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Actual measurement and analysis on microbial contamination in central air conditioning system at a venue in Dalian, China

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Abstract

Actual measurement and analysis were carried out on microbial contamination in central air conditioning system at a venue in Dalian. By studying the microbial contamination in two air handling units with different thermal environments, we found that the fungi and bacteria were common existing on the surface of filter, and the trend of cell density distribution was center > against the wall > corner; The microbial pollution associated in the dust and floating in the air was extremely serious. By comparing the two units, we observed that fungus concentration: Unit A > Unit B, and bacteria concentration: Unit A < Unit B,. And the *candida spp.* accounted for 80 percent of the sample in Unit A; while in Unit B the *cladosporium spp.* occupied up to 50%. At the end of the paper, according to the results of measurement and analysis, the methods of controlling microbial contamination in HVAC system have been proposed.

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Keywords: Central air conditioning system, Air handling unit, Microbial contamination, Actual measurement and analysis, Pollution control;

1. Introduction

More and more city buildings especially the buildings in public places have installed central air-conditioning ventilation systems to improve indoor conditions. However, if the air conditioning system did not get timely

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cleaning, indoor air microbial pollution could be caused by the dust in air conditioning unit equipment, duct in air-conditioning pipe, the suitable thermal environment and other reasons[4,6,9]. Existing research showed that the central air conditioning system with filter, heat exchanger, cold tube, humidifier, the condensate and cooling tower is easy for microorganism growing[1,3,5,10].About the sanitary condition of the air conditioning system, Based on the supervision and inspection of air conditioning and ventilation system in the public places of China's 30 provinces, municipalities and autonomous regions by Chinese Architectural Scientific Research Institute and the Chinese Center for Disease Control and Prevention it was found that more than 90% of the central air conditioning cannot meet China's national health standard[7]. According to the results of the survey in the US and Europe conducted by the U.S. Environmental Protection Agency and the Technical university of Denmark, indoor air biological pollution which caused by air-conditioning ventilation system accounted for 42% ~ 53% [11]. The United States institute of occupational safety and health survey also showed that the proportion of indoor air quality problems caused by the air conditioning system is as high as 50% [2]. Therefore, microbial pollution in central air conditioning system has become a critical issue in the field of indoor air pollution.

In this study, we took a stadium in Dalian as the research object. And the working condition of ventilation air conditioning system was in summer. First, the environment parameters were measured, and microorganisms existing on the filter, on the floor and in the air were collected. After the microbial density was tested and the genome sequence of collected microorganisms was identified, a series of statistical analysis were carried out on these results.

2. Measurement

2.1. Object of study

This study was carried out in September 2014. The object of study is a stadium in Dalian, which covers a total area of 36400 square meters and building area of 17320 square meters. The overground part includes a swimming pool, ball training venues, gymnasium, the lounge room and the clinic. Except them, the shed height of center gymnasium is 19 meters which covers the largest. The underground part consists of a table tennis hall, air conditioning equipment rooms and reservoir area. The whole building is centralized-controlled by the central air conditioning room, which includes two groups of air handling unit equipments: unit A for summer cooling conditions is above the ground, and unit B for winter heating conditions is underground. During the measurement, they were all closed during the sample collection. And the parameters of the two sets are shown in table 1.

Table1. The parameters of the air handling unit

The parameters of air handling unit			
Rated Air Volume (m^3/h)	42000	Fan Full Pressure(Pa)	1350
Current (A)/Tension (V)	568/330	Serial Number	020749
Rated Cooling Capacity(kw)	275.4	Rated Heating Capacity(kw)	310
Motor Power(kw)	30	Net Weight(kg)	3520

2.2. Equipments and materials

(1)Measurement

Tube, tube plug, medical gauze, tweezers, beaker, Petri dishes, Czapek's culture medium, Luria-Bertani culture medium, medical gloves, marker pen, MCH-383SD series of temperature, humidity and CO2 tester, HKM air planktonic microorganism sampler.

(2) Microbial culture

Vertical laminar flow clean bench, Petri dishes, Czapek's culture medium, Luria-Bertani culture medium, rake, Alcohol lamp, pipetting guns, medical alcohol, medical gloves, marker pens, a microbiological incubator. Specially, the Czapek's culture medium is used to separate fungi, and LB culture medium is used for cultivation of bacteria.

2.3. Measurement and Sampling

The actual measurement is divided into two parts: the environment parameter measurement and air unit sampling.

First, the temperature, humidity and CO2 monitor (MCH-383SD, Japan) was installed in air handling units. After the reading is stable, the temperature, humidity, and CO2 concentration were automatically recorded very two minutes. Second, the disinfected planktonic microorganism sampler (HKM series DW-20, China) was installed at the tuyere of filter. After installed the sampler, we loaded medium in the sampler and set the parameter of air flow rate in sampler as 2000L load medium, and the set the air flow rate to 2000L gathering the microorganism in the air. After the sample collection, the Petri dishes must be sealed for preservation. Finally, according to the "Health standard in public places central air-conditioning ventilation systems", we sample the dust by using sterile non-woven on filter surface and floor of unit A and B respectively. Each unit has four sampling points, each of them covers a 15 cm x 15 cm area at the sampling area, which are the center of the filter, against the wall, corners and on the floor.

Ensure the cleanliness of tube in the process of measurement, and confirm the sample will not be destroyed by producing error. Finally, label the collected microbiological sample, and put them in order for follow-up study.

2.4. Experimental procedure

The non-woven fabrics was put into sterile water and stirred to make sure that organic substances on the non-woven was fully dissolved in sterile water. Then, the sample of sterile water dissolved organic substances, 10 times dilution, and 100 times dilution were prepared respectively. The number of colonies was observed by dropping 100 μL of each samples on agar plates.. Each results were calculated from three parallel repeats.

2.5. Genome sequencing

Only a small part of the total microorganisms in ecosystem are culturable. Therefore, the traditional cultivation method could not harvest all the species in ecological samples. Macro genome sequencing is an emerging method to identify the microbial genome which could be directly from environment samples. Macro amplicon sequencing analysis was used in this study. Because the existing research showed that fungal spores are stronger than other microorganisms in the air [4, 8, 12], and fungi dominates the microorganism in air conditioning systems, the method is mainly used to identify fungi in this study. The process is shown in figure 1.

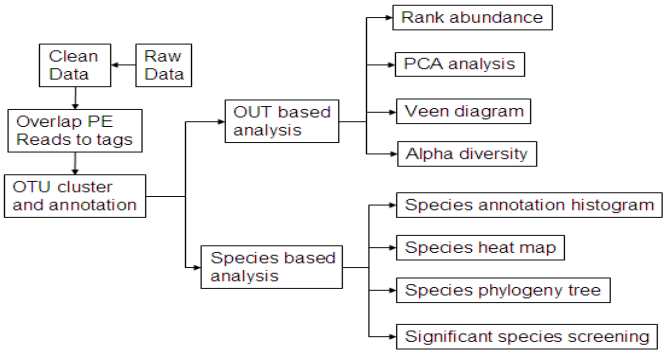

Figure1. Bioinformatics analysis pipeline of Amplicon Sequencing

3. Methods and Results

3.1. Environment parameters in air handling units

Temperature, humidity and CO2 concentration of Unit A and B are shown in table 2. This test about air handling unit environmental factor is gearing up for providing basic data analysis on microbial growth under thermal environment. Table 2.shows that Unit A is in a relatively low temperature and high humidity underground, and Unit B is in a relatively high temperature and low humidity on the ground.

Table2. Environment parameters

Condition	Unit A Closed	Unit B Closed
Temperature（℃）	23.7	25
Relative Humidity（%）	84.4	70.6
CO2 Concentration（ppm）	2282	1826

3.2. Microbial colony analysis

Labelled the samples on beef extract peptone agar and samples on Czapek's ager as MEDIUM-BEP and MEDIUM-CAS, respectively. And numbered the sampling point as 1(center), 2(against the wall), 3(corner), 4(floor), 5(air). The sampling points 1,2,3 are on the filter. In this study, the microbial colonies were classified as fungi and bacteria by using different culture medium for breeding.

(1)Fungi

Figure 2 shows the instance of fungal colony samples. Fungal colony formation density (cfu/ml) at each sampling point are shown in table 3. After conversion, the distributions of fungal density (cfu/cm2) in Unit A and B are shown in table 4 and figure 3.

Figure 2. Instance of fungal colony samples

Table3.Fungal colony formation density

Unit (cfu/ml)	A	B
1-center	280	150
2-against the wall	220	130
3-corner	170	120
4-floor	900	570
5-air	560	490

Table 4.Distributions of fungal colony density

Unit (cfu/cm2)	A	B
1-center	12.5	6.5
2-against the wall	10	5.5
3-corner	7.5	5
4-floor	40	25
5-air	25	22

Based on the results of fungal colony formation in Unit A and B, the fungi are widespread on the filter surface of the air handling units with the order of the centre >against the wall >corner. The comparison between unit A and B indicates that the fungal density in Unit A is more than that in the same sampling point in Unit B. Plus the environmental factors in these units, we found that relatively low temperature and high humidity may be more suitable for fungal growth.

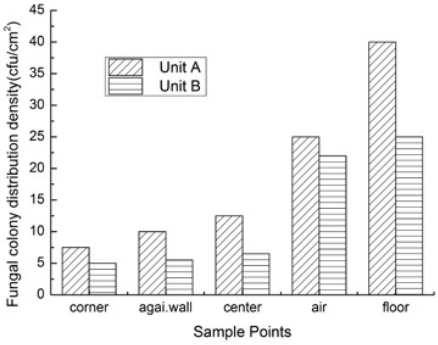

Figure 3.Distribution of fungal colony density (cfu/cm2)

If the air conditioning unit has not been running for a long time, breed a large number of fungus will breed on the filter surface. And if the cleaning and disinfection is not enough before the new run, it will make these fungus into indoor environment and make the pollution of indoor air. Compared with air conditioning filter, the fungus contamination is worse in the floor dust and the air suspension. During the actual measurement, it was found that the unit internal is unprecedented narrow and low intensity of illumination in a closed state. According to the description by technicians, it's easily to trample damage to the underground pipes, which resulting in the disinfection and cleaning work rarely in the unit.

(2)Bacteria

Figure 4 shows the instance of bacteria colony samples. Bacteria colony formation density (cfu/ml) at each sampling point are shown in table 5. After conversion, The distribution of bacteria density (cfu/cm^2) in Unit A and B are shown in table 6 and figure 5.

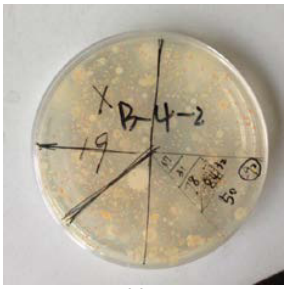

Figure 4.Instance of fungal colony samples

Table5.Bacteria colony formation density

Unit (cfu/ml)	A	B
1-center	20	60
2-against the wall	10	40
3-corner	0	4320
4-floor	780	18640
5-air	30	180

Table 6.Distribution of bacteria colony density

Unit (cfu/cm2)	A	B
1-center	0.9	2.6
2-against the wall	0.5	1.7
3-corner	0	192
4-floor	34.5	828
5-air	1.3	8

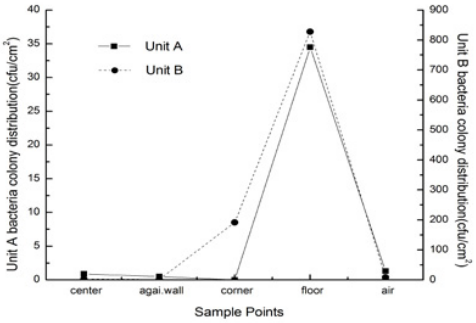

Figure 5.Bacterial colony distribution density (cfu/cm^2)

Based on the analysis on the results of Unit A and B, bacteria were widespread on the filter surface in the air handling units with the order of the centre >against the wall >corner. Except for the corner one in Unit B, which has reached to 192 cfu/cm^2. Interestingly, the bacterial density in the floor dust is extremely high. It should be paid more attention. The comparison between Unit A and B indicates that the bacterial density in unit A is less than that in the same sampling point in unit B. Plus the environmental factor in the units, we found that relatively high temperature and low humidity environment may be more suitable for bacteria growth.

3.3. Macro genome sequencing analysis

In this study, we analysed the samples from the sampling points A1, B1, and B2 by amplicon sequencing information analysis, respectively named A1A, B1A, and B2A.

(1) Principle component analysis (PCA)

PCA analysis is a method used for analysing data and simplifying data collection, which is often used to reduce data dimension and at the same time maintain the key features. e can differentiate the similarity between samples intuitively from PCA analysis diagram, if the closer distance between two samples, the more similar with them [13]. Through calculation, A1A-B1A=8.85, A1A-B2A=10.10, B1A-B2A=5.68 were shown in figure 6.

(2) Samples of microbial components

Figure 7 illustrates the species composition proportion of the three sampling points. Redrawing proportion by removing the strains which were not detected in the sample. The results are shown in table 7. The species with the largest proportion is the dominant fungi.

According to the macro genome sequencing analysis results (figure 6), fungi components in different units at the same sampling are different, and that in the same unit at different sampling points are roughly similar. They are caused by the different environmental conditions. On the center of filter in Unit A, candida accounted for 80%; on the center and against the wall of the filter in Unit B, cladosporium accounted for 50%, and accompanied by alternaria, emericella and other fungus. cladosporium is usually rich in outdoor air, but they will also grow on the indoor surfaces when the humidity is high. Existing research shows that the *cladosporium* spore is an extremely important allergen in the airborne transmission, which could cause asthma attacks or similar respiratory disease in patients with allergic reactions[14]. *Candida* is a kind of conditional pathogenic fungi in the human body.

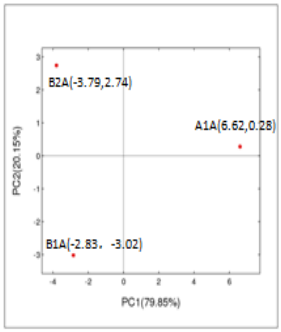

Figure 6. PCA analysis

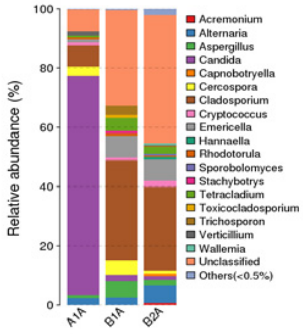

Figure 7. The taxonomic composition distribution in samples of Genus-level

Table7. Species composition proportion

	A1A	B1A	B2A
Alternaria	2.53	3.96	11.02
Aspergillus		7.95	3.34
Candida	80.08	3.10	2.51
Cercospora	3.40	7.26	2.01
Cladosporium	7.86	50.48	51.57
Cryptococcus			4.22
Emericella		11.07	13.12
Tetracladium		6.61	4.45
Trichosporon		4.44	
Others	6.14	5.11	7.75

4. Conclutions and Suggestion

This study selected the central air conditioning system at a stadium in Dalian as the object. Actual measurement was carried out on microbial pollution characteristic. In the two different working conditions of the air handling unit, samples were collected from the filter surface, on the floor and in the air. Combined with the analysis of environmental factors, the results are shown as below:

(1)Fungi and bacteria are widespread on the surface of air conditioning filter, and the trend of microbial densities is center >against the wall>corner. Compared with fungi, the bacterial density is much higher, and there is great difference between the distribution of the densities on different sampling points; Microbial pollution associated in the dust and floating in the air is more serious than that on the surface of air conditioning filter. In contrast, fungi may be more suitable to grow at a relatively low temperature and high humidity environment, while bacteria may prefer high temperature and low humidity environment.

(2) The *candida spp.* accounted for 80 % in Unit A, and the *cladosporium spp.* occupied 50% in Unit B. The two dominant fungus are both deleterious to health, so the timely maintenance and cleaning are required. Today the central air-conditioning system is widely used in buildings, we suggest improving the efficiency of the filter system, setting up microbial processing equipment, paying attention to with the effects of damp on the filter, and strengthening the timely air supply system maintenance and cleaning to build a green living environment.

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