(16)

Introduction

During our evaluation of possible microbial reservoirs in the 25 Sigourney Street building, potted plants were seen in many office areas. Plants in indoor environments have been known to contribute to occupant symptoms especially when over-watering or when excessive fertilizer is applied.

A hypothesis was developed that microbial growth in the potted soil may be a contributing cause of occupant symptoms. Twelve samples were taken from selected potted plant soil on the 19th, 18th, 17th, and 15th floors. The samples were submitted for analysis for cultureable fungal and bacterial spore levels.

Potted Plant Soil Results

Cultureable Fungal Spore Results

The cultureable fungal spore levels in the potted plant soil ranged from 18,563 to 3.2 million colony forming units per gram (CFU/g) and the average was approximately 800,000 CFU/g. There are no standards or guidelines for cultureable fungal spore levels in potted plant soil although the levels in our samples were what we expected to find.

Based upon our experience, cultureable fungal spore levels for soft porous building materials are considered high if levels are more than 100,000 CFU/g of material. Fungal spore levels in soft porous materials between 100,000 and 1,000 are considered moderate and levels below 1,000 are considered low.

Several fungal species known to produce mycotoxins were found in the potted plant soil samples. Acremonium, Aspergillus, Phoma, Fusarium, and Penicillium species are known to cause adverse health effects from ingestion, dermal, and inhalation exposure.

Cultureable Bacterial Spore Results

The cultureable bacterial spore levels in the potted plant soil ranged from 1.8 million to 17 million CFU/g and the average was approximately 6.3 million CFU/g. There are no standards or guidelines for cultureable bacterial spore levels in potted plant soil.

Based upon our experience, cultureable bacterial spore levels for soft porous building materials are considered high if levels are more than 1 million. Cultureable bacterial spore levels were high for all twelve of the potted plant soil samples.

The majority of the cultureable bacterial spores were from bacteria that are associated with outdoor environments (i.e. Bacillus, Micrococcus, Flavobacterium, Methylobacterium, and gram negative bacteria). Actinomycetes were present in two (2) of the samples collected on the 17th floor and it was the predominant bacteria in one of

those samples. When present in air at high concentrations, actinomycetes have the potential to cause hypersensitivity pneumonitis.

Discussion

We have been developing hypotheses for possible causes of occupant symptoms for the building at 25 Sigourney Street. Our first hypothesis was that wallboard along the perimeter of the building was responsible for occupant symptoms, since water incursion had occurred and resulted in fungal growth and amplification. Most of the wallboard remediation was performed in the fall of 2000 and occupant symptoms did not subside.

Another hypothesis was developed that the carpeting that was repeatedly exposed to moisture incursion was contributing to occupant symptoms. Results of carpet dust samples identified the carpets as microbial reservoirs and a recommendation was made to remove carpeting repeatedly exposed to moisture incursion. Plans are in place to remove the carpeting on the 17th floor and other floors that had repeated moisture incursion.

We recommend removal of soft porous building materials when we find fungal spore levels in carpet dust above 100,000 or bacterial spore levels above 1,000,000 and occupants are reporting symptoms typically associated with exposure to bioaerosols. We also recommend removing soft porous materials when occupants are reporting symptoms typically associated with bioaerosol exposure and toxigenic species are one of the predominant species found even though the total fungal or bacterial spore levels are lower than the levels reference above.

Conclusions

Exposure to gram negative bacteria and many of the fungal species found in the potted plant soil are known to cause symptoms that have been reported by building occupants. We have identified potted plant soil as a microbial reservoir and possible cause of occupant symptoms.

Controlling exposures to bioaerosols can be accomplished by source removal or by eliminating the pathway from the source to the occupants. We are recommending that identified microbial reservoirs or sources such as the potted plant soil be removed from the building since eliminating the pathway is not feasible.

Recommendations

1. **Provide the results of the soil samples to the committee members and building occupants.**

2. **Remove potted plants from the building.**

Methods

Twelve potted plant soil samples were collected on May 16th, 2001 at 25 Sigourney Street building. A plastic teaspoon was used to collect potted plant soil from selected floors where building occupants have experienced symptoms reported to be building related. The teaspoons were taken from a new unopened package and one (1) to (2) teaspoons of soil was collected and placed in a zip lock plastic bag. The bags were numbered and the cubicle locations were also noted on the Sample Collection Data Sheet.

The samples were packaged in an insulated shipping container and shipped by overnight courier for next day delivery. The samples were received and analyzed by P & K Microbiology Services, Inc. located in Cherry Hill, NJ. The samples were weighed, diluted, and cultured on agar media at room temperature. The fungal samples were cultured on 2% malt extract agar and the bacterial samples were cultured on tryptic soy agar.

Appendix A

Plant Soil Microbial Sample Results

Tunxis Management
25 Sigourney Street
Hartford, CT

May 16, 2001

Sample ID	Location	Fungal / Bacterial ID	Concentration (CFU/g)	Percentage (%)
1	19th Floor-Room 1936	Fungi		
		Acremonium kiliense	4,321	18
		Aspergillus fumigatus	617	3
		Cladosporium	6,173	26
		Dendrostibella	7,407	32
		Fusarium	617	3
		Penicillium	1,235	5
		Phoma	1,852	8
		sterile fungi	1,235	5
			Total: 23,457	
		Bacteria		
		Bacillus	1,493,210	63
		Flavobacterium	126,543	5
		gram negative bacteria and others	202,469	9
		Pseudomonas sp. non aeruginosa	354,321	15
		Stenotrophomonas maltophilia	177,161	8
			Total: 2,353,704	
2	19th Floor-Zone 4	Fungi		
		Cladosporium	1,198	6
		Epicoccum nigrum	599	3
		Gliocladium viride	1,198	6
		Penicillium	2,395	13
		Phoma	8,383	45
		sterile fungi	1,198	6
		Trichoderma koningii	1,796	10
		yeasts	1,796	10
			Total: 18,563	
		Bacteria		
		Bacillus	73,653	2
		Flavobacterium	171,856	5
		gram negative bacteria and others	392,814	11
		Methylobacterium	1,252,096	35
		Pseudomonas sp. non aeruginosa	834,731	23
		Rhodococcus	270,060	7
		Staphyloccocus	147,305	4
		Stenotrophomonas maltophilia	466,467	13
			Total: 3,608,982	

Appendix A Continued

Plant Soil Microbial Sample Results

Tunxis Management
25 Sigourney Street
Hartford, CT

May 16, 2001

Sample ID	Location	Fungal / Bacterial ID	Concentration (CFU/g)	Percentage (%)
3	18th Floor-Zone 7	Fungi		
		Acremonium strictum	15,845	69
		Aspergillus niger	4,577	20
		Aspergillus versicolor	352	2
		Cladosporium	352	2
		Penicillium	1,761	8
			Total: 22,887	
		Bacteria		
		Bacillus	230,986	12
		gram negative bacteria and others	317,606	17
		Pseudomonas sp. non aeruginosa	389,789	21
		Stenotrophomonas maltophilia	952,817	50
			Total: 1,891,197	
4	18th Floor-Zone 5	Fungi		
		Acremonium strictum	27,097	34
		Cladosporium	3,871	5
		Phoma	47,742	61
			Total: 78,710	
		Bacteria		
		Bacillus	1,005,161	55
		Flavobacterium	105,806	6
		gram negative bacteria and others	185,161	10
		Pseudomonas sp. non aeruginosa	264,516	14
		Rhodococcus	26,452	1
		Staphylococcus	238,065	13
			Total: 1,825,161	

Appendix A Continued

Plant Soil Microbial Sample Results

Tunxis Management
25 Sigourney Street
Hartford, CT

May 16, 2001

Sample ID	Location	Fungal / Bacterial ID	Concentration (CFU/g)	Percentage (%)
5	17th Floor-Zone 2	Fungi		
		Cladosporium	75,000	10
		Phoma	575,000	77
		yeasts	100,000	13
			Total: 750,000	
		Bacteria		
		Acinetobacter	775,000	22
		Flavobacterium	650,000	18
		gram negative bacteria and others	625,000	18
		Pseudomonas sp. non aeruginosa	1,300,000	37
		Stenotrophomonas maltophilia	200,000	6
			Total: 3,550,000	
6	17th Floor-Zone 3	Fungi		
		Acremonium strictum	607,407	73
		Fusarium	75,926	9
		Phoma	75,926	9
		yeasts	75,926	9
			Total: 835,185	
		Bacteria		
		gram negative bacteria and others	2,733,333	15
		Pseudomonas sp. Non aeruginosa	3,340,741	18
		Staphylococcus	8,048,148	45
		Stenotrophomonas maltophilia	3,948,148	22
			Total: 18,070,370	

Appendix A Continued

Plant Soil Microbial Sample Results

Tunxis Management
25 Sigourney Street
Hartford, CT

May 16, 2001

Sample ID	Location	Fungal / Bacterial ID	Concentration (CFU/g)	Percentage (%)
7	17th Floor-Zone 4	Fungi		
		Acremonium strictum	5,660	16
		Aspergillus fumigatus	2,830	8
		Aureobasidium pullulans	943	3
		Cladosporium	1,887	5
		Fusarium	1,887	5
		Penicillium	3,774	11
		Phoma	13,208	38
		Trichoderma harzianum	1,887	5
		yeasts	2,830	8
			Total: 34,906	
		Bacteria		
		Actinomycetes	6,343,397	83
		Bacillus	309,434	4
		Flavobacterium	193,396	3
		gram negative bacteria and others	386,792	5
		Methylobacterium	116,038	2
		Pseudomonas sp. non aeruginosa	309,434	4
			Total: 7,658,491	
8	17th Floor-Zone 5	Fungi		
		Acremonium strictum	80,392	3
		Exophiala jeanselmei	361,765	11
		Penicillium	40,196	1
		Rhodotorula glutinis	1,688,235	53
		yeasts	1,045,098	33
			Total: 3,215,686	
		Bacteria		
		Acinetobacter	120,588	5
		Bacillus	321,569	13
		Flavobacterium	40,196	2
		gram negative bacteria and others	844,118	33
		Methylobacterium	884,314	34
		Pseudomonas sp. non aeruginosa	160,784	6
		Stenotrophomonas maltophilia	200,980	8
			Total: 2,572,549	

Appendix A Continued

Plant Soil Microbial Sample Results

Tunxis Management
25 Sigourney Street
Hartford, CT

May 16, 2001

Sample ID	Location	Fungal / Bacterial ID	Concentration (CFU/g)	Percentage (%)
9	17th Floor-Zone 6	Fungi		
		Acremonium strictum	11,864	18
		Aspergillus fumigatus	847	1
		Cladosporium	28,814	45
		Fusarium	847	1
		Penicillium	18,644	29
		Phoma	2,542	4
		Trichoderma koningii	847	1
			Total: 64,407	
		Bacteria		
		Actinomycetes	277,966	2
		Flavobacterium	4,447,458	25
		gram negative bacteria and others	1,250,848	7
		Methylobacterium	555,932	3
		Micrococcus luteus	3,891,526	22
		Pseudomonas sp. non aeruginosa	1,806,780	10
		Rhodococcus	694,915	4
		Stenotrophomonas maltophilia	4,864,407	27
			Total: 17,789,830	
10	17th Floor-Zone 7	Fungi		
		Alternaria alternata	3,604	9
		Cladosporium	5,405	14
		Fusarium	2,703	7
		Penicillium	5,405	14
		Phoma	12,613	32
		Stachybotrys chartarum	2,703	7
		sterile fungi	1,802	5
		Trichoderma koningii	901	2
		yeasts	4,505	11
			Total: 39,640	
		Bacteria	3,250,451	34
		Bacillus	443,243	5
		Flavobacterium	590,991	6
		gram negative bacteria	295,496	3
		Methylobacterium	4,136,937	44
		Pseudomonas sp. non aeruginosa	738,739	8
		Stenotrophomonas maltophilia	Total: 9,455,856	

Appendix A Continued

Plant Soil Microbial Sample Results

Tunxis Management
25 Sigourney Street
Hartford, CT

May 16, 2001

Sample ID	Location	Fungal / Bacterial ID	Concentration (CFU/g)	Percentage (%)
11	15th Floor-Zone 7	Fungi		
		Cladosporium	8,547	31
		Fusarium moniliforme	9,402	34
		Phoma	7,692	28
		yeasts	1,709	6
			Total: 27,350	
		Bacteria		
		Bacillus	770,940	17
		Flavobacterium	1,191,453	26
		gram negative bacteria and others	350,427	8
		Pseudomonas sp. non aeruginosa	2,067,521	46
		Rhodococcus	140,171	3
			Total: 4,520,513	
12	15th Floor-Zone 1	Fungi		
		Cladosporium	12,800	23
		Fusarium	43,200	76
		Trichoderma harzianum	800	1
			Total: 56,800	
		Bacteria		
		Acinetobacter	98,400	3
		Bacillus	492,000	16
		gram negative bacteria and others	2,099,200	70
		Micrococcus luteus	65,600	2
		Stenotrophomonas maltophilia	229,600	8
			Total: 2,984,800	

CFU/g - Colony Forming Units per Gram
MEA- Malt Extract Agar (Used to collect cultural fungi samples)
TSA- Tryptic Soy Agar (Used to collect bacterial samples)

Appendix C

Total Fungal Spore Counts

25 Sigourney Street
Hartford, CT

November 3, 2000

Sample Number	Location	Presumptive Fungal ID	Fungal Structures /m^3
A-1	16th Floor Zone 7	Asp./Pen-like Basidiospores	357 357 Total 714
A-2	17th Floor Zone 3	Basidiospores	911 Total 911
A-3	19th Floor Outside	Ascospores Basidiospores Cladosporium Rusts	340 11,550 3,737 340 Total 15,966
A-4	19th Floor Room 1937	Asp./Pen.-like Basidiospores Cladosporium	685 343 343 Total 1,371
A-5	6th Floor	Basidiospores Cladosporium	344 344 Total 389

ID = identification
m^3 = cubic meters of air

Appendix C

Total Fungal Spore Counts

25 Sigourney Street
Hartford, CT

November 3, 2000

Sample Number	Location	Presumptive Fungal ID	Fungal Structures /m^3
A-1	16th Floor Zone 7	Asp./Pen-like Basidiospores	357 357 Total 714
A-2	17th Floor Zone 3	Basidiospores	911 Total 911
A-3	19th Floor Outside	Ascospores Basidiospores Cladosporium Rusts	340 11,550 3,737 340 Total 15,966
A-4	19th Floor Room 1937	Asp./Pen.-like Basidiospores Cladosporium	685 343 343 Total 1,371
A-5	6th Floor	Basidiospores Cladosporium	344 344 Total 389

ID = identification
m^3 = cubic meters of air