utilization of the NIST98 reference standard library used in conjunction with retention time comparisons to reference standards. Standard and test compound retention times and concentrations were determined through the use of a quantitation database prepared using 3 and 5 point calibration curves. Only those MVOCs for which standards could be obtained are reported. With respect to the 14 compounds listed in Table 1, eleven (~80%) could therefore be included. An additional 9 compounds were included in the quantification method, based on their MVOC status ascribed by Wessen and Schoeps (1996).

The reference MVOCs included in the study consisted of: 1-butanol (Sigma, >99.9%), 2-pentanol (Fluka Chemika, >98%), 2-heptanone (Sigma, >99.9%), 3-methylfuran (Absolute Standard, purity not stated), 3-octanone (Acros Organics, >99%), 3-octanol (Fluka Chemika, >97%), 1-octen-3-ol (Fluka Chemika, >98%), 2-octen-1-ol (Absolute Standard, >92%), 2-nonanone (Acros Organics, >99%), borneol (Fluka Chemika, 95%), and geosmin (Sigma, >97%). The additional 9 MVOCs included were: ethyl butyrate (Absolute Standard, >95%), 2-methyl furan (Aldrich, >98%), 2-pentylfuran (Absolute Standard, >98%), 2-methyl-1-propanol (Aldrich, >99.5%), fenchone (Absolute Standard, 99%), α- and γ-terpineol (Sigma, >95%), carveol (source, purity not available), and thujopsene (Absolute Standard, >97%). The limits of detection (LOD) for these MVOCs ranged from approximately 0.1–2 μg m^{-3}, with limits of quantification (LOQ) roughly double that range.

2.5. Bioaerosol assessment

Aerotek N6 (single stage) impactors were used to collect bioaerosol samples (Aerotek Instruments, Phoenix, AZ). Samplers were run for 3 min at 28.3 L min^{-1} for a total sampled volume of 84.9 L. Malt extract agar (MEA) was utilized as the growth media of choice for common indoor air fungi (Hung et al., 2005). Three (3) bioaerosol samples were taken at each household. One was from a central location in the basement, one from the main floor living quarters (excluding the kitchen), and one from outside. An alcohol wipe was used to decontaminate samplers after each sampling run. Sample mean and standard deviation was calculated for the three locations and reported as an estimate of the coefficient of variation.

Immediately after collection, samples were transported in a cooler to a laboratory refrigerator, where they were stored from 1 to 3 d until overnight shipment to the analytical laboratory. An AIHA-EMLAP accredited laboratory was utilized to culture, quantitate, and identify molds from the plates.

3. Results

3.1. Environmental measures

Using a paired *t*-test, temperatures were not found to be statistically different between upstairs and downstairs locations ($p > 0.05$). Temperatures upstairs averaged 73.0 °F compared to 73.1 °F down. Likewise, relative humidity was essentially the same in both airspaces (70.7% upstairs average compared to 69.1% down). During the 3 months of the sampling campaign, outside daily temperatures averaged 79.5 °F, and the average relative humidity was 70.6%.

3.2. MOW scores

All three of the individual MOW predictor scores were significantly elevated for basement areas as compared with upstairs locations ($p < 0.007$, minimum; paired *t*-test). The average Mold Extent score in basements averaged 1.9 compared to a predictably lower score of 0.9 for upstairs living quarters. Closely mimicking these results, the Water Extent score for all basements was 2.0 as compared with upstairs values of 0.9. The Organic Volume scores between the locations were also different, averaging 2.7 for basements and 1.6 for upstairs areas sampled (data not presented). Supporting individual MOW score differences, average MOW scores were significantly different between the Basement and Main Floor locations ($p < 0.0001$; Table 7). While bias alone on the part of the assessor could account for MOW score differences, photographs of all locations were taken and the assessor's score assignments independently verified by the second author.

Table 6
Summary statistics for three bioaerosol sampling locations.

	Range (CFU m^{-3})	Geometric mean	Geometric standard deviation	Coefficient of variation	Number of useable samples
Outside	428–3932	968.8	909.0	0.939	14
Main floor	59–2051	450.8	25.9	0.057	22[a]
Basement	83–2871	569.0	29.2	0.051	23

[a] One sample was too numerous to count.

Table 7
MOW scores ranked by basement values for 23 homes and their associated bioaerosols.

Location code	Basement average MOW score	Main floor average MOW Score	Basement bioaerosol (CFU m^{-3})	Main floor bioaerosol (CFU m^{-3})
27NM	1	1.3	1222	440
191ES	1.3	1.3	333	118
46EP	1.3	1.3	83	1124
9NS	1.3	1.0	212	384
85MW	1.7	1.3	323	352
121MO	1.7	1.3	2871	201
18AP	2	1.3	745	1112
12SM	2	1.3	468	351
14SM	2	1.3	544	–[a]
11TP	2	1.3	262	271
22AP	2.3	1.0	335	399
35AP	2.3	1.0	213	119
35BW	2.3	–[b]	1508	59
44EP	2.3	1.3	1059	636
14EW	2.3	1.3	216	1724
68MW	2.3	1.3	1703	421
19NM	2.3	1.3	1681	2051
28CB	2.7	1.3	610	519
107EW	2.7	1.3	1470	1227
25HU	2.7	–[b]	1189	729
11OA	2.7	1.3	193	164
27UT	2.7	1.0	497	1107
73MW	3	1.0	979	717
Averages[c]:	1.95*	1.25*	569	451

[a] Too numerous to count.
[b] Missing value.
[c] MOW averages are arithmetic, bioaerosol averages are geometric; "*" values differ significantly at $p < 0.001$.

3.3. MVOCs

MVOC occurrence (total number of times each MVOC was detected above baseline) was tabulated for both locations (basement and upstairs) and is presented in Fig. 1. The most frequently detected compound was 2-heptanone, with 10 of the targeted 19 compounds seen in the majority (15/23; 65%) of homes. Most of the MVOCs were found with similar frequency in both sampling locations, although it appears from Fig. 1 that MVOCs tended to occur in basements slightly more frequently than in upstairs locations.

Table 3 presents a ranked summary of the normally distributed (Kolmogorov-Smirnoff test) MVOC concentrations in both locations. Concentrations ranged from the LOQ to a maximum of 37.3 μg/m^3. For the MVOCs quantitated, the mean basement concentration was 12.3 μg/m^3 while the upstairs average was 25% lower at 9.1 μg/m^3. Values were compared by location using a two-tailed two sample test assuming equal variance. No single MVOC was significantly higher in basement locations as compared with the main floor living space, although elevation of MVOCs in basements is evident in the Table 3. However, if the less conservative one-tailed test for significance is employed, 2-octen-1-ol did show significant elevation ($p < 0.040$) and both 1-octen-3-ol and 3-octanone tended toward significant elevation in basement locations ($p < 0.095$ and $p < 0.065$, respectively). Allowing for the one-tailed test in this instance is methodologically defensible since it would not be expected that main floor MVOC concentrations would be higher than basement values.

3.4. Molds

All bioaerosol data was useable with the exception of that from one home, where both sampling location plates were overloaded. Data fit a lognormal model distribution using Q–Q plots and the Kolmogorov-Smirnoff test ($\alpha = 0.01$); thus the geometric mean and geometric standard deviation were calculated. The estimate of the coefficient of variation for the upstairs and downstairs locations was very high, at 0.87 and 0.85, respectively, and only slightly lower (0.76) for the outdoor samples. The twenty-three (23) mold genera and 9 distinct species that were detected in the bioaerosol sampling are listed in Table 4, while the ranked occurrence of the predominant genera are shown in Table 5. The mold genera and concentrations determined in sampled homes were generally not remarkable and consistent with the findings of others (Hung et al., 2005). The same was true for outside air comparison bioaerosols (data not presented). The most frequently detected molds were seen in roughly two-thirds of all study locations. *Cladosporium* was ubiquitous and was seen in all but one of the homes, and also had the highest average concentrations detected (227 cfu/m^3). This was followed by *Penicillium* spp., found in 21 of the 23 homes (91%) with typical levels of 165 cfu/m^3. The *Basidiomycetes* occurred almost as often (20/23 homes, 87%) with mean levels of 150 cfu m^{-3}. *Aspergillus* spp. were detected in 30/46 instances (65%) at average concentrations of 97 cfu m^{-3}. Indoor concentrations were generally lower than outside, and main floors averaged lower bioaerosol concentrations than basements as was intuited for these non-problematic homes (Table 6). The geometric mean bioaerosol concentration in the 23 basements was 569.0 CFU m^{-3} as compared with the main floor geometric mean of 450.8. However, a paired t-test for the differences between the upstairs and downstairs bioaerosol concentrations revealed no significant concentration differences ($p = 0.39$). It should be noted that no samples were taken in duplicate, or on more than a single day, and thus inter-sampling variability as well as daily variation cannot be described.

4. Discussion

4.1. Environmental factors

Although basement relative humidity was lower overall by about 1.5 m units (i.e., percent units), it seems somewhat surprising in light of conventional expectations that the average relative humidity levels between the basement and upstairs locations were not greater. This is especially so given the elevated Water Extent predictor averages (1.9 downstairs versus 0.9 upstairs). One possible explanation for this outcome was the presence of central heating, ventilation, and air conditioning (HVAC) in the majority of the homes studied (n = 21%, or 91%). As noted elsewhere in the discussion, the 3 month study period encompassed a cooler and much wetter summer than is typical for the study location. Observations were not made as to whether air conditioning or heating was in use at the time of sampling, only if it existed, but it is logical to anticipate lower than typical usage. Thus we cannot be definitive about the effect HVAC systems or open windows might have had, both on our bioaerosol numbers or on MVOC concentrations. HVAC systems typically exhibit poorly sealed ducts, loose floor penetrations, filter housings, and returns, and so could be expected to have some influence on basement environmental conditions even if not intentionally servicing the airspace of the downstairs location, or in fact even running. This explanation is consistent with the MVOC occurrence data, which indicated no distinct differences (with exceptions as noted) upstairs versus down (Table 3). A second explanation includes the possibility of a true humidity difference between the two locations, but that our sample size of only 23 homes was insufficient to precisely detect it. Finally, it must be allowed that a simple measure such as relative humidity alone may not effectively or accurately describe the propensity for mold colonization in some environments or situations.

4.2. MOW scores

From the MOW score data, it is evident that basement areas are much more likely to demonstrate mold—and any attendant MVOCs—than are upstairs locations. The MOW predictor results enable the determination of discrete values on what has long been intuitively appreciated, and serve to confirm the soundness of the study design of contrasting the two study locations for MVOC occurrence. Basements are seldom as clean as the main floor living areas of the home, and so our findings of an elevated Mold Extent score (i.e., more visible mold growth) in these basement locations might have been expected. The elevated Water Extent scores, in the absence of significantly elevated relative humidity, is more intriguing. A plausible explanation for the difference (in addition to the discussion of relative humidity, above) concerns the influence of external weather conditions during the study period of June through August. Specifically, water intrusion immediately following rainfall events was the likely cause for the elevated Water Extent scores. In an average year, the study location would have received 11.6 in. of rainfall (National Climatic Data Center, 2007). During the period of this study, however, more than twice that amount of rain was recorded (26.5 in.), with June described as the 13th wettest in over 100 years and August noted as the wettest on record. Clearly there was much water in and around the homes during the study period, and our data indicate that water could even be found in the basements of the non-problematic homes studied.

4.3. Organic volume measure

Since many objects are stored undisturbed for long periods in basements and are therefore susceptible to continuous but slow mold colonization, the elevated Organic Volume predictor scores are of particular interest and potential utility. The Organic Volume predictor averaged more than 50% higher in basements than upstairs (2.5 versus 1.6). Viewed in light of the other two elevated predictors, and the observation that the presence of "musty odors" was noted in 12/23 (52%) of the basements, these results lend support to the concept of a "mass effect" for MVOCs. That is, colonization of larger volumes of organic materials in basements with elevated Organic Volume scores can be responsible for some higher MVOC concentrations. This result lends further reinforcement to the validity of the Organic Volume predictor as employed in this study.

4.4. MVOCs

These results, in conjunction with other investigators' findings, serve to confirm the status of 3-octanone as an MVOC with potential for serving as a practical indicator of elevated mold presence ($p < 0.065$, one-tail). This MVOC was found in elevated concentrations in basement areas known to have elevated moisture and excessive growth substrate despite sometimes unremarkable airborne mold levels or visual indications of mold presence. We can cautiously expand this group of MVOC indicators to include 2-octen-1-ol ($p < 0.040$) and 1-octen-3-ol ($p < 0.095$), both of which were arguably found at elevated levels in the presence of elevated mold predictors. In fieldwork characterizing schools and commercial environments suspected of mold contamination, 1-butanol was found at average levels of 17 $\mu g\ m^{-3}$ in almost half of all locations, while 2-heptanone was detected at an average concentration of 1.9 $\mu g\ m^{-3}$ in approximately a third of such environments (Snips, 2002). Although they did not show statistical significance in this study, the elevations of 2-heptanone and 1-octen-3-ol are clearly seen in Table 3. Furthermore, the results for these 8-carbon MVOCs reinforce others observations about the relative general importance of the "C8" compounds as MVOC indicators (Wilkins and Larsen, 1995).

Schleibinger et al. (2005) performed laboratory studies comparing MVOC production by genus/species on a variety of substrates, and reiterated the findings of others that MVOC production is inconsistent under controlled laboratory conditions. They concluded that, based on the low emission rates seen and extrapolation of those production rates to indoor air concentrations, only extensive microbial contamination would be detectable through the use of MVOCs. Our results contradict this finding, at least in part. None of the homes studied was heavily contaminated yet elevated MVOC levels were observed in basements for some of the specific MVOCs quantitated. Given the higher Mold Extent scores in the basements and the prescribed, objective criteria on which scoring was based, we found that under field conditions the specific MVOCs quantitated here can in fact demonstrate elevated concentrations in the absence of extensive visible mold contamination.

4.5. Bioaerosols

The variety of molds identified in this study is similar to the results of another, larger cross-sectional study of 64 homes. Of the 23 genera shown in Table 4, about half (12) are categorized as Moisture Indicating Fungi (MIF) in that work (Mahooti-Brooks et al., 2004). The authors found that basements with a musty odor, water sources, or mold had a two- to three-fold increase in fungal bioaerosol as compared to basements lacking those descriptors (our results show no significant average bioaerosol elevations). One plausible explanation for the increases in MIF seen in this study would be an elevated Mold Extent and/or Organic Volume score. Also of note is the finding of these moisture-indicating fungi under the environmental conditions selected in this study. Moisture indicating fungi, as signaled by higher predictor scores, may help explain the elevated MVOCs detected in this work.

5. Conclusions

In homes free of mold-related complaints, MVOCs were nevertheless sometimes significantly elevated in basement areas. The MOW scoring scheme presented in this paper is a first attempt to quantitate the association between those areas and the MVOCs found in them. Future work should examine problematic locations to obtain MVOCs and MOW scores for comparison, to better assess the meaning of elevated MVOC concentrations as well as validate the MOW scoring approach. When the less conservative one-tailed test for significance is employed, 2-octen-1-ol showed a significant elevation ($p < 0.040$) and 1-octen-3-ol as well as 3-octanone could arguably be considered significantly elevated in basement locations ($p < 0.090$).There exists at this time a need to better elucidate the potential roles these specific MVOCs can play in assessing IAQ via chemical indicator methods, which are demonstrably quicker than culture methods.

As clearly demonstrated in this study, bioaerosol concentration data and visual inspections may be inadequate for precisely defining the extent of airborne mold contamination. Much is presently known about the high variability of CFU m^{-3} in numerous domestic and commercial environments. In an overwhelming number of such studies, the correlations between total CFU m^{-3} indoor air and adverse health effects is unacceptably low and of dubious value. Owning to this inherent variability attempting to find correspondence between total CFU m^{-3} and MVOCs is not advised. Until validation for the use of evolving approaches such as ANN (LeBouf et al., 2010) in conjunction with real-time instruments (Tiebe et al., 2009) can be accomplished, an approach employing MOW scoring represents a new avenue for further evaluation. Such efforts involving fungal biomass research might more productively focus on correlating the genera of mold present to the speciated MVOCs also present, including the MIF. The correlation of those molds, especially the MIF group, and those MVOCs may prove significant and useful as part of a more rapid mold assessment tool for investigators of mold-impacted homes or businesses.

There is a dearth of studies published in the peer-reviewed literature relating to MVOC concentrations under field conditions. The majority of work to date has been done in laboratories where the generation of specific MVOCs by specific microbial genera or species was reported. The small number of MVOC field-based papers published are quite dated, employed extremely small group of subjects, or pertain to non-residential environments. This study provides new and additional baseline values of a definitive group of MVOCs in 23 non-problematic residences, from areas reasonably expected to demonstrate elevated MVOC concentrations. Future work along these lines might attempt to further associate these MVOCs with the variables responsible for their generation and accumulation, serving as the essential basis for differentiating via direct chemical methods a problem from a non-problem environment.

Acknowledgements

The authors wish to thank Jennifer Sirko and Roy Nalazek for their proofing and review of this manuscript. This study was supported by a Grant from the Ohio University Research Committee.

References

Ahearn, D.G., Crow, S., Simmons, R.B., Price, D., Mishra, S., Pierson, D., 1997. Fungal colonization of air filters and insulation in a multi-story office building: production of volatile organics. Curr. Microbiol. 35, 305–308.

Bayer, C.W., Crow, S., 1992. Odorous Volatile Emissions from Fungal Contamination. In: Proceedings of IAQ '92, American Society of Heating, Refrigerating and Air Conditioning Engineers, Atlanta GA, pp. 99–104.

Blackmold.awardspace.com. MVOCs (Microbial Volatile Organic Compounds). <http://blackmold.awardspace.com/mvocs.html> (accessed 05.07.10).

Borjesson, T., Stollman, U., Schnurer, J., 1992. Volatile metabolites produced by six fungal species compared with other indicators of fungal growth on cereal grains. Appl. Environ. Microbiol. 58, 2599–2605.

Burge, H.A., 1989. Airborne allergenic fungi. Classification, nomenclature, and distribution. Immunol. Allergy Clin. North Am. 9, 307–319.

Dillon, H.K., Heinsohn, P.A., Miller, J. (Eds.), 1996. Field Guide for the Determination of Biological Contaminants in Environmental Samples. American Industrial Hygiene Association, Fairfax, VA. pp. 21–35.

Elke, K., Begerow, J., Oppermann, H., Kramer, U., Jermann, E., Dunemann, L., 1999. Determination of selected microbial volatile organic compounds by diffusion sampling and dual-column capillary GC-FID—a new feasible approach for the detection of an exposure to indoor mould fungi? J. Environ. Monit. 1, 445–452.

Fiedler, K., Schutz, E., Geh, S., 2001. Detection of microbial volatile organic compounds (MVOCs) produced by moulds on various materials. Int. J. Hyg. Environ. Health 204, 111–121.

Fischer, G., Dott, W., 2003. Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene. Arch. Microbiol. 179 (2), 75–82.

Flannigan, B., McCabe, E.M., McGarry, F., 1991. Allergenic and toxigenic microorganisms in houses. J. Appl. Bacteriol. 70, 61S–73S.

Gao, P., Martin, J., 2002. Volatile metabolites produced by three strains of *Stachybotrys chartarum* cultivated on rice and gypsum board. Appl. Occup. Environ. Hyg. 17 (6), 430–436.

Hachem, C., Fazio, P., Rao, J., Bartlett, K., Chaubey, P., 2009. Identification and transport investigation of microbial volatile organic compounds in full-scale stud cavities. Build. Environ. 44, 1691–1698.

Home Air Check, 2011. Prism analytical technologies, Inc. Mt. Pleasant, MI. <http://www.homeaircheck.com/our-products> (accessed 07.06.11).

Hung, L.L., Miller, J.D., Dillon, H.K. (Eds.), 2005. Field Guide for the Determination of Biological Contaminants in Environmental Samples, second ed. American Industrial Hygiene Association, Fairfax, VA. pp. 161–171.

Jelen, H.H., Mirocha, C.J., Wasowicz, E., Kaminski, E., 1995. Production of volatile sesquiterpenes by Fusarium sambucinum strains with different abilities to synthesize trichothecenes. Appl. Environ. Microbiol. 61, 3815–3820.

Land, C.J., Hult, K., Fuchs, K., Hagelberg, S., Lundstrom, H., 1987. Tremorgenic mycotoxins from *Aspergillus* fumigatus as a possible occupational health problem in sawmills. Appl. Environ. Microbiol. 53, 787–790.

LeBouf, R.F., Schucker, S.A., Rossner, A., 2010. Preliminary assessment of a model to predict mold contamination based on microbial volatile organic compound profiles. Sci. Total. Environ. 408, 3648–3653.

Li, D., Kendrick, B., 1995. Indoor aeromycota in relation to residential characteristics and allergic symptoms. Mycopthologia 131, 149–157.

Mahooti-Brooks, N., Storey, E., Yang, C., Simcox, N.J., Turner, W., Hodgson, M., 2004. Characterization of mold and moisture indicators in the home. J. Occup. Environ. Hyg. 1, 826–839.

Matysik, S., Herbarth, O., Mueller, A., 2008. Determination of volatile metabolites originating from mould growth on wall paper and synthetic media. J. Microbiol. Methods 75, 182–187.

Matysik, S., Herbarth, O., Mueller, A., 2009. Determination of microbial volatile organic compounds (MVOCs) by passive sampling onto charcoal sorbents. Chemosphere 76, 114–119.

McJilton, C.E., Reynolds, S.J., Streifel, A.J., Pearson, R.L., 1990. Bacteria and indoor odor problems—three case studies. Am. Ind. Hyg. Assoc. J. 54, 545–549.

Miller, J.D., 1992. Fungi as contaminants in indoor air. Atmos. Environ. 26A, 2163–2172.

Miller, J.D., Laflamme, A.M., Sobol, Y., Lafontaine, P., Greenhalgh, R., 1988. Fungi and Fungal Products in Some Canadian Houses. Int. Biodeterior., 103–120.

National Climatic Data Center. Selected US City and State Extremes: August 2007. National Climatic Data Center, Asheville NC, USA, <http://www.ncdc.noaa.gov/oa/climate/research/2007/aug/augext2007.html> (accessed 22.12.11).

New York City Department of Health and Mental Hygiene, 2002. Guidelines on Assessment and Remediation of Fungi in Indoor Environments. <http://www.nyc.gov/html/doh/html/epi/moldrpt1.shtml#exec> (accessed 22.12.11).

Pasanen, A.L., Lappalainen, S., Pasanen, P., 1996. Volatile organic metabolites associated with some toxic fungi and their mycotoxins. Analyst 121, 1949–1953.

Pope, A.M., Patterson, R., Burge, H., 1993. Indoor Allergens: Assessing and Controlling Adverse Health Effects. National Academy Press, Washington, DC.

Ryan, Timothy J., 2011. Chapter 4.4, Microbial volatile organic compounds. In: Flannigan, B., Samson, R., Miller, J.D. (Eds.), Microorganisms in Home and Indoor Work Environments, second ed. CRC Press, Boca Raton, FL.

Samson, R.A., 1992. Mycotoxins: a mycologist's perspective. J. Med. Vet. Mycol. 30, 9–18.

Schleibinger, H.W., Wurm, D., Moritz, M., Bock, R., Ruden, H., 1997. Sick Building Syndrome and HVAC System: MVOC from air filters. Zentralbl. Hyg. Umweltmed. 200, 137–151.

Schleibinger, H., Laussmann, D., Brattig, C., Mangler, M., Eis, D., Ruden, H., 2005. Emission patterns and emission rates of MVOC and the possibility for predicting hidden mold damage? Indoor Air 15, 98–104.

Snips. January 3, 2002. Detecting mold, MVOCs in problem buildings. BNP media. Troy MI, USA. <http://www.snipsmag.com/CDA/Archives> (accessed 22.12.11).

Sorenson, W., 1989. Health impact of mycotoxins in the home and workplace—an overview. In: O'Rear, C.E., Llewellyn, G.C. (Eds.), Biodeterioration Research. Plenum Press, New York, pp. 201–215.

Please cite this article in press as: Ryan, T.J., Beaucham, C. Dominant microbial volatile organic compounds in 23 US homes. Chemosphere (2012), http://dx.doi.org/10.1016/j.chemosphere.2012.06.066

Strom, G., West, J., Wessen, B., Palmgren, U., 1994. Health implications of fungi in indoor environments: quantitative analysis of microbial volatiles in damp Swedish houses. Air Qual. Monogr. 2, 291–305.

Tiebe, C., Miessner, H., Koch, B., Hubert, T., 2009. Detection of microbial volatile organic compounds (MVOCS) by ion-mobility spectrometry. Anal. Bioanal. Chem. 395 (7), 2313–2323.

Tobin, R.S., Baranowski, E., Gilman, A.P., Kuiper-Goodman, T., Miller, J.D., Giddings, M., 1987. Significance of Fungi in Indoor Air. Can. J. Public Health 78, S1–S3. U.S. Department of Health and Human Services. Healthy People 2010: Understanding and Improving Health, second ed. U.S. Department of Health and Human Services, Washington DC, USA. <http://www.healthypeople.gov/2010/> (accessed 10.04.11).

U.S. Department of Housing and Urban Development, Office of Healthy Homes and Lead Hazard Control: Healthy Homes Technical Studies Program. Federal Register, 69 (94): 27225–27239. Friday, May 14, 2004.

U.S. Environmental Protection Agency, 2011. Mold Remediation in Schools and Commercial Buildings. Appendix B – Introduction to Molds. <http://www.epa.gov/mold/append_b.html#mVOCs> (accessed 10.11.11).

Wessen, B., Schoeps, K.O., 1996. Microbial volatile organic compounds–what substances can be found in sick buildings? Analyst 121, 1203–1205.

Wilkins, K., Larsen, K., 1995. Variation of volatile organic compound patterns of mold species from damp building. Chemosphere 31, 3225–3236.

Zeringue Jr., H.J., Bhatnager, D., Cleveland, T.E., 1993. C15H24 volatile compounds unique to aflotoxigenic strains of *Aspergillus* flavus. Appl. Environ. Microbiol. 59, 2264–2270.

Please cite this article in press as: Ryan, T.J., Beaucham, C. Dominant microbial volatile organic compounds in 23 US homes. Chemosphere (2012), http://dx.doi.org/10.1016/j.chemosphere.2012.06.066