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Microbiome in an Office Building Using a Cooling Trench as an Outdoor Air Duct

Mizuki Niimura^{1,*} and U Yanagi¹

¹Kogakuin University

Abstract. Subterranean temperature at a depth of 10 m is almost equal to the average outdoor air temperature of the same area. Therefore, if a building cooling trench is used as an outdoor air duct, outdoor air can be cooled in summer and warmed in winter. This energy-saving technique is often used in Japan. However, since the relative humidity in a cooling trench is high, microbe numbers tend to increase in summer. The present study sought to characterize the microbiome status in the cooling trench of such an office building in Japan. Specifically, we performed a metagenomic analysis in which we analyzed DNA directly upon collection from the environment, without intervening cultivation. The results showed the presence of bacteria of the genera *Pseudomonas*, *Lactobacillus*, *Nesterenkonia*, *Staphylococcus*, *Deinococcus*, *Acinetobacter*, *Enhydrobacter*, and *Corynebacterium*. Bacteria of the genera *Nesterenkonia*, *Deinococcus*, *Enhydrobacter*, and *Corynebacterium* predominated on the surface of the trench. Notably, bacteria of the genus *Nesterenkonia* constituted >50% of the organisms on the surface of the downstream end of the cooling trench. Principal coordinate analysis was used to compare bacterial inhabitants of outdoor air, indoor air from 2nd- and 3rd-floor offices, and the region downstream of the cooling trench. The results suggested that the microbiome of air in this cooling trench influenced indoor air within the building.

1 Introduction

The subterranean temperature at a depth of 10 m is almost equal to the annual average outdoor air temperature of the area. The utilization of geothermal temperature control has been proposed for several applications, including use with heat pumps, heat conduction, circulation of air, and heat piping¹. The cooling trench contained in the structure of an air circulation system allows air to pass into spaces made in underground concrete structures, providing heat exchange and an outdoor load.

Although subterranean heat originally was used in Japan as a source of heating for combatting snow accumulation in areas of heavy snowfall, the spread of ZEB (Net Zero Energy Buildings) in recent years has led to the use of subterranean heat as a mechanism for reducing air-conditioning cooling loads. If a building cooling trench uses as an outdoor air duct, outdoor air can be cooled in summer and warmed in winter. This energy-saving technique now often is used in Japan.

However, high humidity is a problem in Japan. In summer, the outdoor air temperature can exceed the dew point temperature of a cooling trench surface, resulting in dew condensation on the surface of such a trench, leading in turn to a humid internal environment, suitable for the microbes.

The present study sought to better understand the microbiome status in the environment of a building using a cooling trench. In this study, both traditional cultivation

methods and metagenomic analysis (directly analyzing DNA collected from the environment, without cultivation) were performed.

2 Method

2.1 Measurement site

The investigation site was an office building with an S-structure, including one underground floor and three stories above ground, the structure had a total floor area of 1,384 m². This building employed subterranean heating technology, slab thermal storage, and a radiation air-conditioning system.

2.2 Measurement

Measurements were carried out for the period of 9:30 to 10:30 AM on August 3, 2017 after completion half a year. The measured parameters were as follows: air temperature, relative humidity, carbon dioxide concentration, airborne particles, airborne microbes, and adherent microbes. Six measurement sites were selected: outdoor, upstream of the cooling trench, center of the cooling trench, downstream of the cooling trench, indoors in a second-floor office, and indoors in a third-floor office. The following methods were used to measure the various parameters.

(1) Temperature, relative humidity, CO₂ concentrations
Air temperature, relative humidity, and CO₂ concentrations were recorded once per minute during the

measurement period, using a data logger (TR-76Ui, T&D Co.).

(2) Airborne particles

The concentrations of suspended particles were measured using a P611 optical particle counter (Airy Technology Co., Ltd.). The P611 simultaneously detects particles in six size ranges: 0.3-0.5 μm , 0.5-0.7 μm , 0.7-1.0 μm , 1.0-2.0 μm , 2.0-5.0 μm , and $>5.0 \mu\text{m}$. The maximum measured particle density is 14,000 particles/L, the sample flow rate is 2.83 L/min, and a long-life laser diode is used as the light source. The measurement interval was set to 1 min.

(3) Microbes

Airborne live fungi and bacteria were sampled by a one-stage impactor (type MBS-1000). The sampling volume was 250 L. SCD culture media were used in this study. Culture conditions for SCD culture media were 32 $^{\circ}\text{C}$ for two days, respectively. Separately, airborne microbes were obtained using an air pump (Air Check XR5000, SKC, Inc., and filter (PTFE 0.3 μm Filter, SKC, Inc.). The sampling volume was 180 L (3 L/min \times 60 min).

Surface microbes also were collected. Extraction by swab was performed using a swab kit (Bode Secur Swab, Bode Cellmark). Adherent microbes were collected by swabbing of the surface of the cooling trench as well as surfaces in a second-floor office and a third-floor office.

For details of DNA extraction, amplification and sequencing by next generation sequencer, please refer to the previous report .

3. Results and discussion

3.1 Temperature, relative humidity, and CO2 concentration

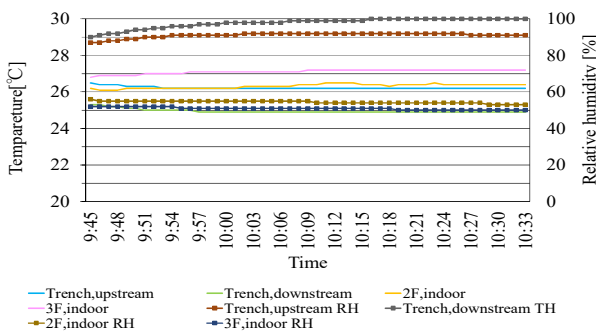


Fig. 1. The results for temperature and relative humidity

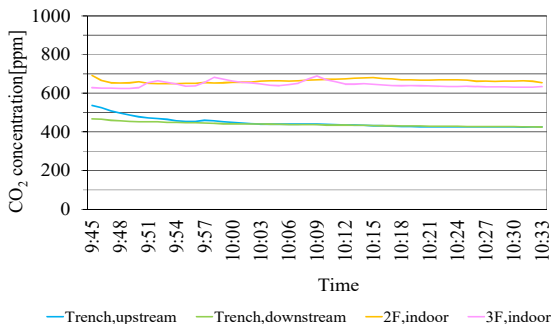


Fig. 2. The results for CO2 concentration

Fig. 1 and Fig. 2 show the results of the temperature, relative humidity, and CO₂ concentration measurements. Indoor air temperature in the second-floor and third-floor offices was 26.0-27.0 $^{\circ}\text{C}$, and CO₂ concentration was 600-700 ppm. These results satisfied the Japanese ‘Law for Environmental Health in Buildings’.

3.2 Live bacteria concentration

Fig. 3 show the results for densities of live airborne bacteria in the outdoors, the upstream/center/downstream of the cooling trench, the second-floor office, and the third-floor office. The level of airborne bacterial was highest inside the cooling trench, where bacteria achieved a concentration of 2500 cfu/m³. These data indicated that bacteria accumulate and/or replicate within the cooling trench. Notably, despite the high bacterial levels in the cooling trench, the airborne bacterial concentrations at the indoor sampling sites remained below the AIJ cut-off of 500 cfu/m³ (Standard for Design and Maintenance on Indoor Air Pollution by Microbe, 2013).

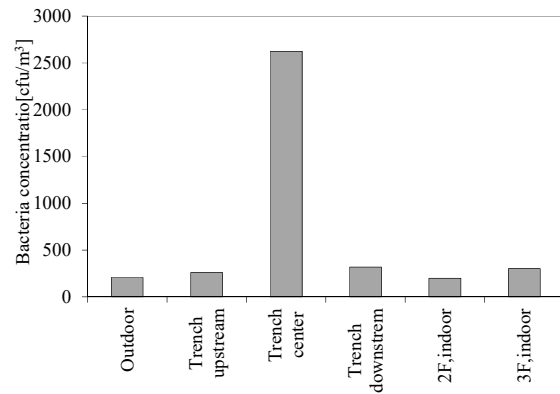


Fig. 3. Airborne bacterial density

3.3 16S rRNA analysis

3.3.1 Generic characteristics of detected bacteria

Table 1 shows the numbers of detected bacteria (classified by phylum, class, order, family, and genus) with relative abundances exceeding 1%. Compared to the airborne bacteria, more kinds of adherent bacteria were detected.

Table 1. Number of detected bacteria by phylum, class, order, family, and genus

| Measuring place | | Phylum | Class | Order | Family | Genus |
|-----------------|--------------------|--------|-------|-------|--------|-------|
| Air | trench, upstream | 7 | 10 | 16 | 15 | 17 |
| | trench, downstream | 6 | 8 | 15 | 20 | 24 |
| | second floor | 5 | 8 | 12 | 14 | 15 |
| | third floor | 8 | 12 | 18 | 23 | 23 |
| Surface | trench, upstream | 7 | 13 | 18 | 26 | 30 |
| | trench, downstream | 5 | 8 | 10 | 17 | 19 |
| | second floor | 6 | 14 | 23 | 36 | 43 |
| third floor | 7 | 15 | 25 | 37 | 44 | |

3.3.2 Relative abundance of bacterial genera

Fig. 4 through Fig. 6 show relative abundances of bacterial genera for all samples, quartile diagrams of the top ten genera, and genus reads of each sample, respectively. Both Fig. 5 and Fig. 7 indicated that

bacteria of the genera *Pseudomonas*, *Lactobacillus*, *Nesterenkonia*, *Staphylococcus*, *Deinococcus*, *Acinetobacter*, *Enhydrobacter*, and *Corynebacterium* had not only high relative abundances, but also more reads. Notably, bacteria of the genera *Pseudomonas* and *Lactobacillus* were detected in high numbers from all sampling sites. Additionally, *Nesterenkonia* bacteria were detected at abundances of >50% on the surface of the cooling trench.

3.3.2 Relative abundance of bacterial genera In the quartile diagram of detected bacteria, few of the top 10 genera in the total bacteria (airborne + adherent) were classified as outliers. When the airborne and adherent bacteria were analyzed using separate quartile diagrams, few of the top 10 genera of adherent bacteria ranked as outliers, in contrast to the case with the airborne bacteria (excepting the *staphylococci*).

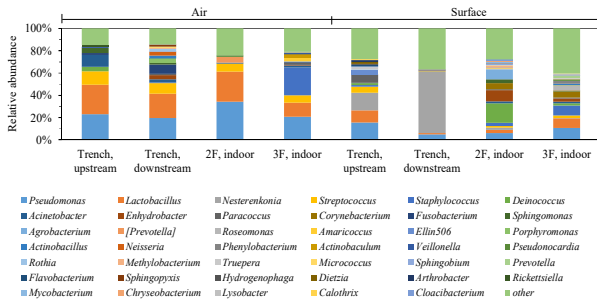


Fig. 4. Relative abundances of bacterial genera for all samples

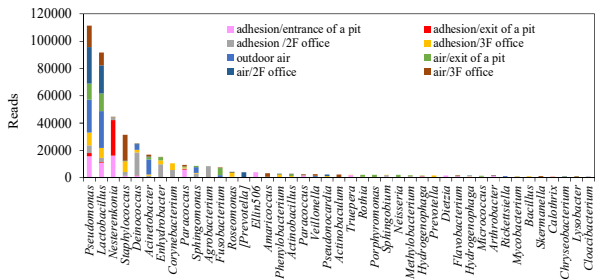


Fig. 6. Genus reads of each sample

3.3.3 Characteristics of detected bacteria

Fig. 8 and **Fig. 9** show source of detected bacteria and the ratio of gram-negative and -positive organisms among the detected bacteria, respectively. Regarding the apparent sources of the detected bacteria, some 40% to 50% of the airborne bacteria were species considered to be human- or animal-associated, and another 20% to 40% were species considered to be of general environmental origin. On the other hand, the characteristics of the adherent bacteria differed depending on the sampling site. In past studies, bacteria associated with salty soil have been detected only rarely in office spaces. However, in the present study, salt soil bacteria constituted approximately 50% of the bacteria detected on the surface of the downstream end of the cooling trench. This observation is thought to reflect the origin of the concrete material used in constructing the trench.

Regarding the ratio of gram-negative and -positive microbes among the detected bacteria, gram-negative organisms were more abundant in all samples, with the exception of the air samples from the third-floor office. Notably, gram-negative organisms constituted 50% or more of the bacteria detected in the second floor office and from the downstream end of the cooling trench.

Members of the genus *Nesterenkonia* the largest proportion of those recovered from the surface of the downstream end of the trench; members of this genus also were recovered from the upstream end of the trench. *Nesterenkonia* are actinomycetes and hence are gram-positive bacteria.

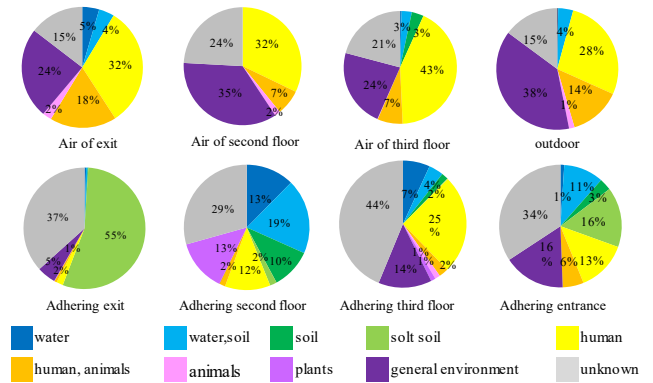


Fig. 7. Source of detected bacteria

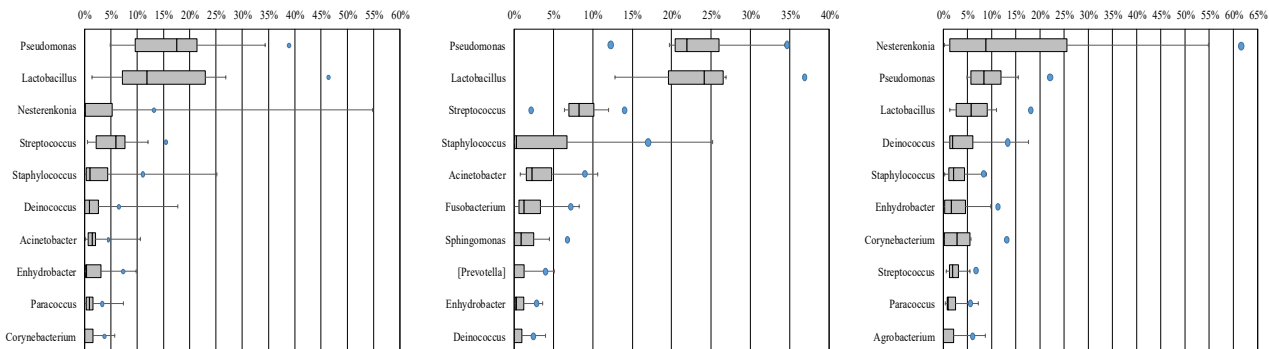


Fig. 5. Quartile diagrams of detected bacteria: all (left), airborne (center), adherent (right)

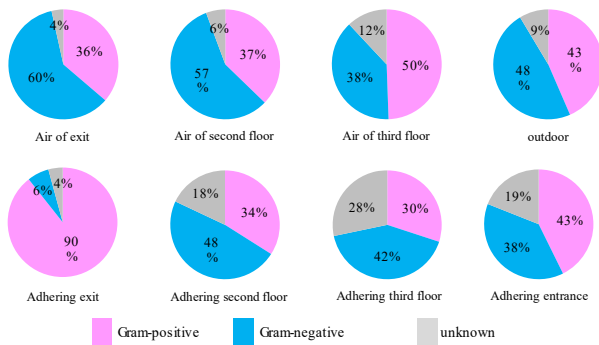


Fig.8. The ratio of gram-negative and -positive organisms among the detected bacteria

3.3.4 Principal coordinate analysis (PCoA)

Fig. 9 shows the principal coordinate analysis results. Unlike principal component analysis, main coordinate analysis is used to visualize multidimensional data by plotting in two or three dimensions. Similar flora were detected in the air samples from outside, from the second- and third-floor offices, and from the trench exit. In contrast, the adherent bacteria plotted to a different position, suggesting that the adherent organisms represented a microbial flora distinct from the airborne species. Based on physical proximity, airborne microbes on the second and third floors presumably would reflect the content of the outside air, but not that of the microbial flora on the surface of the cooling trench. Thus, we infer that that the population of bacteria on the surface of the cooling trench was greatly affected by the content of the outside or office air.

4 Conclusions

In this work, we sought to clarify the actual nature of the bacterial flora in a building's cooling trench and to assess the influence of a building's indoor environment on the constituents of the trench's microbiome. The identity of the observed microorganisms was described primarily based on the result of 16S rRNA analysis. Our results suggested that the temperature, relative humidity, and the CO₂ concentration in the offices of a building that used a cooling trench met the standards specified by the Japanese 'Law for Environmental Health in Buildings'. The concentration of airborne bacteria inside the cooling trench was 2500 cfu/m³, exceeding the AIJ standard of 500 cfu/m³. However, the densities of airborne bacteria in the office spaces remained below this standard value. The airborne bacterial flora differed from the adherent bacterial flora, and the number of bacterial species that constituted outliers was larger in the airborne fraction than in the adherent fraction. Furthermore, bacteria of the genera *Pseudomonas*, *Lactobacillus*, *Nesterenkonia*, *Staphylococcus*, *Deinococcus*, *Acinetobacter*, *Enhydrobacter*, and *Corynebacterium* were detected at high frequencies from all the sites sampled in the present study. Notably, bacteria of the *Nesterenkonia* genus predominated on the surface of the cooling trench.

Acknowledgment

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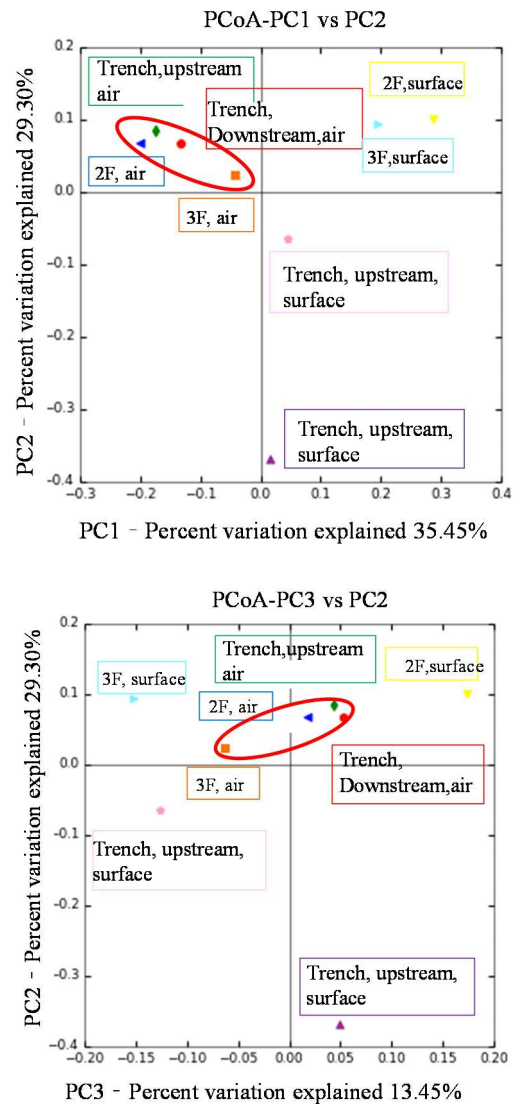


Fig. 9. Principal coordinate analysis results

References

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