DEVELOPMENT OF AN INVESTIGATION METHOD FOR EVALUATION OF MICROBIOLOGICAL INDOOR AIR QUALITY

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ABSTRACT
An investigation method to evaluate microbiological air quality has been developed; it involved a compiling building inspections checklist and occupants questionnaires, choosing sites, sampling frequency and analysis procedures. This method has been applied in a representative building with HVAC system and open-space offices. Air samples and microclimate parameters were collected at the same time.

Airborne microorganisms levels ranged from 50 CFU·m⁻³ to 500 CFU·m⁻³. A high fungal concentration (>1000 CFU·m⁻³) was found associated with a winter flood event. Statistical analysis showed a significant seasonal and daily variation of airborne microorganisms. *Staphylococcus* and *Micrococcus* were the most common bacterial genera and *Penicillium* was the most widespread fungal genus. Samples collected at HVAC system levels demonstrated an overall good microbiological removal efficiency of the device, except for unusual events (flood).

This study highlights the importance, for air quality control, of a preventive approach based on microbiological risk analysis and critical point control.

INDEX TERMS
Bioaerosols, HVAC, airborne bacteria, airborne fungi, health effects

INTRODUCTION
Indoor air quality is becoming an increasingly important issue for occupational and public health (Clarke and Nikkel, 1995). Health effects related to indoor air microbiological quality have increased, with a tendency to some energy saving measures provided for buildings such as tightly sealing, air recirculation and introduction of HVAC (Heating Ventilation Air Conditioning) (Nathanson, 1995). In addition, ageing population, the increasing number of sensitive individuals and a tendency to spend more time indoors (90% of their time) further worsen this problem (Seltzer, 1995).

HVAC modifies air quality and can affect indoor microorganisms concentration (Parat, Perdrix, Fricker-Hidalgo, et al., 1997). Airborne microorganisms may be responsible for specific infections, Building Related Illnesses (Legionella’s disease, aspergillosis, hypersensivity pneumonitis, etc.) and can be involved in Sick Building Syndrome (Stetzenbach, 1997).
Currently, the problem is dealt using a retrospective approach by performing specific analyses in indoor environments following occurrence of complaints or deleterious health effects. The objective of this study was to develop a suitable method to enable preventive controls to be carried out inside buildings using HVAC systems, in order to avoid microbiological environmental problems. The protocol, which has been developed for the evaluation of microbiological risks, was tested in a case-study. Indoor air microbiological contamination and parameters that might influence indoor air microorganisms, were monitored, as well as HVAC influence, seasonal, weekly and diurnal variations.

METHODS

Investigation protocol
A protocol to evaluate microbiological risks related to indoor environment was developed both by considering the available literature (EPA, 1991; Clarke and Nikkel, 1995) and by a theoretical analysis of microbiological problems arising within buildings. This protocol was designed using a sequence of steps.

Case-study

Sampling site
The case-study was carried out in a representative office building (35 years old), located in a busy and suburban zone of Turin, with a central HVAC system and open-space offices.

The HVAC system was composed as follows: air removal from the ceiling via vent ducts, fresh outdoor air intake from the roof, and a duct that brings outdoor air to the Air Treatment Unit (ATU, 4 years old). In the ATU, fresh air (50% minimum) and recycled air were mixed and filtered (85% removal for ≥ 3 μm particles as ASHRAE 52-76), heated or cooled, humidified or dehumidified and distributed through a duct network to vent ducts placed on the false ceiling. Heating and cooling devices (fancoils) were distributed in the open spaces, underneath the windows, in order to heat or cool indoor recycled air. The windows were locked and building occupants could only regulate the fancoil by switching it on/off.

Microbiological analyses
Airborne bacteria and fungi were collected using an impactor sampler. Each sample was collected in triplicate, at a volume rate of 250 L for bacterial and 200 L for fungal counts. Total bacterial counts were performed on Trypticose Soy Agar (TSA) supplemented with cycloheximide. Rose Bengala agar supplemented with chloramfenicol was used for fungal load evaluation. Samples were incubated for 24 h at 37 °C and 48 h at 22 °C for bacterial counts and for 4-8 days at 24 °C for fungal enumeration.

Upon correction, based on the sampler manufacturer instructions, the mean value of the three samplings was calculated and results were expressed in colony forming units per cubic metre (CFU·m⁻³). The consistency of triplicate counts was evaluated using $\chi^2$ index to confirm the suitability of the mean value of the three measurements.

The most widespread bacterial colonies were identified using a method based on bacteria ability to metabolise 96 different carbon sources. Fungi were identified using standard taxonomic keys. Tap water and water samples collected in the air humidification tank of the ATU were analysed for Legionella spp. (ISO 11731, 1998) and total bacterial load (22°C and 37°C counts) (EN ISO 6222, 1999).
**Microclimatic evaluations**

Besides microbiological samples, microclimate parameters (temperature, relative humidity, air flow and comfort index) were also recorded. They were collected using a mobile environmental monitoring station which was equipped with a number of probes, according to ISO 7726 (ISO 7726, 1998): globe thermometer, psychrometer (with forced ventilation) and hot wire anemometer.

**Statistical analyses**

All the parameters considered in the case-study were analysed using Analysis of Variance (ANOVA) in order to evaluate whether bacteria (22°C and 37°C counts) and fungi indoor concentrations were related to some independent variables, namely season, day of the working week, morning or afternoon and microclimatic parameters.

**RESULTS**

**Investigation protocol**

Figure 1 shows the flow chart referred to the investigation method developed for microbiological risk analysis in indoor environment.

**Case-study**

As for the first two steps of the investigation model, the walkthrough in the building did not show any particular risk condition or actual problem; also, the occupants interview revealed a low percentage (5%) of aspecific complaints (occasional manifestations of sneezing, coughing, eye burning sensation). Consequently, it was decided to perform a preventive evaluation of indoor microbiological quality by setting up a monitoring program. Therefore, an investigation was carried out in three open-space offices, selected on the basis of their different position as to the cardinal points. Indoor air was sampled for microbiological analysis at 1,5 m from the floor, at the level of the workstation, in the centre of the open-spaces. At the same time, microclimatic parameters were examined too. In each site, air was collected twice a day (at 9.00 a.m. and 4.30 p.m.), three times a week (Mondays, Wednesdays and Fridays) and during different months (December 2000, March 2001, June 2001 and December 2001). Since an unusual flood occurred during winter sampling 2000, sampling was repeated in 2001. Furthermore, additional evaluations were performed once a week (on Wednesdays) in order to evaluate HVAC influence on indoor microbiological load and to control tap water microbiological quality. The ATU removal efficiency was analysed comparing bacterial and fungal load of outdoor air collected at the intake points and ventilation shafts. In the same day microbiological analysis were also performed on fancoil air flow to evaluate their role on air contamination and *Legionella spp.* and total bacterial counts at 22°C and 37°C were assessed in humidification water samples. Table 1 summarises weekly mean values (calculated from Monday, Wednesday and Friday sampling) of indoor microbiological contamination for the selected samples. Concentration values of bacterial and fungal load in all offices ranged from 50 CFU·m\(^{-3}\) to 500 CFU·m\(^{-3}\), pointing out medium-low contamination levels (Commission of European Communities, 1993). In contrast, during December 2000 a fairly high fungal contamination was recorded (>1000 CFU·m\(^{-3}\)). This finding could be associated to the flood occurred in that period.

Except for fungal air contamination measured during December 2000 flood, fungi and bacteria trend levels observed outdoors showed to be increasingly higher from winter to summer. Such results are consistent with those reported by other Authors (Pastuszka, Paw, Lis, et al., 2000).
Risk Assessment for Microbiological Contamination of Indoor Air

**Walkthrough** using a check-list information about:
- HVAC system
- hot and fresh water distribution system
- bioaerosol sources

**Occupant interview** using a questionnaire information about:
- location and type of complaints

**microbiological risk sources (??)** YES **symptoms or complaints (??)**

NO **is there an explanation for the causes (??)** YES

collect additional information:
- further inspection of risk sources
- managing of water distribution and HVAC systems
- development of hypotheses to explain problems

NO

**preventive evaluation**

microbiological analyses at different standard of investigation

**Figure 1.** Flow chart for microbiological risk analysis in indoor environment.

Air measured at the level of ATU air diffusers reveals a lower bacterial and fungal concentration than outdoors, showing a microbiological load removal efficiency ranging from 49% to 54%. This evaluation was obtained excluding the unusual sampling of December 2000 in which ATU was not able to remove outdoors fungal contamination (0%).

As for microflora identification, results revealed *Micrococcus* and *Staphylococcus* as the most widespread bacterial genera; *Penicillium* was the most common fungal genus followed by *Cladosporium, Aspergillus* and other few genera.

Microbiological contamination levels evaluated at fancoil air flow level were lower than indoor air values. This finding could lead to suggest that fancoils are not directly involved in indoor contamination but they simply determine air movement.
Table 1. Bacterial and fungal concentration (I = indoor air; * = means of values obtained on Monday, Wednesday and Friday; m = morning; a = afternoon; O = outdoor air)

<table>
<thead>
<tr>
<th>CFU·m⁻³</th>
<th>December 2000 Mean ± SD</th>
<th>March 2001 Mean ± SD</th>
<th>June 2001 Mean ± SD</th>
<th>December 2001 Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria 37°C</td>
<td>I* m 102 ± 65</td>
<td>97 ± 70</td>
<td>198 ± 76</td>
<td>253 ± 222</td>
</tr>
<tr>
<td></td>
<td>a 76 ± 26</td>
<td>65 ± 22</td>
<td>136 ± 77</td>
<td>158 ± 66</td>
</tr>
<tr>
<td>Bacteria 22°C</td>
<td>I* m 72 ± 52</td>
<td>120 ± 80</td>
<td>176 ± 44</td>
<td>271 ± 176</td>
</tr>
<tr>
<td></td>
<td>a 28 ± 17</td>
<td>129 ± 87</td>
<td>134 ± 95</td>
<td>160 ± 81</td>
</tr>
<tr>
<td>Fungi 24 °C</td>
<td>I* m 514 ± 426</td>
<td>321 ± 215</td>
<td>44 ± 20</td>
<td>49 ± 49</td>
</tr>
<tr>
<td></td>
<td>a 872 ± 870</td>
<td>43 ± 23</td>
<td>44 ± 27</td>
<td>39 ± 19</td>
</tr>
<tr>
<td>Bacteria 37°C</td>
<td>O a 114 ± 44</td>
<td>96 ± 17</td>
<td>284 ± 86</td>
<td>248 ± 53</td>
</tr>
<tr>
<td>Bacteria 22°C</td>
<td>O a 189 ± 12</td>
<td>352 ± 57</td>
<td>600 ± 127</td>
<td>308 ± 87</td>
</tr>
<tr>
<td>Fungi 24 °C</td>
<td>O a &gt;3268</td>
<td>145 ± 15</td>
<td>695 ± 145</td>
<td>115 ± 8</td>
</tr>
</tbody>
</table>

*Legionella spp.* was never found in tap and ATU humidification water. Total bacterial load was negligible in tap water, whereas a range between 10x10³ CFU/100mL and 150x10³ CFU/100mL was recovered in humidification water tank, with an increasing trend during hot season.

Microclimatic parameters measured in indoor air and comfort index (Predicted Mean Vote, Predicted Percentage of Dissatisfied) showed an overall condition of microclimatic comfort, except for a cold discomfort condition in summer season, which was due to a high level of air conditioning in operation.

**DISCUSSION**

Indoor/Outdoor ratios (I/O) were calculated on indoor (data not showed) and outdoor values obtained on Wednesday. I/O ratios show that fungal and bacterial at 22°C contamination is higher outdoors than indoors (0.28 ≤ I/O ≤ 0.80). On the other hand, I/O value close to 1 (0.82 ≤ I/O ≤ 1.13) underlines a higher bacterial count within the building than outside. This outcome is probably related to various internal sources, including human activities (Pastuszka, Paw, Lis, et al., 2000). The ANOVA shows a significant difference (p<0.0001) in bacterial and fungal indoor contamination related to seasonal sampling period.

Except for December 2000, ATU would seem to remove microbial load in normal climatic conditions, and hence outdoor air does not affect the indoor air microbial contamination (Parat, Perdrix, Fricker-Hidalgo, et al., 1997). Therefore, the recorded seasonal differences of indoor microbial load depends on various indoor factors, such as contingent environmental sources, humans activities, internal air circulation and, above all, an intermittent use of HVAC system, which varies according to the season. A relevant role in air movement might be attributed to building occupants personal choice to switch the fancoil on/off. Apart from this, no significant differences of microbial air contamination were pointed out during days of working week and among the open space offices. In contrast, a significant daily trend was detected (p<0.05): in fact, when contamination data recorded in the morning were compared to those gathered in the afternoon, the levels collected in the morning showed to be higher. Such a trend could be due to the air movement caused by the daily HVAC system being switched on in the morning and switched off at the end of the working day.
The preventive approach applied in this investigation method for indoor microbiological risk assessment seems to be useful to avoid microbiological contamination problems. In fact, by monitoring air biological quality when no complaints were reported, the ATU system proved to be unreliable whenever outdoor air is heavily contaminated (e.g. flood). It was also speculated that great care of the tank providing water for the ATU system should be taken during cleaning operation.

CONCLUSION AND IMPLICATIONS
This work shows the feasibility of a preventive approach developed for air quality control in buildings equipped with HVAC. Such approach is based on risk assessment and critical control points. Therefore, it is important to carry out preventive action within those buildings, in order to avoid occupant complaints or even health effects. This would be possible by performing microbiological risk analysis and by identifying critical points within the HVAC system. It follows that a control plan could be arranged for critical points (frequency and layout); it would be specifically based upon each building’s characteristics and its intended purpose. In addition to the above, the program would also improve the management and maintenance routine of ATU.

Although this approach may be initially more expensive and time consuming, it proves to be useful by preventing building environmental problems. This is important as, in the case-study, only the monitoring program revealed an inherent risk in two critical points of an HVAC system. In conclusion, the described approach, which strictly refers to biological problems in built environments equipped with HVAC systems, could be applied in indoor overall risk surveying, including physical and chemical hazards.

REFERENCES