

**Validation of a Sponge Processing Method for Characterizing Microbes in the
Bullitt Center**

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Abstract

Validation of a Sponge Processing Method for Characterizing Microbes in the Bullitt Center

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Background: The Bullitt Center is a six-story, 50,000 square-foot green building that emphasizes energy efficiency and environmental performance based on design and operation. Microorganisms found in indoor surface environments most often come from human sources or by transport from the outdoor environment. Quantity and location of surface microbes can change due to re-aerosolization with human activity and building air movement. Few studies have been performed on surface microbes in green buildings. The aims are 1) Validate a sponge processing method based on literature review and 2) Characterize microbial populations from surfaces in the Bullitt building.

Methods: Three processing methods (shaker table, hand massage, and stomacher 400 circulator) were evaluated for microbial recovery from 3M™ sampling sponges with 10 ml neutralizing buffer. Sponges were spiked with 1 to 3E8 *E. coli* B cells and cells recovered by each method. DNA from recovered cells was extracted (MO BIO PowerSoil™ DNA Isolation Kit), quantified by nanodrop (ND-3300) using picogreen, and *E. coli* specific targets amplified by qPCR (with fluorescent probe). The best processing method was determined by examining both percent recoveries of CFU and C(t) values. The Bullitt surface samples were processed by the most efficient elution method followed by DNA extraction, nanodrop quantification of nucleic acid, and Illumina 16S metagenomic sequencing to characterize the microbes that were present.

Results: Hand massage was determined to be the most effective elution method based on the highest and most consistent calculated percent recoveries of CFUs ranging on average from 32.2-69.3%. C(t) values gave inconsistent results using undiluted nucleic acid, but tighter results using 10-fold dilutions of the sponge extractions ranging from (16.7-35.8% for shaker table, 15.3-42.1% for hand massage, and 25.7-52.7% for stomacher) suggesting the presence of inhibitors. Throughout the study, nucleic acid results ranged from (0-5121.5 ng/ml). The highest nucleic acid values were observed in June ranging from (78.1-5121.5 ng/ml). Results from Illumina 16S (>10% OTUs) showed changes in microbial populations over time at each location. Results from all 16S data showed that the high bloom conditions for *Streptophyta* were observed in April 2014 with a percent abundance as high as 93.9%. The high bloom conditions for *Sandaracinobacter* were observed in August/November 2013 with a percent abundance as high as 40.1%, then decreases significantly for the rest of the timeline. Data on the top ten microbes based on the number of reads in each sample showed high abundance levels of environment associated microbes and distinct levels of human associated microbes in June.

Conclusion: Validation of hand massage as a sponge processing method was the best compared to the shaker table and stomacher methods and can be used for future studies to characterize microbial populations. Nanodrop and Illumina 16S results provided information in the change of quantity of surface microbes at each location over time in the indoor environment. Seasonality, temperature, humidity, and human occupancy all relate to one another in creating the indoor microbial environment. Both environmental and human associated microbes are present in the indoor microbiome suggesting the environment and humans as major sources. Human occupancy contributes to the characterization of the indoor environment by resuspension of dust that results in microbial similarities at different locations within the same building. More surface sampling and analysis in green buildings needs to be investigated to understand the green indoor microbiome.

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Chapter 1. INTRODUCTION

1.1 INDOOR ENVIRONMENT

In creating the built environment, a diversity of microhabitats has been created inadvertently. These microhabitats have been colonized by many different organisms [8]. Figure 1 shows the sources, dispersal vectors, and environmental factors of microbial communities in the built environment. The most common sources are from direct human shedding and from environmental sources [8]. These common microbiome sources transmit indoor microbes between environments by dispersal vectors such as clothes, dust, and ventilation. Factors such as material, temperature, pH, humidity, season, and location may influence microbial diversity. Conventional and green building studies were reviewed to investigate microbial diversity from human and environmental sources and the impacts environmental factors on them.

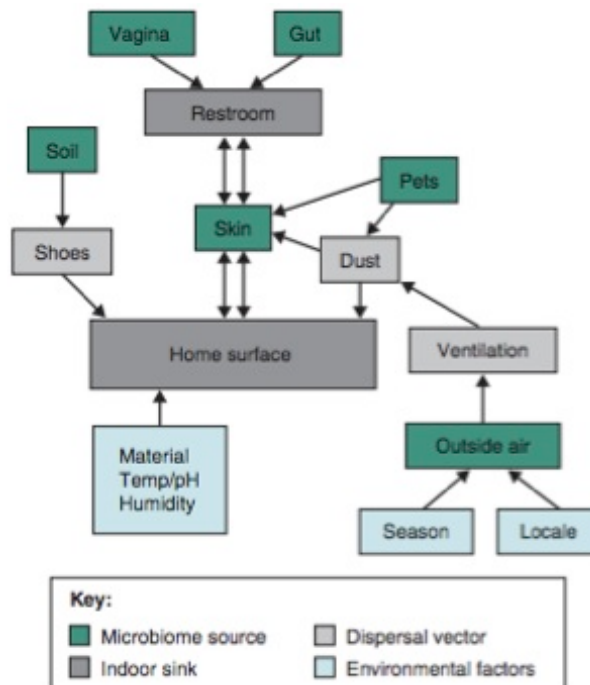


Figure 1: Illustration showing the various sources and transmission routes of hypothetical built environment microbial communities [8].

1.1.1 *Conventional Buildings*

A study was done in a 90 m³ university classroom when occupied and vacant on the first floor of a five-story building to investigate the major sources of microorganisms in the indoor environment [6]. The indoor air, outdoor air, ventilation duct supply air, and floor dust samples were collected on 0.8 um pore-sized, 37 mm diameter polycarbonate track etched filters in samplers for 5-8 hours. The bacterial phylogenetic populations were characterized after using the 454 GS-FLX pyrosequencing platform with multiplex identifiers (MIDs) and 16S rDNA gene sequencing. The total aerosol mass and bacterial genome concentration increased two times more in PM₁₀ compared to PM_{2.5} in indoor air samples. By looking at the twenty major bacterial taxa of each sample type, the comparisons suggest resuspension of the floor dust as an important contributor to bacterial populations in the air that can influence the concentration and character of these microbes. The relative abundances of microbes in the floor dust, indoor air, HVAC filter dust, and ventilation duct supply air samples are shown in Figure 2. Microbes found of environmental origin included *Sphingomonadaceae*, *Rhodobacteraceae*, and *Streptophyta* (chloroplasts from land plants). A high abundance of *Streptophyta* at 45% was found in the HVAC filter dust samples compared to indoor air samples at 2-4%. HVAC filter dust samples also contained only 3% of the following five human associated bacteria: *Propionibacterineae*, *Staphylococcus*, *Streptococcus*, *Enterobacteriaceae*, and *Corynebacterineae*. There were five human associated bacteria comprised 17%, 20%, and 17.5% of the indoor air, floor dust, and ventilation duct supply air samples, respectively. The major microbiome sources are from both humans and the environment. This high abundance of *Streptophyta* in dust samples most likely resulted from being tracked in by humans and outdoor air filtration.

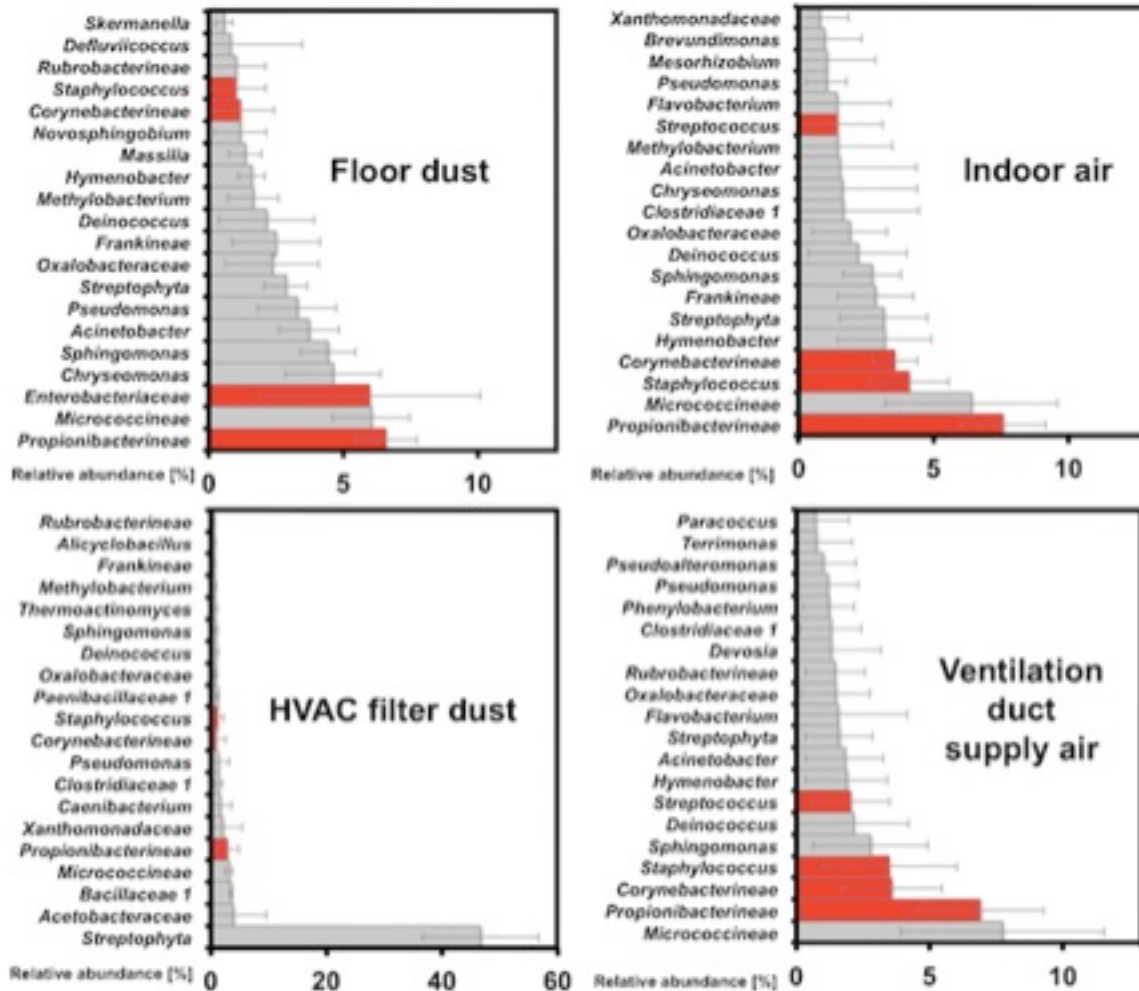


Figure 2: Relative abundance of bacteria in the floor dust, indoor air, HVAC filter dust, and ventilation duct supply air samples. Reprinted from [6].

Lax et al examined microbial interactions between humans and the indoor environment in seven different ethnic families over six weeks by skin and surface sampling [12]. Samples were taken from ten homes (three of the seven families moved), three dogs, and one cat. Skin samples came from hands, feet, and noses. Surface locations were sampled on the kitchen floor, bedroom floor, front door knob, bathroom doorknob, kitchen counter and kitchen light switch. It was found that samples in the same location in different homes differed more than samples in different locations within the same home. The difference suggests residential environment can

influence what microbes are found in the home. Microbes found on surfaces resemble microbes found on human skin such as similarities between hands and doorknobs and feet and floor surfaces. The relative abundance of Firmicutes and Actinobacteria were dominant in human samples while Proteobacteria was dominant in home and pet samples (Figure 3). Firmicutes and Actinobacteria are associated with the human gut and Proteobacteria are associated with mucous that is more likely to come from animals [16]. Microbial colonization grew rapidly of the three families who moved, the microbial communities were similar to their previous homes. The microbial taxa found indoors demonstrates the importance of human and environmental associated bacteria to floor dust that is tracked indoors and then resuspended. Resuspension suggests microbial similarities at different sites within the same home.

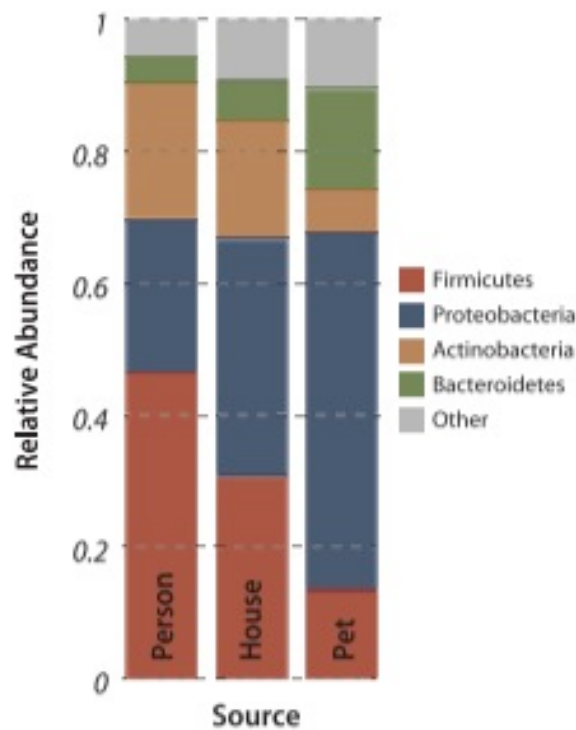


Figure 3: Relative species abundance by source type. From [12]. Reprinted with permission from AAAS.

Characterization of the indoor environment was investigated by taking microbial samples using a HOBO U12-012 sensor and an IR beam people counter placed in ten patient rooms and two nurse stations on two floors of a hospital in Chicago, IL [18]. The data from the microbial samples and IR beam people counter were looked at to see how temperature, humidity, and human occupancy factors affect the growth or survival of microbes in the indoor environment. The air temperature range between patient rooms had only a 7°C difference and less of a difference between the nurse stations. Humidity had a large range that may affect the growth and survival of microbes with variations due to individual rooms depending on the air temperature (as T increases, humidity decreases) and the amount of sunlight, suggesting seasonality can inhibit growth. There were approximately 100 entrances and exits per day in patients' rooms. This number of entrances/exits is comparable to an office-building environment that receives low traffic in comparison to a retail building, which receives high traffic. Human occupancy was the major contributor to the characterization of the indoor environment.

Review of conventional building studies showed that the major microbiome sources are from both humans and the environment. Human occupancy contributes to microbial diversity. The high abundance of *Streptophyta* in the indoor environment resulted from clothes and ventilation dispersal vectors. The human and environmental associated bacteria settle as dust and are constantly resuspended in the air and settle again. The resuspension of microbes in dust causes microbial similarities at different sites within the same home.

1.1.2 *Green Buildings*

What are green buildings? Green buildings are structures that emphasize energy efficiency and environmental performance based on the design and operation. Little has been done on determining what types of microbes are found in a green building. Most studies have focused on bioaerosol concentrations, abundance of culturable microbes, or factors that influence the diversity and composition of the indoor microbiome instead of focusing on indoor microbial concentrations and diversity [9].

A study done in Lillis Hall, a four-story green building as part of the University of Oregon's Biology and the Built Environment Center explored the indoor microbiome by analyzing bacterial communities from surface samples [10]. Surface samples were collected using filters by vacuuming each area for 2 minutes with a shop-Vac 9.4L Hang Up vacuum. After DNA extraction, the bacterial communities of the 155 samples were profiled by Illumina 16S rRNA sequencing and the majority were identified from Proteobacteria, Firmicutes, and Deinococci groups. Samples with *Lactococcus*, *Pseudomonas*, and *Streptococcus* were associated with the centrally located, high occupancy areas and samples with *Methylobacterium*, *Sphingomonas*, and *Streptococcus* were associated with window ventilation. Offices with mechanical ventilation contained different microbes in comparison to offices with a window ventilation source suggesting building design can influence the built environment microbiome. The relative abundance of *Deinococcus*, a gram positive bacteria that is highly resistant to radiation was 1.7 times greater in mechanically ventilated offices whereas the relative abundance of *Methylobacterium* was 1.8 times greater in window ventilated offices (Figure 4).

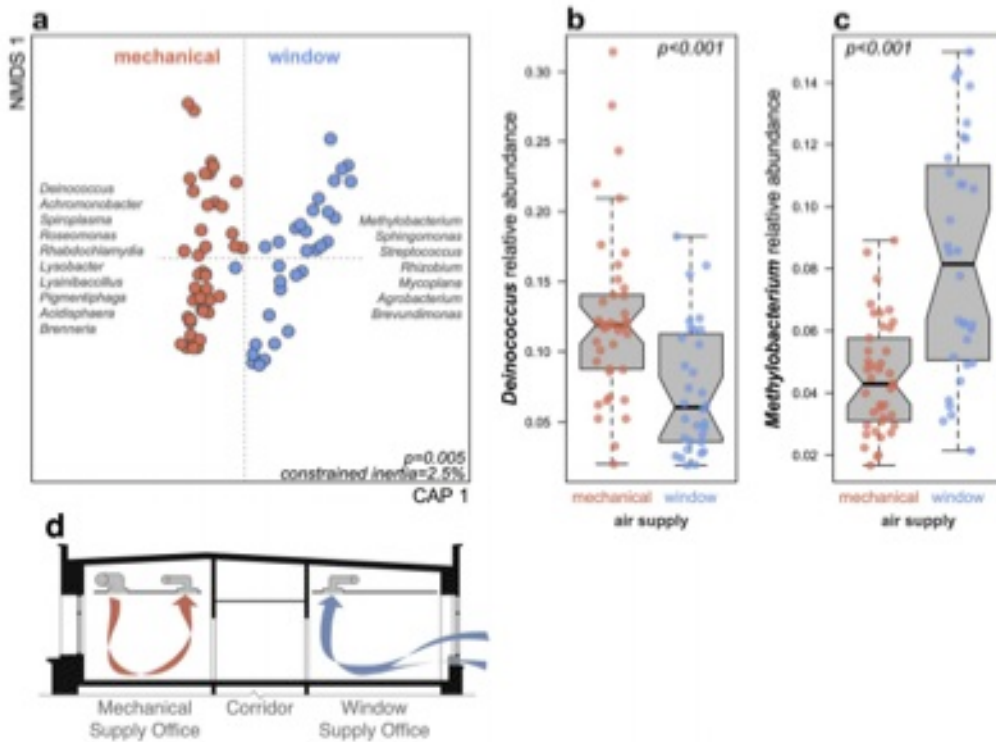


Figure 4: Offices contain significantly different dust microbial communities depending on ventilation source. Reprinted from [10].

Fungal communities were investigated and compared in green roofs and city parks in New York City [14]. This study looked at two native plants in three green roof habitats and soil habitats in five city parks. Twelve planting boxes were placed on each green roof where six boxes were installed with 10 cm of green roof media and the other six boxes were installed with 15 cm of media. Samples were collected only from the boxes with 15 cm of media where the boxes were divided to accommodate two native plant communities, Hempstead Plains and Rocky Summit Grasslands. These plant communities were chosen because they are able to withstand harsh environmental conditions. Samples 10 cm deep were collected with 2.5 cm diameter soil corers and composited into Whirl-Pak bags. Soil samples were collected from parks adjacent to the three green roofs in addition to High Line Park and Central Park. Samples from 0-10 cm collected were composited into one sample. The DNA was extracted using the MoBio PowerSoil

extraction kit, amplified by PCR, quantified with the PicoGreen dsDNA assay, and sequenced using Illumina. Green roof fungal communities were distinct with 54% OTU similarity to city park fungal communities where the most abundant fungal orders from green roofs were *Sordariales*, *Pleosporales*, *Microascales*, and *Glomerales* (Figure 5).

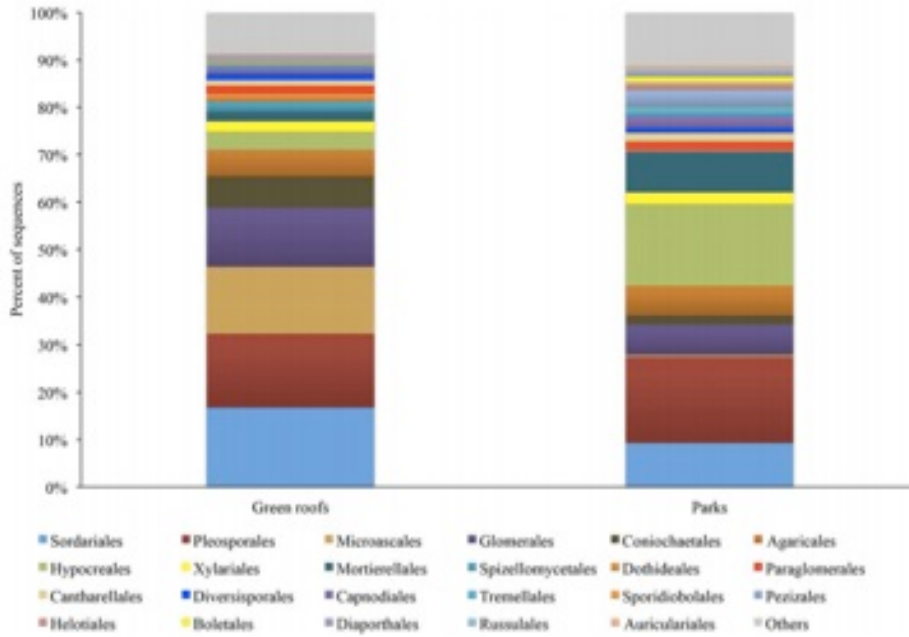


Figure 5: The relative abundance of the most dominant fungal orders detected from green roof substrates and city park soils. Reprinted from [14].

By looking at the sources of how microhabitats are created in the indoor environment, there is a clear relationship between human and environmental sources with microbes. Human occupancy which causes resuspension of floor dust and tracked in from outside is a major contributor to the characterization of the indoor environment. Microbes from different sites within the same home are more similar than microbes found in same sites in different homes. The microbial similarities within the same home, due to the area’s surrounding environment also characterizes the indoor environment. Temperature and humidity are factors that can potentially affect microbial survival in the indoor environment. Building design can influence the built

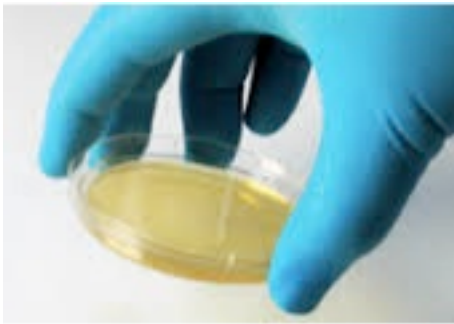
environment microbiome depending on ventilation source. Although microbes in green buildings have not been a major focus in research, characterizing the microbiome in green buildings will help look at similarities and differences of microbial diversity in conventional buildings. The studies found related to green buildings use similar DNA applications by DNA extraction, qPCR, nanodrop, and Illumina sequencing to characterize green building microbes [10] [14].

1.2 SURFACE SAMPLING DEVICES

Many types of surface sampling devices have been used in research. Choosing the right device is key to the particular area being sampled. The following is a list comparing the most commonly used surface sampling devices and Figure 6 shows what each device looks like.

- Contact plates come with various types of agar and are easy to use where the surface of the media is pressed against the surface being sampled [7]. They are low cost at approximately \$15 per sleeve of 10 depending on agar type and plate size, costing about \$1.50 per sample. However, some disadvantages are that contact plates are not suitable for irregular surfaces or surface corners.
- Swabs are suitable for irregular surfaces where contact plates are not useful [7]. They are low cost at approximately \$90 per case of 100, costing about \$0.90 per sample. Disadvantages are that the swabs have small surface areas compared to a larger wipe or sponge.
- Foam spatulas are suitable for irregular surfaces where contact plates are not useful [11]. They are low cost at approximately \$10 per bag of 50, costing about \$0.20 per sample. Disadvantages are that the spatulas have small surface areas compared to a larger wipe or sponge.

- Pre-moistened wipes are convenient to pick up dust off of surfaces easily and have large surface areas compared to swabs [17]. They are low cost at approximately \$10 per bag of 100, costing about \$0.10 per sample.
- Sponge-sticks come in wet or dry formats, wet being more convenient to pick up dust off of surfaces easily [17]. The sponges of the sponge-sticks also have large surface areas compared to a swab, measuring 1.5 x 3 inches. The stick can be broken off and allows sampling without directly handling the sponge, which makes it easier to reach into tight spaces and around objects. They are low cost at approximately \$150 per case of 100, costing about \$1.50 per sample.



A



B



C



D



E

Figure 6: Surface Sampling Device:s (A) contact plate, (B) sponge-stick, (C) spatula, (D) wipe, (E) swab). Reprinted from <http://dinovomed.com/>, <http://solutions.3m.com/>, www.bluethundertechnologies.com, <http://www.drug-aware.com/>, www.amazon.com.

1.2.1 *Conventional Building Studies on Surface Sampling Devices with Microbial Recovery*

Previous research can also help with the decision of which device is most effective in recovery of surface microbes. Although there are few studies on surface sampling of microbes in green buildings using the devices mentioned in the previous section, percent recoveries of microbes from conventional buildings found in the literature would hopefully help determine which sampling device and subsequent processing method would be best used to recover microbes. Table 1 shows microbial recoveries using various sampling devices, processing methods and processing times from the literature.

One study placed ten-milliliter samples of *B.atrophaeus* in SDW or *P. agglomerans* in 0.01 M PBS in a bioaerosol deposition system placed above glass and steel test surfaces [13]. The aerosolized *B.atrophaeus* was allowed to settle for 16 hours in the test chamber and *P. agglomerans* was allowed to settle for 1 hour only on glass surfaces. Tips of the foam spatulas cut to a width of 2.5 cm, macrofoam (dry) sponge wipes cut to 3 x 5 cm, and polyester swabs were used to swab surfaces. The macrofoam sponge wipes and spatula tips were placed in stomacher bags in 10 milliliters of SDW (sterile distilled water) for extraction of *B.atrophaeus* or PBS (phosphate buffered saline) for extraction of *P. agglomerans* and hand massaged for 1 minute. Polyester swabs were placed in 5 milliliters of SDW and sonicated in an ultrasonic bath for 12 minutes or vortexed for 1 min. Deposition of spores from the a glass surface as a function of aerosol exposure using a spatula resulted in an average recovery of 9.9% of *B.atrophaeus* and 0.001% of *P. agglomerans*. Deposition within the test chamber resulted in an average recovery of 7.9% using sponge wipes and swabs.

Another study looked at the evaluation of two surface sampling methods for detection of *Erwinia herbicola* [2]. Seven types of surfaces (metal file cabinet, glass computer monitor, nylon

cushion, vinyl tile, wood laminate, plastic seat, and finished concrete) were swabbed with a sponge and a macrofoam swab premoistened in 0.01 M PBS and TBS with 0.1% Tween 20 respectively. The sponge was placed in a stomacher bag in 30 milliliters of 0.01 M PBS and hand massaged for 1 minute. The swab was placed in 9 milliliters of TBS with 0.1% Tween 20 in a 50 milliliter tube and vortexed for 1 minute. After DNA extraction and qPCR amplification, average recovery resulted in 0.7-47.3% (\pm 0.2-4.2%) from sponges and 0.8-52.2% (\pm 0.1-8.3%) from swabs, range depending on surface material.

Recoveries of bacteria from wipes using different extraction solutions and processing methods were looked at in another study [3]. *E. coli*, *B. cereus*, and *B. thailandensis* were cultured and approximately 10^9 CFU were inoculated onto polyester-rayon blend wipes premoistened with 1 milliliter of neutralizing buffer. Cells were left for 1 hour to promote adherence to wipes then wipes were placed into 50 milliliter conical tubes containing 30 milliliters of various extraction solutions. All organisms were vortexed, sonicated, or both at various times. Average recovery resulted in 40.5-60.6% for *E. coli*, 65.9-81.3% for *B. cereus*, and 81.6-98.3% for *B. thailandensis*, depending on extraction solution.

Bacillus anthracis spores were sampled from surfaces in a validation study of various sponge processing methods [19]. Three milliliters of the *bacillus anthracis* culture was placed on steel coupons. The cells were left on the steel coupons for 2 hours to dry then sampled with various premoistened sponges. The sponge heads were placed in stomacher bags and the handles were removed aseptically. Ninety milliliters of PBS with 0.02% Tween 80 were placed in each bag with the sponge head and processed in a Stomacher 400 Circulator at 260 rpm for 1 minute. Sponge heads were squeezed to obtain excess liquid and sponge heads were removed. The liquid was poured into 50 milliliter conical tubes and centrifuged at 3,500 x g for 15 minutes. Average

recovery resulted in 26.0-36.3%(\pm 5.6-15.5%) for *Bacillus anthracis*; the highest percent recovery was from sponge-sticks in PBST.

Table 1: Microbial Recoveries form Conventional Buildings

Sampling Device	Processing Method	Processing Time	Percent Recovery	Microbe
Foam spatula, Macrofoam sponge wipe, Polyester swab [13]	Hand Massage, Sonicate or vortex, Sonicate or vortex	1 min 1 min 1 min	Spatula: 9.9, Swabs & wipes: 7.9	<i>B. atrophaeus</i>
Foam Spatula [13]	Hand Massage	1 min	0.001	<i>P. agglomerans</i>
Sponge, Macrofoam swab [2]	Hand Massage, Vortex	1 min 1 min	0.7-47.3 0.8 – 52.2	<i>E. herbicola</i>
Polyester-rayon blend Wipe [3]	Vortex, Sonicate, Both	(10 sec, 2 min, 10 min), (1 min, 2 min, 5 min), (1 min+1 min, 2 min+2min)	40.5-60.6	<i>E. coli</i>
Polyester-rayon blend Wipe [3]	Vortex, Sonicate, Both	(10 sec, 2 min, 10 min), (1 min, 2 min, 5 min), (1 min+1 min, 2 min+2min)	65.9-81.3	<i>B. cereus</i>
Polyester-rayon blend Wipe [3]	Vortex, Sonicate, Both	(10 sec, 2 min, 10 min), (1 min, 2 min, 5 min), (1 min+1 min, 2 min+2min)	81.6-98.3	<i>B. thailandensis</i>
Sponge-stick [19]	Stomacher 400	1 min	26.0-36.3	<i>B. anthracis</i>

Based on the percent recoveries determined for the organisms in the literature reviews mentioned above, it was difficult to determine which sampling device was the best for microbial recovery. Each sampling device is coupled with various processing methods and times (hand massage [2] [13], sonicate [3] [13] or vortex [2] [3] [13], and stomacher 400 [19]) and made evaluation difficult.

1.3 STUDY OBJECTIVE

Determine types of microbes found in a green building by characterizing the indoor microbiome and compare results to microbes found in conventional and green building studies.

For my thesis, I chose a surface sampling device that can directly collect dust off of surfaces. Based on the advantages and disadvantages of the different surface sampling collection types, sponge-sticks (3M sponges with 10 ml neutralizing buffer in sealed sponge bags) were chosen as the surface sampling collection type for this thesis project (Figure 7). This is due in large to the sponge having a large surface area and the stick allows sampling without directly handling the sponge. Since there have been few studies on the determination of microbial types in green buildings, sample processing methods were chosen from the literature review in conventional buildings to test which processing method is the best to use.



Figure 7: 3M Sponge-stick in sealed sponge bag

1.4 AIMS

1. Validate a sponge processing method based on literature reviews.
2. Characterize microbial populations from surfaces in the Bullitt building.

Aim #1 was achieved by testing three of the different processing methods from Table 1 using *E. coli B* and calculate percent recoveries by CFUs and C(t) values. Aim #2 was achieved by using the best processing method based on the calculated percent recoveries from Aim #1. That method was used on the Bullitt sponges to characterize microbial populations from surfaces in the Bullitt building.

Chapter 2. METHODS: LAB SAMPLING

Various processing methods [2] [3] [13] [19], for getting the microbes off the sponge-sticks were used as a test to determine which gives the best percent recoveries. To accommodate the size of the sponges, the shaker table was substituted in place of using a sonicator or vortexer because the sponges couldn't fit on them. The methods were tested on sponges spiked with a known microbe, *E. coli B*, the DNA was extracted, amplified by qPCR, and quantified by nanodrop (Figure 8).

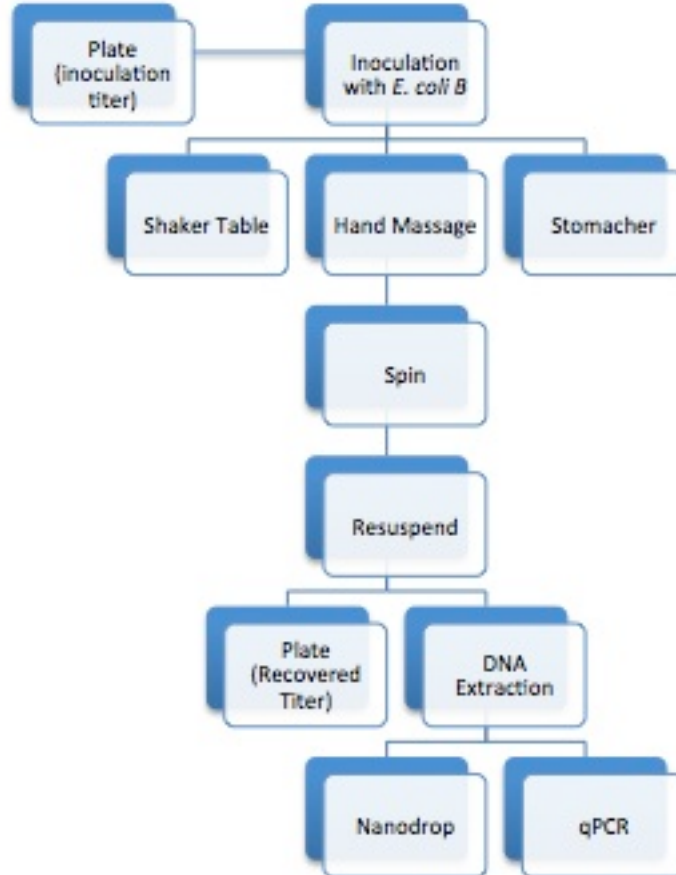


Figure 8: Lab Sampling: Methods Flow Chart

2.1 OVERNIGHT CULTURE

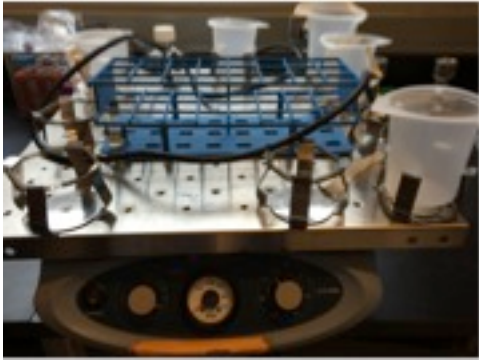
E. coli B culture was grown overnight from a freezer stock at 37°C.

2.2 SERIAL DILUTIONS AND PLATING (INOCULATION TITER)

Ten fold dilutions of the overnight were made in PBS from -1 to -7 and PBS was used as the negative control. Duplicates of 100ul dilutions of -5 to -7 and the negative control were plated onto nutrient agar plates, and then placed in the 37°C incubator overnight to determine the inoculation titer.

2.3 PROCESSING METHODS

Sponges were taken out of bags and spiked using a pipette to add droplets of overnight culture onto the sponge surface with 1 to 3E8 *E. coli B* cells in duplicates of each of the three methods. The sponge heads were placed back into the sponge bags, the sticks removed aseptically, bags sealed, labeled, and processed by one of the three methods (Figure 9). The same elution buffer was used for each method. PBS is a wash buffer and the tween is a detergent that is added to promote more effective washings. The percentage of tween in the media that best recovers cells was chosen based on an ongoing study of filter samples from the Bullitt building where different tween percentages in the media were tested.



A



B



C

Figure 9: (A) Shaker Table, (B) Hand Massage, and (C) Stomacher 400 Circulator

1. Shaker Table, (T)

Thirty milliliters of PBS with 0.01% tween was added to submerge each sponge in the sponge bag. The sponges were placed onto a shaker table and shaken at 300 rpm for 15 minutes.

2. Hand Massage, (H)

Thirty milliliters of PBS with 0.01% tween was added to submerge each sponge in the sponge bag. The sponges were hand massaged with both hands for 15 minutes.

3. Stomacher 400 Circulator, (S)

Ninety milliliters of PBS with 0.01% tween was added to submerge each sponge in the sponge bag. The sponges were processed at 260 rpm for 1 minute. The Stomacher 400 Circulator is a paddle blender that provides a homogenized sample for analysis. The curved shape of the

paddles creates a washing effect driving more organisms in suspension. The Stomacher requires volumes between 80-400 millileters, so 90 millileters of PBS with 0.01% tween was added with the sponge in the sponge bag (the least volume amount in a multiple of 30).

After bubbles were allowed to settle, the sponge heads were squeezed to obtain excess liquid, and the sponge heads were removed from the bags and discarded. The liquid from each bag was poured into a 50 ml conical tube (2 x 50 ml conical tubes for stomacher samples), tubes balanced, and centrifuged at 5,000 x g for 10 minutes. The supernatant was discarded and the pellet resuspended in 300ul PBS (2 x 150ul PBS for stomacher samples). When discarding the supernatant there were a few drops left in the tube, so for all samples to be equal for later percent recovery calculations, the volume was brought up to 400 ml for each sponge sample.

2.4 SERIAL DILUTIONS AND PLATING (RECOVERED TITER)

Ten-fold dilutions of the samples from (-1 to -6) were made; duplicates of 100ul of each dilution (-3 to -6) were plated onto nutrient agar plates, and placed in an incubator at 37°C overnight to determine the recovered titer.

2.5 DNA EXTRACTION

DNA was extracted using the MO BIO PowerSoil DNA Isolation Kit protocol [Appendix A], which was also used in a study on indoor bacterial communities [10]. This kit is known for isolating genomic DNA from all environmental samples including those that contain high humic acid content such as compost, sediment, and manure soil types. According to MO BIO, the isolated DNA has a higher level of purity due to the humic substance and PCR inhibitors removal. For this protocol, typically 0.25 grams of a soil sample is added to the PowerBead tube.

Since the samples obtained after the processing methods are in liquid form, 100ul of the sample is added to the PowerBead tube and approximately 650ul of the supernatant is transferred to a clean 2 ml collection tube in step 7. After the samples go through cell lysis, inhibitor removal, the binding and washing of DNA, 100ul is eluted (Figure 10).

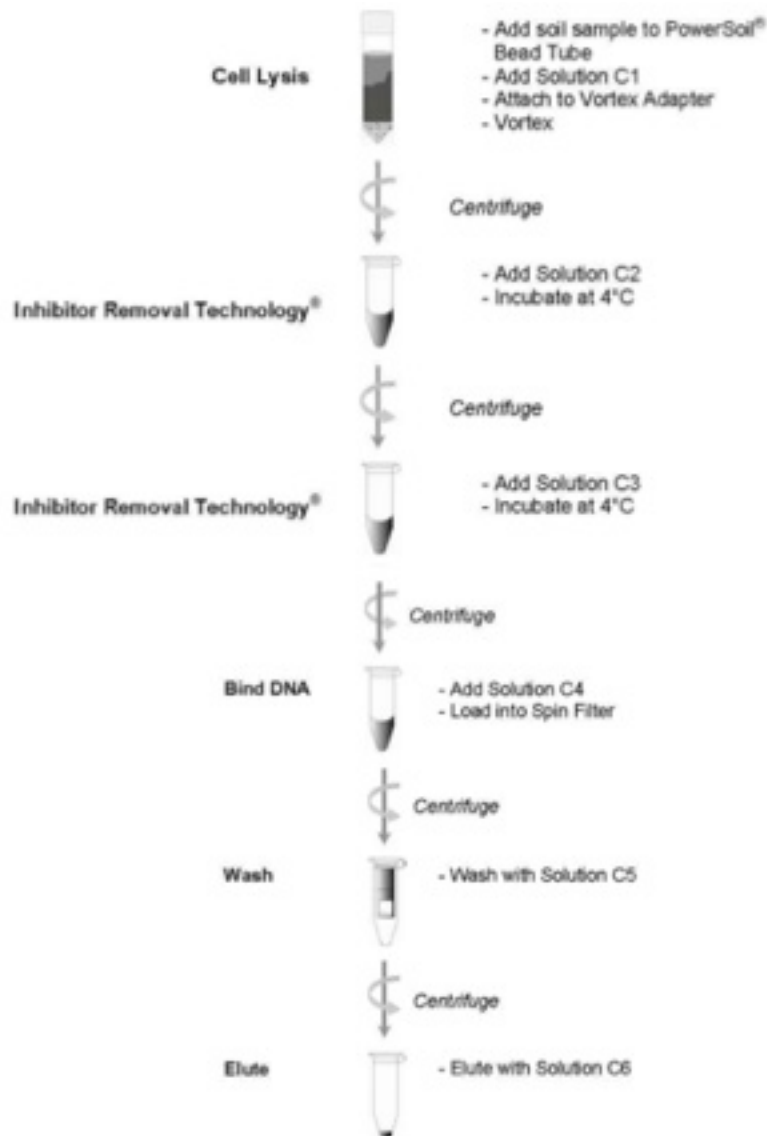


Figure 10: Flow chart summarizing PowerSoil DNA Isolation protocol. Adapted from [15].

2.6 QUANTITATIVE PCR

E. coli specific targets were amplified by qPCR with fluorescent probe in duplicates for each sample. The master mix includes volumes of the iQ supermix (12.5ul/rxn), forward primer (2.25ul/rxn), reverse primer (0.75ul/rxn), probe (0.50ul/rxn), water, and DNA (5ul/rxn) to make 25ul per reaction. In the final stages of running the last of the samples on qPCR, 2ul/rxn of DNA was used. The iQ supermix contains iTaq DNA polymerase, deoxyribose nucleoside triphosphate (dNTPs), and buffer optimal for qPCR. The primers, probe, and cycling sequence protocol were designed and tested to amplify *E. coli* specific targets [5]. The probe for *E. coli B* is 200nM and the primers for *E. coli B* are the forward primer (900nM) and reverse primer (300nM).

Typically samples go through 40-45 cycles. The samples went through 40 cycles instead of 45 cycles of the classic thermal profile as shown in Figure 11. Step 1 is the initialization step at 50°C for 2 minutes to reduce non-specific amplification. Step 2 is the initial denaturation step at 95°C for 10 minutes where the dsDNA is melted into single strands by disruption of the hydrogen bonds between complementary bases. Step 3 is the denaturation step at 95°C for 15 seconds. Step 4 is the anneal and extension step at 60°C for 1 minute where the probe and primers anneal to the ssDNA template and the DNA polymerase attaches to the template and incorporates complimentary nucleotides. The DNA polymerase allows the extension of the primers with the complimentary nucleotides to the DNA template to yield a dsDNA.

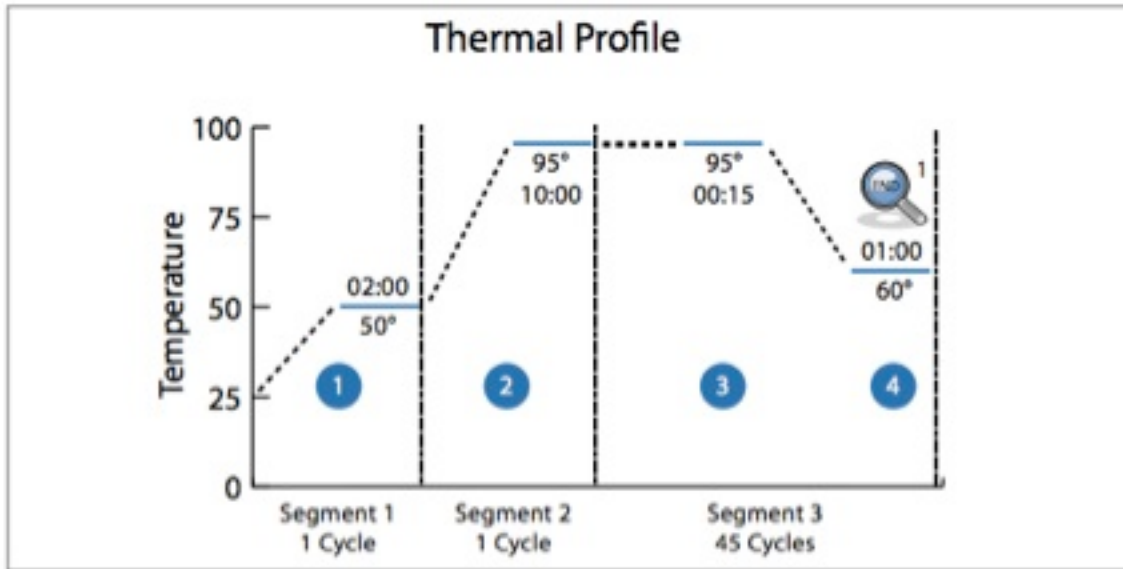


Figure 11: Classic thermal profile for qPCR. Reprinted from [4].

The chemistry behind TaqMan is the probe uses a fluorophore or reporter dye during PCR to detect specific PCR products as it is accumulated as shown in Figure 12. The probe contains the reporter dye on the 5' end and a quencher on the 3' end. The close proximity of the quencher reduces the reporter dye's fluorescence. The polymerization/strand displacement step involves the probe annealing downstream from one of the primer sites when the target sequence is present. The probe is then cleaved by the 5' nuclease activity of the Taq DNA polymerase as the primer is extended to the end of the template strand. Increase in fluorescence intensity is proportional to the amount of amplicon produced as each additional reporter dye molecule is cleaved from its respective probe.

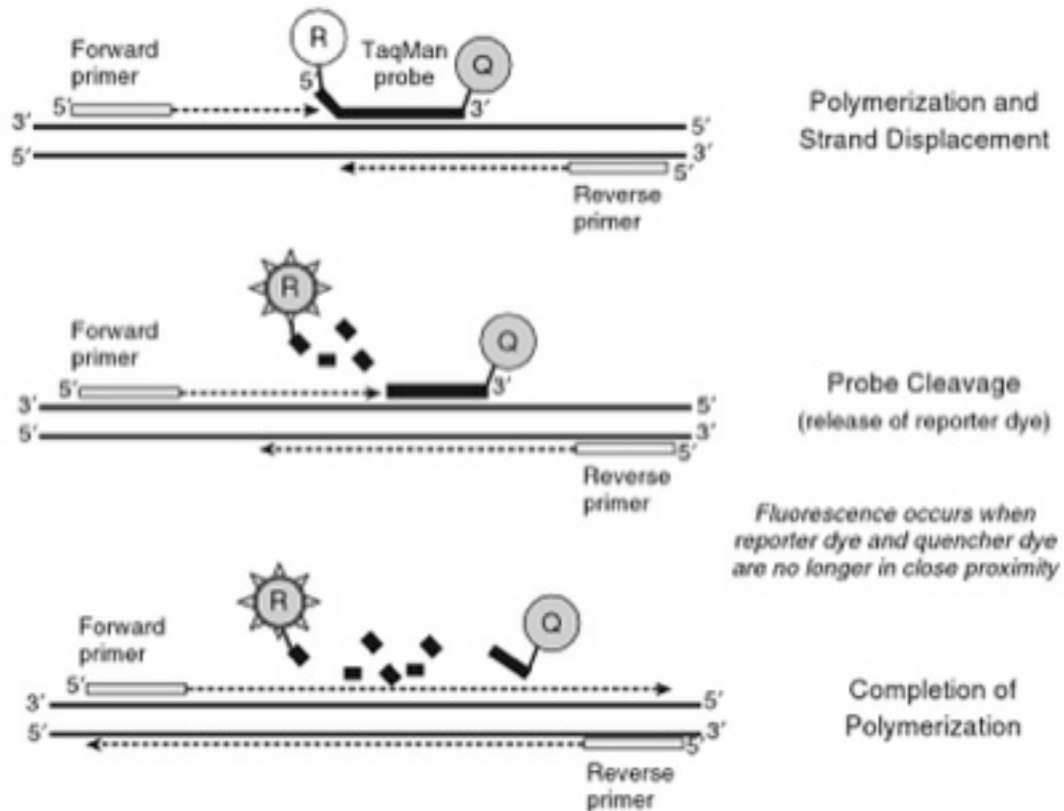


Figure 12: Schematic of TaqMan assay. Reprinted from [1].

2.7 NANODROP: PICOGREEN ASSAY FOR dsDNA

The PicoGreen Assay for dsDNA protocol [Appendix B] on the nanodrop (ND-3300) was used to detect and measure microbial DNA. PicoGreen dye is a fluorescent nucleic acid stain for quantitating dsDNA. The 1X TE buffer is made up of Tris and EDTA, which solubilizes the DNA and prevents degradation. The detection range using the ND-3300 is 1 ng/ml – 1000 ng/ml. The samples were combined with PicoGreen and compared to a standard curve to quantify DNA.

Chapter 3. RESULTS: LAB SAMPLING

The best processing method was determined by examining both percent recoveries of CFU and C(t) values. The CFU percent recoveries indicated the amount of viable *E.coli* recovered. Recovered CFUs after undergoing each of the processing methods, CFUs were compared to the CFUs in the initial inoculation onto the sponge. The C(t) percent recoveries indicated the total microbial DNA recovered. The CFUs calculated to be in 5 ul of DNA used for qPCR was translated to log CFU, inserted into the standard curve equation to obtain the C(t) and compared to the C(t) of each method. Percent recoveries by C(t)s are expected to be higher than percent recoveries by CFUs because C(t) recovery indicates the total cells recovered whereas CFU recovery indicates the viable cells recovered.

3.1 CALCULATIONS BY CFUS (COLONY FORMING UNITS)

The titer of each dilution (-5, -6) from the undiluted *E. coli B* sample and weighted titer was calculated by Equations 1 and 2 from the colony plate counts. The percent recovery for each processing method was calculated by Equation 3.

Equation 1: Titer (CFU/ml)= average CFU count * dilution * dilution factor

Equation 2: Weighted titer (CFU/ml)= [(Sum of all CFUs)/0.22]*lowest dilution

where 0.22 is the volume plated

Equation 3: Percent Recovery (%) = (CFUs recovered/CFUs applied)*100%

The amount of cells recovered was calculated by taking the weighted titer of each method dilution sample (-5, -6) and multiply that by the final volume of resuspended *E. coli B* in PBS

(400ul). The amount of cells applied was calculated by taking the weighted titer of the overnight (-6, -7) and divide that by a factor of 10 to obtain the amount of cells in 100 ul used to spike a sponge.

Table 2: Percent Recoveries by CFUs

Sample ID	12/27/13	1/17/14	1/30/14	2/7/14	5/9/14	Average Recovery (Standard Deviation)
T	19.1% (0.19)	6.4% (2.7)	40.1% (3.5)	19.4% (0.66)	60.2% (1.1)	29.0% (20.1)
H	47.0% (6.9)	32.2% (0.49)	69.3% (8.9)	56.8% (1.0)	64.1% (2.1)	53.8% (14.4)
S	25.3% (5.6)	30.7% (0.40)	45.3% (7.2)	29.2% (2.4)	57.7% (2.9)	37.6% (13.2)

T = Shaker Table, H = Hand Massage, S = Stomacher

*2 trials were completed for each method and a replicate of each were plated

Table 2 shows the calculated percent recoveries of *E. coli B* by CFUs for each processing method with respective date and the comparison of the average recoveries of each processing method. The standard deviations were calculated from individual trials of each method. The raw data of CFUs are shown in Table 9 [Appendix C]. Hand massage was determined to be the most effective method based on the highest viable recoveries of CFUs with an average of 53.8% across all data and standard deviation of 14.7. A Kruskal-Wallis rank sum test was used to perform a comparison between these groups.

3.1.1 *Kruskal-Wallis Test*

The Kruskal-Wallis test is a rank-based nonparametric test used to determine statistically significant difference between 2 or more groups. In statistics, a significance level can be set at

variable levels, but often at $\alpha = 0.05$. If the $p\text{-value} < \alpha$, the test suggests that the difference between the groups is statistically significant. Here we chose 0.1.



Figure 13: Kruskal-Wallis Test of average CFU percent recoveries for each date by recovery method

The boxplots in Figure 13 show the average CFU percent recoveries for all dates by recovery method. The results show that the $p\text{-value}$ is 0.09348, which is greater than a 0.05 significance level, making the difference between recovery methods not statistically different. However, if the significance level is set at 10%, $\alpha = 0.10$, making the difference between recovery methods statistically different. The lower the significance level, the smaller the chance of the pattern in the data is not true.

3.2 RECOVERY LOSS

The hand massage method was determined as the best processing method based on CFU percent recoveries, the percent recoveries of the hand massage method ranged from approximately 32.2-69.3%, which was similar to the range of percent recoveries from the conventional building literature review in Table 1. We still wanted to investigate the possible causes of recovery loss due to the wide range of recoveries. A test by adding two types of positive controls in addition to the hand massage method samples were investigated. The same

lab sampling method for hand massage was used following the flow chart in Figure 8 with a few changes. For the hand massage processing method, 100 ul of the -3 and -4 dilutions of the overnight culture of *E. coli B* were spiked onto sponges, hand massaged, and directly plated. The positive controls were represented by adding 100 ul of the -3 and -4 dilutions of *E. coli B* directly into 1.5 ml tubes and the volume brought up to 400 ul. A second set of positive controls was made to see if recovery loss occurred from loss in the supernatant due to centrifugation. The 100 ul of the -3 and -4 dilutions of *E. coli B* were added directly into 30 ml PBS with 0.01% tween in 50 ml conical tubes, centrifuged at 5,000 rpm for 10 minutes, pellet resuspended in PBS, volume brought up to 400 ul, and samples plated onto nutrient agar plates.

3.2.1 *Calculations by CFUs for Recovery Loss*

The plate count data showed that the ten-fold dilution of each -3 dilution sample was not countable. Calculations by plate counts were calculated again using Equations 1 through 3 with a modification to Equation 2. Similar to Equation 2, Equation 4 is used to calculate the weighted titers of the hand massage, positive control, and positive control with centrifugation samples. Since only the 100-fold dilutions of each -3 dilution sample were countable by plates and not the ten-fold dilutions, the sum of all plate counts was divided by 0.2 instead of 0.22 as in Equation 2 to represent the volume plated.

Equation 4: Weighted titer (CFU/ml)= [(Sum of all plate counts)/0.2]*lowest dilution

where 0.2 is the volume plated

Table 3 shows the calculated percent recoveries of *E. coli B* by CFUs for the hand massage method, positive controls, and positive controls with centrifugation.

Table 3: Percent Recoveries by CFUs for Recovery Loss

Sample ID	6/19/14	7/29/14	Average Recovery (Standard Deviation)
PC_-3	149.6%	77.1%	113.4% (51.3)
PC_-4	130.0%	-----	130.0%
H1_-3	142.8%	17.5%	80.2% (88.6)
H1_-4	110.2%	-----	110.2%
H2_-3	71.1%	18.9%	45.0% (36.9)
H2_-4	136.0%	-----	136.0%
PCC_-3	30.2%	-----	30.2%
PCC_-4	42.1%	-----	42.1%

PC = Positive Control, H = Hand Massage, PCC = Positive Control w/Centrifugation, ----- = did not run

The percent recoveries by plate counts in June showed that the positive controls and hand massage samples are comparable whereas the positive controls with centrifugation samples had significantly low percent recoveries at the -3 dilution, meaning high loss of recovery. Another test for recovery loss was done in July with just the positive controls and the hand massage samples since the positive controls with centrifugation samples had very low percent recoveries, they were not included in this test for recovery loss. Overall, from this data centrifugation is a possible contributor to recovery loss and that suggests the reason why there is a huge range of percent recoveries for each processing method in Table 2.

3.3 CALCULATIONS BY C(T)S (CYCLE THRESHOLD)

For each processing method sample, the amount of CFUs in a volume of 100ul of *E. coli* *B* used to spike a sponge is divided by four from the volume of a sample being brought up to 400ul with PBS. This result is the amount of CFUs in 100ul as the input extraction, which equals

the output extraction since 100ul DNA is eluted in the end. The amount of CFUs in the output extraction is divided by twenty to obtain CFUs in a volume of 5ul of DNA used for qPCR. In later experiments, the amount of CFUs in the output extraction was divided by fifty to obtain CFUs in a volume of 2ul of DNA used for qPCR. The log CFU is calculated by taking the log of the result. The log CFU represents the “x” on the axis of the standard curve plot and is inserted into the standard curve equation to obtain the “y” which is the C(t) value. Percent recovery was calculated by using Equation 5.

$$\text{Equation 5: Percent recovery (\%)} = 100\%/2^n,$$

where n = the difference between the actual C(t) of each method and expected C(t) value calculated from the standard curve

Table 4: Percent Recoveries by C(t)s

Sample ID	12/27/13	1/30/14	2/7/14	5/9/14	Average Recovery (Standard Deviation)
T	7.1%	ND	ND	-----	7.1% (7.6)
T(-1)	19.4%	16.7%	17.7%	35.8%	22.4% (9.9)
H	0.0%	ND	ND	-----	0.0%
H(-1)	42.1%	15.3%	29.3%	30.4%	29.3% (11.4)
S	2.5%	16.0%	2.2%	-----	6.9% (6.5)
S(-1)	27.6%	25.7%	33.0%	52.7%	34.7% (18.6)

T = Shaker Table, H = Hand Massage, S = Stomacher
 (-1) = 10-fold dilution, ND = no data, ----- = did not run

*Each method ran in duplicates

Table 4 shows the calculated percent recoveries of the undiluted and 10-fold dilution of *E. coli B* by C(t)s for each processing method with respective date and the comparison of the average recoveries of each processing method. The standard deviations were calculated from the

individual replicates. The raw data of C(t)s are shown in Tables 10 and 11 [Appendix D]. C(t) values gave inconsistent results using undiluted nucleic acid, the majority of them with no data, but tighter results using 10-fold dilutions of the sponge extractions (averaging recoveries with standard deviations at 22.4% (9.9) for shaker table, 29.3%(11.4) for hand massage, and 34.7%(18.6) for stomacher), suggesting the presence of inhibitors. Due to the data being nonparametric, a Kruskal-Wallis rank sum test was used to perform a comparison between these groups.

3.3.1 *Kruskal-wallis test*

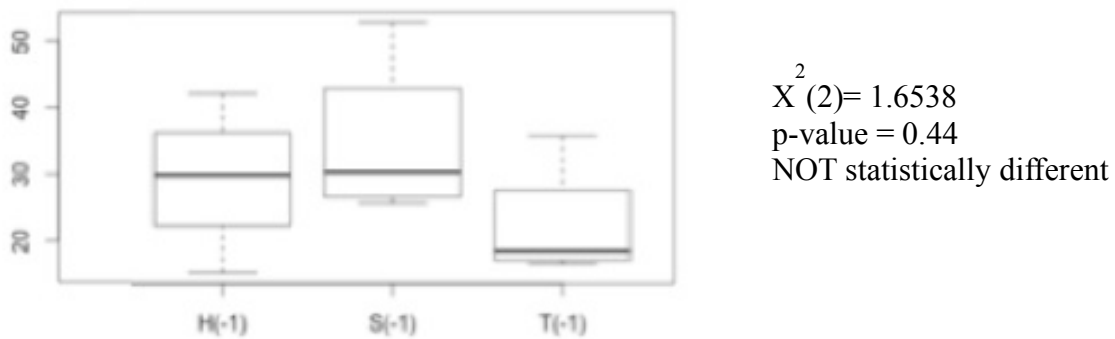


Figure 14: Kruskal-Wallis Test of average C(t) percent recoveries at 10-fold dilution for each date by recovery method

The boxplots in Figure 14 show the average C(t) percent recoveries at a 10-fold dilution for each date by recovery method. The results show that the p-value is 0.44, which is greater than 0.05, indicating the difference between recovery methods is not statistically different. Although there is no statistical difference, but because of the inhibition, the difference between recovery methods could have been statistically different if we had ran a 100-fold or 1000-fold dilution.

3.4 INHIBITION

Before making and running 10 fold dilutions of each sample on qPCR, the majority of the samples showed no C(t) values. To be sure whether DNA was present or not, DNA levels in samples were quantified using nanodrop. Since DNA was present by nanodrop in quantification, possible inhibition was thought to be the problem in qPCR. To get past the inhibition, a 10-fold dilution of the samples was made and run on qPCR where C(t) values were obtained.

Chapter 4. METHODS: FIELD SAMPLING

Now that aim #1 has been evaluated, aim #2 was achieved by using hand massage on the Bullitt samples. For field sampling, surfaces in the Bullitt building were swabbed with the same 3M sponge sticks with 10 ml neutralizing buffer, hand massaged, DNA was extracted, quantified on nanodrop, and sent out for Illumina 16S metagenomic sequencing to characterize the microbes (Figure15).



Figure 15: Field Sampling: Methods Flow Chart

4.1 STUDY SITE

The study took place in the Bullitt Center (www.bullittcenter.org), the world's greenest commercial building achieving the goals of the Living Building Challenge. The Living Building Challenge is the most motivated standard for sustainability utilizing self-sufficient energy and water for at least twelve continuous months, meet rigorous standards for green materials, and for

the quality of its indoor environment. To be certified as a Living Building, the structure is required to meet the following seven performance areas:

- Site: Location supports a pedestrian, bicycle, and transit transportation.
- Water: The building will use roof-collected rainwater stored in an underground cistern.
- Energy: The building must produce as much electricity as it uses.
- Health: Have inviting stairways, operable windows, and features to promote walking and resource sharing to promote health.
- Materials: Not contain any “Red List” hazardous materials.
- Equity: Workstations are within thirty feet of operable windows to offer access to fresh air and natural daylight.
- Beauty: Features to make the building and surrounding neighborhood look beautiful.



Figure 16: The Bullitt Center. Reprinted from <http://www.bullittcenter.org/>

The Bullitt Center is a six-story, 50,000 square-foot building in the Capitol Hill area (Figure 16). The most important feature relating to the indoor microbiome is the Bullitt Center’s operable windows. About 82% of the building gets natural daylight. The windows automatically open when the Building Management Systems (BMS) senses the building needs fresh air and automatically closes at angles for shading from direct sunlight.

4.2 SURFACE SAMPLE COLLECTION

The sampling was conducted over one year from March, 2013 through April, 2014. All samples were collected with 3M™ Sponge-Sticks in 10 mL neutralizing buffer by swabbing various areas with both sides of the sponge. The sponge was placed back into the sponge bag, the stick was discarded, the sponge sealed in the sponge bag, and stored in the refrigerator until ready to use a validated processing method (hand massage).



Figure 17: Bullitt Sponge Sample

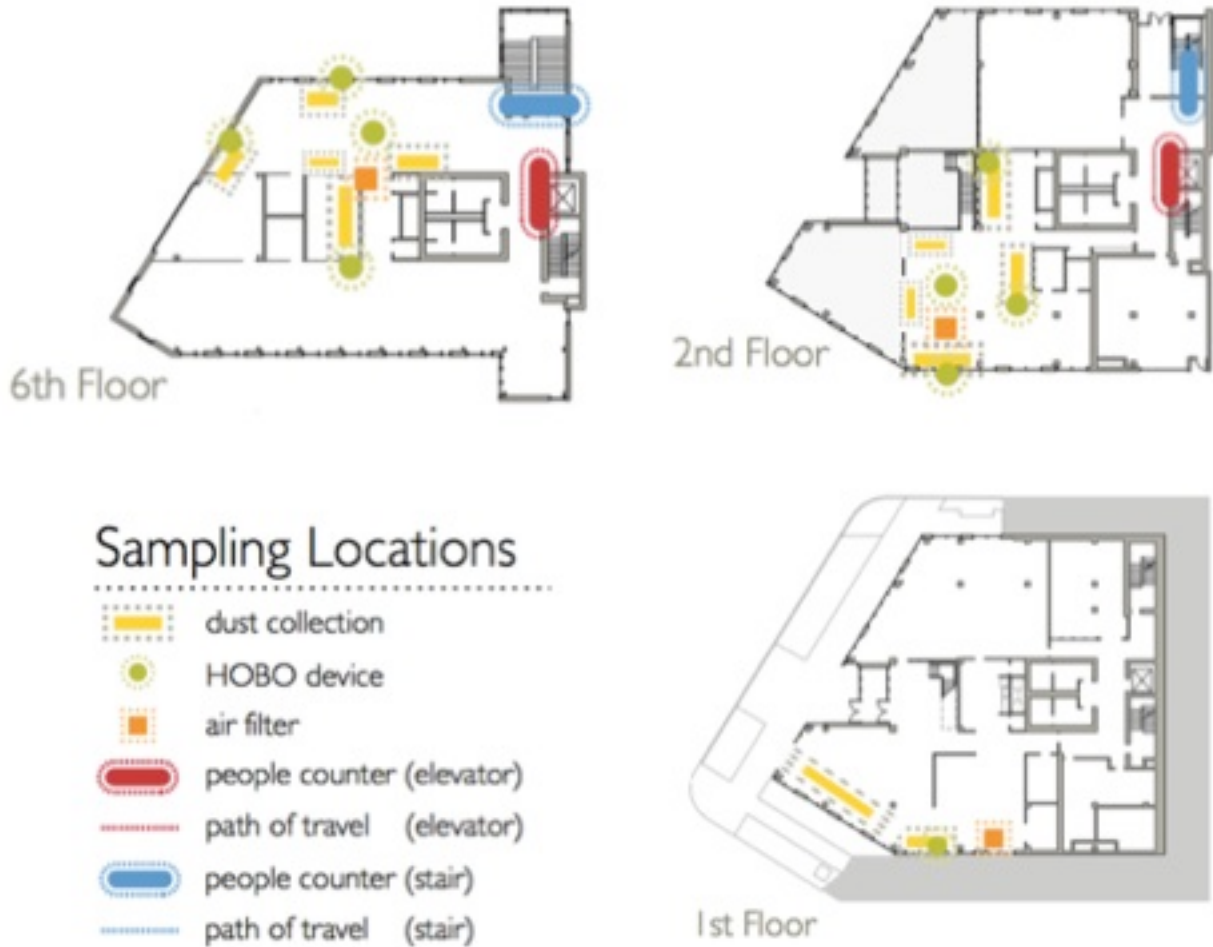


Figure 18: Bullitt Center Data Collection Locations. Adapted from Heather Burpee.

Figure 18 shows the different sampling locations on the first, second, and sixth floors of the Bullitt Center represented by the rectangles. The lower floor window sill was the only location sampled on the first floor. The second floor locations sampled were the top of bookshelf, top of window ledges (two desks and next one back, top and floor), top of window ledge in conference room, front ledge of space overlooking first floor, and top of ledge next to entryway door. The sixth floor locations sampled were the top of junction boxes in conference room with west conference room floor board, west window sill, north window sill (two window sills closest to dog bed), white beam sill above small conference room, and metal and white edges above kitchen entrance.

DATA COLLECTION TIMELINE

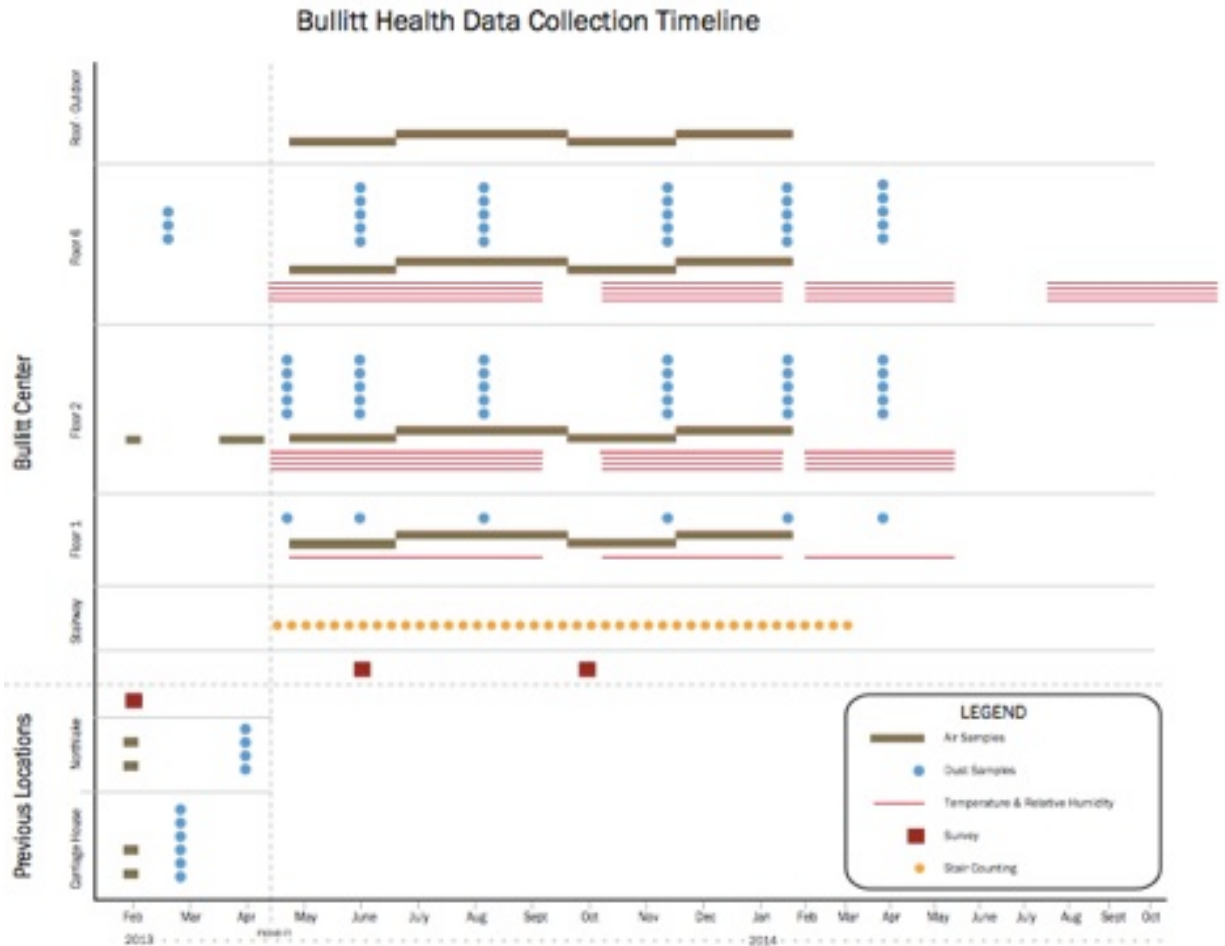


Figure 19: Bullitt Center Data Collection Timeline. Adapted from Heather Burpee.

Figure 19 shows the data collection timeline at the Bullitt Center where the large dots on each floor and previous locations represent the surface samples. The vertical axis represents the old and new buildings of surface sampling. The horizontal axis represents the timeline of when surface samples were collected at each building. The timeline shows sampling of the IDL and Bullitt at previous locations before the Bullitt Center opened in April. The IDL is the integrated design lab at UW's Department of Architecture who rent out a space within the Bullitt as well.

Tables 5 and 6 show the total area sampled, sampling locations, collection dates, and the number of sponges sampled at each location from the Bullitt Center and the old Bullitt and IDL buildings. A total of 74 sponges were sampled with most locations sampled more than once over time. One location was sampled on the first floor at six different dates. Five locations were sampled at six different dates on the second floor. Three locations were sampled at six different dates and two locations at 5 different dates on the sixth floor. The first set of sponges was stored at 4°C and developed mold so subsequent sponges were stored at -80°C. Two sponges went missing possibly due to either being misplaced or accidentally thrown out. Two additional sponges were thrown out due to contaminated PBS buffer.

Table 5: Sponge Stick Sample Collection in Old Bullitt and IDL Buildings

Sampling Location	Collection Dates	No. of Sponges Sampled	Total Area Sampled (sq. in.)
Lower floor window sill	5/1/13, 6/12/13, 8/15/13, 11/26/13, 1/30/14, 4/10/14	6	2562.7
Top of bookshelf	5/1/13, 6/12/13, 8/15/13, 11/26/13 ⁺ , 1/30/14 ⁺ , 4/10/14 ⁺	6	1890 1093
Top of window ledges (two desks, top and floor)	5/1/13, 6/12/13, 8/15/13, 11/26/13 (42S) [^] , 1/30/14, 4/10/14	6	580
Top of window ledge in conference room	5/1/13*, 6/12/13, 8/15/13, 11/26/13, 1/30/14 [#] , 4/10/14	6	988
Front ledge of space, overlooking 1 st floor	5/1/13*, 6/12/13, 8/15/13, 11/26/13 (43S) [^] , 1/30/14, 4/10/14	6	1960
Top of ledge next to entryway door on 2 nd floor	5/1/13*, 6/12/13, 8/15/13, 11/26/13, 1/30/14, 4/10/14	6	1281.25
(1) Top of junction boxes in conference room; (2) W. conference room floor board	3/6/13, 6/12/13, 8/15/13, 11/26/13, 1/30/14, 4/10/14	6	36
W. window sill	3/6/13*, 6/12/13, 8/15/13, 11/26/13, 1/30/14, 4/10/14	6	625.25
N. window sill (two window sills closest to dog bed)	3/6/13*, 6/12/13, 8/15/13, 11/26/13, 1/30/14, 4/10/14	6	1142.25
White beam sill above small conference room	6/12/13, 8/15/13, 11/26/13, 1/30/14, 4/10/14	5	603
Metal and white edge above kitchen entrance	6/12/13 [#] , 8/15/13, 11/26/13, 1/30/14, 4/10/14	5	1169.5

⁺ = decreased due to picture frames * = moldy sponge samples
[#] = missing sponges [^] = sponges with contaminated PBS

Table 6: Sponge Stick Sample Collection in Old Bullitt and IDL Buildings

Sampling Location	Collection Dates	No. of Sponges Sampled	Total Area Sampled (sq. in.)
Upstairs conference room, top of light	3/6/13*	1	936
(1) Shelf over fireplace; (2) window sills	3/6/13*	1	805.3
Top of refrigerator	3/6/13*	1	750
Stair, bottom lower railing	3/6/13*	1	223.5
Left bottom shelf in entrance/reception room	3/6/13*	1	393.75
Downstairs reception top of light closest to door	3/6/13*	1	936
Top of light above Heather's desk	4/24/13*	1	204
Top of three pillars	4/24/13*	1	621
Cross beam in conference room	4/24/13*	1	1265
Window sills in main room	4/24/13*	1	1230

*= moldy sponge samples

Chapter 5. RESULTS: FIELD SAMPLING

After using the hand massage processing method and extraction of DNA as described in the Lab Sampling: Methods section, the samples were run on nanodrop using the PicoGreen Assay for dsDNA protocol to ensure a measureable quantity of DNA was extracted before sending out for sequencing [Appendix B].

5.1 DNA CONCENTRATIONS

The DNA concentrations of the Bullitt Sponges by location over time showed that the majority of the highest concentrations of each location were from the month of June ranging from (78.1-5121.5 ng/ml). Since overall for each location every sampling date except for June was low by comparison, data was logged and shown graphically on a log scale as shown in Figure 20. The negative controls in Figure 21 showed low background DNA concentrations (ranging from 0-14.5 ng/ml); so negative controls were sent out for sequencing to determine the composition and possibly source of the DNA.

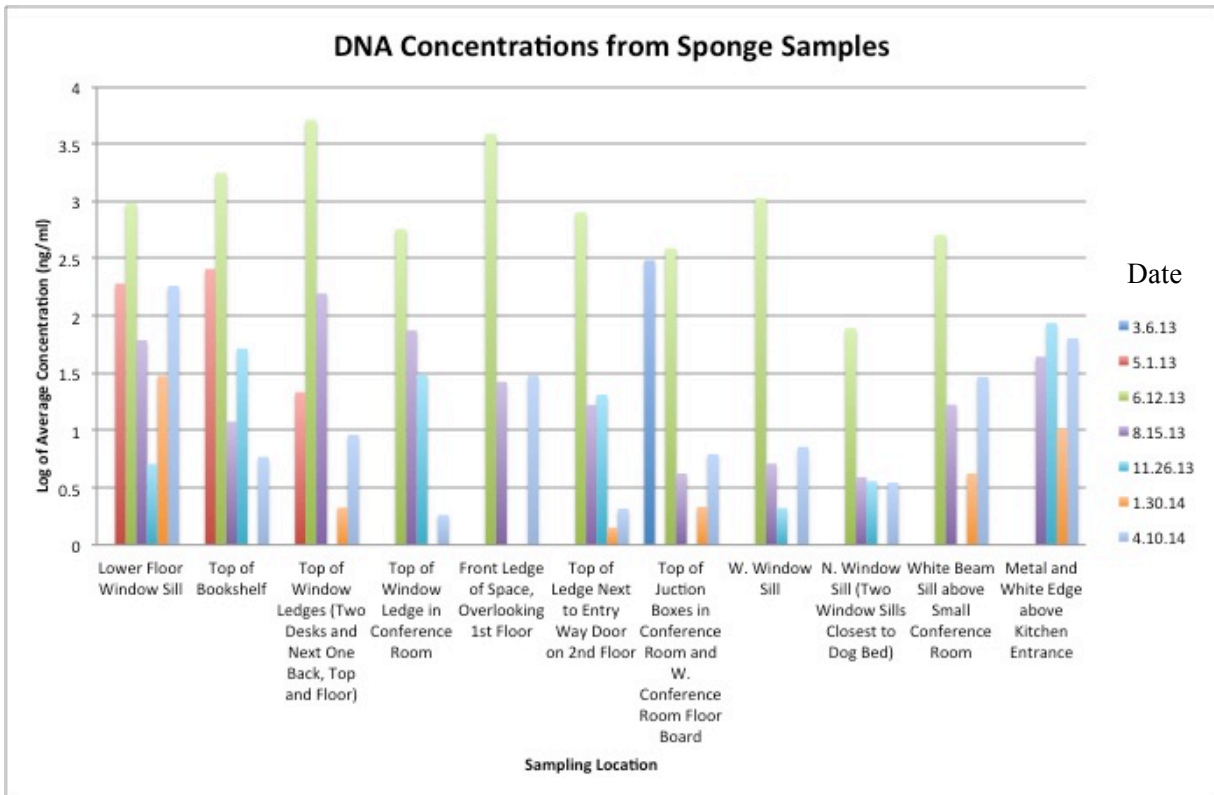


Figure 20: Bullitt Sample Log DNA Concentrations

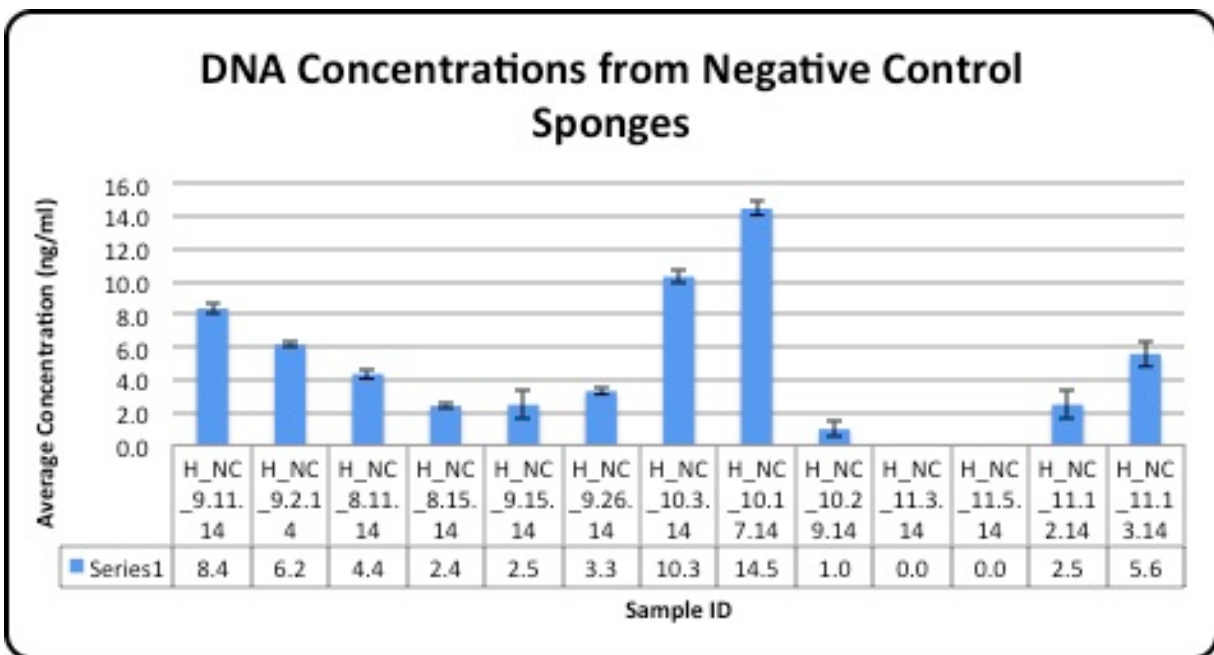


Figure 21: Negative Control DNA Concentrations

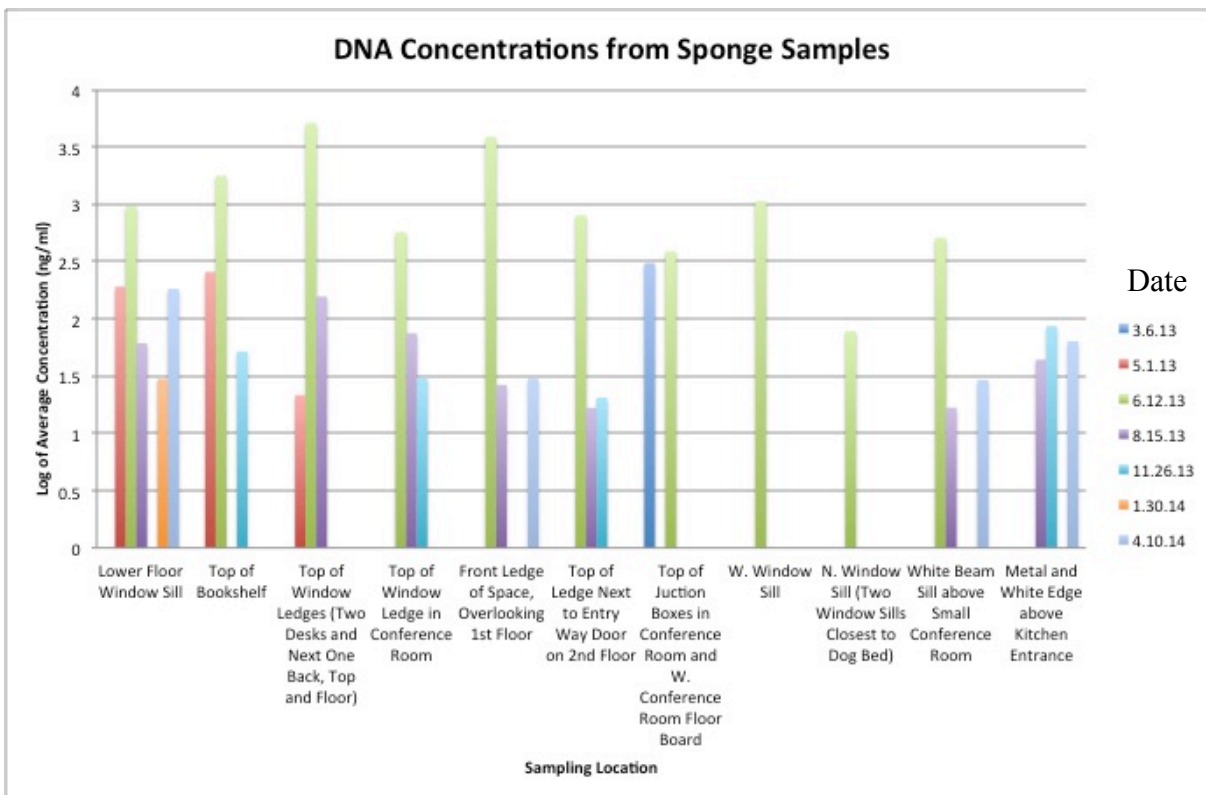


Figure 22: Bullitt Sample Log DNA concentrations excluding samples ≤ 14.5 ng/ml

Any of the DNA concentrations from the Bullitt sponges at and below 14 ng/ml were taken out, due to it being possibly background DNA from the negative controls as shown in Figure 22. Due to the month of June having the highest DNA concentrations in almost all locations, a Kruskal-Wallis rank sum test was used to perform a comparison between months.

5.1.1 *Kruskal-Wallis Test*

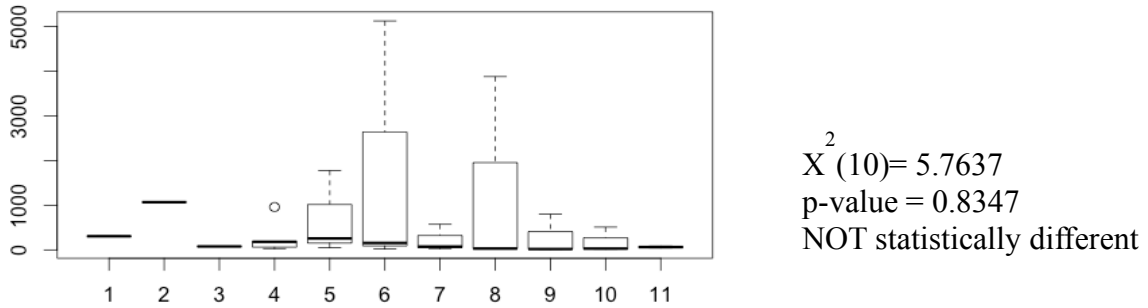


Figure 23: Kruskal-Wallis Test of average DNA concentrations excluding samples ≤ 14.5 ng/ml by location

The boxplots in Figure 23 show the average DNA concentrations excluding samples ≤ 14.5 ng/ml by location. The results show that the p-value is 0.8347, which is greater than 0.05, making the difference in DNA concentrations by locations not statistically different.

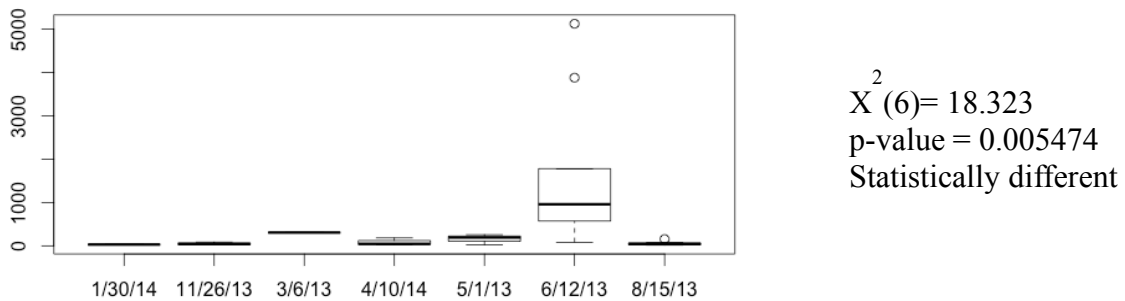


Figure 24: Kruskal-Wallis Test of average DNA concentrations excluding samples ≤ 14.5 ng/ml by date

The boxplots in Figure 24 show the average DNA concentrations excluding samples ≤ 14.5 ng/ml by date. The results show that the p-value is 0.005474, which is less than 0.05, making the difference in DNA concentrations by location statistically different.

5.2 ILLUMINA 16S METAGENOMIC SEQUENCING

Bullitt samples excluding those ≤ 14.5 ng/ml were sent out for sequencing. Illumina 16S metagenomic sequencing is useful for identifying microbial classifications present in a sample. The Illumina involves sequencing the 16S ribosomal RNA gene for taxonomic classification. The 16S ribosomal gene contains 9 variable regions interspersed with conserved regions. The variable regions where the universal primer pairs are used to target specific conserved regions where read 1 and read 2 overlap in the middle are V1-V3 (27F – 534R) and V4 (515F – 806R). After this paired end sequencing, the read pairs are joined (the middle or overlap area has less of a high quality), so some of those reads in the overlap region are thrown out. Careful steps are made to throw out low quality data and keep only high quality data. Initial samples were sent out for sequencing to determine which primer pairs would give the most informative data. The V1-V3 region best differentiated bacterial species to the genus level.

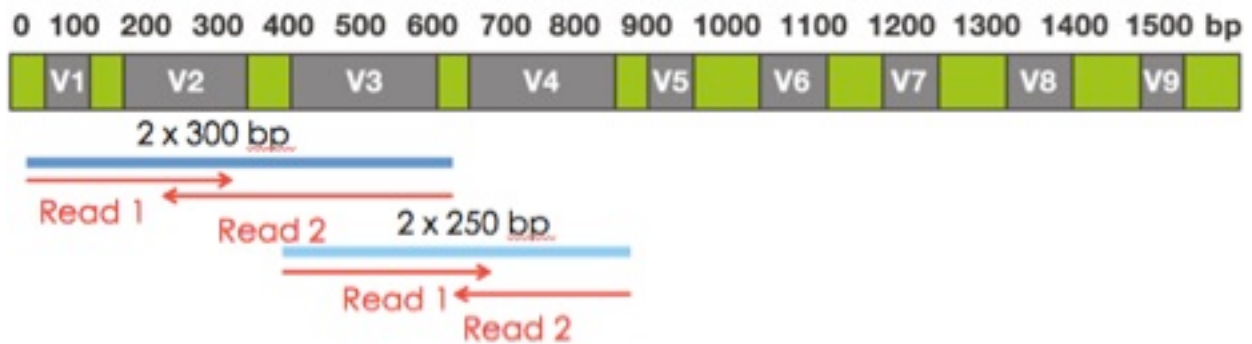


Figure 25: 16S ribosomal gene

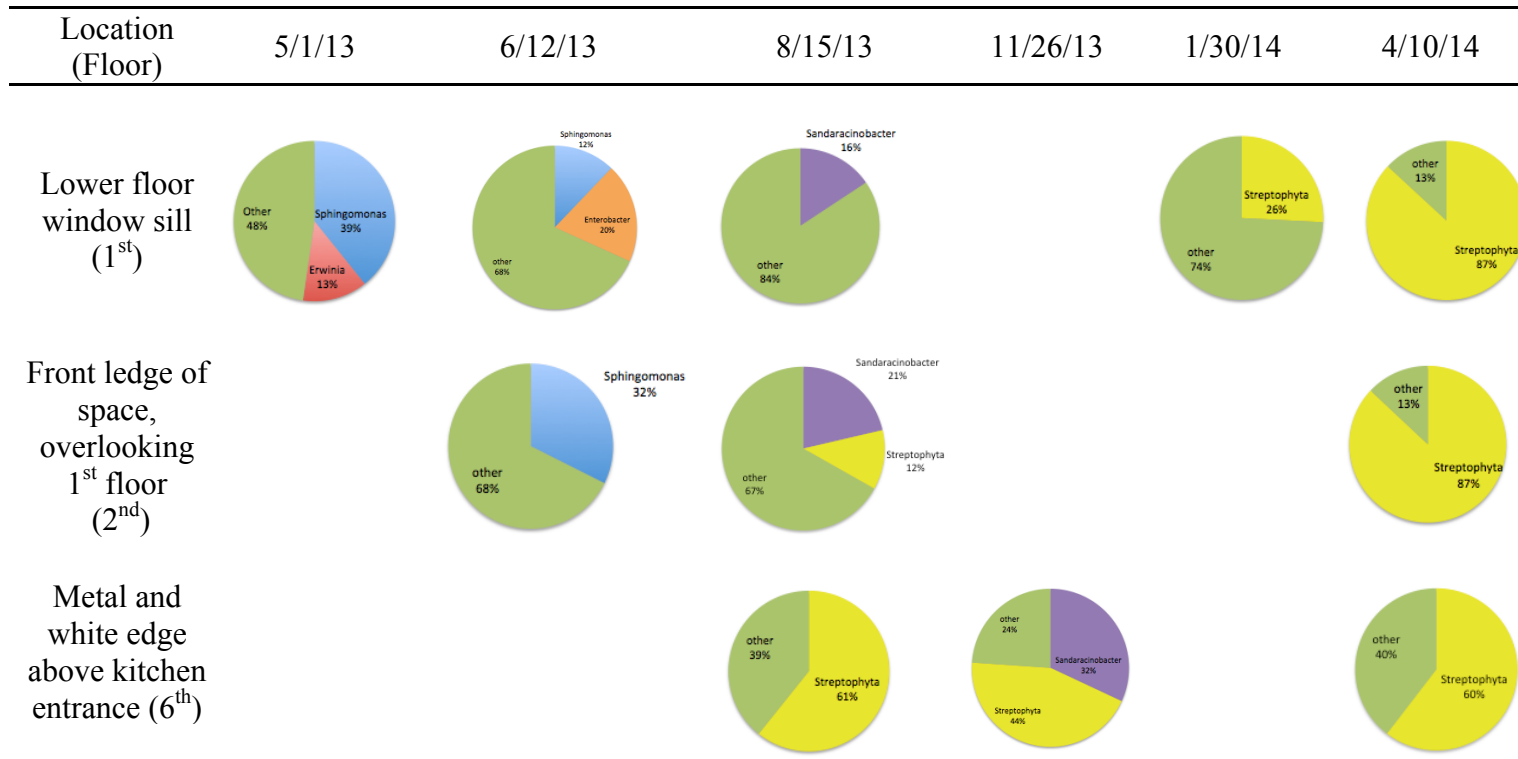
A cluster of species that have similar 16S rRNA sequences are defined as OTUs (Operational Taxonomical Units). Since there were so many OTU groups (over 2000), data of the major microbes that were at least 10% present in each sponge sample were examined. The data was analyzed by taking the sum of the reads by genus classification, dividing that by the total reads per sample, and reporting the microbes present at or greater than 10%.

Pseudomonas was present in the negative control sponges so any other sponge samples that had *Pseudomonas* as a major microbe in the pie charts in Table 7 were not included as a major microbe and considered as other. Table 7 shows pie charts of the major microbes at one different location on each floor. The full pie chart data of the major microbes at all locations are shown in Table 12 [Appendix E]. Over time, for each location and floor, the presence of *Streptophyta* (yellow) increases and the *Sandarincobacter* microbe (purple) increases then decreases. From observation of the pie charts, both *Streptophyta* and *Sandaracinobacter* appear in the middle of the timeline suggesting lower levels in the earlier dates that are not observed because of the 10% cutoff. Analyzing the data again, this time with no cutoff, the presence of *Streptophyta* and *Sandaracinobacter* was observed in the earlier dates at very low levels. The high bloom conditions for *Streptophyta* were observed in April 2014 with a percent abundance as high as 93.9% (Figure 26). The high bloom conditions for *Sandaracinobacter* were observed in August/November 2013 with a percent abundance as high as 40.1%, then decreases significantly for the rest of the timeline (Figure 27). The decrease in *Sandaracinobacter* abundance suggests natural die-off of the microbe in contrast to *Streptophyta*.

From investigating the top 10 microbes with the most reads in each sample, the majority of samples had a higher abundance of environmental associated bacteria compared to human associated bacteria. The data in Table 8 shows that the samples with data had higher percentages

of environmental associated microbes based on reads in each sample. The rest of the samples had no data due to no sampling retrieved, missing, moldy, contaminated sponge samples and sample data taken out because of low levels of background DNA. The samples in June had a distinct high percentage of human associated microbes compared to other dates.

Table 7: Pie Charts of Different Locations on 1st, 2nd, and 6th Floors



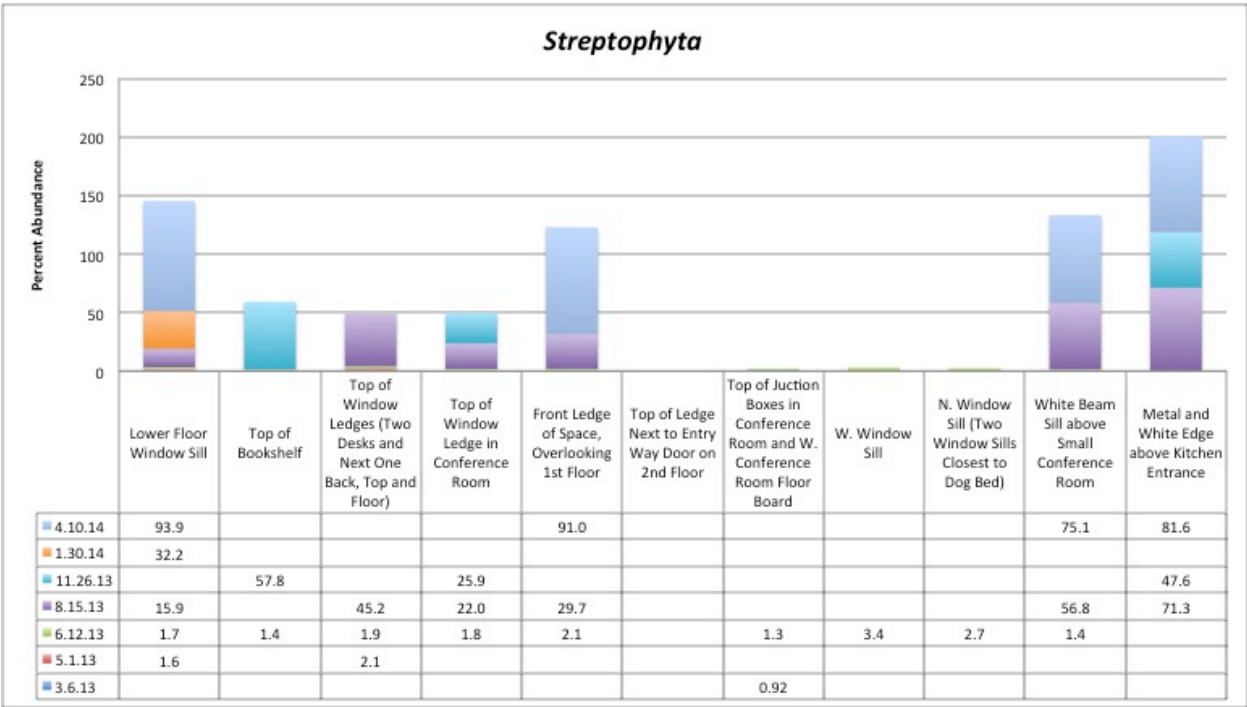


Figure 26: Abundance of *Streptophyta*

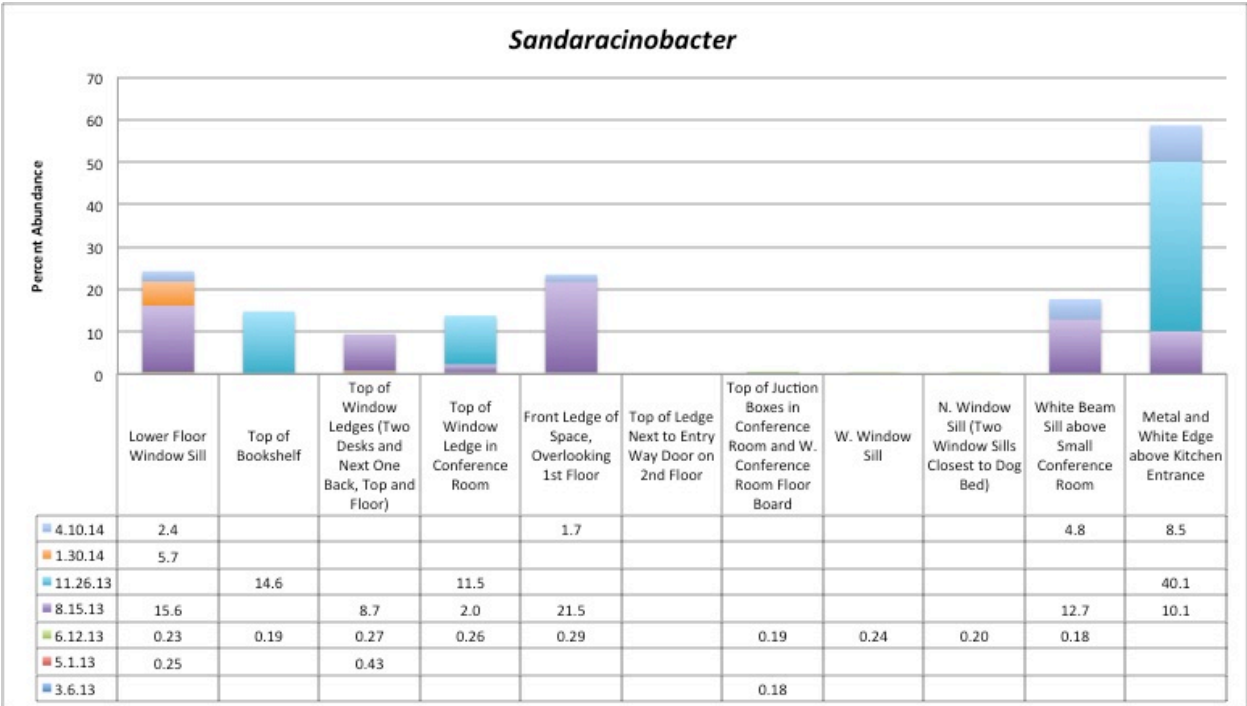


Figure 27: Abundance of *Sandaracinobacter*

Table 8: Attribution of Top 10 Genre to Source (Human or Environmental) and Overall Percentage Composition

Location	3.6.13 Environment, Human	5.1.13 Environment, Human	6.12.13 Environment, Human	8.15.13 Environment, Human	11.26.13 Environment, Human	1.30.14 Environment, Human	4.10.14 Environment, Human
Lower Floor Window Sill	-----	81.5, 3.0	42.6, 19.5	59, 3.1	^	57.6	97.3, 0.08
Top of Bookshelf	-----	-----	3.1, 0.59	^	82.8	-----	^
Top of Window Ledges (Two Desks and Next One Back, Top and Floor)	-----	84.2	68.3, 3.1	72.9	ND	^	^
Top of Window Ledge in Conference Room	-----	ND	54.8	57.2	60	ND	^
Front Ledge of Space, Overlooking 1st Floor	-----	ND	39.5, 9.5	69.2, 1.7	ND	-----	94.4, 0.21
Top of Ledge Next to Entry Way Door on 2nd Floor	-----	ND	-----a	64.7	61.8, 1.5	^	^
Top of Junction Boxes in Conference Room and W. Conference Room Floor Board	96.6	-----	97.8	^	-----	^	^
W. Window Sill	ND	-----	30.6, 21.4	^	^	-----	^
N. Window Sill (Two Window Sills Closest to Dog Bed)	ND	-----	56.7	^	^	-----	^
White Beam Sill above Small Conference Room	-----	-----	46.4	82.3, 1.8	-----	^	85.5, 0.22
Metal and White Edge above Kitchen Entrance	-----	-----	ND	89.6	93.5	^	92.2

----- = no sampling, ND = no data due to missing, contaminated, and moldy sponges, ^ = data taken out due to background DNA

Chapter 6. DISCUSSION

The results of the lab samples of this study showed that when validating a sponge processing method, the best method was inconclusive based on the calculated percent recoveries by C(t) values. C(t) values gave inconsistent results using undiluted nucleic acid, but tighter results using 10-fold dilutions of the sponge extractions ranging from (16.7-35.8% for shaker table, 15.3%-42.1% for hand massage, and 25.7-52.7% for stomacher) suggesting the presence of inhibitors. For a tighter 3.32 C(t) value between dilutions especially for the standard curve or obtaining duplicate C(t) values within 0.5 C(t)s of each other, 2 ul of DNA was used instead of 5 ul per reaction. Although the Kruskal-Wallis test showed that there is no statistical difference between the methods at a significance level of 5%. Hand massage was determined to be the most effective method based on the highest and most consistent viable recoveries ranging on average from 32.2-69.3% with an average of 53.8%(14.4) compared to shaker table at 29.0%(20.1) and stomacher at 37.6% (13.2). This is comparable to the percent recoveries from the literature in Table 1, but depends on the microbe investigated, processing method, and sampling collection device [2] [3] [13] [19]. However, the percent recoveries of the hand massage method ranged from approximately 32.2-69.3%, which shows some loss of recovery. The recovery loss test included hand massage, positive controls, and positive control with centrifugation samples. The positive controls and the hand massage samples were comparable (7.1-15% for the -3 dilution and 110.2-136.0% for the -4 dilution) while the lowest percent recoveries came from the positive controls with centrifugation (3.0% at the -3 dilution and 42.1% at the -4 dilution), at least 4 times less recovery showing that is a possibility where recovery loss came from.

Looking at the advantages and disadvantages of each of the different sampling collection types [2] [3] [13] [19] from Table 1, the 3M sponge-sticks were used. The sponge's large surface area to pick up a large amount of dust and its stick to prevent direct handling of the sponge made it the best choice for sampling in the Bullitt Center. The results of the field samples of this study showed that the highest DNA concentrations of the Bullitt sponges by location over time were observed in June ranging from (78.1-5121.5 ng/ml). The concentrations from the negative controls showed up as high as 14.5 ng/ml and were suspected as contamination. Bullitt samples with DNA concentrations of ≤ 14.5 ng/ml were taken out of this study. Sequencing of the negative controls found that the contamination came from *Pseudomonas*. The contamination was thought to have come from in the lab environment or buffers used. Although the Kruskal-Wallis test showed that there is no statistical difference between the average DNA concentrations by location, the test showed that there is a statistical difference between the DNA concentrations by date suggesting seasonality has a role in the types of microbes found indoors. Seasonality coupled with temperature and humidity suggests different microbial diversity depending on the amount of sunlight a space has. The Bullitt Center receives 82% natural daylight with warmer temperatures in the summer, suggesting greater abundances of microbes.

Results from Illumina 16S (>10% OTUs) showed changes in microbial populations over time at each location. Illumina sequencing of the DNA showed a dominance of environmental associated bacteria, specifically *Streptophyta* and *Sandaracinobacter*. *Streptophyta* had high blooms at 93.9% in April 2014 while *Sandaracinobacter* had high blooms in August/November 2013 at 40.1%. *Streptophyta* is a photosynthetic blue-green colored bacterium that has a phylum classification of cyanobacteria/chloroplast and *Sandaraciniobacter* is a photosynthetic purple bacterium that has a phylum classification of Proteobacteria. Since both *Streptophyta* and

Sandaracinobacter are photosynthetic, the expectation of both microbes was to have high blooms in April 2014 suggesting die-off of *Sandaracinobacter* due to the abundance decrease after November 2013. The common phylum classifications of the top 10 microbes were environment-associated: Proteobacteria, Actinobacteria, Cyanobacteria/Chloroplast, and human-associated: Bacteroidetes, and Firmicutes. Data in June shows a higher varied microbial diversity, both environment and human associated microbes compared to other dates.

Streptopyhta dominated most of the samples from the Bullitt Center and is comparable to the university classroom study where *Streptophyta* had an abundance of 45% in the dust samples. *Streptophyta* most likely entered the building by being blown in through ventilation in the summer when the windows are mostly operated or carried in by humans and dominate the building space by optimal indoor temperatures and convert sunlight into energy for growth and survival. This is comparable to the hospital study where factors such as temperature, humidity, and human occupancy contribute to the growth and survival of microbial communities. In comparison to the mechanically and ventilated window office building study, *Sphingomonas* was the only specific microbe that matched with the green building study suggesting window ventilation as a possible dispersal vector. *Sandaracinobacter* was the only Bullitt Center microbe that stood out in the middle of the timeline and has not been shown as a notable microbe in either conventional or green building studies.

Chapter 7. CONCLUSION

The sponge-stick is a promising sample collection device for sampling large and irregular surfaces compared to swabs, wipes, spatulas, and contact plates. Viable recoveries of *E. coli B* using hand massage were significantly greater than recoveries using the shaker table and the stomacher processing methods. Viable recoveries were similar to the literature, but low due to recovery loss most likely from centrifugation. Total recoveries of *E. coli B* by C(t)s were similar and therefore inconclusive of which processing method was the best. Total recoveries were lower than viable recoveries due to inhibition. Characterization of the indoor microbiome in the Bullitt Center, specifically *Streptophyta* most closely resembled the indoor conventional microbiome in the university study. Seasonality, temperature, humidity, and human occupancy all relate to one another in creating the indoor microbial environment. Both environmental and human associated microbes are present in the indoor microbiome suggesting the environment and humans as major sources. Human occupancy contributes to the characterization of the indoor environment by resuspension of dust that results in microbial similarities at different locations within the same building.

7.1 FUTURE RECOMMENDATIONS

The results of the lab sampling portion of the study emphasize the need to improve the viable recoveries by CFUs and total recoveries by C(t)s with improvements to recovery loss and inhibition. As expected, total recoveries should be higher than viable recoveries. Running a 100-fold dilution test to further eliminate inhibition could obtain higher total recoveries or C(t)s may not show meaning a 10-fold dilution is the best to eliminate as much of the inhibition as possible. The loss in viable recoveries is most likely due to pouring off the supernatant after centrifugation. To obtain tighter and more consistent viable recoveries, increasing the

centrifugation speed may help with the cells in suspension to stick tighter to the bottom of the tube and not be lost when pouring off the supernatant.

Analysis of the Illumina 16S data of microbes present > 10% in each sample showed distinct microbes that stood out at certain dates within the timeline. However, with a 10% cutoff, we were not able to observe the low abundance levels of the distinct microbes in other dates where the microbes seemed to not be present. From analyzing the individual data of *Streptophyta* and *Sandaracinobacter* with no cutoff percentage, we were able to observe the low abundance levels. No cutoff percentage would help analyze all the data. The analysis of the top ten microbes did not clearly distinguish the abundances of environment and human associated microbes. Some of the data at a specific location and time showed no corresponding human associated microbe abundances because the human associated microbes were not a part of the top ten microbes for certain samples. Sorting all the Illumina data by phylum classification and going through labeling which are environment and human associated would result in complete abundance level results. The data in this study is preliminary and we remain in the very early stages of understanding the indoor microbiome in green buildings. More surface sampling in green buildings is needed to make a clear statement on the comparisons between the microbial diversity in green buildings versus conventional buildings. Determining these microbial taxa is important to investigate the abundances of human vs. environmental associated microbes. Further research is necessary to look at the influence of temperature, humidity, and human occupancy on the microbial communities found indoors that help characterize the indoor environment. This data can help further the study to investigate the survival and impact of the microbial communities.

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APPENDIX A

MO BIO PowerSoil™ DNA Isolation Kit Protocol

1. To the PowerBead Tubes provided, add 0.25 grams of soil sample.
2. Gently vortex to mix.
3. Check Solution C1. If Solution C1 is precipitated, heat solution to 60°C until dissolved before use.
4. Add 60 µl of Solution C1 and invert several times or vortex briefly.
5. Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog# 13000-V1-24) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. Note: If you are using the 24 place Vortex Adapter for more than 12 preps, increase the vortex time by 5-10 minutes.
6. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x g for 30 seconds at room temperature. CAUTION: Be sure not to exceed 10,000 x g or tubes may break.
7. Transfer the supernatant to a clean 2 ml Collection Tube (provided). Note: Expect between 400 to 500 µl of supernatant. Supernatant may still contain some soil particles.
8. Add 250 µl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.
9. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.
10. Avoiding the pellet, transfer up to, but no more than, 600 µl of supernatant to a clean 2 ml Collection Tube (provided).
11. Add 200 µl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes.
12. Centrifuge the tubes at room temperature for 1 minute at 10,000 x g.
13. Avoiding the pellet, transfer up to, but no more than, 750 µl of supernatant into a clean 2 ml Collection Tube (provided).
14. Shake to mix Solution C4 before use. Add 1200 µl of Solution C4 to the supernatant and vortex for 5 seconds.
15. Load approximately 675 µl onto a Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Discard the flow through and add an additional 675 µl of supernatant to the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Note: A total of three loads for each sample processed are required.
16. Add 500 µl of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x g.
17. Discard the flow through.
18. Centrifuge again at room temperature for 1 minute at 10,000 x g.
19. Carefully place spin filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the Spin Filter.
20. Add 100 µl of Solution C6 to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog# 17000-10).
21. Centrifuge at room temperature for 30 seconds at 10,000 x g.
22. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application. No further steps are required.

APPENDIX B

PicoGreen Assay for dsDNA and Standard Curve Protocol

1. Dilute PicoGreen Dye stock two-hundred fold (198ul 1X TE and 2ul dye) and place in a 1.5 ml amber tube (PG working solution).
2. Thaw the standard and unknown dsDNA samples. Once thawed, mix each solution.
3. Prepare serially diluted dsDNA standards at 2X the final concentration in 1.5 ml tubes. Starting standard is (2ul standard and 98ul TE).
 - a. Place 10ul starting standard in a 1.5 ml tube labeled 2000.
 - b. Place 10ul of 2000 and 10ul TE in a 1.5 ml tube labeled 1000.
 - c. Place 4ul of 1000 and 16ul TE in a 1.5 ml tube labeled 200.
 - d. Place 5ul of 200 and 15ul TE in a 1.5 ml tube labeled 50.
 - e. Place 8ul of 50 and 12ul TE in a 1.5 ml tube labeled 20.
 - f. Place 10ul of 20 and 10ul TE in a 1.5 ml tube labeled 10.
 - g. Place 4ul of 10 and 16ul TE in a 1.5 ml tube labeled 2.
 - h. Place 10ul of TE in a 1.5 ml tube labeled 0 (assay buffer).
4. Place one volume of each standard and equal volume of PG working solution in 1.5 ml amber tubes.
 - a. Place one volume of 2000 standard and equal volume of PG working solution in an amber tube labeled 1000 (Standard #7).
 - b. Place one volume of 1000 standard and equal volume of PG working solution in an amber tube labeled 500 (Standard #6).
 - c. Place one volume of 200 standard and equal volume of PG working solution in an amber tube labeled 100 (Standard #5).
 - d. Place one volume of 50 standard and equal volume of PG working solution in an amber tube labeled 25 (Standard #4).
 - e. Place one volume of 20 standard and equal volume of PG working solution in an amber tube labeled 10 (Standard #3).
 - f. Place one volume of 10 standard and equal volume of PG working solution in an amber tube labeled 5 (Standard #2).
 - g. Place one volume of 2 standard and equal volume of PG working solution in an amber tube labeled 1 (Standard #1).
5. Place one volume of each sample and equal volume of PG working solution in 1.5 ml amber tubes.
6. Place one volume of 1X TE and equal volume of PG working solution in a 1.5 ml amber tube labeled NC (reference).
7. Vortex and spin all samples, allow to equilibrate at room temperature for 5 minutes.

Standard Curve

1. Clean both sampling pedestals with 2ul of nuclease free deionized water.
2. Open upper arm and firmly blot the two pedestals with a dry lab wipe. Make sure there are no traces of lint on the pedestals before continuing.
3. Open the operating software. Click on the Nucleic Acid Quantitation button and select the PicoGreen method.

4. Add 2ul of assay buffer (no dye, no sample) to the lower pedestal. Lower the arm and click F3 or the Blank button. When the measurement is complete, lift the arm and use a dry laboratory wipe to blot the buffer from both the bottom and upper measurement surfaces. Use a fresh aliquot of buffer to verify a proper baseline.
5. Under Measurement type, click on the Standards tab. Highlight the Reference standard.
6. Mix the reference solution (assay buffer and dye, no sample) briefly and transfer 2ul of the solution onto the lower pedestal. Lower the arm and click F1 or the Measure button. A pop up window will ask for confirmation of the units. (Recommended ng/mL or pg/uL)
7. Measure up to 5 replicates of the reference solution using a fresh 2ul aliquot for each measurement.
8. Select Standard 1 to enter a value. Enter values for up to 7 standards.
9. Mix the standard solution briefly and transfer 2ul onto the lower pedestal. Lower the arm and click F1 or the Measure button. Measure up to 5 replicates of each standard using a fresh 2ul aliquot for each measurement.
10. Once the standard curve is completed, select the Standard Curve Type (Interpolation, Linear, 2° polynomial, 3° polynomial) that best fits the standards data set.
11. Click on the Sample tab under Measurement Type, and enter the unknown samples' respective ID information. If a dilution of the unknown sample was made, enter the dilution factor in the box below the sample ID window.
12. Add 2 ul of the sample and use the F1 key or click the Measure button to initiate the measurement cycle. Use a fresh aliquot of sample for each measurement.
13. Clean both sampling pedestals with 2ul of nuclease free deionized water.
14. Open upper arm and firmly blot the two pedestals with a dry lab wipe.

APPENDIX C

Table 9: CFUs of *E. coli B*

Sample ID	12/27/13	1/17/14	1/30/14	2/7/14	5/9/14
0 (-6)	188, 230	287, 275	103, 113	164, 178	200, 260
0 (-7)	17, 18	33, 38	7, 10	16, 27	20, 38
T1 (-5)	108, 86	58, 60	115, 107	87, 90	392, 328
T1 (-6)	5, 18	7, 7	13, 13	8, 6	35, 35
T2 (-5)	99, 94	29, 36	111, 90	76, 91	330, 364
T2 (-6)	8, 13	4, 3	9, 9	8, 7	37, 38
H1 (-5)	228, 204	194, 260	159, 169	250, 248	389, 346
H1 (-6)	22, 23	26, 35	22, 17	25, 30	45, 30
H2 (-5)	250, 275	212, 187	191, 198	244, 244	384, 373
H2 (-6)	33, 29	50, 55	28, 23	33, 19	41, 51
S1 (-5)	94, 122	170, 247	138, 130	116, 151	362, 324
S1 (-6)	15, 11	24, 40	10, 15	13, 17	31, 57
S2 (-5)	106, 171	195, 255	98, 111	105, 139	365, 289
S2 (-6)	30, 24	11, 29	13, 12	14, 7	31, 35

T = Shaker Table, H = Hand Massage, S = Stomacher

*Replicates of each sample were plated

APPENDIX D

Table 10: C(t)s of *E. coli* B at 10-fold dilution

Sample ID	12/27/13	1/30/14	2/7/14	5/9/14
(-1) of 0	23.99	22.65	22.86	22.39
(-1) of 0	23.77	22.15	22.45	21.01
(-1) of 1	26.5	25.4	25.36	N/A
(-1) of 1	26.11	25.23	25.51	N/A
(-1) of 2	29.06	27.92	28.4	26.21
(-1) of 2	29.54	27.82	28.53	27.02
(-1) of 3	32.7	31.48	30.82	28.15
(-1) of 3	33.28	31.71	30.82	28.71
(-1) of 4	35.77	32.6	33.35	-----
(-1) of 4	35.86	33.14	32.9	-----
T1 (-1)	28.29	27.64	26.82	23.87
T1 (-1)	28.13	27.78	26.58	23.97
T2 (-1)	27.34	26.07	27.25	24.16
T2 (-1)	27.4	26.38	26.79	23.81
H1 (-1)	26.63	26.34	26.13	23.45
H1 (-1)	27.37	26.19	25.96	23.74
H2 (-1)	26.75	29.81	26.39	23.68
H2 (-1)	26.59	26.5	26.32	23.97
S1 (-1)	26.69	25.53	27.05	24.45
S1 (-1)	26.51	25.47	27.05	24.44
S2 (-1)	28.44	27.43	25.39	24.43
S2 (-1)	28.42	27.44	25.47	24.71

T = Shaker Table, H = Hand Massage, S = Stomacher, (-1) = 10-fold dilution,
 ----- = did not run

Table 11: C(t)s from undiluted *E. coli B*

Sample ID	12/27/13	1/30/14	2/7/14
0	19.31	20.86	19.83
0	19.12	20.71	19.51
-1	25.87	24.34	23.24
-1	22.15	24.55	23.31
-2	25.64	28.36	28.69
-2	31.74	28.28	28.62
-3	29.18	32.44	30.55
-3	30.21	32.07	30.53
-4	35.78	37.88	34.85
-4	33.95	37.91	34.45
T1	27.45	N/A	N/A
T1	28.53	N/A	N/A
T2	24.87	N/A	N/A
T2	25.41	N/A	N/A
H1	39.11	N/A	N/A
H1	N/A	N/A	N/A
H2	N/A	N/A	N/A
H2	N/A	N/A	N/A
S1	36.24	N/A	26.03
S1	35.04	N/A	27.38
S2	25.82	25.34	N/A
S2	27.07	25.67	34.39

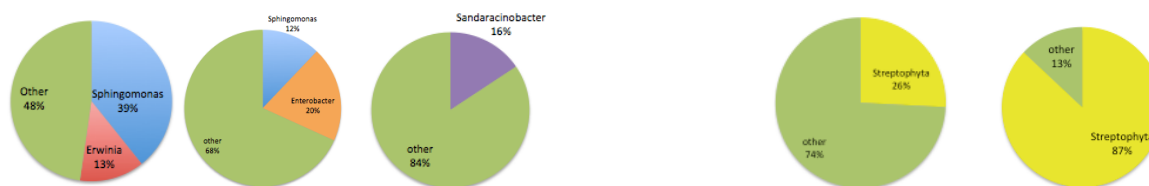
T = Shaker Table, H = Hand Massage, S = Stomacher

APPENDIX E

Table 12: Pie Charts of Major Microbes at All Locations on 1st, 2nd, and 6th Floors

Location	3/6/13	5/1/13	6/12/13	8/15/13	11/26/13	1/30/14	4/10/14
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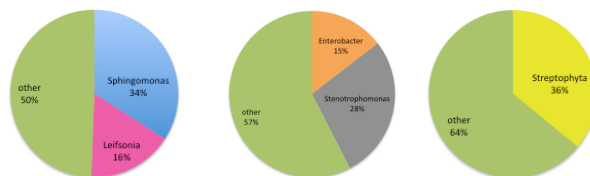
Lower Floor
Window Sill



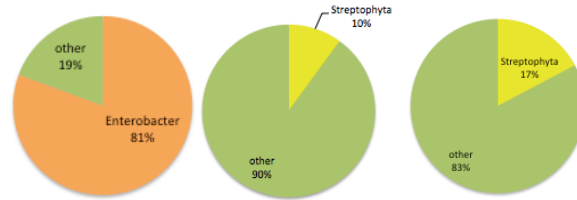
Top of
Bookshelf



Top of Window
Ledges (Two
Desks and Next
One Back, Top
and Floor)



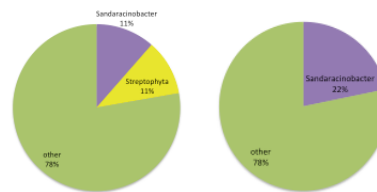
Top of Window
Ledge in
Conference
Room



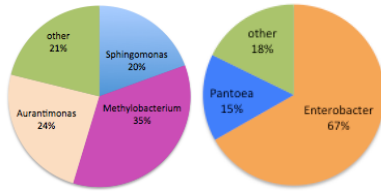
Front Ledge of
Space,
Overlooking 1st
Floor



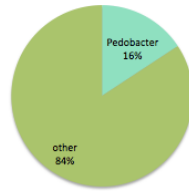
Top of Ledge
Next to Entry
Way Door on
2nd Floor



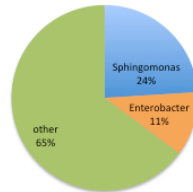
Top of Junction
Boxes in
Conference
Room and W.
Conference
Room Floor
Board



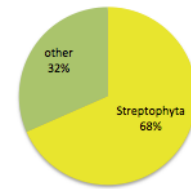
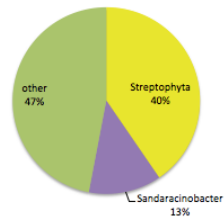
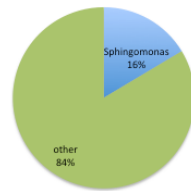
W. Window Sill



N. Window Sill
(Two Window
Sills Closest to
Dog Bed)



White Beam Sill
above Small
Conference
Room



Metal and White
Edge above
Kitchen
Entrance

