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## **APPLYING PULSETEST METHOD TO DETECT THE POSSIBLE SOURCES OF BIOLOGICAL CONTAMINANTS IN ONE OFFICE**

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### **ABSTRACT**

The PulseTest method was used to detect the possible sources of biological contaminants in one office room. Different pressure conditions are created via closing and/or opening the air vents. This helps to examine the possible pollution sources as surface emissions, structural leaks emissions, supplied air and outdoor air origins. Microbial concentrations were determined with RCS Microbial air sampler (TC+SDX strips). One set of TC strips was incubated at 37°C to detect potentially pathogenic microbes. The other strips were incubated following supplier instructions. Particulates matter (PM) with a cut-off size of 2 µm were monitored. No moulds grew on the TC strips. Airborne microbial concentrations were very low (<35 CFU/m<sup>3</sup>) but correlated with the PM concentrations. It was difficult to detect sources of microbial pollution as concentrations were so low.

### **INTRODUCTION**

In Europe, many people have jobs where they spend 8-hour a day in an office room. Poor indoor air quality (IAQ) in the office building may severely affect workers health, comfort, and productivity. The presence of biological and chemical contaminations and inadequate ventilation are the leading causes of poor IAQ in office buildings /1,2/.

The occurrence of fungal contaminants in damp buildings is associated with allergy, asthma, rhinitis, bronchitis and respiratory tract infections /3/. When no apparent microbial growth can be observed, reliable detection methods are essential to locate the possible origins of contamination.

The PulseTest is a method that was previously applied to detect volatile organic compounds and particulate matter in one office room /4/. The sources of pollution were detected by creating different pressure conditions in the office room. The different pressure scenarios focused on emissions originating from surfaces, structural leaks, supplied air and outdoor air.

The study aimed at identifying the possible sources of biological contaminants in one office room using the PulseTest method.

### **MATERIAL AND METHODS**

The study took place in one office room of a four-storey building (built in 1959, renovated in 1997) located in the Helsinki capital region on 11.12.2020 (9h-16h). The 23 m<sup>2</sup> one-person office room (≈ 60 m<sup>3</sup>) with openable windows is ventilated with one

supply and one extract air vents (mechanical supply and extract ventilation system with F7 filter: efficient for particles  $\geq 1 \mu\text{m}$ ).

Outdoor conditions on 11.12.2020 (9h-16h) recorded by the nearest weather stations were: weather station 1 (one km west of sampling location, next to a busy road; one data point/hour) average  $\text{PM}_{10}$  concentration  $15.5 \mu\text{g}/\text{m}^3$ , average  $\text{PM}_{2.5}$  concentration  $9.3 \mu\text{g}/\text{m}^3$ ; weather station 2 (3 km west of the sampling location; one data point/ 10 minutes) average relative humidity 86% RH and average temperature  $-0,4 \text{ }^\circ\text{C}$ .

### The PulseTest

In the PulseTest, different pressure conditions are created to detect possible sources of pollution. The different pressure conditions are generated by closing and/or opening the air vents with ventilation valves. The PulseTest was performed in one day when the ventilation operates at full power. The office room was empty except during sampling and when changing the room's conditions. Particulate matter concentrations were continuously monitored (one data point/min). Airborne microbial sampling was performed using RCS® High Flow Touch at specific times (describe below). All the instruments were placed in the middle of the room with the sampling inlet at 1,5 m high (average adult breathing zone).

The PulseTest comprises five phases:

- **Phase I (normal operating condition)** represents exposure under the ventilation system's normal operating condition. The microbial sampling was done after 45 min. The air vents status was as follows: supply vent: open, extract vent: open.
- **Phase II (neutral phase):** It gives an "on hold" picture of the status of the biological load of the room (emissions from surfaces and everything that is in the room). The microbial sampling was done after 1h. The air vents status was as follows: supply vent: close, extract vent: close.
- **Phase III (negative pressure):** It represents emissions from structural leaks. The microbial sampling was done after 30 min. The air vents status was as follows: supply vent: close, extract vent: open.
- **Phase IV (positive pressure):** It represents emissions from the supplied air. The microbial sampling was done after 1h. The air vents status was as follows: supply vent: open, extract vent: close.
- **Phase V (window ventilated phase):** It represents emissions from the outdoor air. The microbial sampling was done after 15 min. The air vents status was as follows: supply vent: close, extract vent: open, and one opened window.

### RCS® High Flow Touch

Airborne microbial concentrations were determined using RCS® High Flow Touch (Merck, Germany) microbial air sampler and two types of strips. Tryptic Soy Agar (TC) strips were used for the total airborne viable count (pH 7,3; Tryptic Soy Agar, Soybean Casein Digest Agar, lecithin and polysorbate 80) and Sabouraud Dextrose Agar (SDX) strips were used for airborne yeast and moulds count (pH 6,9; Sabouraud Dextrose Agar with chloramphenicol and chlortetracycline antibiotics). Four TC strips (2\*100L, 2\*200L) and 2 SDX strips (1\*100L, 1\*200L) were collected at each phase. Field blank samples (2 TC strips, 1 SDX strip) were acquired in the middle of the PulseTest, and the

strips were inserted in the RCS without turning on the microbial sampler and incubated similarly to the samples. Outdoor samples of 50L were acquired at the end of the PulseTest (2\*TC strips, 2\*SDX strips). The TC strips were incubated in the dark for five days at 33°C (half of the strips) and 37°C (half of the strips) before counting. The SDX strips were incubated in the dark for seven days at room temperature ( $\approx 21^\circ\text{C}$ ). The sampling head was autoclaved before the PulseTest. During the PulseTest, the instrument was fixed on the tripod with the sampling head facing toward the ceiling.

### Particulate matter.

An aerosol monitor (MIE-pDR 1500 with 1-4  $\mu\text{m}$  cyclone inlet, ThermoFischer), set to 2  $\mu\text{m}$  cut-off size, represented the airborne concentration of fungal spores.

## RESULTS

### Airborne microbial concentration

Only yeasts/bacteria grew on the TC strips. No pathogenic mould strains were detected on the TC strips at 37°C. Concentrations were higher for the strips incubated at 33°C than at 37°C (cf. Table 1). Highest to lowest concentrations for the strips incubated at 33°C were as follow: II (neutral phase) > I (normal condition) > III (negative pressure) > V (window ventilated phase) > IV (positive pressure).

Table 1. Airborne total count (TC strips) during the PulseTest on 11.12.2020.\*

	concentration (CFU/m <sup>3</sup> ) after 5 days incubation at 33°C			concentration (CFU/m <sup>3</sup> ) after 5 days incubation at 37°C		
	for the 100L samples	for the 200L samples	average	for the 100L samples	for the 200L samples	average
phase I	40	15	27.5	10	15	12.5
phase II	30	40	35	10	0	5
phase III	30	5	17.5	10	15	12.5
phase IV	0	10	5	0	20	10
phase V	20	0	10	10	5	7.5
outdoor†	0 (50L)	0 (50L)	0	-	-	-

No growth on the TC field blanks strips. \*Only yeasts/bacteria grew on the incubated TC strips.

†outdoor sampling volume was 50L.

The SDX strips showed very low concentrations of yeasts and moulds (cf. Table 2). The concentrations during phases I-IV (<10 CFU/m<sup>3</sup>) were 3-10 times smaller than outdoor (phase V). Excluding window ventilated phase (phase V), the concentrations were similar during phase I (normal operating conditions), III (negative pressure), and IV (positive pressure), and the highest during phase II (neutral phase). The indoor/outdoor mould ratios (I/O ratio) were 1.

Table 2. Airborne yeasts and moulds (SDX strips) during the PulseTest on 11.12.2020.

	concentration (CFU/m <sup>3</sup> ) after 7 days incubation at RT*			I/O ratio‡
	for the 100L samples	for the 200L samples	average	
<b>phase I</b>	0	5	2.5	0.04
<b>phase II</b>	10	10	10	0.17
<b>phase III</b>	0	5	2.5	0.04
<b>phase IV</b>	0	5	2.5	0.04
<b>phase V</b>	20	45	32.5	0.54
<b>outdoor†</b>	20 (50L)	100 (50L)	60	-

No growth on the SDX field blank strip. The SDX strips contain chloramphenicol and chlortetracycline antibiotics. \*The room temperature was  $\approx 21^{\circ}\text{C}$ . †outdoor sampling volume was 50L. ‡ indoor/outdoor mould ratio.

### Particulate matter concentration

During the RCS sampling, the highest concentration of particulates with a cut-off size of 2  $\mu\text{m}$  was from window ventilated phase (phase V) (cf. Table 3). Beside phase V (window ventilated phase), the highest concentrations of particulates during RCS sampling were detected at phase II (neutral phase) and phase III (negative pressure). The lowest concentration was detected during phase IV (positive pressure).

Table 3. The concentration of particulates 1-4  $\mu\text{m}$  during the PulseTest on 11.12.2020.

	concentration ( $\mu\text{g}/\text{m}^3$ )*		
	average	STD	
<b>phase I</b>		4.35	0.13
	<b>during RCS sampling</b>	4.43	0.12
<b>phase II</b>		5.32	0.38
	<b>during RCS sampling</b>	5.69	0.16
<b>phase III</b>		5.37	0.23
	<b>during RCS sampling</b>	5.20	0.22
<b>phase IV</b>		3.71	0.56
	<b>during RCS sampling</b>	3.13	0.12
<b>phase V</b>		9.75	0.74
	<b>during RCS sampling</b>	9.89	0.22

Outdoor average PM<sub>2.5</sub> concentration 9.3  $\mu\text{g}/\text{m}^3$  (one data point/hour) from the nearest weather station (one km west of sampling location, next to a busy road) on 11.12.2020 from 9h-16h.

\* D<sub>50</sub>=2  $\mu\text{m}$  (1-4  $\mu\text{m}$  cyclone inlet).

### DISCUSSION

The concentration of airborne microbial contaminant was extremely low (<35 CFU/m<sup>3</sup>). However, it seems that excluding window ventilated phase (phase V), the highest particulates and mould concentrations originated from the room indoor air and its surfaces (phase II, neutral phase) and structural leaks (phase III, negative pressure).

The lowest concentrations of particulates and moulds during phase IV (positive pressure) were expectable as the supplied air is filtered from  $\geq 1$   $\mu\text{m}$  contaminants, and the pressure condition pushes contaminants away from the room. The absence of air

filtration caused increased particulates concentrations during phase III (negative pressure) and phase V (window ventilated phase).

There was some correlation between the concentration of particulates matter and the RCS sampling, the higher the PM concentration, the higher the microbial concentration. This seems consistent as most of the fungal spore have sizes between 2-5  $\mu\text{m}$  /5/.

The absence of mould strain growth on the TC strips (33°C) may indicate that no viable spores were collected due to the small sampling volume ( $\leq 200$  L).

Since there was no obvious source of contamination in the office room, the results' interpretation was difficult.

A few parameters may have influenced the RCS sampling results, too low sampling volume, sampling head oriented toward the ceiling. This study was limited to a one-day test during the winter season; however, the production of fungal spores varies with the season and the time of the day /6/.

## CONCLUSIONS

It was challenging to detect microbial pollution sources since airborne microbial concentration was as low as  $<35$  CFU/m<sup>3</sup>. However, airborne microbial concentrations were correlated with the PM concentrations. In the future, it would be meaningful to repeat at least two-day tests at different seasons and on a larger scale.

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