Fungi, Bacteria, Nano-particulates, Mycotoxins and Human Health in Water-Damaged Indoor Environments

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Abstract

Nine types of biocontaminants in damp indoor environments from microbial growth are discussed: (1) indicator molds; (2) Gram negative and positive bacteria; (3) microbial particulates; (4) mycotoxins; (5) volatile organic compounds, both microbial (MVOCs) and non-microbial (VOCs); (6) proteins; (7) galactomannans; (8) 1-3-b-D-glucans (glucans) and (9) lipopolysaccharides LPS (endotoxins). When mold species exceed those outdoors contamination is deduced. However, there are no current recommendations by the EPA, OSHA, NIOSH, WHO and the Medical and Toxicology professions as to what constitutes a safe level of indoor molds and bacteria and their toxins in a water-damaged indoor environment. The thrust of his review is to discuss the role of fungi and their toxins on the health of occupants of damp indoor spaces.

Introduction

Damp or wet building materials occur from a variety construction defects, roof leaks, HVAC condensation, water intrusion from floods, hurricanes, leaking appliances and plumbing, poorly designed foundations, e.g. basement walls that allow water seepage from wet soils, slope of the building lot leading to water accumulation under concrete slabs. We have been involved in homes with cracked cement slabs, bent aluminum window framing, highly contaminated wall cavities, poorly installed roofing, improperly sealed fireplaces, to mention a few. All of these situations lead to both hidden as well visual signs of fungal and bacterial growth. For simplicity, “water intrusion” will be used as an encompassing term [1-18].

Signs of Microbial Growth

Signs of water intrusion include, but are not necessarily limited to: (1) water stains on ceilings, walls and around windows; (2) increased moisture content using a moisture meter with penetrating electrodes on dry wall (e.g. wall cavities), doll space, attics and carpeting; (3) visible fungal growth on surface of dry wall, insulation, e.g. crawl space attic, clothing, shoes and other wearing apparel, bedding, under side of carpeting; (4) musty odor from microbial volatile organic compounds; (5) The E.P.A. cautions that approximately 50% of the fungal growth can be hidden, therefore hidden from view. The identification of airborne mold spores only reveals what is present at the time of testing, not 24/7. Airborne mold testing does not necessarily reveal hidden mold, e.g. wall cavities, attic, under carpeting, ventilation ducts.

Water activity

Water concentration of substrate upon which fungi and bacteria grow dictates the species of fungi that are present. Thus, the water concentration is defined as water activity ($a_w$) as follows: Water activity is the partial vapour pressure of water in a substance divided by the standard state partial vapour pressure of water. The standard state is most often defined as the partial vapour pressure of pure water at the same temperature.

Using this particular definition, pure distilled water has a water activity of exactly one. Higher $a_w$ substances tend to support more microorganisms. Bacteria usually require at least 95+, and fungi at 0.7 $\geq$ 0.95. The fungi that are present at different water concentrations are listed in Table 1.

Xerophilic ($a_w < 0.8$)

Species of Aspergillus and Penicillium, and Eurotium amstelodami and other fungi grow at this water activity. Several of these species produce mycotoxins that include Ochratoxin A, Aflatoxins, Sterigmatocystin, Allatoxins and Gliotoxin. Some species of Aspergillus (fumigatus, niger, terreus and flavus are potential human pathogens, particularly $A$. fumigatus.

Mesophilic ($a_w 0.8-0.9$)

Species of Alternaria, Cladosporium, Phoma and Ulocladium as well as Epicoccum nigrum. This group of fungi also produces mycotoxins, but they are not as toxic as those produced by the xerophilic and hydrophilic fungi.

Hydrophilic ($a_w >0.9$)

The fungi at this water concentration in include Chaetomium globosum, species of Fusarium, Trichoderma, Stachybotrys chartarum, Memnoniella echinata and Rhizopus stolonifer. Trichothecenes are produced by Fusarium, Trichoderma, and Stachybotrys and Memnoniella, while Chaetomium produces chaetoglobosins A and C. All of these mycotoxins are very toxic to humans and animals.
Bacteria
A variety of bacteria have been identified in water damaged indoor environments. They require \( a_w \) of \( \geq 0.95 \). They include several species Gram positive *Bacilli* and *Cocci* (*Streptococcus, Micrococcus*, and *Staphylococcus*) as well as Gram negative bacteria. Several of these bacteria are pathogens, while the Gram negative bacteria release endotoxins into the indoor environment. Other bacteria that have been identified in water-damaged indoor environment are the Actinobacteria: species of *Streptomyces, Mycobacterium* and *Nocardia*. The Actinobacteria produce exotoxins, e.g. Valinomycin, a mitochondrial poison. The nontuberculin Mycobacteria can cause Mycobacterium Avium Complex (MAC) in humans as reviewed by Griffin et al and published on the website of the American Thoracic Society.

Particulates
Particulates shed from molds include spores, fragments of mycelia and nano-particulates. Of the mold particulates the greatest concern is the nano-particulates at or less than 0.3 microns shed from mold colonies. Field studies of water-damaged homes have shown concentrations of nano-particulates in indoor dust that are at least 1000 times or greater than the indoor air mold spore counts [12-18]. These particulates contain 1, 3-beta glucans, a variety of fungal proteins that include substrate enzymes as well as mycotoxins.

The translocation of nano-particulates with their attached toxins occurs by two mechanisms: the nervous system and the surfactants of the alveoli. The nano-particulates enter the surfactants of the lungs and are then transported across the alveolar cell membranes and enter the systemic circulation. In the alveoli they are taken up by alveolar macrophages and alveolar Type I cells. They cause the generation of reactive oxygen species and nitrogen species, release of proinflammatory cytokines and injury to nuclear DNA. Morphological changes include emphysema and granulomatous and fibrotic lesions [19-21]. The other mode of transportation is via the olfactory neurons. Nano-particulates attach to the nasal mucosa and are transported up the olfactory nerve through the cribiform plate and enter the hypothalamus/pituitary axis and spreading throughout the brain [22-25].

Mycotoxins
Mycotoxins produced by various fungi have been identified in damaged building materials, wall cavities, and dust samples from HVAC ducts (return and supply air) and refrigerator compressor (Table 2).

The fungi present in the dust samples were identified by ERMI-36 and are listed in Table 2. *Stachybotrys chartarum* was identified in all dust samples along with other mycotoxin producing fungal species (work in progress).

### Table 1: This table lists the species of fungi and their water activity requirements for growth.

<table>
<thead>
<tr>
<th>Colonizer Group</th>
<th>( a_w ) Range</th>
<th>Classification</th>
<th>Fungal Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Colonizers (storage</td>
<td>&lt; 0.80</td>
<td>Xerophilic/ Xerotolerant</td>
<td><em>Penicillium chrysogenum and Aspergillus versicolor</em>: the most common ones;</td>
</tr>
<tr>
<td>fungi)</td>
<td></td>
<td></td>
<td>A. fumigatus, niger, sydowi, ustus, Eurotium spp., P. brevicaespactum, commune,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>corylophilum, pallans, variola, Paecilomyces Wallemia sebi</td>
</tr>
<tr>
<td>Secondary Colonizers</td>
<td>0.80-0.9</td>
<td>Mesophilic</td>
<td>*Alternaria spp., Cladosporium spp., Epicoccum nigrum, Phoma spp., and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ulocladium spp.*</td>
</tr>
<tr>
<td>Tertiary Colonizers</td>
<td>&gt; 0.9</td>
<td>Hydrophilic</td>
<td><em>Chaetomium globosum, Fusarium, Mononiva echinata, Rhizopus stolonifer, Stachybotrys chartarum, Trichoderma spp. (T. atrovirede, T. citrinoviride, T. harzianum, and T. longibrachiatum)</em></td>
</tr>
</tbody>
</table>

### Table 2: HVAC ducts, Refrigerator compressor and HVAC Air Filter.

This table summarizes the concentrations of mycotoxins in ppb detected in HVAC ducts, dust from the refrigerator compressor and one home with a dirty filter in the intake of the HVAC duct.

Mycotoxins and molds have been identified in urine samples from patients with ME/CFS and in autopsy materials from deceased individuals exposed to fungi in their water-damaged homes [25-34]. Three of the case studies will be briefly reviewed below.
Milk, placenta and umbilical cord.

The mother was pregnant during the trimester.

ppb); third child

year old woman who was exposed to molds in a water-damaged exposure (Table 5).

This species of molds in the highest concentration are listed. Note that although Stachybotrys chartarum was not detected in home I.D. 2, it is noted

The table summarizes the detection of trichothecenes, aflatoxins and ochratoxin A present in bulk samples taken from the master bath, master bedroom (sandal) and crawl space. The reported date are in ppb per mycotoxin, NP: Not Present, Limit of Detection: Trichothecenes (0.2 ppb); Aflatoxins (1.0 ppb); Ochratoxin A (2.0 ppb).

Figure 1 and Table 6 are from reference 33. This case involved a 55 year old woman who was exposed to molds in a water-damaged office building. She developed a mass in her sphenoid sinus that lead to severe headaches and fatigue.

Diagnostic imaging revealed a mass that six different pathologists diagnosed as a malignancy. Sphenoid surgery, radiation, chemotherapy and radiation did not remedy the situation. Slides of the biopsy were sent to Dr. Dumanov (one of the authors). He performed differential staining that revealed fungal growth.

Table 3: This table summarizes the indicator species of molds identified by ERMI-36 in the dust taken from various sources in Table 1. Only the species of molds in the highest concentration are listed. Note that although Stachybotrys chartarum was not detected in home I.D. 2, it is noted that it does not readily shed its spores unless its colonies are disturbed or desiccated.

Table 4: This table summarizes the detection of trichothecenes, aflatoxins and ochratoxin A present in bulk samples taken from the master bath, master bedroom (sandal) and crawl space. The reported date are in ppb per mycotoxin, NP: Not Present, Limit of Detection: Trichothecenes (0.2 ppb); Aflatoxins (1.0 ppb); Ochratoxin A (2.0 ppb).

Table 5: Tables 4 and 5 are from reference 30.

Mycotoxins were present in nasal secretions, urine, mothers breast milk, placenta and umbilical cord. The dog had trichothecenes in its urine and skin lesions. The urine of the newborn was negative for mycotoxins. However, the presence of mycotoxins in the placenta, breast milk and umbilical cord most likely are indicative of fetal exposure (Table 5).

Figure 1 and Table 6 are from reference 33. This case involved a 55 year old woman who was exposed to molds in a water-damaged office building. She developed a mass in her sphenoid sinus that lead to severe headaches and fatigue.

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PCR-DNA test identified *Aspergillus terreus*. She was then seen by Dr. Gray, Benson, Arizona. He treated intranasally with voriconazole and cyclosporine. MRI imaging demonstrated that the mass had resolved. During the antifungal treatment her urine was tested for mycotoxins.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Trichothecenes</th>
<th>Aflatoxins</th>
<th>Ochratoxin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine - Father</td>
<td>NP</td>
<td>NP</td>
<td>18.2</td>
</tr>
<tr>
<td>Nasal Secretion-Father1</td>
<td>NP</td>
<td>0.5</td>
<td>13</td>
</tr>
<tr>
<td>Urine - Mother</td>
<td>NP</td>
<td>NP</td>
<td>18.2</td>
</tr>
<tr>
<td>Nasal Secretion - Mother</td>
<td>1.02</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Urine - Daughter</td>
<td>0.23</td>
<td>NP</td>
<td>28.0</td>
</tr>
<tr>
<td>Nasal Secretion- Daughter2</td>
<td>4.68</td>
<td>NP</td>
<td>3.8</td>
</tr>
<tr>
<td>Urine - Son</td>
<td>0.2</td>
<td>NP</td>
<td>18.9</td>
</tr>
<tr>
<td>Nasal Secretion - Son</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Breast Milk</td>
<td>0.18</td>
<td>0.9</td>
<td>2.7</td>
</tr>
<tr>
<td>Placenta</td>
<td>NP</td>
<td>NP</td>
<td>4.2</td>
</tr>
<tr>
<td>Umbilical Cord</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Urine - New Born</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Urine - Dog</td>
<td>1.49</td>
<td>NP</td>
<td>25.9</td>
</tr>
<tr>
<td>Ear Mass - Dog</td>
<td>23.07</td>
<td>0</td>
<td>2.2</td>
</tr>
<tr>
<td>Liooma - Dog</td>
<td>20.9</td>
<td>0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

**Table 5:** Mycotoxins present in body fluids and tissues of the family and pet dog. The newborn's urine sample was negative with respect to mycotoxins. It is noted that the amniotic fluids were lost with the birth of the baby. Limits of Detection: Trichothecenes (0.2 ppb); Aflatoxins (1.0 ppb) Ochratoxin (2.0 ppb), ND – Not Done, NP – Not Present, 1: *Pseudomonas aureooginosa* and Penicillium were cultured from the nasal secretions. These data represent two different tests, 2: Acinetobacter sp and *Aspergillus fumigatus* were cultured left sphenoid sinus surgical specimens.

Tables 7-9 are from reference 34. This case study is a family of five (parents and three daughters. Prior to renting a home the health history consisted of common colds and annual flu. Upon occupying a water-damaged home all became ill with a variety of symptoms.

<table>
<thead>
<tr>
<th>Date</th>
<th>Aflatoxins (ppb)1</th>
<th>Trichothecenes (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/27/2007</td>
<td>21</td>
<td>1.51</td>
</tr>
<tr>
<td>3/11/2007</td>
<td>5</td>
<td>0.53</td>
</tr>
<tr>
<td>6/3/2007</td>
<td>4</td>
<td>1.38</td>
</tr>
<tr>
<td>7/10/2007</td>
<td>12</td>
<td>3.44</td>
</tr>
<tr>
<td>7/14/2007</td>
<td>9</td>
<td>0.73</td>
</tr>
<tr>
<td>8/21/2007</td>
<td>5</td>
<td>21.3</td>
</tr>
<tr>
<td>12/12/2007</td>
<td>20</td>
<td>2.9</td>
</tr>
</tbody>
</table>

**Table 6:** This table summarizes the mycotoxins detected in the urine on several dates after removal of the fungal aspergilloma. The data show that detoxification probably results from the liberation of stored

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**Figure 1:** This figure shows the *Aspergillus terreus* in the sphenoid sinus of an office worker. She was given an initial diagnosis of cancer of the sphenoid sinus.
mycotoxins, Limit of Detection Aflatoxins (1.0 ppb)’ Trichothecenes (0.2 ppb).

The most concerning to the family was the presence of persistent ME/CFS. ERMI-36 testing of dust samples from the refrigerator compressor/insulation dust identified species of Aspergillus, Penicillium, Trichoderma viride, Chaetomium globosum and Stachybotrys chartarum (Table 7).

Table 7: This table summarizes the results of the ERMI-36 test performed on the dust sample taken from the area of the refrigerator compressor and insulation. The species of each mold is given as the number of spores per milligram of dust.

<table>
<thead>
<tr>
<th>Fungal Species</th>
<th>Number of Spores/mg of dust</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus flavus</td>
<td>8</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>33</td>
</tr>
<tr>
<td>Aspergillus versicolor</td>
<td>78</td>
</tr>
<tr>
<td>Aspergillus ochraceus</td>
<td>151</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>33</td>
</tr>
<tr>
<td>Aspergillus sydowii</td>
<td>10</td>
</tr>
<tr>
<td>Eurotium amstelodami</td>
<td>785</td>
</tr>
<tr>
<td>Penicillium purpurogenum</td>
<td>7</td>
</tr>
<tr>
<td>Aureobasidium pullulans</td>
<td>114</td>
</tr>
<tr>
<td>Penicillium corylophilum</td>
<td>27</td>
</tr>
<tr>
<td>Penicillium crustosum</td>
<td>1,946</td>
</tr>
<tr>
<td>Scopulariopsis chartarum</td>
<td>44</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>12</td>
</tr>
<tr>
<td>Wallemia sebi</td>
<td>25</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>3</td>
</tr>
<tr>
<td>Stachybotrys chartarum</td>
<td>1</td>
</tr>
</tbody>
</table>

Ochratoxin and trichothecenes were identified in the dust sample from the refrigerator and in urine specimens from all family members (Table 8). The diagnosis of ME / CFS was confirmed by sleep monitoring, demonstrating periods of waking, restless and un-refreshing sleep. In addition, a variety of Gram negative and positive bacteria were identified in a dust sample taken from the master bedsheets at 8,400,000 CFU/g (data not shown). Thus, bacteria must also be considered as contributing factors in the ME / CFS.

Table 8: This table summarizes the mycotoxins identified in the dust sample from the refrigerator compressor area and in the urine of the five occupants of the house. The concentrations are in ppb, Urine Ochratoxin References: < 1.8 ppb (negative), 1.8-2.0 (equivocal, 2.0 ppb (positive)). Trichothecene References: ≥ 0.2 ppb (positive).
Sarcoidosis including focal segmental glomerulosclerosis [65,66]. Sinusitis as well as adversely affect biocontaminants. However, the peer-reviewed literature is replete with published papers regarding adverse health effects. A short review of the literature on this subject. Often, the office and/or field nurses are the first individuals to interview and interact with the patient. With respect to the nurse in the field, I highly recommend that awareness of water intrusion and microbial growth that are present in homes and retirement facilities. Therefore, you are encouraged to look for signs of water-damaged and fungal growth. For example, I just had a conversation with a mother, age 32, who has a ten month old infant with chronic rapping cough. Also, her two older children have repeated upper and lower respiratory infections. The mother has ME / CFS as well as chronic sinus-nasal congestion. The rented home has had water intrusion via a faulty roof and plumbing leaks.

Table 9: This table summarizes the results of sleep monitoring events in each family member using FitBit Surge monitor.

<table>
<thead>
<tr>
<th>Event</th>
<th>Father, 36 y</th>
<th>Mother, 34 y</th>
<th>Daughter, 17 y</th>
<th>Daughter, 15 y</th>
<th>Daughter, 7 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep Minutes</td>
<td>477 ± 62.5</td>
<td>508.2 ± 68.1</td>
<td>407 ± 67.7</td>
<td>431.6 ± 45.9</td>
<td>551.4 ± 62.2</td>
</tr>
<tr>
<td>Times Awake</td>
<td>3 ± 1.6</td>
<td>3.4 ± 1.3</td>
<td>2.2 ± 1.3</td>
<td>0.8 ± 1.3</td>
<td>1.5 ± 1.5</td>
</tr>
<tr>
<td>Restless</td>
<td>19.3 ± 3.2</td>
<td>19.6 ± 9.5</td>
<td>13 ± 6.5</td>
<td>9.8 ± 5.3</td>
<td>14.8 ± 6.2</td>
</tr>
<tr>
<td>Un-refreshing Sleep</td>
<td>42.8 ± 6.7</td>
<td>38 ± 20.8</td>
<td>25.2 ± 18.4</td>
<td>32.6 ± 12.2</td>
<td>26.8 ± 12.4</td>
</tr>
</tbody>
</table>

Discussion

A short review of the literature

Three case studies have been briefly reviewed with respect to chronic illness resulting from water-damage and fungal biocontaminants. However, the peer-reviewed literature is replete with published papers regarding adverse health effects on occupants in, water-damaged indoor environments with demonstrable fungal and bacterial growth. These include, but are not necessarily limited to the following.

Upper and lower respiratory infections, bronchitis and lung disease

The health effects include infections, asthma, and hypersensitivity pneumonitis [7-10, 35-40].

Fungal sinusitis

Fungi as well as bacteria in damp indoor environments do cause sinusitis as well as adversely affecting the endocrine functions of the hypothalamus/pituitary axis. In addition, intracranial invasion occurs in immunocompetent patients [41-47].

Sarcoidosis

This is systemic inflammatory illness that can affect one or all organs. It is associated with nano-particulates shed by mold that contain 1, 3-beta glucans, mycotoxins and a variety of antigenic proteins [14,21,25,48-55].

Nervous system

Neurological damage includes the following: decrease in short and long memory in adults and children, autistic spectrum disorder in young children, peripheral neuropathy, loss of balance, facial pain, glossopharyngeal neuralgia, head and neck myalgias, movement disorders, and decreased visual acuity [56-64].

Kidney disease

Kidney disease has been associated with exposure to ochratoxin A produced by several species of Aspergillus. In European Asian counties it is referred to as endemic Balkan nephropathy associated with oral consumption of affected foods. In addition, a recent review of ochratoxin A has associated inhalational exposure to kidney disease, including focal segmental glomerulosclerosis [65,66].

Conclusion

Exposure to water-damaged indoor environments and subsequent fungal and bacterial growth leads to a variety of symptoms that are often overlooked by the medical profession. Most likely this results from the fact that a medical doctor with a busy practice has not kept up with the peer reviewed literature on this subject. Often, the office and/or field nurses are the first individuals to interview and interact with the patient. With respect to the nurse in the field, I highly recommend that awareness of water intrusion and microbial growth that are present in homes and retirement facilities. Therefore, you are encouraged to look for signs of water-damaged and fungal growth. For example, I just had a conversation with a mother, age 32, who has a ten month old infant with chronic rapping cough. Also, her two older children have repeated upper and lower respiratory infections. The mother has ME / CFS as well as chronic sinus-nasal congestion. The rented home has had water intrusion via a faulty roof and plumbing leaks.

References


