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One-Way ANOVA Method to Relate Microbial Air Content and Environmental Conditions

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1. Introduction

During the past few years, people passed most of their lives indoors than ever before. While one part was spent in their homes, the other part was spent in their working environments, such as factories and offices.

As a consequence of spending their lives in these two environments, we found that people developed certain health-related symptoms, such as headache, fatigue, nausea and getting irritated with other people. When these symptoms detected in indoor environments, it is called sick building syndrome (SBS). On the other hand, when these symptoms are stronger and related to their workplace, it is called work risk hazard.

To identify these symptoms, sampling apparatus is employed. There is a growing interest in employing bioindicators that, after tests in the laboratory, can now be employed in real case studies.

In this chapter, a new methodology based on the statistical study of One-Way ANOVA was developed to test bioindicators, such as fungi, in real case studies. With this statistical study it was possible to relate bioindicators with indoor conditions like pets' presence, limited space and presence of localized humidity problems.

2. Problematic indoor ambiances

2.1 Sick building syndrome

The SBS is defined by the World Health Organization (WHO, 1983) as the occurrence of an increased prevalence of no specific symptoms among populations in determined buildings (Thörn, 1998).

Between its more important and common symptoms, we find eyes, nose and throat irritation, mental fatigue, headaches, nausea, skin irritation, irritability and lack of concentration (Hedge *et al.*, 1996; Raw *et al.*, 1996; Gupta *et al.*, 2007).

It is difficult to detect SBS due to its non-specific symptoms that appear with a higher prevalence than the expected value. Consequently, there is no clear consensus at the moment to define SBS and to determine if it is diagnosed by the exclusion of other causes.

On the other hand, subjective detection of SBS is being analyzed in recent research works. This detection is based on surveys and questionnaires about the perception of occupants of an indoor environment, such as that developed previously to analyze indoor thermal comfort (ASHRAE, 2003). All the subjective and objective parameters are analyzed in depth below.

As stated above, there are some parameters that can define SBS. To control these parameters like indoor air temperature, relative humidity and dust, amongst others, are some of the questions that must be asked.

The first question is if there is a need to employ any natural or mechanical ventilation. After a review of recent research works on this topic, it was concluded that there is a prevalence of some symptoms when the mechanical ventilation is working.

These symptoms are, for example, some upper respiratory problems and fatigue. The results showed that these effects can be related to the dust deposition in ducts, humidifiers and chillers of the HVAC system. On the other hand, the air remains clean when it is introduced into the house through natural ventilation, such as doors and windows.

The corrective actuation way to relax these symptoms is by HVAC system corrections and building design improvements. For example, recent research works like that of Kolari *et al.* (2005) showed that duct cleaning will not imply an improvement of indoor air quality, but is related to an improvement of indoor perception of air quality and its relation to nasal symptoms. Furthermore, a clear reduction, in the building, of the volatile organic compounds (VOC), carbon dioxide (CO₂) and fungal spore concentrations reach values below the actual after a duct cleaning process.

At the same time, the percentage of outdoor air that can be introduced into the indoor ambience must be considered. In this sense, it is very important to remember that, in the last few years, to reduce the energy consumption in HVAC system the air changes of indoor air was reduced (Osayintola & Simonson, 2006); consequently, a percentage of indoor air returns to the same indoor ambience mixed with outdoor air with a corresponding increment in CO₂ and moist air humidity.

We must consider that air changes are due to mechanical ventilation and infiltrations of outdoor air. In this sense, the mechanical ventilation must be improved and buildings must be less airtight. What is more, an increment in air changes of indoor air implies an improvement of indoor air quality, as reported by Haghighatt & Donnini, 1999.

Finally, outdoor air is not the only source of contaminants of indoor environments. Other parameters, such as indoor materials contamination must be considered as a source of pollution.

Once the objective parameters to detect the SBS (WHO, 1989) are defined, it is the right moment to define the subjective parameters. Among the main subjective parameters, we must consider job stress and perception of the indoor air quality.

Studies by Hedge *et al.* (1996) showed a clear relationship between SBS and working conditions than with environmental parameters. Furthermore, parameters such as type of job can influence the perception of SBS.

The other parameter, which is perception of indoor air quality, must be considered. In this sense, we find that the perception of indoor air quality depends not only on combustion gases, VOC, dust, particles and but also on parameters like mucous membranes temperature and humidity that can alter these perceptions (Salonvaara & Simonson, 2000 and Simonson *et al.*, 2001). Other parameters such as air movement perception can alter this situation too.

Finally, to control the subjective parameters, we can employ questionnaires that are recognized as a more important tool to define the relationship between objective and subjective perception.

These surveys must cover an area for objective parameters that must be sampled, such as indoor air temperature and relative humidity and, at the same time, the same survey must

be present in another region for the subjective parameters that must question the occupants of a building, such as indoor air perception and job stress.

The SBS detected by symptoms or by some bioindicators must be corrected. In other words, we must consider that SBS is related with building construction and not necessarily with the occupants. Consequently, parameters like air temperature, relative humidity and dust are sampled to define the SBS.

2.2 Objective detection of indoor air quality

Despite the fact that indoor air quality can be sampled with different apparatus like multi-gas samplers, it was learned that, in the past few years, there is an increasing interest in employing some natural indicators and monitors because they present some advantages with respect to most of loggers.

The main advantages of these indicators are based on the fact that they do not need any kind of calibration or energy source. It is the work of fungi to evaluate indoor environments and mosses and lichens to evaluate outdoor ambiances.

From these concepts, we do an initial definition of bioindicator and biomonitor. A bioindicator is an organism that can be used for identification and qualitative determination of human-generated environmental factors.

At the same time, we can define accumulative bioindicators which have the ability to store contaminants in their tissues and are used for the integrated measurement of the concentration of such contaminants in the environment, as a result of the equilibrium process of biota compound intake/discharge from and into the surrounding environment.

Biomonitors are organisms used for the quantity determination of contaminants and can be sub-classified as sensitive and accumulative. The methodology based in biomonitors present the problem of the need of a background level of this contaminant in the environment objective of study.

3. Detection of indoor and outdoor air quality

3.1 Fungi

Owing to its feasibility to be employed in a real case study, once defined, a few examples of biomonitors and bioindicators are explained below. To sample outdoor ambiances, we can employ mosses and lichens, and to sample indoor ambiances we can employ fungi.

Despite the fact that fungi develop in nature some functions, such as recycled energy and nutrients, most of these tasks are not adequate if it is to be developed in indoor environments.

It is due to fungi being related to spores, fungal fragments, mycotoxins and VOCs emissions is the reason for the failing health of the occupants. In this sense, nowadays we find indoor environments that present higher VOCs concentration than in years before and related to the fact that building designs were modified in accordance with energy saving, and consequently, buildings are more airtight and present a low number of air changes.

When most researchers tried to find where fungi were located in indoor environments, the results showed that it developed on walls, roofs and in materials wherever it can be find dust to develop a growing media.

This fungi development increased with higher air temperatures and relative humidity values of over 75%. This is the higher value of relative humidity that must never be passed to prevent fungi developing in indoor environments.

At the same time, fungi emit mycotoxins that are low molecular weight compounds and toxic for animals and men (Cabral, 2010).

3.2 Mosses and lichens

To analyze outdoor air quality, bioindicators, such as lichens and mosses, are employed due to they present some advantages respect traditional sampling methods. For example, these bioindicators were selected as they do not present any seasonal variation and their longevity. Consequently, these bioindicators let us sample indoor conditions for long periods without calibration and without any kind of energy source, which is a clear advantage respect traditional loggers.

Lichens are defined as a symbiotic association (Newbound *et al.*, 2010) of a fungus and an alga, and can be employed to develop a map of all the species detected in a sampling area. Another method is basically in the sampling process of pollutants in the thallium of the lichen.

Other methods are based in the transplantation of native lichens to a place where it will be killed by pollutants after a reduced period of time. Finally, new methods are being developed to define the climate change with different lichens sampling processes over calcareous rocks.

As a function of previous sampling processes, two indexes can be defined based in lichens measurements. The first index is called the index of atmospheric purity and the second is the index of poleotolerance.

Mosses are employed to define, at the same time as lichens, outdoor air quality (Szczepaniak & Biziuk, 2003). Mosses present some advantages as bioindicator as, for example, it can be employed in different regions due to their growth in different environmental conditions, such as industrial and urban areas.

Another advantage of mosses is based on the fact that sampling process is cheap and simple. It will allow a very large number of sites to be sampled, obtaining a better sampling map. At the same time, these natural indicators present some disadvantages. For example, to develop a comparative study with mosses and lichens, the same species of lichens and trees are needed to obtain adequate results to do a comparative study.

On the other hand, fungi growth in indoor environments depends on indoor temperature and relative humidity in all zones of the building. Consequently, one of the methods to control fungi growth in indoor ambiances is to control occupants' habits. For example, most researchers proposed an increment in the air changes during cooking in the kitchens, in the bathroom during mornings and in bedrooms during the nights. This increment in air changes can be obtained by natural ventilation through open windows.

4. Practical case study in flats

Recent research works is related to airborne microorganisms with some infections or allergic disorders (Parat *et al.*, 1997), and some epidemiologic studies have been related to dust mite exposure with some degree of asthma. In general, however, we can say that in ambiances with higher allergen exposure we find higher asthma prevalence (Liu, 2004).

Despite this, there is not always a relationship between fungi development and asthma in children. Consequently, some researchers reached the conclusion that, with actual methodologies, it cannot be done (Jovanovic *et al.*, 2004).

Nowadays, there is very little information on how to prevent allergies, such as environmental hygiene, avoidance of some foods and prevention of contact with some kind of pets.

However, things are more complicated and not the same for all allergens. Although increased exposure to house dust mite allergen is paralleled by increased sensitisation rates, the same is not true for cat allergen.

One possible explanation for the different effects of different allergens may be their biochemical properties: mite allergens, in contrast to cat and dog allergens, contain proteolytic enzymes. It has been shown that, concerning house dust mites, a low-allergen environment can be achieved (Lauener, 2003).

To summarize, we can say that new standards are needed to show the better methodology to sample indoor ambiances and define the effect of indoor pollutants over health to reduce sensitization to these parameters (Lauener, 2003).

Other factors like distance of the building from the source (a nearby park) and supermicrometre particle concentrations will be associated with the concentration levels of fungi in indoor ambiances of occupied buildings (Hargreaves *et al.*, 2003).

At the present time, the only way to guarantee lower mite allergen levels in modern homes in the western world is to remove the carpets and to encase the mattresses and beddings. Furthermore, to reduce this exposure, we must improve IAQ.

The three primary considerations in improving IAQ are (1) evaluation of construction failures that allow moisture into the walls and roofs, (2) poor ventilation, causing excessive humidity and accumulation of gaseous and/or chemical exposure from materials in the living space, and (3) poorly designed or failing HVAC systems that contribute to poor air calculation.

About the two last points, some authors (Parat *et al.*, 1997) have analyzed that massive proliferation of microorganisms may take place in HVAC unit with certain risk factors, such as low efficiency filters, cold mist humidifiers using water recycling, areas in which condensation water remains stagnant, large recirculation of air and faulty or deficient maintenance conditions.

They demonstrated that compared to a naturally ventilated building, a HVAC system which is well designed and well maintained improves the microbiological quality of indoor air and, in consequence, can reduce health hazards for its occupants.

Finally, not all indoor allergens are necessarily equal in their propensity to cause asthma and its related health effects like shortness of breath and coughing. For example, using dust allergen concentration as a proxy for exposure, recent studies have revealed that indoor cockroach allergen exposure, but not mite or cat allergen exposure, is a significant risk factor for asthma (Hens, 2007).

Despite the fact that the data collected on household characteristics varied greatly between the studies and that building materials techniques are very different in different parts of the world, some common themes have emerged.

For example, concentrations of moulds varied hardly between areas (Jovanovic *et al.*, 2004), and neither climatologically conditions nor differences between urban and rural regions exhibited a systematic influence.

In another example (Perfetti *et al.*, 2004), no association was found between the concentration of mite allergens and the environmental characteristics (geographic location, floor above ground, type of ventilation) and no correlation was found between indoor humidity and allergen levels.

We must consider the fact that house dust mites live in an environment where there is no liquid water, and they are dependent on the ambient humidity to absorb water from the atmosphere. To get this, water dust mites can gain it by diffusion through the body or

extract the water vapor from air via hygroscopic crystals in their supracoxal glands, located at the base of their first pair of legs.

The optimum relative humidity for mite growth is 75–95%, at temperatures of 15–30°C, whereas above 70% relative humidity conditions may be optimal for fungal growth (Liao *et al.*, 2004). Relative humidity has a major influence on the survival of mite colonies and therefore levels of mite allergens.

Although laboratory and early field studies suggested that there was a strong relationship between relative humidity and mite allergen levels, this had not been conformed by more recent large-scale studies when other factors have been considered in a multivariate analysis.

For example, freezing and/or dry weather can damage fungi and reduce the spore counts on outdoor samples, but the conditions indoors may be very hospitable to fungal growth non-seasonally (Zhou *et al.*, 2000).

From these studies, we can conclude that outdoor relative humidity influences indoor relative humidity, but other household factors can influence mite allergen levels and that means allergen levels in different geographical areas tend to be influenced by the local climate.

As a result of this, novel techniques have been developed recently, which allows measurements of relative humidity to be made within the mite microhabitat, that is, where it matters, in the depth of the carpet or mattress.

This has revealed that the relative humidity in the carpet may be higher than that in the room air, and that with different types of construction, the differences between room RH and floor RH will vary. This suggests that the relative humidity in the room air does not necessarily reflect the RH in the micro-habitat of the mite – in the depth of the carpet pile.

The object of this chapter was to get information about Spanish apartments' microbial levels and relate it with their characteristics. Results will be useful to get a healthy home, taking into account costs versus energy saving, and improve health outcomes (Bernstein *et al.*, 2008).

4.1 Materials and methods

4.1.1 Apartments

In our case study, different apartments located in the northwest of Spain were selected, in accordance with different criteria defined to obtain realistic comparisons between indoor ambiances.

The first criterion is that, in all apartments, the residents presented some kind of health problems related with the relative humidity, which is typical in this area. The reason for this work is to relate health problems with indoor conditions, in building constructions' humid areas and occupants' habits. In particular, fungi and bacterial growth were sampled in these indoor environments.

All apartments present natural ventilation to remove all indoor air. Despite this, other mechanical ventilation system was located in toilets to reduce the humidity released during bath.

The heating system consists of heat water radiators and is employed only for a few months in the winter season. On the other hand, there is no cooling system, as the temperatures during the summer season are not too high. Consequently, during summer ventilation is enough to reduce indoor temperature.

To obtain adequate comparative results in apartments, sampling process was developed in accordance with the daily life conditions indicated by occupants. Furthermore, all buildings present the same construction and located in the same city. Consequently, outdoor weather conditions were the same for all the buildings.

Outdoor weather conditions were sampled by some weather stations located in a representative zone of the area where the buildings are located. This sampling process was developed by weather stations from MeteoGalicia (2002), with a sampling and frequency of 10 minutes.

On the other hand, humidity and temperature were measured by a 1221 Datalogger, with sensors of temperature and relative humidity, and tinytag Plus 2 dataloggers were employed.

These loggers were located in each apartment, in accordance with the ISO Standard indications. In particular, each sampling point was separated from heat sources conditions and as near as possible of center of gravity each room to obtain representative values of indoor

As explained earlier, a microbiological analysis of indoor ambiances was done. Consequently, two culture media were employed: Trypticase Soy Agar to find the total number of bacteria and Malt Agar was used to define fungi growth.

4.1.2 One-way ANOVA

To compare sampled mean values of temperature and relative humidity for fungi and bacteria, a statistical study of one-way ANOVA was done. This statistical study consists in an analysis of the variance of one factor for a significance level of 0.05.

Furthermore, different statistical studies, such as Duncan and Student-Newman-Keuls *post hoc* analyses, let us define groups of apartments that present the same condition for this level of significance.

In this study, two assumptions were defined. The first is based on the fact that the dependent variable is normally distributed. The second is that the two groups have approximately equal variance on the dependent variable.

In the same study, two hypotheses were considered. The hypothesis null is that there are no significant differences between the groups' mean scores. The alternate hypothesis is that there is a significant difference between the groups' mean scores. Finally, to develop this task, the statistical software SPSS 11.0 was employed. More information on how to employ this software SPSS can be found in their website (SPSS).

5. Results and discussion

At this point, the main results obtained are shown. In particular, Table 1 shows us the main indoor and outdoor air temperature and relative humidity of 25 apartments during the sampling process.

Despite this, we must consider the fact that this table shows the main value of sampled data, and consequently, conclusions about instantaneous values at different hours cannot be obtained from this table.

From Figs. 1, 2 and 3, we can conclude that Coruña, located in the northwest coast of Spain, presents a mild climate. In this sense, we can see that outdoor temperature is not too high in summer and too low in winter season. Mean temperature values of 11°C during winter and 16°C during summer, respectively, can be expected.

Flat	tindoor (°C)	RHindoor	toutdoor (°C)	RHoutdoor
A	20.97	63.45	16.72	74.6
B	24.09	65.09	17.76	88.8
C	19.42	62.1	14.12	82
D	20.38	64.87	17.7	59.6
E	21.46	63.59	17.7	62
F	23.43	65.03	22.28	66.8
G	22.2	70.92	16.24	94
H	19.92	63.73	15.64	75
I	21.24	49.62	18.08	45
J	23.78	55.7	17.76	47
K	25.11	48.09	17.6	58.8
L	23.63	65.58	20.04	73.2
M	22.37	67.19	19.28	78.4
N	21.74	63.65	15.6	74.76
O	24.05	50.31	15.2	74.2
P	20.29	59.22	12.08	88
Q	20.32	62.23	11.84	88
R	20.1	62.66	14.4	89.2
S	17.4	69.88	14.4	87.4
T	19.42	64.79	16.6	72
U	21.13	61.1	15.8	76
V	22.63	65.22	21.4	59.4
W	25.12	61.18	21.64	64.4
X	19.14	64.56	14.84	78
Y	23.83	65.65	21.5	60.8

Table 1. Indoor/outdoor sampled variables.

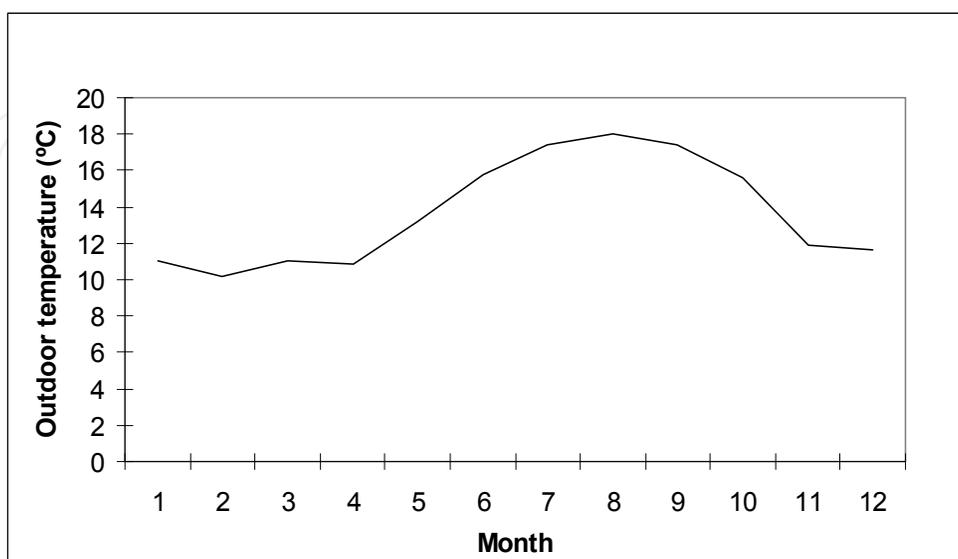


Fig. 1. Outdoor temperature.

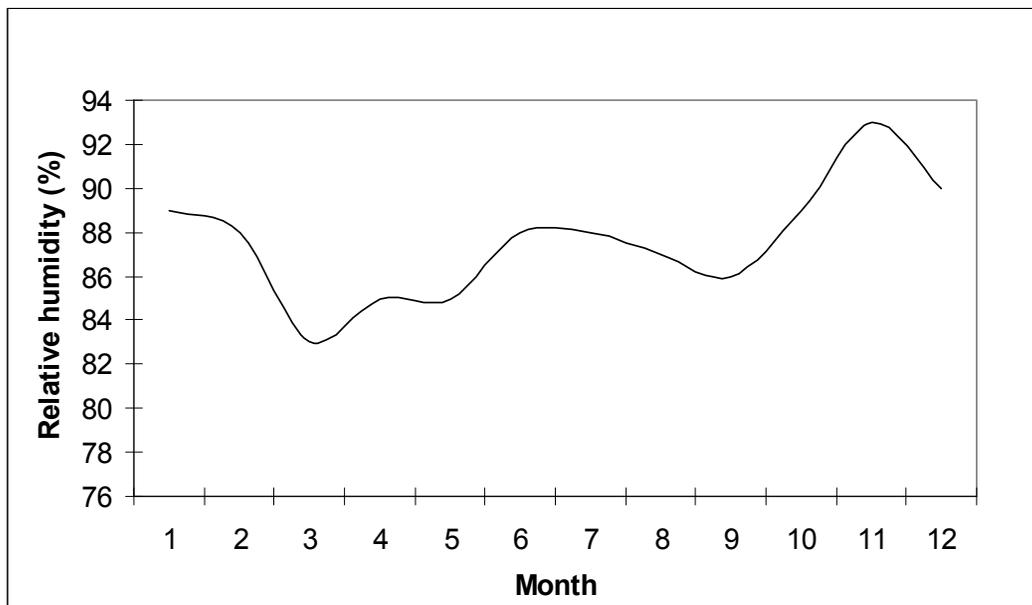


Fig. 2. Outdoor relative humidity.

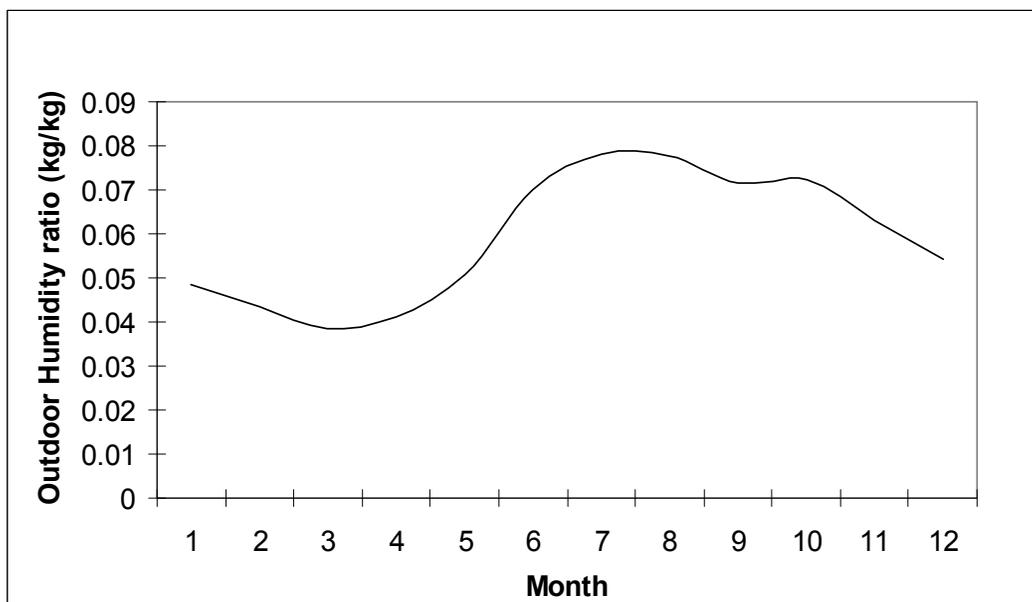


Fig. 3. Outdoor humidity ratio.

On the other hand, outdoor relative humidity showed mean values between 83% in March and 93% in November. Consequently, mean outdoor relative humidity of 86% is obtained throughout the year, as we can see in Fig. 2.

Finally, another way to show the relationship between temperature and relative humidity was to express the outdoor air humidity ratio, as we can see in Fig. 3. This humidity has shown yearly values between 0.04 and 0.08 kg of water per kilogram of dry air.

This high humidity is incremented with different indoor moisture sources. This increment of humidity ratio under temperatures of 21°C will imply relative humidity values over 75% in some daily life periods, as we can see in Fig. 3.

From Table 1, we can conclude that the northwest of Spain present apartments with an indoor mean relative humidity about 62% and temperature of about 21.7°C. Thus, value is relatively high, but not excessively high for a coastal area. However, due to an indoor relative humidity of not more than 75% with an adequate cleaning procedure, development of fungi can be prevented.

Flat	Characteristics
A	Pet
B	Normal
C	Normal
D	Limited space
E	Normal
F	Humidity problems
G	Humidity problems
H	Limited space
I	Normal
J	Pet
K	Humidity problems
L	Limited space
M	Normal
N	Humidity problems
O	Normal
P	Normal
Q	Pet
R	Normal
S	Normal
T	Humidity problems
U	Normal
V	Normal
W	Humidity problems
X	Normal
Y	Normal

Table 2. Observed characteristic.

At the same time, temperature and relative humidity were sampled and different characteristics of each apartment flood were considered (Table 2). This Table shows us parameters like pets' presence, limited space and presence of localized humidity problems in the walls and roofs that were considered.

Finally, if none of previous commented parameter was detected, then the apartment was considered normal.

Once Tables 1 and 2 have shown us the main value of temperature, relative humidity and apartment characteristics, it is the right moment to show the results of fungi and bacteria growth, as we can see in Figs. 4 and 5.

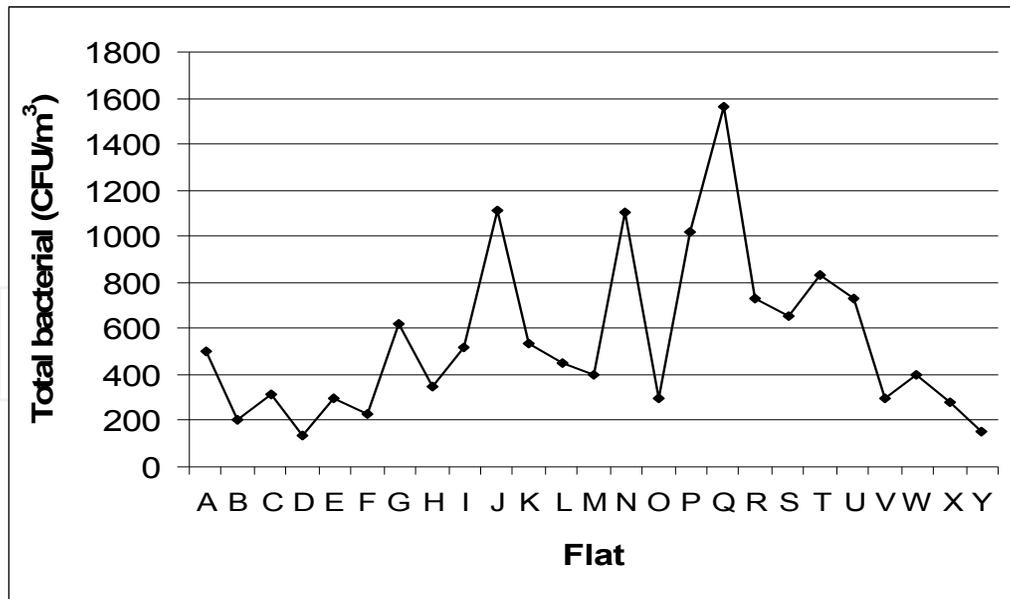


Fig. 4. Total bacteria sampled (CFU/m³).

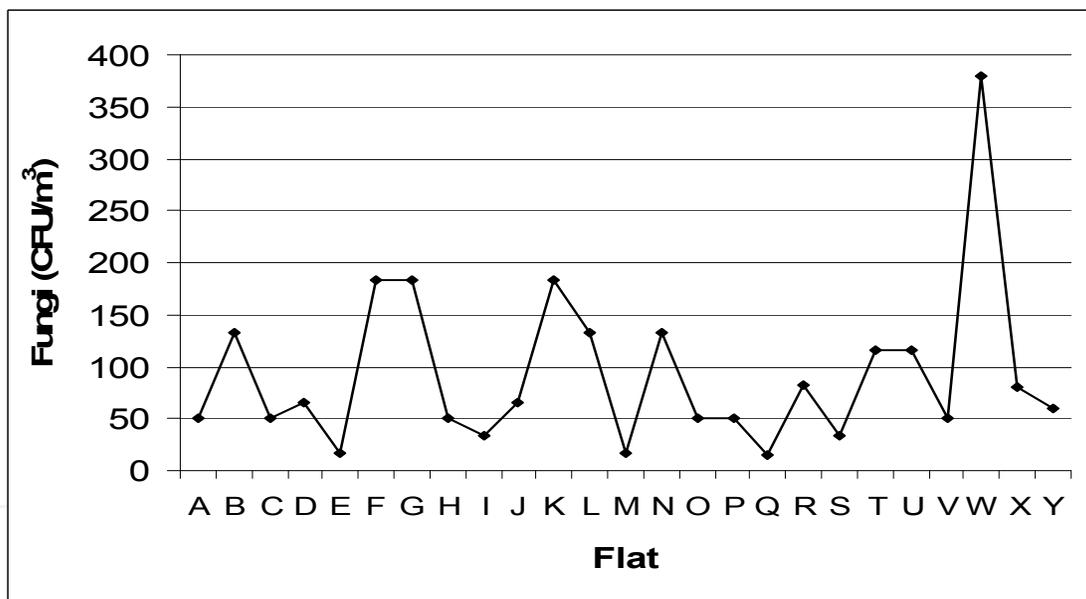


Fig. 5. Total fungi sampled (CFU/m³).

After fungi and bacteria growth in these indoor environments are sampled to relate these pollutants with indoor relative humidity, like in most of laboratory studies (NTP 335, 2008), it is necessary that this is applied to real case studies. In particular, in this chapter a relationship between fungi and bacteria with the particular parameters detected in each building, reflected in Table 2, was proposed.

From this study, we see that there is no possibility to obtain an adequate linear regression between humidity and fungi reflected by a correlation factor below 0.9, see Figure 6.

It is related with the fact that, in real buildings, there are other parameters that does not influence in most laboratory studies, but in real case studies it can alter the situation. It is the case of the presence of pets, moisture sources and moisture-damaged walls and roofs.

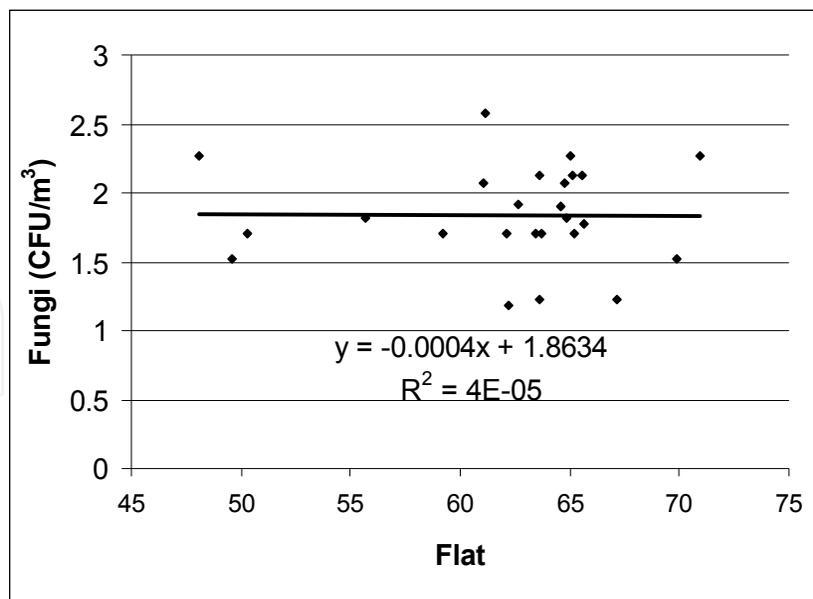


Fig. 6. Fungi linear regression.

To relate these indoor conditions with sampled indoor parameters, it was proposed to develop one-way ANOVA analyses with different *post hoc* studies to define which groups of indoor environments experience the same evolution with time.

In particular, the Duncan *Post hoc* analysis with mean total bacteria and fungi was developed as we can see in Tables 3 and 4.

From the one-way ANOVA analysis, we can concluded that apartments having pets showed the same indoor air bacteria evolution with time and, consequently, can be separated as an independent group, as we can see in Table 3.

A	J	Q	B	C	E	I	M	O	P	R	S	U	V	X	Y	D	H	L	F	G	K	N	T	W
Group 1: Pets			Group 2: Normal, limited space and humidity problems																					

Table 3. One-way ANOVA and Duncan *post hoc* with mean total bacterial (CFU/m³).

A	J	Q	B	C	E	I	M	O	P	R	S	U	V	X	Y	D	H	L	F	G	K	N	T	W
Group 1: Normal, limited space and pets															Group 2: Humidity problems									

Table 4. One-way ANOVA and Duncan *post hoc* with mean fungi (CFU/m³).

On the other hand, it was concluded that there exists a clear different indoor air fungi developed in apartments that present some humidity problems on walls and roofs with respect to others, as we can see in Table 4.

6. Conclusions and future research works

This research work tried to relate indoor air conditions with fungi and bacteria growth. In this sense, objective and subjective parameters were considered. So, parameters like indoor

and outdoor temperature and relative humidity were sampled in relation to bacteria and fungi growth. At the same time, parameters such as presence of pets and humidity problems in walls and roofs were considered too.

The results showed us that it is not easy to relate fungi growth with indoor air relative humidity like in laboratory studies. It is owing to the fact that there are some factors that can alter this situation. Furthermore, pets' presence was related to the increment in bacteria in indoor air, and humidity problems were related with fungi developed in a statistical way.

In conclusion, we can say that one-way ANOVA is an interesting tool to be employed by engineers to approach real case studies with laboratory conclusions.

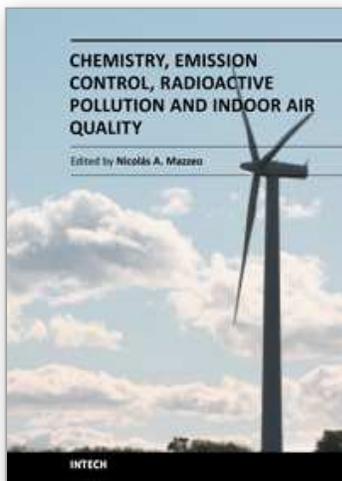
7. Acknowledgement

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The atmosphere may be our most precious resource. Accordingly, the balance between its use and protection is a high priority for our civilization. While many of us would consider air pollution to be an issue that the modern world has resolved to a greater extent, it still appears to have considerable influence on the global environment. In many countries with ambitious economic growth targets the acceptable levels of air pollution have been transgressed. Serious respiratory disease related problems have been identified with both indoor and outdoor pollution throughout the world. The 25 chapters of this book deal with several air pollution issues grouped into the following sections: a) air pollution chemistry; b) air pollutant emission control; c) radioactive pollution and d) indoor air quality.

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